
# *Proceedings of 24th Asian-Pacific Weed Science Society Conference October 22-25, 2013, Bandung Indonesia*

## *"The Role of Weed Science in Supporting Food Security by 2020"*

*Baki Hj Bakar*
*Denny Kurniadie*
*and Soekisman Tjitrosoedirdjo*
*(Editors)*

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*in Collaboration with*
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# PREFACE

Very warmth welcome to the 24th APWSS Conference, Bandung, Indonesia. We sincerely hope that you will have a very productive and intellectually stimulating conference week. With delegates from 17 countries, and a wide range of presentation topics and materials, we are confident that this conference will serve as an excellent platform to exchange ideas and develop research links and promote cooperation among delegates within the weed science fraternity.

The conference theme ”The Role of Weed Science in Supporting Food Security by 2020” should display a strong bond and meaningful tri-partite cooperation between the Asian Pacific Weed Science Society (APWSS), Faculty of Agriculture, Padjadjaran University, Bandung and the Weed Science Society of Indonesia (WSSI). We are all aware that food security, or rather food insecurity and food safety are high on the agenda among the key issues and priorities among policy makers, food industry players, agriculturalists and agriculturists alike globally, and in the Asia Pacific, in particular. This is the 3rd time that Indonesia is hosting the APWSS conference, since the last one in 1991 in Jakarta

We also hope that you will make effort to maximize opprtunities for networking, for the benefit of weed science development in region.

We wish to thank Buddhi Marambe, Michael Renton, Diedre Lemerle, Abul Hashem, Steve Walker, Edison Purba, M. Chozin, Marwat Khan, C. Chinnusamy, A.N. Rao, N.T. Naduraju, Y. Fujii, Hisashi Kato-Noguchi, Soekisman Tjitrosoedirdjo, Aurora Baltazar, A. Abeysekera, A.R. Sharma, and Do-Soon Kim, *inter-alia,* for helping to review the papers.

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## PROFILE OF APWSS FELLOW

### *Emeritus Prof. Dr Muhammad Soerjani*

Muhammad Soerjani was born on 15th October 1932 to a family of teachers in Tulungagung, Java. Like many parents in the pre-independence Indonesia, his father was so proud of him and had a high expectation of him to become an engineer like Soekarno, his father's idol, the first president of Republic of Indonesia. His beloved mother passed away in his early childhood, and his schooling was shouldered by his big family, in Madiun, Blitar, Malang and Tulungagung. He completed his secondary education, albeit a little late, due to the struggle for independence followed by the war of attrition, in 1952. The political situation at that time demanded him to defend the Indonesian independence and as a patriot, he joined the army. He, then, enrolled in the Faculty of Agriculture, Gajahmada University. While he was a student he made himself available as a volunteered teacher at the Agricultural Vocational School in Palembang, South Sumatera. His volunteering work earned him a recognition from the Department of Agriculture, sequencially issuing him a decree installing him as a government employee. In 1960 he won a scholarship to complete his university education at the Faculty of Agriculture, he went back to Gajahmada University, graduating in 1964. While as a wasan engineer (an Indonesian title of agricultural degree) was alright for Muhammad Soerjani, but not as a civil engineer as expected by his father. Muhammad Soerjani was active in student's movement, being a member of Students Guild, and an editor of university's student digest.

Upon his graduation from Gajahmada, Muhammad Soerjani was appointed as a research assisstant at the Indonesian Institute of Science by Dr O.Soemarwoto, then a professor at the Faculty of Agriculture, Gajahmada University. This appointment moved M. Soerjani from his employment at the Department of Agriculture to the Indonesian Istitute of Science, attached to Bogor Botanical Garden. Muhammad Soerjani took special interest in the physiology of Alang-alang [Imperata cylindrica], and under the direction of Prof Dr. Otto Soemarwoto, his first research finding was presented at the 9th Congress of Pacific Science Association in Tokyo 1966. Prof. Dr. Otto Soemarwoto proposed a Regional Centre for Tropical Biology (BIOTROP) to SEAMEO (Southeast Asian Minister of Educations Organisation). He was made the first director of the regional centre. Muhammad Soerjani read for a doctorate degree programme at Gajahmada University, supervised by Prof. Dr Otto Soemarwoto. He was sent to Reading University, UK, to take a special course in Biostatistics. He obtained another scholarship from British Council to continue his I.cylindrica research work in WRO (Weed Research Organisation), Oxford, and then to CABO (Centrum voor Agrobiologisch Onderzoek voor Landbouwgewassen) in Wageningen, the Nederlandsand, he was to acquire the techniques and skill in autoradiography in ITAL (Instituut voor Toegepaste Atomoisch-energie voor Landbouwgewessen) in Benekom, the Nederlands. Upon returning to Indonesia, those research findings during his stay in UK, and the Nederlands were embodied into a desertation for his doctoral degree in Gajahmada University in 1970. He was then appointed as the Program Manager of Tropical Pest Biology Program in BIOTROP.

The programme of Tropical Pest Biology dwelled upon weeds and ectoparasites, the two subjects that were left neglected by other institutes in the region. Dr Muhammad Soerjani developed the program on weeds intelligently. He devised and promulgated a long-term programme by inviting world authorities on weed science from UK, including the director of WRO, IBC, (Institute of Biological Control) scientists from IPPC Oregon State and California University, USA), from Japan (Tsukuba University), the Nederlands ( NUFFIC, KIT) from France, Australia, etc. The written programme was regional and very comprehensive, consisted basically of 3 activities, (1) to do research on the biology and management of selected weeds, (2) to train young scientists in the region how to do research, and to guide and encourage them to do research in their own country, and to (3) present research finding at the regional scientific meetings or symposia. The research topics were in line with BIOTROP goals, i.e. to identify critical biological problems the solution of which will enhance the economic gain of the region. Imperata cylindrica and Eichhornia crassipes (waterhyacinth) were selected the first 2 weed species that should be managed properly.

The trainings were carried out with instructors from USA, UK, Japan or the Nederlands and participated by participants from the regions, Malaysia, Thailand, Vietnam, Laos Cambodia, Philippines, of course Indonesia. The trainings were oraganized not only in Bogor Indonesia, but laso other SEAMEO member countries such as Malaysia, Thailand, Philippines so were the symposia; activities on weed control and management were indeed plentiful. In its early development there were short-term (6 weeks) and long-term (9 months) training courses. In the long-term training courses participants are obliged to write research proposal and carried out them in the fields. To stimulate research activities in their own countries funds were provided and research report were submitted to BIOTROP. This programme has been instrumental in developing research institutes in the region such National Research Institute of Biological Control of Weeds Thailand with the first director in Dr. Banpot Napompeth, the alumni of BIOTROP; many universities in Indonesia adopted the curricula of BIOTROP weed science training course into weed science curricula given to their students of agricultural sciences. Many weed scientists in the Southeast Asian region, are alumni of BIOTROP undertaking weed science programme of which was designed by Dr.M. Soerjani, inter-alia, Prof. Dr. Baki Hj Bakar, Dr. Anwar Ismail of Malaysia, Dr Umporn Suwunamek, Prof Dr. Banpot Napompeth of Thailand, Dr Soetikno, Dr Soekisman, Dr. Jody Munandir of Indonesia, and many more.

Dr Muhammad Soerjani has contributed considerably to the awareness of weeds and the accompanying impact upon policy makers, in inspiring young scientists interested in weed science and carrying out research and other activities on weeds, developing research organisation dedicated to weed science, and above all providing many alternatives of weed control for farmers to reduce the cost of agricultural production. At BIOTROP itself the output was considerable, and BIOTROP is enjoying a good reputation of institution with strong committment to weed science. Monumental books such as WEEDS OF RICE by Dr Muhammad Soerjani as a leading author, or Aquatic Weeds of South East Asia written by Soerjani and Pancho are among books about weeds beside other documents in the form of internal reports, proceedings of seminar or symposia, BIOTROPIA publications, (BIOTROP journal that published research results of BIOTROP researchers) are available in the BIOTROP library, where students or training participants can satisfy their interest on weeds

Dr.M.Soerjani also invited foreign researchers to do research works at BIOTROP such as, Mr. D.Robson of WRO, Dr. Ikushima of Chiba, Prof. Dr. J. Pancho of UPLB, J. Eussen of the Nederlands and others, beside producing data in accordance with their hyphotheses they also provided direct training for staff assigned to be the counterparts of those visiting scientists.

The positive impact of BIOTROP programme on weeds control not only on governmental institutions, but also on professional association, such as Weeds Science Society in Indonesia, in the Philippines, Thailands and elsewhere emerged in the period of BIOTROP dessiminating information on weeds. These professional organisations were responsible in dessiminating information on weed management, and more in integrating stakeholders interested in weed management from farmers, , extension workers to retailers of herbicides to agrochemical companies and policy makers. The Indonesian Weed Science Society, for example, is given a prestigious position to assess the standards and quality of herbicides sold in the Indonesian market. Dr Muhammad Soerjani was the person resposible for the initiation, then the eventual formation of Indonesian Weed Science Society (WSSI) in March 7, 1970. He established the charter of the WSSI and promulgated the rules and regulation and established a newsletter, “Weeds” to accommodate publications of information on weeds to be dessiminated to members of WSSI. He was the first President of Weed Science Society of Indonesia, and organised a succesful First Indonesian Weed Science Conference Januari 29-31, 1971 only 8 months after its inaguration. It was at this conference that Dr Muhammad Soerjani clearly stated the objectives, among others, promoting the advancement of weed science, with good ethics and fellowship, coordinated activities, published research results for every one to share the information, and it was expected that WSSI could function as a liason between its members and APWSS.

Dr Muhammad Soerjani was intrumental in developing the Asian Pacific Weed Science Society since its birth in Hawaii, and such contribution to the development of APWSS has been considerable. Together with his colleges Dr. Marcos Mega of the Philippines, Dr Barnes of Malaysia, Lez Matteuz of New Zealand, Dr Roy Nishimito of Hawaai, Dr. Larry Burril of Oregon State University, Dr Muhammad Soerjani were actively nurtering the society. He was involved in the 2nd (in Kuala Lumpur), 3rd (in Manila), 4th (in Rotorua, New Zealand), 5th (Tokyo) APWSS conference and he was the president of APWSS and organized successful 6th APWSS conference in Jakarta Indonesia in 1989. Dr Muhammad Soerjani was involved in the formulation of the APWSS charter including organisational memberships. He was offered by FAO to take a post as FAO weed expert in Rome, but he declined the offer as he was the president of APWSS and in the preparation of organizing the 6th APWSS conference. He was and still is a nationalist and a patriot, and also a man of his words, characterizing his wit and wisdom.

The untiring involvement of Dr Muhammad Soerjani with weeds at BIOTROP and elswhere came to a halt when he was called upon by the Directorate General of Higher Education, Minister of Education to move from the Indonesian Institute of Science to the Department of Education and Culture and seconded to the famous University of Indonesia, at the Faculty of Science and Mathematics. Greatly inspired by his mentor, Prof. Dr. Otto Soemarwoto who led an Institute of Ecology at Pajajaran University, Dr Muhammad Soerjani also developed the Center for Environmental Studies at the University of Indonesia. He was concentrating more on the environmental problems in cities rather than those in agricultural or forest ecosystems. He was instrumental in issuing laws related to environmental management No 23/1997 which formulates that environment as a unit of space with all its materials, energy, forces, condition and living organisms including human and their behaviours in affecting life sustainability and welfare including other living organisms. He later on revised the concept of environment being a much wider, and should be understood in the context of cosmology, in line with Islamic teaching , that environment is not limited to only on earth, but the whole universe that God the Al-Mighty has created.

This award of the Fellow of Asian Pacific Weed Science Society is very fitting to Dr Muhammad Soerjani, a man who has spent considerable time and energy to develop weed science in the region as we see today.

**Dr Soekisman Tjitrosoedirdjo**
SEAMEO BIOTROP, Bogor

**SPEAKERS PROFILE**

**Dr Steve Adkins** is the leader of the Tropical and Subtropical Weed Research Unit within the School of Agriculture and Food Science and the Professor of Plant Physiology at the University of Queensland. Steve received his Honours in Botany and Zoology in 1977 from the University of London, UK and his PhD in 1981 from the University of Reading, UK. He completed a series of postdoctoral fellowships at the University of Saskatchewan, Canada and has since worked at Murdoch University before coming to the University of Queensland in 1988.

Steve is the president of the Asian-Pacific Weed Science Society and principal research scientist on many tropical weed projects at Uqin the fieldsof plant ecology, weed biology, and seed physiology. He has supervised more than 50 PhD and MSc students in plant and weed science and produced over 300 research publications. He has spoken about weed biology and management at conferences in over 30 countries around the word. He has been a member of the Asian Pacific Weed Science Society for over 25 years.

**Dr. Aurora M. Baltazar** is adjunct professor of weed science at the Crop Protection Cluster, University of the Philippines Los Banos (UPLB) where she taught weed science and crop protection courses until she retired in January 2012. She obtained her BS and MS in Agriculture from UPLB and her PhD from the North Carolina State University. She worked as postdoctoral research associate at the University of Arkansas where she was involved in the first studies to confirm the resistance of barnyardgrass to propanil and determined strategies for control of propanil-resistant barnyardgrass. She also conducted studies on selectivity of postemergence grass herbicides which served as basis in management strategies using grass herbicides for control of emerged grasses in rice.

From 2001 to 2003 she was the Asian Site Coordinator of the USAID-funded IPM CRSP (IPM Collaborative Research Support Program), a collaborative program among Virginia Tech, UP Los Banos, and Philippine Rice Research Institute. Through the IPM CRSP weed research program, she and her group have developed cost-reducing weed management strategies to help increase income of rice-vegetable farmers in tropical rainfed farms and documented the increase in populations of a lowland ecotype of purple nutsedge in rice-vegetable areas in the Philippines. Her collaborative research work with weed scientists at the International Rice Research Institute has helped determine how a lowland ecotype of purple nutsedge has developed morphological and physiological mechanisms to adapt to the flooded environments of lowland rice. She served as APWSS president in 2001-2003 and is currently on the APWSS Executive Committee.

**Dr. Yoshiharu Fujii** is an alumnus of Kyoto University where he graduated with BSc, MSc and PhD degrees. Currently, a professor at the Tokyo University of Agriculture and Technology, Dr Y. Fujii was a senior research scientist at the National Institute of Agro-Environmental Sciences, heading, *inter-alia*, the Allelopathy Laboratory, and as Research Group Leader, Risk Assessment of Alien Species for Biodiversity National Institute for Agro-Environmentl Sciences. His principal research interest focuses on Plant Ecology with emphasis on allelopathy, screening of allelopathy and identification of allelochemicals in action, utilization of allelopathy for agriculture. He was a recipient of Life Time Achivement Award, International Allelopathy Foundation for 2009-2011. An active scientist in the weed science fraternity in Japan andworldwide, Dr Y. Fujii was the President of International Allelopathy Society for YEAR. He published regularly in high impact research journals in Japan and elsewhere.

**Dr Albert Fischer** is the current holder of the Melvin D. Androus Endowed Professorship for Weed Science in Rice and is Professor of Weed Ecophysiology in Rice Systems at the Department of Plant Sciences of the University of California in Davis. He is President of the International Weed Science Society (IWSS). He graduated in Agricultural Sciences at the University of Uruguay and received his MS and PhD in Crop and Weed Science from Oregon State University in the USA. Before coming to UC Davis, he had conducted research on weed biology at North Dakota State University, USA. From 1989 to 1996 he was Senior Scientist for Rice and Weed Ecophysiology at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia. He was professor and researcher in Weed Science at the Autonomous University of Chapingo in Mexico and prior to that, he conducted extension and research in Weed Science for the Ministry of Agriculture of Uruguay.

His strategic and applied research addresses critical weed challenges to California rice production, such as the evolution of herbicide resistance in major weeds and the recent appearance of weedy forms of rice. His program works towards: a) understanding the physiological, biochemical and molecular mechanisms endowing herbicide resistance in key weeds of rice, b) elucidating effects of landscape and crop management on the genetic structure and the spread of herbicide resistant weeds in rice, c) developing knowledge on weed biology and the ecophysiology of rice-weed interactions, and d) developing novel weed management concepts for rice. His program integrates a wide range of research activities whereby basic science supports novel concepts for field-level solutions. He currently teaches courses on plant physiology, herbicide mechanisms of action and herbicide resistance in weeds, and weed science. Has published 71 scientific refereed papers and more than 60 scientific documents.

Having gained his PhD from Massey University in Soil Science (environmental), **Dr Trevor James** research interest now covers all aspects of weed management. This includes general and specific (environmental, noxious and herbicide resistant) weed control, seed ecology with special interest in dynamics of the soil seed bank and its implications for weed management, and the impacts and control of weeds in arable and horticultural crops and in pasture. Another important part of his work is around herbicide use and safety, which includes the interaction of herbicides and the environment (leaching, movement and persistence) and maximising their efficacy through use of adjuvants and improved application systems. Some of Dr James' key achievements include the publication of books on common weeds, on grasses, sedges and rushes and very recently on weed seeds.

**Dr Hisashi Kato-Noguchi** is a professor at the Department of Applied Biological Sciences, Faculty of Agriculture, Kagawa University, Japan. He is an expert in allelopathy and allechemicals research. His main research interest is in allelopathy, focusing especially on allelopathic chemical communications. Dr Kato-Noguchi is also interested in searching for new allelopathic substances in tropical and subtropical plants. His research group have already found that there is chemical communication between rice crop and barnyardgrass plants. They also have found several new compounds that act as allelopathic substances..

There are still many plants unexplored, some of which may contain unknown allelopapthic substances. Dr Kato-Noguchi's research group welcomes cooperative research to investigate those allelopathic substances and to find the solutions for sustainable weed management strategies by expanding our knowledge of allelopathy.

**Dr. Kwang-Ho Park** is a Professor and Vice President, Korea National College of Agriculture and Fisheries (KNCAF), Ministry of Agriculture, Forestry and Rural Affairs, Korea. His main research interest is in rice crop science and weed management research. Dr. Kwang-Ho Park's awards include, *inter-alia*, the Daesan Foundation for Rural Culture & Society Award (locally known as a Novel Prize in Agriculture) in Korea (2011), the Korea Government Award (2005), and the Korea Agricultural Science and Technology Award (2002).

**Dr. A. R. Sharma** obtained his B.Sc. Agric degree from Himachal Pradesh Agricultural University, Palampur (1981); M.Sc. from Punjab Agricultural University, Ludhiana (1983); and Ph.D. from Indian Institute of Technology, Kharagpur (1988). He served as Scientist at the Central Rice Research Institute, Cuttack (1987-1996); Agronomist at the Punjab Agricultural University, Ludhiana (1996-1998), Senior Scientist at the Central Soil and Water Conservation Research and Training Institute, Dehradun (1998-2001); and Principal Scientist and Professor of Agronomy at the Indian Agricultural Research Institute, New Delhi (2001-2012). Dr. Sharma has made outstanding research contributions in the field of tillage and weed management, conservation agriculture and nutrient management. He has published more than 150 research articles and presented about 50 papers in various seminars/symposia. He was conferred ICAR Jawaharlal Nehru Award, KRIBHCO Award for Outstanding Research, ISCA Pran Vohra Award, CRRI Best Worker Award, IARI Hooker Award, and FAI Dhiru Morarji Memorial Awards and a recipient of the prestigious Fellowship of National Academy of Agricultural Sciences (2004), Indian Society of Agronomy (2009), and Indian Association of Soil and Water Conservationists (2010). Dr. Sharma joined as Director, Directorate of Weed Science Research, Jabalpur, Madhya Pradesh, India in March 2012.

**Dr Soekisman Tjitrosoedirdjo** completed his primary and secondary educations in Klaten, Central Java, Indonesia, and graduated from Veterinary Technical School in 1961. He first employment by the government of Indonesia as a technical assistant up to 1966. He won the prestigious Colombo Plan Scholarship to continue his study to the tertiary level, and matriculated at Leederville Technical School in Perth, in 1968 and enrolled at Faculty of Agriculture, University of Western Australia, and obtained his B Sc. (Agric.) degree (Hons) in 1972.

He was employed by BIOTROP (Regional Centre for Tropical Biology) as a researcher in Weed Science, and won a scholarship to continue his study in Weed Science and enrolled at the Faculty of Agriculture, University of the Philippines at Los Banos in 1976 and graduated with an MS degree in 1978. He organised a lot of training programmes in Weed Control and Management in the region, and recently also on the Management of Invasive Alient Plant Species under BIOTROP program. He won another prestigious Mombuso Scholarship, enabled him to enroll at the Faculty of Science and Technology, Kobe University of Japan in 1988, as a doctorate student under the supervision of Prof. Dr.S.Matsunaka, and obtained his PhD degree in 1991 with the dissertation entitled "*Use of Herbicides to Establish Agricultural Production Systems in Imperata-dominated lands, in Indonesia*".

He was also employed by Bogor Agricultural University at the Faculty of Science and Mathematics teaching Plant Physiology and Weed Science. He was a member of Silviculture Intensive under the Minister of Forestry. He retired from BIOTROP and Bogor Agricultural University in 2007. He is currently active as a member of Panel Expert of Indonesian Pesticide Commission, under the Minister of Agriculture, a Member of Working Group, which formulates policies and regulations of Invasive Alien Species Management in the Ministry of Environment. He is also an expert consultant for Invasive Alien Plant Species in the Center of Conservation and Rehabilitation Research and Development, Ministry of Forestry, Indonesia.

**Dr. N. T. Yaduraju** took graduate and post-graduate degrees from the University of Agricultural Sciences, Bangalore, India and PhD from the Reading University, UK. He undertook research and teaching in agronomy, weeds and weed management at the Indian Agricultural Research Institute, New Delhi (1976 to 2000). He served as the Director, National Research Centre for Weed Science (NRCWS) at Jabalpur, India (2000 to 2005), where he set up of world-class facilities for weed science research and provided leadership at the national level. He has over 200 research publications and is the Fellow of the Indian Society of Weed Science (ISWS) and a recipient of the ISWS Gold Medal. As National Coordinator (2006-2010), National Agricultural Innovation Project (NAIP) – a World Bank-funded project implemented by the Indian Council of Agricultural Research (ICAR), he tried to build a network of highly reputed and competent professionals in ICT4D in agricultural information management and KM and facilitated the development and implementation of several projects worth over $ 50 million. Currently, Dr. Yaduraju is working at ICRISAT as Principal Scientist for ICT4D.

# *Keynote Paper and Invited Plenary Papers*

## SOME PRESENT PROBLEMS AND FUTURE APPROACHES TO WEED MANAGEMENT IN THE ASIAN-PACIFIC REGION: SUPPORTING FOOD AND ENVIRONMENT SECURITY BY 2020

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**ABSTRACT** The prevention and management of weeds has been a continuous problem throughout the history of food production and native ecosystem protection in the Asian-Pacific region. Despite improvements in the approaches used, weeds still reduce productivity and profitability by unacceptable amounts, and upset the balance of our natural ecosystems. The Asian-Pacific region has considerable strengths in many aspects of weed science technology and training. However various issues, such as the rapidly increasing requirement for food due to an ever growing population, the ever increasing influx of new weeds as international travel and trade increases, the acceptance of climate change and its likely impacts upon weed distribution and abundance, and the advent of special threats such as the evolution of weeds that are resistance to herbicides and to the adoption of reduced tillage agriculture, have all necessitated the re-evaluation of our current approaches to weed management. In this paper I attempt to provide a brief summary of some of the present problems facing food production and environment protection in the Asian-Pacific region and review some of the future approaches we may need use to manage these weed threats. The weed challenges now facing our regions agriculture and land management will require food and land systems that are safe for the community and the environment, sustainable and profitable in both the short- and long-term, and adaptable for use in all situations. In the past the vision and the objective of achieving good weed science outcomes in the Asian-Pacific have been primarily achieved through information exchange at conferences, workshops and training courses, through our newsletter and international journal and some bilateral research collaborations. The time has come for an increased level of international collaboration to meet these new challenges.

***Keywords:*** population growth, climate change, invasive weeds, herbicide resistance, Asian-Pacific, biotechnology, computer technology

## INTRODUCTION

By 2050 the world's population is expected to reach 9.0 billion requiring a further 70% improvement in food production. This will place an unprecedented demand upon both natural and agricultural ecosystems and their ability to convert the natural resources of sunlight, water, air and soil nutrients into food, fibre and feed for at least another 2.0 billion people. The crucial issue is how we can meet this challenge while faced with the limitation of little new land available and in the expectation that our chosen methods will not have unsustainable environmental consequences. The growing demand for food, fibre and energy must be balance with the need for biodiversity conservation and we need to do this under an unpredictable and changing climate.

Weeds are the largest limitation to food and fibre production around the globe. In the Asian-Pacific region the prevention and management of weeds has been a continuous battle in the history of agriculture and natural ecosystem management. Despite continually advancing our technology for weed management, weeds still reduce productivity and profitability, and



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upset the natural balance of our ecosystems. The Asian-Pacific region has considerable strengths in many aspects of weed science and technology. However, various issues, such as the rapidly increasing population, the appearance of new weed threats, an acceptance of climate change, and concerns for the environment have necessitated the re-evaluation of our current approaches to weed management.

The challenge facing Asian-Pacific weed scientists and land managers is to develop weed management procedures for food and natural land systems that are effective and profitable, in both the short- and long-term, sustainable and safe for the community and the environment, and adaptable to cover all management requirements. In this paper I consider the four main challenges to food production and environment protection in the Asian-Pacific region (*viz*. a rapidly increasing population, emerging new weed problems, climate change, and unsustainable farming practices), and then provide a brief inventory of future approaches and options that may be used to meet these challenges.

## THE PRESENT PROBLEMS

The present problems within the Asian-Pacific region that are causing concern to food and fibre production and threaten the environment, come from several sources of which the following are important; a) a rapidly increasing population that is living longer and demanding a higher quality of food, b) a growing threat from new weed problems and other agricultural pests, c) a changing climate which is manipulating the size and scope of the weed and pest problems within our agricultural and natural ecosystems, and d) the certain non-sustainable approaches being used to manage weeds. The challenge facing Asian-Pacific weed scientists and land managers is one of developing weed prevention and management procedures for land protection and food security systems that are effective and profitable, in both the short- and long-term, sustainable and safe to the community and the environment, and adaptable so they can be used in most situations. The following sections will focus on these four main problems facing land managers and researchers today.

### The increasing Asian-Pacific population

It has been estimated that the world population of approximately 7.0 billion will rise to 9.0 billion by 2050, which is 180,000 more people per day, with the largest increases being in Africa and the Asian-Pacific region. It has been estimated that crop and animal production will need to be increased by 70% to meet the 2050 population demand and to achieve this we will need to either find 9% more land area to put into agriculture or for us to become more productive on the land we presently use. Within the Asian-Pacific region the present population of 4.2 billion will rise to 6.0 billion by 2050 with the greatest population growth being in India and China; each country predicted to reach 1.4 billion by 2025. This regional population increase will be the result of both an increase in the birth rate and because people are living longer. Over the last decade, the life expectancy for both women and men has increased in every country of the Asian-Pacific region with the largest increases seen in Nepal, where the life expectancy for women has increased by 4.1 years and for men by 3.7 years.

How are we going to meet this increased food production target? Marris (2008) has identified five possible approaches we might use to improve food production in the next 20 to 50 years. The first approach is one being considered by an Australian plant pathology team who are looking to develop better stem rust resistance in wheat (*Triticum aestivum* L.) Continues protection from old and new stem rust strains could potentially save 19% of the world's wheat harvest from being lost to disease. The second approach is one being followed by an American agro-ecology team who are looking to turn wheat into a perennial crop. Such a longer-lived crop would reduce seasonal land preparation, preventing soil erosion while the deeper rooting potential of the perennial plant would mean the crop would require less fertilizer and water inputs, turning traditional cropping land into highly productive perennial



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grassland. The third approach is one being developed by a Chinese plant physiology team who have discovered that drought stressing plants can convince them to throw extra resources into reproduction as their death may be imminent. An approach called 'deficit irrigation' is being used to increase the yields of certain crops, especially in situations where irrigation water is also becoming scarce. The forth approach is one being followed by an American biotechnology team who are, through genetic manipulation, turning cassava (*Manihot esculenta*Crantz) into a super food producing more protein and a wide range of micronutrients for feeding 250 million people in Africa. The crop will grow on poor soil, requires little labour to produce and is tolerant to drought. The final approach considered is one of a UK molecular biology team attempting to improve, using genes from maize, the photosynthetic mechanism of rice (*Oryza sativa* L.), effectively changing it from a C3 plant into a C4 supercharged plant with 50% greater seed production. It is interesting to note that since weeds are considered to be the single largest limitation to crop and animal production world-wide, not one of the possible approaches considered by Marris (2008) to raise food production by 2050 was one requiring the skills of a weed scientist. Perhaps the contribution from weed scientist will not come, not from any one large change in our approach to weed management, but from many smaller improvements that are more orientated towards site-specific outcomes. Before considering possible new improvements to food and fibre production and environmental protection through weed science, I would like to consider the diversity of the weed problems we face within the Asian-Pacific region.

**The growing threat from weeds**

***(a) Historical weed problems:*** Historically, there have been many weed problems develop within the Asian-Pacific region. For example, within our cropping systems two well established problem weeds have been barnyard grass (*Echinochloa crus-galli*(L.) Beauv) and canary grass (*Phalaris minor* L.). These weeds continue to threaten the productivity of our rice and wheat cropping systems, respectively and have done so for much of the past 30 years. Alternatively, in our aquatic systems, weeds such as water hyacinth (*Eichhornia crassipes*(Mart.) Solms), salvinia(*Salvinia molesta* L.) and alligator weed (*Alternanthera philoxeroides* Griseb.) have also threatened productivity and biodiversity and new approaches have had to be developed to manage them. In pastures and non-agricultural land weeds such as blady grass (*Imperata cylindrical* L.), giant sensitive bush (*Mimosa pigra* L.) and lantana (*Lantana camara* L.) have also posed considerable threats and have been the subject of many investigations (Rahman *et al*. 2012). However, as we now enter an era of significantly increased movement of commodities and people with our region and to and from other parts of the world, a much greater threat looms from the accidental introduction of new weeds, and under climate change several native and sleeper weeds may also emerge as new threats.

***(b) The new threats:*** Many serious weeds that have arrived in the Asian-Pacific region in recent times are still expanding their distribution and abundance. Colonisation of new habitats through certain natural and human-created interference is a common feature of these weeds that have recently arrived into our region. In addition some native plants and previously unimportant weeds are of increased importance as the stresses of population growth and climate change take effect.

**Weedy Rice** For a number of countries in the Asia-Pacific region, weedy or red rice has now emerged as a new and significant weed. First noted in 1988, this weed has now become a problem in rice production in Malaysia, Thailand, China, Vietnam etc. Weedy rice is a form of cultivated rice (*Oryza sativa* L.) but having a taller stature and a shorter life span, and also having weaker culms, few, small seeds that shatter easily. The origin of weedy rice is not certain. It may have originated from the degradation of cultivated rice or may have come from the crossing between cultivated rice and a species of wild rice. In most rice production



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regions, weedy rice has become a problem following the transition from transplanting to direct seeded rice production. In Vietnam the average yield loss due to weedy rice ranges from 15 to 17%. The close similarity between weedy rice and the commercial varieties prevents the use of herbicides and the only effective approach to reduce weedy rice infestations is through integrated management.

**Mile-a-minute** Mile-a-minute (*Mikania micrantha* Kunth. ex. H.B.K) is regarded to be one of the most invasive plants worldwide (Lowe *et al*., 2000) and ranked among the World's top 100 worst invasive species. For many countries in the Asian-Pacific region, mile-a-minute has rapidly become one of the most damaging weeds of plantation and field crops and the environment. Outside of its native range in tropical America, mile-a-minute has spread with the aid of human intervention to locations which include China, India, Sri Lanka, Bangladesh, Philippines, Indonesia, Taiwan, Brunei, Malaysia, Singapore, Thailand, the Pacific Islands, Nepal, Bhutan, Vietnam and recently to Australia. The increase in the rate of urban development and human interference has been implicated as one reason as to why mile-a-minute has spread so rapidly, between and within countries in the Asian-Pacific region. Human interference and development in certain parts of Nepal has lead to the rapid establishment of this weed in many of the countries protected areas bordering India causing considerable declines in biodiversity, and where it is known locally as the 'forest killer'. The occurrence of natural disasters, such as cyclones and floods has contributed to its spread in south Asia. It is widespread in the Pacific Islands where it is a major weed of sugarcane (*Saccharum officinarum* L.), taro (*Colocasia esculenta* (L.) Schott) and banana (*Musa* spp.) crops (Macanawai*et al*., 2012). Losses due to mile-a-minute can range from 15 to 100% and only through integrated control approaches, utilizing biological, physical and chemical control methods can reductions of mile-a-minute infestation be effectively achieved.

**Oxytropis** Qinghai Province is located in the north of the Asian-Pacific region and on the Qinghai-Tibetan plateau. At 36 million ha, it is one of the largest rangelands in China, carrying 20 million yak and sheep which produce 20 million L of milk and 120 million kg of meat each year. The average altitude of the rangelands is 4,000 m above sea level creating a unique plateau ecosystem, with well adapted native vegetation. However, in recent years this region has been influenced by a changing climate and over utilization of the grasslands. There are 224 species of poison plant, from 10 families, to be found on the Qinghai rangelands. The most serious being five Oxytropis species (*Oxytropis ochrocephala* Bge., *O. kansuensis* Bge., *O. glabra* Dc., *O. m elanocalyx* Bge., *O. falcate* Bge). The area of rangelands contaminated by these toxic weeds is now 26 million ha of which 13 million ha is affected by Oxytropis. The area infested is increasing and is thought to be related to the change in climate experienced by the region. Each year approximately 27,000 sheep and 1,900 yak are poisoned with 6,000 sheep and 630 yak dying from these toxic plants. It is estimated that the economic loss from Oxytropis is in the order of $2.8 million per year. Integrated control approaches are in development, but as the weed species are native little opportunity exists for biological control. Cultural management to help protect the landscape from the effects of overstocking and climate change are among those approaches that are most likely to be helpful.

**Parthenium Weed** There are many examples of weeds that are still extending their range within the Asian-Pacific region. One significant weed is parthenium weed (*Parthenium hysterophorus* L.), a serious weed of all types of natural and agro-ecosystem that continue to spread from a number of accidental sites of introduction in India, Australia, and the south China/Vietnam region (Adkins and Shabbir, 2013). Parthenium weed is an aggressive invasive weed, threatening the natural and agro-ecosystems in over 14 countries in the Asian-Pacific region. Parthenium weed is inflicting losses to crops and pastures, degrading the biodiversity of natural plant communities, causing human and animal health hazards and



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seriously inflicting economic losses to people and their interests in many countries of our region. Several of its biological and ecological attributes contribute towards its invasiveness. Various management approaches (*viz*. cultural, mechanical, chemical and biological control) have been used to minimize losses caused by this weed but most of these approaches are ineffective, uneconomical and/or possess several limitations. Although, chemical control using herbicides and biological control utilizing exotic insects and pathogens has been found to contribute to the management of the weed, the weed remains a major and growing problem. Losses due to parthenium weed range from 15 to 80% in up to 30 crops and 10 to 100% in rangelands and only through integrated control approaches, utilizing biological, physical, cultural and chemical control methods can reductions of parthenium weed infestation be effectively achieved.

**Merremia vine** In central Vietnam at the Son Tra (920 ha) and the Nam Hai Van (1,100 ha) nature reserves, a serious invasive merremia woody vine**s**pecies (*Merremia eberhardtii* (Gagnep.) T.N.Nguyen) has recently appeared and has been rapidly spreading across these and other protected areas since 1999 (Le *et al.* 2012). In the near-by Ba Na Nui Chua nature reserve, nearly 300 ha of forest are affected by a second merremia species (*M. boisiana* (Gagnep.) Ooststr.). In all locations these woody vines act, in the main, by climbing up and covering the forest canopy, killing the trees and plants below by preventing them from obtaining sunlight. The presence of these invasive plant species also heightens the risk of forest fires as their large, thick leaves catch fire easily, even when green. This latter species is also highly invasive in southern China where serious forest damage has been reported on Hainan Island. Additional, but less intense damage has been reported from Yunnan and Guangxi Provinces in China. The cause of this recent problem in Vietnam and southern China has been put down to climate change, mostly to do with an increasing ambient temperature and atmospheric $CO_2$ concentration. According to Chinese scientists, merremia vine has three possible centres of origin; firstly in southern China, secondly in Malaysian Sarawak/eastern Indonesian Kalimantan, or thirdly in northern Vietnam that border Laos and Guangxi Province, China. Forestry losses due to merremia vine can range from 15 to 100% and integrated control approaches are in development, but as the weed species are native to these regions of infestation little opportunity exists for biological control.

## The growing threat from climate change

***(a) Impact of temperature rise:***

The present world atmospheric temperature has increased 1.0$^{o}$C since 1900 with half of this rise coming in the past 30 years. One prediction is that the world atmospheric temperature will rise by a further 2 to 4$^{o}$C by 2050 and possibly higher in certain parts of the world. The Asian-Pacific region is expecting a 4 to 6$^{o}$C rise by 2100. In the long term, gradual warming of the climate within the Asian-Pacific region may lead to many weeds of tropical parts extending their ranges into more temperate parts of our region. This kind of response to climate change may also result in the loss of certain cool-temperate weeds from the more northerly and southerly parts of our region. Species currently restricted to the lowlands could be expected to move into land at higher altitudes. For temperature sensitive plants such as parthenium weed this shift may be significant and there is already some evidence that this weed is invading into higher altitudinal areas in countries such as Pakistan, Bhutan and Nepal. Frost-intolerant species such as Siam weed (*Chromolaenaodorata* L.) can also be expected to shift their ranges significantly, further south and further north. There is a high risk that some weed species at present not considered high priorities and are currently limited by temperature may show increased spread with a rise in mean temperature.



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***(b) Impact of atmospheric $CO_2$ increase:***

The present atmospheric $CO_2$ concentration at the International Observatory, Mauna Loa in Hawaii is closing in on 400 ppm. One prediction is that this atmospheric $CO_2$ concentration will hit 800 ppm by 2050 and possibly higher in certain highly industrialized parts of the Asian-Pacific region. At this present point in time the Asian-Pacific region is accounting for approximately 50% of the world's total $CO_2$ emissions with the total $CO_2$ emissions standing at about 12 billion tons per annum. In 2009, China was the single largest emitter of greenhouse gases worldwide, emitting 6.8 billion tons of $CO_2$, which was 1.1 billion tons more $CO_2$ than from all of North America. Increased atmospheric $CO_2$ concentration can be expected to influence the invasiveness of a number of weed species, particularly those operating a C3 carbon metabolism, a form of photosynthesis that can greatly benefit from increased $CO_2$ concentrations. This will be especially noticeable in communities dominated by native or agricultural plants operating a C4 photosynthesis which will not show an increased growth under the same conditions. As a result, invasive weeds such as parthenium weed (Navie *et al*. 2005) and cropping weeds such as wild oats (*Avena fatua* L.) will be even more competitive in the raised $CO_2$ environment (O'Donnell and Adkins 2001), and this will be in addition to the temperature and rainfall effects which are also likely to benefit these weeds. However some crops and pasture grasses, may also benefit from an increased $CO_2$ concentration, and show increased growth and therefore will be better able to suppress the growth of certain weeds. Species that are already present in the Asian-Pacific region could, through elevation of $CO_2$ concentration become more serious weeds in regions where they were not previously present or problematic before.

***(c) Impact of changed rainfall patterns:***

Changes in rainfall pattern in the Asian-Pacific region are less certain. In the tropical regions there is expected to be an increase in rainfall especially during the monsoon season, with reduced rainfall in the more southerly and northerly regions. In many areas, an increase in extreme events, including droughts, floods, and severe storms, may be expected. Without good information on changed rainfall patterns, the impact on weed problems is difficult to predict. Reduced rainfall may limit or reduce the distribution of many weeds such as lantana and the vine species like merremia. Reduced rainfall will also reduce the growth of many crops and pastures, reducing canopy cover and increasing patchiness which in turn will favour weed invasion. Increased drought periods interspersed with occasional very wet seasons, will promote weed invasion, because established vegetation, both native and crop, will be weakened, leaving areas for invasion. For example, the appearance and rapid spread of parthenium weed in wet seasons following drought has been observed in a number of locations around the globe. More severe cyclones will both disperse weed seeds through wind and floods, and also open up gaps for weed invasion in areas of undisturbed native vegetation.

***(d) Overall impact of climate change:***

Overall, climatic change of the kind expected within the Asian-Pacific region is expected to induce changes in where crops are grown and where native vegetation can be supported. Species, whether crop, native or weed with efficient dispersal mechanisms by bird, wind, water or by human activities, are likely to make these range changes more rapidly. Invasive plants generally have well developed seed dispersal mechanisms, and are likely to spread rapidly into new areas, quickly exploiting the new climatic conditions that favour new plant establishment. Climate change can therefore be expected to favour invasive plants over established native vegetation, especially if accompanied by an increase in extreme conditions such as droughts alternating with wet seasons. There is a strong possibility that certain 'sleeper weeds' may be favoured by a changing climate and could rapidly establish across the landscape once certain environmental limitations for growth are overcome.



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### The growing threat from other sources

There are a number of new developments in the way in which we live our lives and go about land and agricultural management that are causing new and special threats to food and fibre production in the Asian-Pacific region. Two that come immediate to mind are the increased travel, transport and tourism pastimes that have lead to an unprecedented increase in the number of new weeds being introduced to our region and the over use of chemicals for weed management that has lead to herbicide resistance.

#### *(a) Herbicide resistance*

Prior to the 1940's most weed management around the globe was undertaken using either a physical or a cultural approach. However, from the time of discovery of synthetic, selective herbicides there has been a considerable shift away from these more traditional approaches to ones reliant upon herbicides. This widespread adoption of the newly available chemical weed management techniques has led to changes in weed abundance, their distribution and diversity all within a relatively short period of time. This shift to chemical farming has not only promoted a species shift to more herbicide-tolerant weeds but also to the evolution of herbicide-resistant weeds. For example, there are now 403 biotypes of weeds, from 218 species that are resistant to one or more herbicides, present in 66 crops in 61 countries, and the number of new cases of herbicide resistance is increasing by 25% per year. In the Asian-Pacific region there are now 183 biotypes recorded as being herbicide resistant in 12 countries with Australia having 62, Japan 18, Malaysia 17, New Zealand 15 and China with 34, 9 of which are weeds found in rice production systems (Heap, 2013). The appearance of herbicide resistant weeds is likely to increase in the Asian-Pacific region as the commercially available herbicide tolerant crops such as cotton, canola, and maize, are more widely grown. These new crops having resistance to non-selective herbicides such as glyphosate, are expected to provide farmers with a greater capacity to manage otherwise hard-to-kill weeds such as weedy rice. However, as has been the case in the USA where glyphosate–tolerant crops have been grown for 16 years, many new herbicide resistant weeds are expected to evolve under this new chemical management system.

#### *(b) Global transfer of weeds*

Another new, but significant, threat to food production and the environment in the Asian-Pacific is that of globalization. Today more than ever before people and products are being moved around the globe, and with this are spread many new weeds. For example in 2011, with 36% of the total world merchandise exported, the Asian-Pacific region surpassed Europe to become the top exporter of merchandise. In addition, the total share of world merchandise imported was approximately 37%. The rapid spread of weeds by human-induced mechanisms is now seen to be the most important mechanism of weed spread, greater than that of most natural mechanisms, including those of water, wind or animals. Human-induced spread is now considered to be the main reason for new weed incursions globally (Mack and Lonsdale, 2001).

## FUTURE APPROACHES TO WEED MANAGEMENT

As our senior scientists have reminded us, much has happened in weed science within the Asian-Pacific region in the past 30 years (Rahman *et al*. 2012). In recent times there have been the new exciting developments in herbicide chemistry and application technology, the use of herbicide-tolerant crops and the move towards site specific, integrated sustainable weed management approaches. However, there has also been the disappointing discovery of herbicide resistance, a growing concern for the environment as well as human and livestock human health. We are now entering a new era where new challenges are arising more rapidly due in part to our rapid population growth, the continued movement of new weeds into our



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region and the impact of climate change. In future, to meet these new challenges weed scientists in our region will need to promote research in the following areas;

1) The development of new integrated management approaches for important crop weeds (such as weedy rice and barnyard grass) which are threatening the productivity of rice.
2) The development of new integrated management approaches for the new or rapidly spreading invasive alien species (such as parthenium weed and mile-a-minute) which are threatening a wide number of crops as well as native community biodiversity.
3) The development of practical and economic strategies for the management of herbicide resistant weeds.
4) The development of sustainable weed management strategies for the newly adopted herbicide tolerant crops to stop the potential emergence of 'super weeds' such as a herbicide-resistant weedy rice.
5) An understanding of the impact of climate change on weeds (such as C3 weeds) and the further development of their management strategies.
6) The development and use of computer-based expert systems for the identification and solving of complex weed management problems.
7) The development of new physical methods of weed management including precision agriculture technology through greater use of guidance systems, GIS, GPS and remote sensing technology.
8) The development of new biological management options including the use of suppressive crops and forage plants, natural products, and classical biological control methods for emerging weed problems.
9) The development of site specific weed management options, using expert systems to identify and map problem weeds and to minimising herbicide use.
10) An understanding of the mechanisms, then prevention of the expansion of invasive alien species due to increased international trade, transport and tourism
11) The development of effective technology transfer to end users for a faster uptake of improved management options.
12) Education of the next generation of weed scientists for the Asian-Pacific region.

The earlier section focused on the four main problems facing agriculture and land managers and researchers today, now I provide a brief inventory of some of the approaches that may be of use in meeting these challenges in the future.

**Biotechnology in weed science**

***(a) Herbicide tolerant crops***

The growing of transgenic crops remains controversial in some parts of the world, however by 2011 there were 160 million ha of transgenic crops being grown by approximately 17 million farmers in 29 countries. In the case of transgenic herbicide tolerant crops, a single, non-selective herbicide application can be to be used to control all the weeds within the crop and so herbicide tolerant crops, have the potential to significantly increase food quality, to provide for an approximate 20% reduction in the use of herbicides and for the wider use of no-till agricultural systems which may help in soil moisture and nutrient conservation.

Some concerns have been raised about herbicide tolerant crops. For example, there is the possibility for these crops to transfer, through wide hybridisation, the genes conferring herbicide tolerance to taxonomically close weedy relatives. Although research has shown that this possibility is with a low risk with most crops, some concern exists with herbicide tolerant rice if it were grown in locations were weedy rice is present. The debate has intensified recently as it has been shown that weedy rice plants containing genes for glyphosate resistance would be more fit than those without the genes, producing greater levels of



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tryptophan, and consequently having higher rates of photosynthesis and producing more seed (Wang *et al*. 2013).

A second concern is that herbicide tolerant crops and their management come with a risk of selecting weed species resistant to the herbicide concerned, though arguably this risk is no greater than that posed by using the same herbicide for fallow weed control. Therefore, for each herbicide tolerant grown crop in Australia, there has been a management plan developed, which acts as a safeguard to help prevent the overall risk of herbicide resistance developing from overusing a single mode-of-action herbicide.

Within the Asian-Pacific region six countries (India, China, Myanmar, Philippines, Australia and Pakistan) are growing transgenic crops over 16.8 million ha which represents 11% of the world production. Of these countries India has 9.4 million ha under cotton and China 3.5 million ha under cotton, tomatoes, papaya and capsicum. While in Australia glyphosate tolerant cotton varieties have been grown for some years, and more recently glyphosate tolerant canola and glufosinate tolerant cotton crops have become available.

### *(b) Other uses*

Beyond the development of crops tolerant to herbicides, biotechnological approaches may help develop weed management in other ways; 1) in the identification of weed species from morphologically similar plants, 2) the determination of the origin of a new weed invasion and its genetic similarity to populations of the same weed in other parts of the world, 3) in the detection of herbicide-resistant weeds in the field and 4) in the modification of biological control agents to allow for improved virulence or better weed consumption in the field.

## Computing Technology

The recent developments in computing technology has made many kinds of modelling approach available to the weed scientist, and this will help in the more rapid development of efficient and environmentally sound weed management practices. A number of commercially produced computer programs and simulation models are available to help identify weed species, to predict the outcomes of weed-crop interactions and weed seed bank dynamics, to determine the distribution, abundance and spread of weeds, to predict the rate of evolution of herbicide resistance, as well as the fate of herbicides in the environment. The following are a few examples of how computing technology may impact upon weed management in the Asian-Pacific region in the future.

### *(a) LucID*

Weed identification can be improved using a software system called LucID. LucID keys have been developed for a range of weed flora within the southern part of the Asian-Pacific region and offer benefits over traditional keys including ease of use and improved accuracy, since weed characteristics can be analysed in any order convenient to the user. The keys developed so far are multi-media expert systems designed specifically to help users make the correct identification of weeds. The key itself uses a relatively basic set of weed characters so that a great deal of botanical knowledge is not required to use the key. Many vegetative, seedling and seed characters are involved so that identification is easier if, as is often the case, floral characters are not present on the weed specimen. All characters present within the key are defined, and their different states illustrated and described. Each species then has an information sheet attached so that once an identification is made it can be checked and this includes photos of different stages of the weed’s life cycle as well as text descriptions including control recommendations. The key is now available for smart phones which are increasing in number in the Asian-Pacific region, with mobile phones in our region presently accounting for more than 50% of the world’s mobile-phone operators.



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***(c) Climex***

The climate matching software, CLIMEX is an important tool for the exploration of the effects of climate on the distribution of an invading weed. The software allows for the simultaneous matching of the native range of a weed to that of a potential new home. CLIMEX is a dynamic model based on weekly responses of the weed to climate which are recorded as growth indices which are in turn used to describe the potential growth of the weed during favourable climatic conditions at that location (Sutherst *et al*. 2007). Eight stress indices (cold, wet, hot, dry, cold-wet, cold-dry, hot-wet, and hot-dry) are also used to predict the ability of a weed to survive under unfavourable conditions. The growth and stress indices are combined to calculate an eco-climatic index, which is then used as a measure of the favourability of a given locality for a species to establish and grow. These values for locations range from 0 (unsuitable) to 100 (maximum suitability). The compare locations function of CLIMEX was used to develop a distribution model for parthenium weed and one of its biological control agents the Mexican beetle (*Zygogramma bicolorata* Pallister) in a number of locations world-wide and the output being used to develop an integrated management plan for this weed in eastern Africa, Pakistan and Nepal.

***(d) Population dynamics models:***

Population dynamics models can also be used to investigate the evolution of traits in weed populations. In particular, models of the evolution of herbicide resistance (Thornby *et al.* 2009) have been useful in analysing how management practices and weed characteristics contribute to the speed of evolution of resistance to herbicides in specific weed cases, and in specific cropping systems. These types of models will continue to be useful for the development and refining of weed management strategies using herbicides.

***(e) Virtual plants***

Virtual plants are computer simulations of the structural development and growth of individual plants in three-dimensional space (Room *et al.* 1996). They are created in a model that contains rules for plant growth, and can be used to try out management ideas before testing them in field experiments. A range of environmental and other events, such as biological control agent attack and crop planting geometry, can be simulated. Recent work using the virtual plant approach includes an investigation of the competitiveness traits of chickpea cultivars to determine the crop architecture best able to suppress the growth of weeds (Cici *et al.* 2008). One further study used the three-dimensional computer modelling technique to develop a probabilistic model of the kinds of air turbulence that develops during pesticide spray application to plants with differing architectures to investigate the influence of plant architectures and crop geometry upon the pesticide spray application process (Dorr *et al.* 2006)

***(f) Biological control:***

A computer tool has been developed to help predict the risks and benefits from releasing a classical biological control agent into the field. In one use of the tool, quarantine testing had indicated that a beetle being evaluated for the management of cat's claw creeper (*Dolichandra unguis-cati* (L.) Gentry) would feed, if only marginally on a non-target native species. However, fitness data obtained for the insect feeding in its native range, where both target and non-target species co-occur, was used to predict the risk to the non-target species in the introduced range. Data showed that risk to the non-target species only became great when weed populations had significantly declined in number. Mapping of the range of the target and non-target species identified regions where an unacceptable feeding risk might occur and where acceptable control to be obtained. By considering both risk and benefit simultaneously it was possible to make a more objective decision on the release of the insect.



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***(g) Weed seed bank dynamics:***
The Weed Seed Wizard is a computer simulation tool that uses location-specific management inputs and site-specific weather data to predict and monitor changes in weed seed banks in arable land and estimates the losses in crop yield caused by the emergence of weed populations from the seed bank. The tool helps inform a land manager of the best weed management choices available to obtain the best management of a number of weed and to manage herbicide resistance. The Weed Seed Wizard is a scenario-exploring tool that is easy to use and provides farm consultants and farmers with the opportunity to explore the potential impacts of adopting various weed management strategies. It allows the user to test and verify existing rules of thumb and explore new ones, allowing the user to decide on the 'fit' of a new practice within their specific system and location (Renton *et al.* 2008).

**New management methods**
**(a) *Reduction in herbicide use***
For various reasons, notably those of herbicide resistance and the growing public demand for organically grown produce, there has been a push for land managers to use less herbicide in their weed management practices. To achieve reductions in herbicide use, a number of options could be used, 1) biological control including the use of bio-herbicides, living mulches and ground covers, 2) the use of natural plant products with herbicidal properties, 3) the use of suppressive (competitive and/or allelopathic) crop or pasture plants, 4) physical weed control delivered in the form of precision tillage and 5) precision chemical spray application technology.

## CONCLUSIONS

As identified by Rahman *et al*. (2012), the Asian Pacific Weed Science Society has played an extremely important role in connecting a very large number of weed scientists, from a large number of countries, over a large number of years, by providing a vibrant forum for the exchange of weed science information and ideas. However, we are now entering a period of unprecedented population growth and climate change, and wherethe growing demand for food, fibre and energy must be balance with the need for biodiversity conservation. The Society must rise to these new challenges and assist further in the immediate years, by 1) the setting up study groups to focus in on the key threats to food and land protection in our region such as those of weedy rice, herbicide resistance and new invasive weed introductions, 2) by the organising of regional workshops on these key threats then, 3) supporting the publication of the findings of these workshops within special issues of our national and regional weed science journals, 4) by encouragement of the younger scientists to seek higher degree education in weed science and related topics, and 5) being a voice in the identification of new emerging issues of weeds and creating long-term solutions to these new weed problems. As identified by Rahman *et al.* (2012) the Society must take on an active role in making the public aware of the weed factors that will endanger our food production and land protection systems and identify the ways in which these threats can be mitigated.

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# IMPLICATIONS OF WEEDS AND WEED MANAGEMENT ON FOOD SECURITY AND SAFETY IN THE ASIA-PACIFIC REGION

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**ABSTRACT** The food price crisis of 2007 and 2008 caused widespread food shortages and food and nutrition insecurity the world over. Home to the largest number of poor and undernourished people in the world, the Asia and Pacific region (APR) was at the epicenter of the crisis and was hit extremely hard. Although food prices have eased since then, recent studies indicate that food prices will remain high and volatile in the future. Reducing the existing large crops yield gaps is one of the appropriate approaches to meet the growing regional food security demands. Crop yield gap reduction is possible by optimizing crop productivity through identification and alleviation of major impediments such as weeds, which are more adapted to wide range of environments. Weeds continue to cause yield losses ranging from 10 to 60% depending on the crop and associated environment. Appropriate weed management has the potential to ensure food security by enhancing productivity and increasing profitability of farmers by cutting costs. Judicious selection, integration and proper application of herbicides will guarantee consumers the safety of foods they consume. However, impact of globalization, climate change, genetically modified crops and other recent trends, also have an impact on weeds and weed management. Severe labour scarcity, shortage of water for agriculture, emphasis on organic and conservation agriculture, are redefining the way we address weed problem. The solutions adopted by the developed countries may not suit the vast majority of the countries in the APR. It is time to evolve APR's own strategies and approaches. Besides these technological challenges, APR countries have to grapple with the problems of different sort such as the ignorance of vast majority of farmers about the weed problem, the inadequate capacity of the extension personnel and the insensitive administrators and policy makers. The weed scientists in APR countries have a daunting job at their hands to deal with this multitude of problems. Optimal weed management solutions, to meet the food security and safety needs, could be evolved from networking and collaboration with weed scientists from the developed countries in the region as well as from the other parts of the world.

***Keywords:*** Yield gap, integrated weed management, food security, climate change, Asia Pacific region.

## INTRODUCTION

Agriculture in the Asia and Pacific region (APR), accounts for 11% of the gross domestic product (GDP) and over 50% of total employment. In 2009, there were 1 billion hungry people in the world, an increase of about 100 million over the previous year. In the APR alone, the number of under nourished people increased by over 60 million in 2009 to 642 million. Food security has once again become a major issue for the world as the world population increased from 3.5 billion in 1970 to 8 billion in 2011 and is expected to reach 9 billion by 2050. Increasing population is putting pressure both on available cultivatable land and on yields required. In 2011, 925 million people suffered from chronic malnutrition, on average 16% of the population in the developing world, and this can be expected to worsen if there is no increase in world food production. With approximately 3.5 billion people, APR is



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home for 58% of the world's population. Reducing the existing large crops yield gaps is one of the appropriate approaches to meet the growing regional food security demands. Sustainable food security is a critical issue for countries in the region. As both the world's key supplier and largest consumer of food, what Asia does for its food security will have significant effects on ensuring sustainable global food security. With this realization, the Asian Development Bank (ADB), FAO and the International Fund for Agricultural Development (IFAD) joined forces to tackle widespread hunger and build food security throughout the APR and the details are available in the publication *Food For All - Investing in Food Security in Asia and the Pacific - Issues, Innovations, and Practices* (ADB, 2011).

In order to meet the demands of growing population, the agriculture in the next decade will have to sustainably produce more food from less area of land through more efficient use of natural resources with minimal impact on the environment (Hobbs et al., 2008). FAO calculated that food production must rise by 70% and most of this will have to come from increased yields per hectare of arable land (McFadyen, 2012).

The reduction of current yield losses caused by pests, including weeds, is a major challenge to agricultural production (Popp et al., 2013). Bakar (2010) rightly stated that the story of agriculture is also the story of weed interference. Weeds continue to be a perennial and constant threat to agricultural productivity, despite decades of research on development of modern weed control practices aimed at their elimination (Oerke and Dehne, 2004). Weed management has become more important today for increasing food crop productivity, keeping in view of food security and safety. The majority of the countries in the APR are developing countries and the work of weed management on farmland is generally underestimated by the public; many agricultural scientists and technologists. In this paper, we have tried to discuss the problem of weeds and their management in some select countries in the Asia- Pacific region in the background of their socioeconomic status and the challenges that are being faced in balancing food security and safety with sustainability of production systems.

***i) Socio-economic status***

In the world, Asia Pacific is the most populous region in which China is the most populous country followed by India (Table 1). However, by 2023 India is projected to have higher population (1456 million) compared to 1387 million of China. The population dependent on agriculture varies from as little as 2.4% in Japan to as high as 64% in Vietnam. Barring Australia, New Zealand, Japan and South Korea, in almost all the countries the population is mostly agrarian. Despite this, the contribution of agriculture to the total national GDP is less than 20% in all countries with the exception of Vietnam. This is because the majority of the farmers are small, marginal and resource poor who would mostly follow subsistence farming. About 87% of world's 500 million small holder farmers (operating less than 2 ha of crop land) are in the APR. People living below poverty line (BPL), is above 20% in Bangladesh, India, Pakistan, Philippines, Sri Lanka. China has done exceedingly well in keeping BPL population at 2.8% despite over 60% of the population dependent on agriculture. Similarly the undernourished population is over 20% in Bangladesh, India, and Pakistan. The gross trade is highest in China and Japan but the exports are higher than imports in Australia, India, Indonesia, Malaysia, New Zealand, Thailand and Vietnam.

***ii) Agriculture in Asia Pacific region***

India, with 169 million ha, has the highest area under agriculture in the region followed by China (Table 2). Bangladesh has the highest percentage (agricultural land to total land mass) followed by India. The lowest is with New Zealand followed by Australia. The percent irrigated area is over 50% of the cropped are in Pakistan, Sri Lanka, New Zealand, Japan, China and Bangldesh. The pesticide consumption is less than10 kg/ha in all countries except China, Japan and Philippines. The highest fertilizer consumption is noticed in New Zealand,



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Malaysia and China. The highest tractors numbers are reported from India, followed by China and South Korea.

The area, production and productivity of major food crops is given in Table 3. Rice is the main staple in the Asia and the Pacific region, providing almost 39 % of calories, followed by Wheat.Wheat is growing much faster than rice and it now makes up 19.2 percent of total calorie supply. Maize is Asia's third most important grain, but about 60 percent is now being used as animal feed. In developing Asia, rice availability is equated with food security and closely connected with political stability. Changes in rice availability and hence prices have caused social unrest in several countries, most recently during food crisis of 2008 (Timmer, 2011). The World Bank estimated that an additional 120 million people were pushed into poverty as a result of that crisis. It is often said that in Asia *Rice is Life* (Zeigler and Dobermann, 2011). The share of rice to cereals declined by 5% in South Asia and SE Asia, the decrease was over 13 % in East Asia (Table 4). Despite rapid transformation of Asian economies, agriculture remains very important. This is mostly because of large number of small farmers in Asia can not be moved to urban industrial and service jobs in just a few decades, even with rapid economic growth (Timmer, 2011). However, from a food security perspective, the overall importance of rice to Asian consumers is gradually declining (Table 5) The share of rice fell by 0.25% per year between 1960 and 1990, and by 1.00% per year from 1990 to 2007. This is an evidence of changing food habits. Open trade and globalization of tastes, there is preference for wheat, meat and dairy products, fruits and vegetables. Projecting forward, global rice consumption is expected to rise from the 441 mmt consumed in 2010 to about 450 mmt in 2012, before declining to just about 360 mmt in 2050 (Timmer, 2011). Another major force altering the food equation is shifting rural-urban populations and the resulting impact on spending and consumer and preferences. With an income growth of 5.5 %per year in South Asia, annual per capita consumption of rice in the region is projected to decline to its 2000 level by 4% by 2025. At the same time consumption of milk and vegetables is projected to increase by 70% and of meat, eggs and fish by 100 % (Kumar etal 2007).

***iii) Challenges in agriculture***

Feeding the increasing population is a big challenge particularly in the developing countries. According to the FAO, the food production must rise by 70% by 2015 and most of this will have to come from increased yields per hectare of arable land (McFadyen, 2012). This is most daunting considering the shrinking quantity and quality of resources, impact of globalization and changing food habits, climate change impacts, diversion of land for cultivation of crops for biofuel, industrial and urbanization demands etc.

Weeds have been one of the major constraints in production of crops. A substantial quantity of crop harvests is lost each year, due to untimely and inadequate weed control. Poor appreciation of the damage the weeds cause in crop production is most prevalent in most of the developing countries in APR. The following section discusses how better understanding of weeds and their management could help in addressing some of the challenges facing agriculture with respect to food security and food safety.

## MAJOR WEEDS AND LOSSES

### a) Major weeds

Weed communities of APR are floristically diverse because of variation in: agro-ecosystems in the region, seasonal crop management patterns at the farm level, spatial heterogeneity, soil type, fertility levels, water availability and other agro-ecological factors and have been adequately documented by many weed scientist from the region (Moody, 1989; Azmi and Baki, 2003; Zhang, 2003; Gogoi et al., 2005; Rao et al., 2007). Changing cropping patterns and agricultural practices have altered the floristic composition and the competition by weeds. The dominance of *Phalaris minor* in wheat- rice system (Yaduraju and Gopinath, 2005) and



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of weedy rice under direct-seeded rice cultivation (Rao et al., 2007; Chauhan, 2013) are some of the examples. Impending effects of climate change will bring in its own share of changes not only on composition of weeds, but also on the crop-weed competition and weed management.

**b) Weed losses**

Weeds are the main constraints in achieving higher crop production. To farmers, the most tangible losses due to weeds are those of crop yields and quality which have bearing on food security and safety, respectively. Baki, (2006) reported that those losses in the APR, range from 10 to 25%. The extent of crop yield losses due to weeds, vary depending on the crop and associated agro-ecological factors. Even with existing crop protection measures, approximately one-third yield losses occur globally (Bruce, 2012). Weeds contribute most to these losses. Global estimated loss potential of weeds in rice, wheat and maize indicate that weeds account for 46.2 % to 61.5% of potential losses and 27.3 to 33.7% of actual losses caused by all pests together (Table 6). The crop losses of Rs 900 billion ( US$ 2 billion) per annum in India to insect pests, diseases and weeds (Singhal, 2008). Zhang (2003) reported that in China, weeds are responsible for an average reduction of crop yields of 12.3–16.5%.

The economic impact of weeds in Australian winter cropping systems has been estimated in terms of an economic surplus loss of $1.3 billion (Jones et al. 2005). This surplus loss represented 17% of the gross value of Australian grain and oilseed production in 1998-99, and was comprised primarily of yield losses from residual weeds and herbicide costs. Sinden et al. (2004) have reported weed losses Australian agriculture in the range of $3.4 to4.4 billion per annum.

Most estimates take in to account only the losses in yield. However, if the cost of weed management, reduced efficiency of inputs, losses in quality, disease and pest occurrences (weeds being the alternative hosts of many diseases and pests) are taken into account, the figures can be quite monumental (Baki,2004).

Given the projected increase in demand for food by 2050, sustainable ways of preventing these losses are needed. Reducing crop yield losses due to weeds is one of the most promising measures to improve food security in the coming decades.

## WEED MANAGEMENT

Depending on the socio-economic conditions of the country, various methods of weed control are practiced. The objective of this paper is not to give a review of the various methods adopted in different countries. An attempt is however made to discuss some of them in the light of challenges the agriculture is facing today with respect to food security and safety.

Manual weeding is still the most predominant method of weed removal in many countries in the Asia and the pacific region. However, it is not only tedious, time-consuming and inefficient but is increasingly becoming uneconomical as well. Use of draught animals for intercultural operations is also coming down. Wages have gone up many-fold over the past two decades. In India the wages were less than $0.5 in 1970s are currently not less than $4 to $5 per person per day. In other words, one time hand weeding of an hectare rice which used to cost $10, costs now a minimum of $80. Poverty alleviation programs introduced in some countries to promote inclusive growth in economy have also contributed to the scarcity of labour for farm work. The introduction of National Rural Employment Guarantee scheme in India has been implicated for skyrocketing of wages and non-availability of labour for agricultural operations. The scheme guarantees by law 100 days of employment in a year for at least one member in the family living below poverty line (BPL). This flagship program which cost the exchequer nearly Rs 400 billion (USD 9 billion) annually has proved very successful and has benefitted a vast number of rural workforce (40 million households in 2010). Another scheme recently passed by the Indian Government- the National Food Security Bill proposes to ensure that every BPL family in the country will be entitled to 25 kg



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wheat (at Rs 3 = 5 cents) or 35 kg rice (at Rs 2 = 3.3 cents) per month. This ambitious scheme expected to cover 67 % o the population will cost the government a whopping over US$ 200 billion per annum.

As farm wages have increased due to economic growth and certain countries government policies in Asia, herbicides have increasingly been substituted for hand weeding (Naylor, 1996). Wage rates for farm workers in South East Asia have steadily increased; the average wage rate today is 5-10 times greater than what was prevalent in the 1970s. Between 100-200% increases in the current labor price are realistic expectations within 5-10 years (Beltran et al., 2012). Farmers are left with little choice but to reduce labor and production costs, particularly for the most labor-intensive tasks, such as manual weeding. Hence the herbicide use increased over years even in India and China (Table 7). In China, the area treated with herbicides has increased from less than one million hectares in the early 1970s to more than 60 mha in 2000 (Zhang 2001). In 1973 in China, it was estimated that rice crop losses due to weeds were 40% even though the crop was hand weeded several times. In 1988 with increased adoption of herbicides, the loss of rice to weeds was estimated at 8% (Moody, 1991).

In India, herbicides constitute 18% of the total pesticides (41,350MT) in 2004.Among field crops herbicide use in India is maximum in rice (Raju and Gangwar, 2004) and 57% area of wheat is under herbicide use in Punjab, Haryana, western Uttar Pradesh and Uttarakhand.

In Malaysia weed management is herbicide-based with no less than 70% of the pesticide market or RM276 millions annually for the past two decades (Baki, 2005). About US$4.10 million is spent annually on herbicides for rice alone, and this amounts to approximately 7% of the total expenditure on herbicides (Karim et al., 2004). In Philippines,96-98% of rice farmers use herbicides. The majority of farmers supplement herbicide application with hand weeding; 35% perform one additional hand weeding while 45% do two hand weedings. Three additional hand weedings are carried out by 15% of farmers (Gianessi and Williams, 2012).

In Pakistan, about 40% area of wheat, 20% in rice, 30% each in maize and cotton and 35% in sugarcane is treated with herbicides (Marwat and Azim- personal communication). In Bangladesh, the loss in rice yield in farmers' fields due to poor weed control is reported to be 43-51% (Rashid etal, 2012). The yield gap between herbicide use and hand weeding is as high as 1mt/ha with30% of farmers losing in excess of 500 kg/ha in the absence of herbicides (Ahmed et al., 2001). Pre-emergence herbicides in rice are 38-46% cheaper than one hand weeding (Mazid et al., 2001). The herbicide application gave 116% higher net income than hand weeding due to increased yield and lower cost (Rashid et al., 2012).

In the Philippines, the proportion of rice farmers using herbicides increased from 14% in 1966 to 61% in 1974 (De Datta and Barker, 1977).Today 96-98% of Philippine rice farmers use herbicides (Beltran et al, 2012). A recent study determined that with increased labor cost, herbicide application in rice fields is superior to manual weeding even at the lowest weed density by US$ 25-54/ha (Beltran et al, 2012)). At the highest weed density and highest labor cost, herbicide application is approximately 80% (about US$ 200/ha) more profitable than hand weeding. In Korea, the rice area treated with herbicides which was 27% in 1971 (Wang, 1971), went up to 65% in 1977 and currently entire area is treated with herbicides (Kim, 1981).

The trend of using more herbicide in rice production has been observed in Vietnam. There are about 37 compounds or proprietary mixtures formulated in 79 commercial products available for use in Vietnam. Vietnam used 5,000 tons of herbicides (19.4% of total pesticides) costing US$ 18 million, with rice herbicides contributing 89%. (Tuat et al., 2002).

In Nepal, herbicide use is yet to take off with 91% of rice farmers practicing manual weeding and only about 2% reported to have used butachlor (Regmi et al., 2009). About 7% did no weeding at all, particularly in lowland fields. In wheat, *Terai* farmers generally did not weed the crop but about 59% of the farmers removed weeds manually for animal feed. Only



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17% of the farmers used chemicals and 23% did not weed. In the hills, manual weeding was a common practice with 98% of the farmers following the practice.

Herbicides worth over $1.25 billion is used in Australia each year, covering more than 90% of the cropped area of wheat, barley, oats, sorghum and canola – the important food crops in the country (Michael Widderick, personal communication). In New Zealand, the total pesticide consumption is about 3,700 MT per annum with herbicides taking the highest share (68%) followed by fungicides (24%) and insecticides (8%) (Manktelow et al., 2005). They reported that herbicide imports increased by 42%, between 1999 and 2003

## CHALLENGES, OPPORTUNITIES AND IMPLICTIONS

### i) Higher resource use efficiency

One of the challenges facing agriculture is to produce more with limited resources. Enormous quantities of applied nutrients are wasted through inadequate and inappropriate agricultural practices. Timely weeding is critical for healthy crop growth as it will shift crop-weed competition in favour of crop. But it is seldom practiced. The fertilizers are simply broadcasted and placement of fertilizers is seldom practiced. Same is true with irrigation. Water will be the most limiting resource for agriculture in many countries. Simple interventions such as drip irrigation or supply of water in alternative furrows would reduce weed infestation substantially. Conservation agriculture by allowing the crop residues to remain on ground not only reduces the demand for water but also reduces soil erosion. Zero tillage is another resource conservation technology which also results in saving of water by 10-15%, besides effecting 15-20 % control of the major weed - *Phalaris minor*.

Rice requires huge quantities of water. Scheduling irrigation at alternate drying and wetting (AWD) cycle will result in saving of 15-40 % water. A simple technique developed at IRRI is helping thousands of farmers in Bangladesh, Vietnam and Philippines, in recording higher profits by observing the safe interval between wetting and drying cycles (Zeigler and Dobermann, 2011).

Direct-seeding of rice (DSR) is another approach to save water, labor and energy requirement. Several experiments carried out have demonstrated that the productivity of DSR is comparable to puddle transplanted rice, if weeds are managed adequately (Rao and Ladha, 2011; Kumar and Ladha, 2011; Rao and Nagamani, 2013). However, the weed management will be great challenge under DSR, particularly the problem of weedy rice (Rao et al., 2007; Chauhan, 2013).

### i) Conservation agriculture

In APR, smallholder farmers normally use tillage for the purpose of weed management and to facilitate planting of crops. However, conservation agriculture (CA) is gaining popularity word over and is considered as a sustainable method crop production. It ensures cultivation of crops with minimum or no disturbance of the soil and maintaining crop residues on the surface. In the last decade, farmers in the rice-wheat farming system in the Indo-Gangetic plain of Bangladesh, India and Pakistan have adopted minimum tillage practices widely. Since being introduced in the early 1990s, zero tillage for wheat has been adopted rapidly by more than one million farmers. Farmer’s wheat yields have been reportedly improved and production costs have decreased by an average of $ 65 per hectare with additional benefits being water conservation, saving of fossil fuel and reduced use of herbicides. However, there are issues that need to be addressed with respect to effective weed management. The performance of herbicides and integration of mechanical methods are the key ones.

Australian grain growers have been reducing their use of cultivation since the 1970s with 44% of the nation’s crop in no-till by 2001 (D’Emden and Llewellyn 2006). The falling price of the predominant knockdown herbicide, glyphosate, had a significantly positive effect on the adoption of no-till with 78% of the farmers practicing no-till in 2008. The use of no-till means that the seed is sown with minimum soil disturbance, reducing evaporation and



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increasing yields. In addition, no-till systems allow for earlier planting. Research demonstrated that using herbicides instead of tillage resulted in 27 mm of extra water in the soil profile and an increase in grain yields of 15-25% (Wylie, 2008). Agricultural green house gas (GHG) emissions can be curbed by decreasing fuel use by field equipment. Each gallon of diesel fuel burned by a tractor is estimated to release 10,180 grams of CO2 (EPA, 2011). In a wheat-fallow system in semi-arid subtropical Queensland, Australia, practicing zero tillage reduced fossil fuel emissions from machinery operation by 2.2 million g CO2/ha over 33 years or 67 kg CO2/ha/year (four to five tillage operations with a chisel plough to 10 cm during fallow each yearwere replaced by one herbicide spray).

**ii) Organic agriculture**

Organic food is increasingly being sought after by the health conscious public even at the cost of higher market prices. Weed management is a great challenge in organic farming. Acute shortage of labour, higher wages, unavailability of draught animals have made non-chemicals methods uneconomical. Further, in many countries of the Asia and the Pacific, wherein view of population pressure, increasing food production is priority, practicing organic agriculture on a large scale is highly unrealistic. The problem is much more serious in advanced countries like Australia, New Zealand and Japan. Non-chemical methods such as flaming, steaming, and new implements with sophisticated machine guidance and weed detection technologies are used for managing weeds. Use of a single biocontrol agent to control a wide variety of weeds is impossible. Designer crops which resist weeds is not in sight yet. Similarly, research on allelopathy, despite pursuing it for many years, has not yielded any practical solution till date.

**iii) Climate change**

Global climate change is a topic of serious discussion world over. There are many studies to indicate that the climate change would impact agriculture in a big way. Climate change manifested by droughts, floods, rise in sea levels etc would affect cropping pattern and crop productivity. Cereal yields are set to decrease up to 30% by 2050 in South Asia, in East Asia, for 10 raise in temperature expected by 2020s, water demand for irrigation would increase by 6-10% or more. Around 12 per cent of GHG emissions come from agriculture and the Asia-Pacific is responsible for around 40% of global agricultural emissions (Rosegrant et al., 2008). The APR is likely to face the worst impacts on cereal crop yields. Loss in yields of wheat, rice and maize are estimated in the vicinity of 50%, 17%, and 6% respectively by 2050 (http://www.ifpri.org/publication/impact-climate-change-agriculture-factsheet-asia). This yield loss will threaten the food security of at least 1.6 billion people in South Asia.

Very limited research has been conducted on weeds and their management under climate change in APR. Under elevated CO2, changes in temperature and precipitation patterns, both weeds and crops may be affected similarly depending on their photosynthetic pathway. It may be noted that 14 of the world's worst weeds are C4 plants (Holm et al. 1977), while around 76% of the harvested crop area in 2000 were grown with C3 crops (Monfreda et al. 2008). If the hypothesis is right that C3 crops would benefit more from elevated CO2 than C4 weeds, losses due to C4 weeds might decrease (Patterson and Flints 1980; Coleman and Bazzaz 1992; Ziska 2003). However, high temperatures due to global warming may decrease reproductive output despite an increase in CO2. In drought situations C4 weeds might also have advantages over C3 crops under elevated CO2 (Ward et al. 1999). C4 crops might out-compete better growing C3 weed in drought situations, and at higher temperatures utilizing mycorrhiza (Tang et al. 2009).

Much more research is needed to understand different factors involved in the climate change and their effect on crops, weeds and crop weed competition and weed control measures. Development of crop and weed management practices that are better adapted to changing climate is important for food security in the Asia - Pacific region.



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**iv) Alien invasive weeds**

Globalization-increased trade, tourism and travel has enhanced the risk of introduction of alien invasive weeds. The negative impacts of such invasions on biodiversity, environment, agriculture and health of humans and animals is well documented. The total loss to the world economy as a result of invasive non-native species has been estimated at 5% of annual production (Pimentel et al. 2001).The total annual cost of dealing with INS worldwide is estimated to be in the hundreds of billions of dollars, including costs of control, detrimental effects on human health and losses in agricultural production and ecosystem services (Sastroutomo and Hong, 2007).

An estimated 20-30% of all introduced species worldwide cause a problem (Pimentel et al. 2001). List of invasive weed species of Australia (DiTomaso, 2012 ), India (Reddy, 2008), Malaysia (Baki, 2004 ), Indonesia (Tjitrosoedirdjo, 2005), China (Xu, 2012);tropics (Yaduraju and Kathiresan, 2003); pacific (Sherley, 2000) and south and south east Asia (Pallewatta et al., 2003) are available. Several recent studies have been undertaken to estimate the economic impact of INS in a number of countries, which indicate that the cost of INS to a country's economy can be very high, but the estimates vary widely.

A synthesis of literature (Ziska and George, 2004) on impact of climate change on invasive weeds indicated that: a) invasive, noxious weeds on the whole have a larger than expected growth increase to both recent and projected increases in atmospheric CO2 relative to other plant species, b) rising CO2 can preferentially select for invasive, noxious species within plant communities; c) initial observations suggesting that control of such weeds may be more difficult in the future.

Yaduraju and Kathiresan (2003) attempted to identify the number of potential invaders in some of the countries in the APR. Matsui et al. (2005) have developed an internet data base to facilitate sharing of information among countries in the APR Asian and Pacific region, and to easily accumulate and search data on various species existing in each country. Thus the measures such as establishing early-warning mechanism, strengthening the management of invasive species and quarantine of alien species, establishing scientific system of introduction and improving people awareness are necessary to control the invasion of non native weed species.

The first line of defence against invasion is prevention, which by and large depends on legislation backed up by inspection procedures. But, unfortunately this has not been put into practice in many countries with the exception of Australia and New Zealand. The developing countries in the region should seek help from these two countries in capacity building in weed risk analysis and other relates issues. It has been reported from Australia, that every dollar spent on prevention activities, between $ 25.60 to 38.30 of benefits are provided (Table 8). Based on these findings, the estimated expenditure of $ 46.0 m million on weed management initiatives in 2005-06 is expected to generate a net benefit overall to Queensland of between $152 and 249 million. It is time other countries generate such data to impress the policy makers.

**v) Herbicide resistant weeds**

Herbicide resistance is a global phenomenon. The incidence of resistant weeds is more in developed countries like Australia, where herbicides have been in use for long. Among crops, wheat and rice have more herbicide resistant weeds than maize. The shift in method of rice establishment to direct-seeding, increased herbicide use and continuous use of similar herbicides is resulting in weeds resistance in rice of developing countries also.

Herbicides with novel modes of action are needed to combat the evolution of resistance to currently available herbicides (Kim, 2001). As the extent and cost of herbicide-resistant weed populations increases, farmers are being urged to invest in practices to prevent, or at least delay, further resistance development.



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**vi) Herbicide tolerant crops (HTCs)**

Genetically modified crops have become extremely popular since their introduction in 1996. Currently grown on over 170 mha area in 29 countries involving over 17 million farmers of which about 15 million are small and resource poor (ISAAA, 2012). Tolerance to herbicides is the most predominant trait contributing nearly 70% of the total area. India with 10.8 mha and China with 4.0 mha are ranked 5$^{th}$ and 6$^{th}$ in terms of total area under GM crops. Pakistan, Philippines, Australia and Myanmar are the other countries in the region growing GM crops. However, herbicide tolerant crops (Cotton & Canola) are cultivated only in Australia. Despite the predominance of HTCs in USA, Brazil, Argentine and Canada, they have not yet been commercialized in other Asian countries. In India, despite the strong support by the academia, approvals for GMCs including HTCs, are facing stiff opposition by the anti-GM groups. However, with the proposed introduction of Golden rice in Philippines and Indonesia and of bio-tech maize in China, the GM crops adoption prospects in Asia look brighter (ISAAA, 2012).

There is strong resistance to the technology in several countries. The impact of HTCs on biodiversity, development of super weeds and health risks are the major apprehensions coming in the way of their commercialization. There is ample evidence to demonstrate that the technology has indeed helped in recording yield increases- mostly realized due to better weed management. Safety of GM foods is again an issue hotly debated. But in USA- the country with the largest area under GM crops- consumption of GM food for over 16 years has not led to any health issues. The protagonists argue that GM crops would indeed auger well to deal with food security and safety.

Clearfield rice - a imidazolinone (IMI) resistant rice derived from conventional breeding technique, has been in cultivation in Malaysia mainly for managing weedy rice (Sudianto et al., 2013). It is under testing stage in Vietnam. The possible evolution of resistance to ALS-inhibitor herbicides in weedy rice and the risk of weedy rice acquiring resistance to herbicide following introgression of resistant gene from the HT rice are the major concerns that need to be addressed adequately. The risk of gene transfer may be higher in the centre of origin of a crop or when the crop and a related weed species are grown under a cropping system which promotes evolutionary selection (Mallory-Smith 2000). These are the reasons why release of GM soybean in east Asia and rice in India and China might be a danger where these crops have originated (Kim, 2001). Weed science community should engage in awareness and educational activities involving the public and other stakeholders and influence policy makers in taking informed decisions.

## CONCLUSIONS

"The Asia and the Pacific region is still home to some 578 million hungry people, approximately two-thirds of the world's hungry, so it is high time to move out of comfort zones and forge new partnership, collaborative arrangements and net works with the single objective of achieving food for all", said ADB president Haruhiko Kuroda (ADB, 2011). "Gross annual investments of $ 120 billion are required for primary agriculture and downstream services- in a responsible manner and focussed on rural areas through pro-poor programs and livelihood activities for poor and small farmers" - Said Diouf, the Director General of FAO.

The scientific solutions used in the developed world may not suit the vast majority of the developing countries in the APR. Hence new location specific strategies and approaches are to be evolved in APR.. Creating awareness is urgently needed among the vast majority of farmers, extension personnel and the insensitive administrators and policy makers, regarding the losses caused by weeds and the need for weed management at the critical period. The inadequate capacity of the extension staff needs to be alleviated by proper training of the trainers. The region has wealth of innovative technology and good practices- along with an



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abundance of natural and human resources. The challenge is how to harness and channelize these assets to ensure food security and safety to all, through better weed management and optimised crop productivity. In addition to chemical component, future research emphasis must be made equally on several non-chemical components of integrated weed management, which are neglected by public research institutes and are of not interest to private sector. Networking and collaboration with weed scientists from the developed countries in the region as well as from the other parts of the world are to be encouraged for evolving optimal weed management solutions to meet the food security and safety needs. Future weed management strategies for Asian pacific region should take in to consideration the present and future economic, social, and environmental concerns for reducing the detrimental effects of existing and invading weed species on food security and food safety.

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**Table 1.** Agricultural development in Asia Pacific region (2009)

| Country | Total Population (million) | Population dependent on Agriculture (%) | Agri. GDP (%) | Agri. Trade (billion US$) | | Urbanization (%) | BPL (%) |
|---|---|---|---|---|---|---|---|
| | | | | Import | Export | | |
| Australia | 22.0 | 4.0 | 2.5 | 7.8 | 23.6 | 97 | 12.8 |
| Bangladesh | 151.0 | 47.2 | 19.0 | 3.9 | 0.3 | 26 | 40.0 |
| China | 1336.0 | 62.0 | 12.1 | 59.2 | 32.2 | 37 | 2.8 |
| India | 1189.0 | 49.3 | 17.5 | 7.8 | 16.7 | 28 | 25.0 |
| Indonesia | 245.0 | 38.6 | 14.4 | 8.6 | 17.7 | 42 | 13.3 |
| Japan | 126.0 | 2.4 | 1.4 | 46.0 | 2.3 | 79 | 15.7 |
| South Korea | 48.0 | 5.2 | 2.5 | 14.9 | 2.6 | 83 | 15.0 |
| Malaysia | 29.0 | 13.0 | 10.2 | 8.9 | 17.7 | 58 | 5.1 |
| New Zealand | 4.4 | 8.0 | 5.6 | 2.6 | 13.5 | 86 | NA |
| Pakistan | 176.0 | 43.6 | 20.4 | 3.7 | 2.0 | 33 | 24.0 |
| Philippines | 101.0 | 34.7 | 14.9 | 5.6 | 3.1 | 59 | 32.9 |
| Sri Lanka | 22.0 | 43.8 | 13.4 | 1.6 | 1.2 | 23 | 23.0 |
| Thailand | 67.0 | 42.6 | 11.6 | 5.1 | 18.0 | 20 | 9.6 |
| Vietnam | 87.0 | 64.0 | 22.1 | 4.5 | 5.6 | 25 | 11.0 |

DES: Dietary Energy Supply

**Source:**

**1.** Selected Indicators of Food and Agricultural Development in the Asia-Pacific Region 1999-2009.

**2.** http://www.nationmaster.com/



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**Table 2.** Agricultural statistical data of Asia-Pacific countries

| S. No. | Country | Agricultural land (million ha) | Irrigated land as % of Agricultural land | Consumption (kg/ha) | | Agricultural Tractors ('000 Numbers) |
|---|---|---|---|---|---|---|
| | | | | Pesticides | Fertilizers | |
| 1. | Australia | 44.4 (5.8)* | 5.7 | 8.0 | 34 | 315 |
| 2. | Bangladesh | 8.7 (66.8) | 58.0 | 9.8 | 165 | 5 |
| 3. | China | 122.5 (13.1) | 52.3 | 27.6 | 468 | 2064 |
| 4. | India | 169.3 (56.9) | 36.8 | 6.6 | 189 | 3149 |
| 5. | Indonesia | 37.1 (20.5) | 18.1 | 3.0 | 189 | 70 |
| 6. | Japan | 4.6 (12.7) | 54.4 | 26.5 | 278 | 2028 |
| 7. | South Korea | 1.7 (18.0) | 47.6 | 13.4 | 480 | 244 |
| 8. | Malaysia | 7.6 (23.1) | 4.8 | 4.0 | 930 | 43 |
| 9. | New Zealand | 0.5 (2.0) | 82.0 | 4.7 | 1720 | 77 |
| 10. | Pakistan | 21.2 (27.5) | 93.7 | 2.5 | 163 | 470 |
| 11. | Philippines | 10.3 (34.5) | 14.8 | 18.0 | 131 | 63 |
| 12. | Sri Lanka | 2.2 (35.1) | 84.0 | 0.9 | 284 | 22 |
| 13. | Thailand | 18.8(37.0) | 34.0 | 2.1 | 131 | 830 |
| 14. | Vietnam | 9.4 (30.4) | 48.9 | 2.0 | 287 | 163 |

* Percentage of total land area

**Source:**

FAO Statistical Yearbook 2013

Selected Indicators of Food and Agricultural Development in the Asia-Pacific Region 1999-2009.

http://www.fao.org/nr/aquastat



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**Table 3.** Food grain production and Productivity of Asia-Pacific Countries (2010)

| S.No. | Country | Rice | | | Wheat | | | Maize | | | Pulses | | | Oil crops | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | P | Y | A | P | Y | A | P | Y | A | P | Y | A | P | Y |
| 1 | Australia | 0.02 | 1.90 | 9.5 | 13.51 | 22.14 | 1.6 | 0.07 | 0.399 | 5.7 | 1.75 | 1.92 | 1.1 | 2.05 | 1.03 | 0.5 |
| 2 | Bangladesh | 11.8 | 49.35 | 4.2 | 0.38 | 0.91 | 2.4 | 0.22 | 1.32 | 6.0 | 0.24 | 0.22 | 0.9 | - | - | - |
| 3 | China | 30.12 | 197.22 | 6.5 | 24.25 | 115.18 | 4.7 | 29.88 | 164.34 | 5.5 | 2.79 | 4.47 | 1.6 | 27.98 | 16.19 | 0.6 |
| 4 | India | 36.95 | 120.62 | 3.3 | 28.52 | 80.71 | 2.8 | 8.30 | 19.92 | 2.4 | 26.17 | 18.32 | 0.7 | 37.44 | 11.23 | 0.3 |
| 5 | Indonesia | 13.24 | 66.41 | 5.0 | - | - | - | 4.00 | 16.4 | 4.1 | 0.26 | 0.33 | 1.1 | 9.53 | 26.70 | 2.8 |
| 6 | Japan | 1.63 | 10.6 | 6.5 | 0.21 | 0.88 | 4.2 | <0.01 | 0.027 | 2.7 | 0.04 | 0.09 | 2.2 | - | - | - |
| 7 | South Korea | 1.46 | 8.23 | 5.4 | <0.01 | 0.03 | 4.1 | 0.02 | 0.102 | 5.1 | 0.24 | 0.20 | 0.9 | - | - | - |
| 8 | Malaysia | 0.67 | 2.55 | 3.8 | - | - | - | <0.01 | 0.052 | 5.2 | - | - | - | 4.25 | 19.12 | 4.5 |
| 9 | New Zealand | - | - | - | 0.04 | 0.32 | 8.1 | 0.02 | 0.224 | 11.2 | 0.01 | 0.03 | 2.9 | - | - | - |
| 10 | Pakistan | 2.36 | 7.23 | 3.1 | 9.13 | 23.73 | 2.6 | 1.05 | 3.57 | 3.4 | 1.47 | 0.83 | 0.6 | 3.30 | 0.99 | 0.3 |
| 11 | Philippines | 4.35 | 15.77 | 3.6 | - | - | - | 2.66 | 6.916 | 2.6 | 0.08 | 0.06 | 0.8 | 3.55 | 2.13 | 0.6 |
| 12 | Sri Lanka | 1.06 | 4.3 | 4.1 | - | - | - | 0.05 | 0.11 | 2.2 | 0.02 | 0.02 | 1.0 | - | - | - |
| 13 | Thailand | 10.99 | 31.6 | 2.9 | <0.01 | 1.00 | 1.0 | 1.04 | 4.264 | 4.1 | 0.18 | 0.17 | 0.9 | 1.08 | 1.73 | 1.6 |
| 14 | Vietnam | 7.51 | 40.00 | 5.3 | - | - | - | 1.12 | 4.48 | 4.0 | 0.40 | 0.40 | 1.0 | - | - | - |

**A:** Area in million ha. **P:** Production in million tonnes **Y:** Yield in tonnes per ha.

**Source**: FAO Statistical Yearbook 2013; Selected Indicators of Food and Agricultural Development in the Asia-Pacific Region 1999-2009.



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**Table 4.** Rice in Asian Agriculture

| Category | Rice as % of Cereal Production | | | Rice as a % of Agriculture | | |
|---|---|---|---|---|---|---|
| | 1961 | 1980 | 2007 | 1961 | 1980 | 2007 |
| World | 24.6 | 25.6 | 28.1 | 5.3 | 6.2 | 6.0 |
| East Asia | 56.2 | 53.2 | 43.0 | 18.9 | 20.2 | 8.3 |
| South Asia | 60.9 | 56.7 | 55.2 | 20.0 | 19.8 | 15.2 |
| Southeast Asia | 90.6 | 88.2 | 85.9 | 40.2 | 37.6 | 32.0 |
| Africa | 9.3 | 11.9 | 15.2 | 1.5 | 1.9 | 2.3 |

**Source:** Timmer, (2011).

**Table 5.** The changing role of rice in food consumption (Calories from Rice as % of total)

| Country | 1970 | 1990 | 2007 |
|---|---|---|---|
| Bangladesh | 75.1 | 75.2 | 69.8 |
| China | 38.7 | 33.4 | 26.8 |
| India | 32.4 | 35.2 | 29.9 |
| Indonesia | 54.8 | 55.2 | 48.8 |
| Republic of Korea | 48.6 | 35.6 | 26.8 |
| Philippines | 43.3 | 40.6 | 49.6 |
| Vietnam | 69.2 | 72.6 | 57.8 |

**Source:** Timmer, (2011).

**Table 6**. Global estimated loss potential and actual loss due to weeds in three major crops of Asia Pacific Region.

| Crop | Total loss due to all pests % | | Loss due to Weeds | | | |
|---|---|---|---|---|---|---|
| | Potential | Actual | Potential | % of total pests loss | Actual | % of total pests loss |
| Wheat | 49.8 (44-54) | 28.2 (14-40) | 23.0 (18-25) | 46.2 | 7.7 (3-13) | 27.3 |
| Rice | 77 (64-80) | 37.4 (22-51) | 37.1 (34-47) | 48.2 | 10.2 (6-16) | 27.3 |
| Maize | 65.8 (58-75) | 31.2 (18-58) | 40.3 (37-44) | 61.5 | 10.5 (5-19) | 33.7 |

**Source:** Oerke,( 2006).

**Table 7**. Global rice herbicide sales (million US$), selected years

| .Region | 1980 | 1988 | 1996 | 2007 |
|---|---|---|---|---|
| Japan | 459 | 753 | 703 | 490 |
| China | 19 | 11 | 51 | 125 |
| Republic of Korea | 15 | 37 | 117 | 84 |
| India | 15 | 26 | 28 | 50 |
| Rest of world | 119 | 219 | 196 | 436 |
| Total | 741 | 1,169 | 1,363 | 1,343 |

**Source:** Norton et al ( 2010).



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**Table 8.** Estimated benefit cost ratios for various weed management programs in Queensland, Australia

| | Activity | BCR range (%) |
|---|---|---|
| 1 | Prevention | 25.6 – 38.3 |
| 2 | Eradication (Siam weed as a test case) | 9.9 – 26.8 |
| 3 | Control ( *Acasia*, Rubber wine & Mesquite as case studies) | 1.7 – 3.1 |
| 4 | Research (ex: *Parthenium hysterophorous)* | 13.9 -24.4 |
| 5 | Education & awareness (Weed Buster) | 8.0 -79.9 |
| 6 | Environmental weeds | 1.1 – 1.8 |

**Source:** Sinden et al, (2004).

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# WEED MANAGEMENT IN CONSERVATION AGRICULTURE SYSTEMS – PROBLEMS AND PROSPECTS

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**ABSTRACT** Conservation agriculture has drawn the attention of resource management scientists throughout the globe since early 1970s, following widespread resource degradation problems and rise in energy prices. It is estimated that >125 M ha of the cropped area is under conservation agriculture systems in countries like USA, Canada, Australia, Brazil, Argentina, Australia as well as in some south and central Asian countries. In south Asia including India, some initiatives were undertaken since early 1990s to develop resource conserving technologies in rice-wheat cropping system. Conservation agriculture (CA) technologies involve minimum soil disturbance, providing a soil cover through crop residues or other cover crops, and crop rotations. Weeds are a major constraint in adoption of CA-based technologies. Conservation tillage influences weed infestation, and thus interactions between tillage and weed control practices are commonly observed in crop production. There are reports available that zero tillage increased as well as reduced infestation of certain weed species in different crops. In rainy season when the weed problem is generally more, growing crops with zero tillage required additional measures for effective weed control, including use of non-selective herbicides like paraquat and glyphosate. Zero-till sowing in standing crop residues along with application of herbicides in proper combination, sequence or in rotation led to lower weed population and higher yield than conventional planting. However, changing from tillage-based farming to no-till farming is not easy. No-till incurs a greater risk of crop failure or lower net returns than conventional agriculture, and this perception has seriously hindered its adoption in countries outside north and south America. Yields of no-till crops may be lower by 5-10%, especially on fine-textured and poorly-drained soils. No-till farming demands use of extra N fertilizer and heavy reliance on herbicides. Herbicide-resistant weeds are already becoming common on no-till farms in some countries. The continued practice of no-till is, therefore, highly dependent on development of new herbicide formulations and integrated weed management options.

***Keywords:*** Conservation agriculture, no-till farming, non-selective herbicides, crop residues, rice-wheat system, weed management

## INTRODUCTION

The birth of modern (conventional) agriculture coincided with industrial revolution. The identification of N, P and K as critical factors in plant growth led to the manufacture of synthetic fertilizers. While chemical fertilizers and pesticides have existed since the $19^{th}$ century, their use grew significantly in the early $20^{th}$ century with the invention of Haber-Bosch method for synthesizing ammonia. The rapid mechanization, especially in the form of tractor and combine harvester, coupled with science-driven innovations in methods and resources led to efficiencies enabling outputs of high quality produce per unit area and time. The contribution of Norman Borlaug and other scientists since 1940s towards development of crops for increased yields further accelerated the modern agriculture and initiated the era of `Green Revolution'. However, the growth of conventional agriculture thus attained was on the basis of capital depletion and massive additions of external inputs e.g. energy, water, chemicals etc.



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**Conventional agriculture systems**

The transformation of 'traditional animal-based subsistence farming' to 'intensive chemical and tractor - based conventional agriculture' has led to multiplicity of issues associated with sustainability of these production practices. Conventional crop production technologies have inculcated: (i) intensive tillage to prepare fine seed- and root-bed for sowing to ensure proper germination and initial vigour, improve moisture conservation, control weeds and other pests, mixing of fertilizers and organic manures, (ii) mono-cropping systems, (iii) clean cultivation involving removal or burning of all residues after harvesting leading to continuous mining of nutrient and moisture from the soil profile; and bare soil with no cover, (iv) indiscriminate use of pesticides, and excessive and imbalanced use of chemical fertilizers leading declining input-use efficiency, factor productivity and environmental, ground water, streams, rivers and oceans pollution, and (v) energy intensive farming systems.

**Emerging problems**

Green Revolution contributed to food security through increased food production and reduced volatility of foodgrain prices, and also demonstrated that agricultural development provides an effective means for accelerating economic growth and reducing poverty. But, post-Green Revolution input intensive conventional agriculture production systems have led to several global concerns, such as: (i) declining factor productivity, (ii) declining ground water table, (iii) development of salinity hazards, (iv) deterioration in soil fertility, (v) deterioration in soil physical environment, (vi) biotic interferences and declining biodiversity, (vii) reduced availability of protective foods, (viii) air and ground water pollution, and (ix) stagnating farm incomes.

The current state of production systems management is posing a threat to food security and livelihood of farmers, especially to poor and under-privileged smallholders in vulnerable ecologies. Hence, the agronomic management in conventional crop production systems need to be looked into critically and understood with an overall strategy of: (i) producing more food with reduced risks and costs, (ii) increasing input use-efficiency, viz. land, labour, water, nutrients, and pesticides, (iii) improving and sustaining quality of natural resource base, and (iv) mitigating emissions and greater resilience to changing climates.

**Change in conventional agricultural systems**

Widespread resource degradation problems under conventional system, and the need of reducing production costs, increasing profitability and making agriculture more competitive, have made the conservation issues more imperative. Globally innovations of conservation agriculture-based crop management technologies are said to be more efficient, use less inputs, improve production and income, and address the emerging problems (Gupta and Seth, 2007). Additionally, secondary drivers, such as: (i) availability of new farm machinery, (ii) availability of new biocide molecules for efficient weed, insect-pest and disease control, (iii) ever-decreasing labour force and ever-increasing labour cost, (iv) increasing production costs, energy shortages, erosion losses, pollution hazards and escalating fuel cost, and (v) residue burning, have accelerated change in thinking of researchers, policy makers and farmers to adopt modified methods for cultivation of crops aimed at improving productivity and resource-use efficiency.

**Conservation agriculture - a new paradigm in crop production**

Adequate food production for ever-increasing global population can only be achieved through the implementation of sustainable growing practices that minimize environmental degradation and preserve resources while maintaining high-yielding profitable systems. Conservation agriculture practices are designed to achieve agricultural sustainability by implementation of sustainable management practices that minimize environmental degradation and conserve resources while maintaining high-yielding profitable systems, and also improve the biological



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functions of the agro-ecosystem with limited mechanical practices and judicious use of external inputs. It is characterized by three linked principles, viz. (i) continuous minimum mechanical soil disturbance, (ii) permanent organic soil cover, and (iii) diversification of crop species grown in sequences and/or associations. A host of benefits can be achieved through employing components of conservation agriculture or conservation tillage, including reduced soil erosion and water runoff, increased productivity through improved soil quality, increased water availability, increased biotic diversity, and reduced labour demands.

Conservation agriculture systems require a total paradigm shift from conventional agriculture with regard to management of crops, soil, water, nutrients, weeds, and farm machinery (Table 1).

**Table 1.** Some distinguishing features of conventional and conservation agriculture systems

| Conventional agriculture | Conservation agriculture |
|---|---|
| • Cultivating land, using science and technology to dominate nature | • Least interference with natural processes |
| • Excessive mechanical tillage and soil erosion | • No-till or drastically reduced tillage (biological tillage) |
| • High wind and soil erosion | • Low wind and soil erosion |
| • Residue burning or removal (bare soil surface) | • Surface retention of residues (permanently covered soil surface) |
| • Water infiltration is low | • Infiltration rate of water is high |
| • Use of e*x-situ* FYM/composts | • Use of *in-situ* organics/composts |
| • Green manuring (incorporated) | • Brown manuring/cover crops (surface retention) |
| • Kills established weeds but also stimulates more weed seeds to germinate | • Weeds are a problem in the early stages of adoption but decrease with time |
| • Free-wheeling of farm machinery, increased soil compaction | • Controlled traffic, compaction in tramline, no compaction in cropped area |
| • Mono-cropping/culture, less efficient rotations | • Diversified and more efficient rotations |
| • Heavy reliance on manual labour, uncertainty of operations | • Mechanized operations, ensure timeliness of operations |
| • Poor adaptation to stresses, yield losses more under stress conditions | • More resilience to stresses, yield losses are less under stress conditions |
| • Productivity gains in long-run are in declining order | • Productivity gains in long-run are in incremental order |

**Prospects of conservation agriculture**

Conservation agriculture systems are being advocated since 1970s but it is only in the last 2 decades that the area has been increasing rapidly. This has been accelerated due to development of efficient farm machinery and availability of effective herbicides coupled trained manpower, which have resulted in reduced production costs and higher profitability, besides several indirect benefits. Presently, about 125 M ha area is practiced following the concepts and technologies for conservation agriculture; the major countries being USA, Brazil, Argentina, Canada and Australia (Table 2).

Farmers of the developing countries have also initiated to practice some of the conservation agriculture technologies. For example, presently resource conservation technologies are practiced in >3 M ha under the rice-wheat based system in the Indo-Gangetic plain. The major CA-based technology being adopted in this region is zero-till (ZT) wheat in the rice-wheat system; and it is now foreshadowing the age-old concept, popularly known as "more you till and more you harvest".



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**Table 2.** Global adoption of conservation agriculture systems

| Country | Area (M ha) | % of Global Area |
|---|---|---|
| USA | 26.5 | 21.2 |
| Brazil | 25.5 | 20.4 |
| Argentina | 25.5 | 20.4 |
| Australia | 17.0 | 13.6 |
| Canada | 13.5 | 10.8 |
| Russian Federation | 4.5 | 3.6 |
| China | 3.1 | 2.5 |
| Paraguay | 2.4 | 1.9 |
| Kazakhstan | 1.6 | 1.3 |
| Others | 5.3 | 4.2 |
| **Total** | **124.8** | **100.0** |

**Source:** FAO (2012)

Adoption and spread of ZT wheat has been a success story in north-western parts of India due to: (i) reduction in cost of production by 2000-3000 per ha, (ii) enhance soil quality i.e. soil physical, chemical and biological conditions in the long-term, (iii) enhance C sequestration and build-up in soil organic matter, (iv) reduce incidence of weeds, such as *Phalaris minor* in wheat, (v) enhance water- and nutrient-use efficiency, (vi) enhance production and productivity, (vii) advance sowing date, (viii) reduce greenhouse gas emission and improve environmental sustainability, (ix) avoid crop residue burning, loss of nutrient, environmental pollution, reduces serious health hazard, (x) provide opportunities for crop diversification and intensification, (xi) enhance resource use efficiency through residue decomposition, soil structural improvement, increased recycling and availability of plant nutrients, and (xii) surface residues as mulch control weeds, moderate soil temperature, reduce evaporation, and improve biological activity.

**Weed problems in CA**

Weeds are the major constraints in CA-based systems. Tillage affects weeds by uprooting, dismembering, and burying them deep enough to prevent emergence, by moving their seeds both vertically and horizontally, and by changing the soil environment and so promoting or inhibiting weed seed germination and emergence. Any reduction in tillage intensity or frequency may, therefore, influence the weed infestation. The composition of weed species and their relative time of emergence differ between CA systems and soil-inverting conventional tillage systems. Some weed seeds require scarification and disturbance for germination and emergence. Their germination and emergence may be accelerated by the type of equipment used in soil-inverting tillage systems than by CT machinery.

Shifts in weed populations from annuals to perennials have been observed in CA systems. Perennial weeds are known to thrive in reduced or no-tillage systems. Most perennial weeds have the ability to reproduce from several structural organs other than seeds. For example, Bermuda grass (*Cynodon dactylon*), nutsedge (*Cyperus rotundus*) and Johnson grass (*Sorghum halepense*) generally reproduce from underground plant storage structures: stolons, tubers or nuts and rhizomes, respectively. Conservation tillage may encourage these perennial reproductive structures by not burying them to depths that are unfavorable to emergence or by failing to uproot and kill them. Weed species shifts and losses in crop yield as a result of increased weed density have been cited as major hurdles to the widespread adoption of CA. Crop yield losses in CA due to weeds may vary depending on weed dynamics and weed intensity. However, the recent development of post-emergence broad-spectrum herbicides provides an opportunity to control weeds in CA. Crop yields can be similar for conventional and conservation tillage systems if weeds are controlled and crop stands are uniform (Mahajan *et al.*, 2002). Results of on-farm trials at several locations in Haryana, India revealed that



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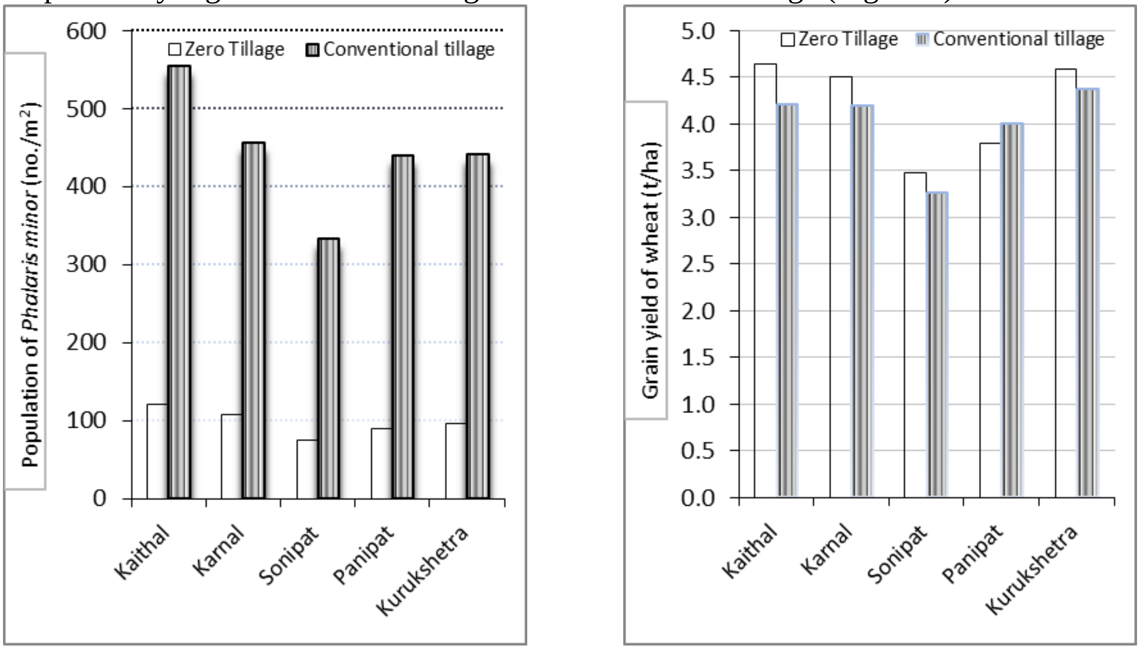
population density of *Phalaris minor* was considerably lower and grain yield of wheat was comparatively higher under zero tillage than conventional tillage (Figure 1).




**Source:** Gupta and Seth (2007)

**Fig. 1.** Effect of tillage on wheat yield and population of *Phalaris minor* at different locations in Haryana, India.

In the Vertisols of Jabalpur, India, zero-tillage significantly increased the population of *Vicia sativa* but reduced the population of *Chenopodium album* compared with conventional tillage. Higher yields of pea and linseed were recorded under ZT with herbicide application, which also proved to be more profitable than conventional tillage (Table 3).

**Table 3.** Effect of tillage and weed control on weed growth and yield of winter crops after rice at Jabalpur, India.

| Winter crops | Pendimethalin @ 1.0 kg/ha | | Weedy check | |
|---|---|---|---|---|
| | Zero tillage | Conventional tillage | Zero tillage | Conventional tillage |
| *Chickpea* | | | | |
| Seed yield (kg/ha) | 1592 | 2027 | 1450 | 1680 |
| Net returns | 16432 | 21039 | 15529 | 16385 |
| *Pea* | | | | |
| Seed yield (kg/ha) | 2227 | 2010 | 1508 | 1258 |
| Net returns | 23199 | 16075 | 13092 | 5739 |
| *Linseed* | | | | |
| Seed yield (kg/ha) | 1087 | 982 | 654 | 785 |
| Net returns | 8227 | 3037 | 2346 | 1286 |

**Source:** Mishra and Singh (2011).

In CA systems the presence of residue on the soil surface may influence soil temperature and moisture regimes that affect weed seed germinationand emergence patterns over the growing season.This shows that under CA system, farmers have to change the timing of weed control measures in order to ensure their effectiveness. Soil surface residues can interfere with the application of herbicides, so there is a greater likelihood of weed escapes if residue is not managed properly or herbicide application timings or rates are not adjusted.



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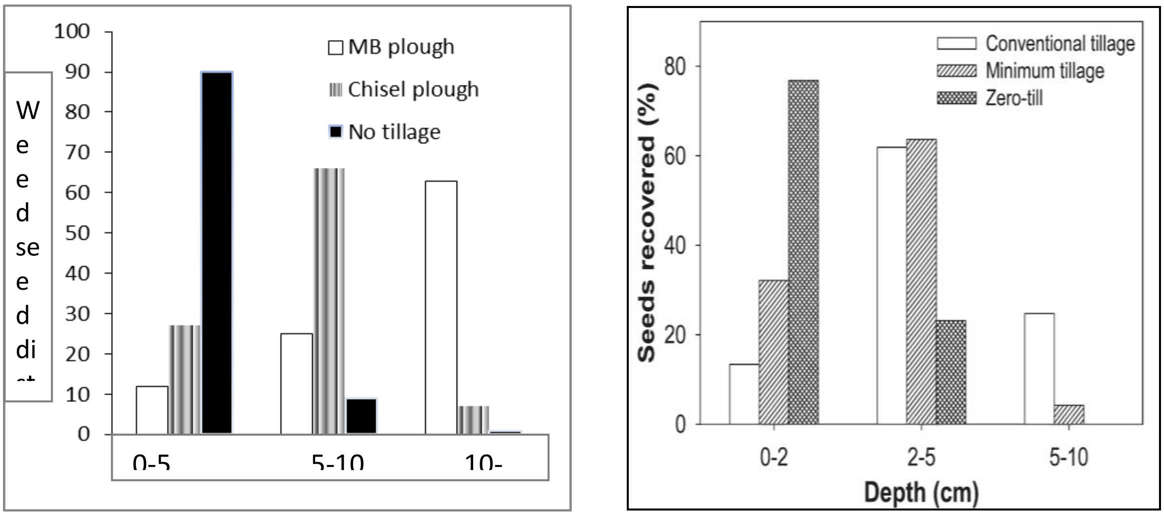
**Weed seed bank dynamics**

The success of CA system depends largely on a good understanding of the dynamics of the weed seed bank in soil. A soil weed seed bank is the reserve of viable weed seeds present in the soil. The seed bank consists of new seeds recently shed by weed plants as well as older seeds that have persisted in the soil for several years. The seed bank in the soil builds-up through seed production and dispersal, while it depletes through germination, predation and decay. Different tillage systems disturb the vertical distribution of weed seeds in the soil, in different ways (Figure 2). Moldboard ploughing buries most weed seeds in the tillage layer, whereas chisel ploughing leaves most of the weed seeds closer to the soil surface. Similarly, depending on the soil type, 60-90% of the weed seeds are located in the top 5 cm of the soil in reduced or no-till systems (Swanton *et al.*, 2000). As these seeds are at a relatively shallow emergence depth, they are likely to germinate and emerge more readily due to suitable moisture and temperature than those seeds which are buried deeper in conventional systems.

**Weed management**

There is a need to gain understanding on weed management as it is the major hindrance in CA-based crop production systems. Weed control in CA is a greater challenge than in conventional agriculture because there is no weed seed burial by tillage operations. The behaviour of weeds and their interaction with crops under CA tend to be complex and not fully understood. CA often causes weed shift resulting in increase in the density of certain weeds. The weed species in which germination is stimulated by light are likely to be more problematic in CA. In addition, in the absence of tillage, perennial weeds may also become more challenging in this system. Hence, effective weed control techniques are required to manage weeds successfully. In the past, attempts to implement CA have often caused a yield penalty because reduced tillage failed to control weed interference. However, the recent development of post-emergence broad-spectrum herbicides provides an opportunity to control weeds in CA. Various approaches being employed to successfully manage weeds in CA systems include: preventive measures, cultural practice (tillage, crop residue as mulches, intercropping, cover cropping, competitive crop cultivars, planting geometry, sowing time, nutrient management etc.), use of herbicide-tolerant cultivars, and herbicides.




**(a)** **(b)**

**Fig. 2.** Effect of tillage systems on vertical seed distribution of weed seeds.
**Source:** (a) Swanton *et al.* (2000), (b) Chauhan and Johnson (2009)

**Preventive measures**

Weed seeds resembling the shape and size of crop seeds are often the major source of contamination in crop seeds. Contamination usually happens during the time of crop harvesting if the life cycles of crops and weeds are of similar duration. Preventive measures



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are first and the most important steps to be taken to manage weeds in general and especially under CA as the presence of even a small quantity of weed seeds may cause a serious infestation in the forthcoming seasons. The various preventive measures include: (i) using weed-free crop seed, (ii) preventing the dissemination of weed seeds/ propagules from one area to another, (iii) using well-decomposed manure/ compost so that it does not contain any viable weed seeds, (iv) inspecting nursery stock/ transplants to prevent transplanting of weed seedlings from nursery to main field, (v) removing weeds near irrigation ditches and fence rows prior to flowering, (vi) mechanically cutting the reproductive part of weeds prior to seed setting, and (vii) implementing stringent Weed Quarantine Laws to prevent the entry of alien invasive and obnoxious weed seeds/ propagules in the region.

**Cultural practices**

A long-term goal of sustainable and successful weed management is not to merely control weeds in a crop field, rather to create a system that reduces weed establishment and minimizes weed competition with crops. Further, since environmental protection is a global concern, the age-old weed management practices, viz. tillage, intercultivation, intercropping, mulching, cover crops, crop rotation/diversification and other agro-techniques, which were once labeled as uneconomical or impractical should be relooked and be given due emphasis in managing weeds under CA. One of the pillars of CA is ground cover with dead or live mulch, which leaves less time for weeds to establish during fallow or a turnaround period. Some other common problems under CA include emergence from recently produced weed seeds that remain near the soil surface, lack of disruption of perennial weed roots, interception of herbicides by thick surface residues, and change in timing of weed emergence. Shrestha *et al.* (2002) concluded that long-term changes in weed flora are driven by an interaction of several factors, including tillage, environment, crop rotation, crop type, and the timing, and type of weed management practice.

Laser land leveling is an integral component of CA as it provides uniform moisture distribution to the entire field and allows uniform crop stand and growth, leading to lesser weed infestation. On the other hand, unleveled fields frequently exhibit patchy growth of crops. The areas with sparse plant populations are zones of higher weed infestation. Weed management in laser leveled field is relatively easier and requires less labour and time for manual weeding operation due to lesser weed infestation than unleveled one. A reduction of 75% in labour requirement for weeding operation was reported due to precision land leveling. Reduction in weed population in wheat after 30 DAS was recorded under precisely leveled fields in comparison to traditional leveled fields (Jat *et al.*, 2009).

**Chemical weed management**

Herbicides are integral part of weed management in CA. Use of herbicides for managing weeds is becoming popular as it is cheaper than traditional weeding methods, requires less labour even to tackle difficult-to-control weeds, and allows flexibility in weed management. However, for the sustenance of CA systems, herbicide rotation and/or integration of weed management practices is preferable as continuous use of a single herbicide over a long period of time may result in the development of resistant biotypes, shifts in weed flora, and negative effects on the succeeding crop and environment. In CA, the diverse weed flora that came up in the field after harvesting of preceding crop must be killed by using non-selective herbicides like glyphosate, paraquat, or ammonium-glufosinate. Non-selective burn-down herbicides can be applied before or after crop planting but prior to crop emergence in order to minimize further weed emergence.

Unlike in conventional system, crop residues present at the time of herbicide application in CA systems may decrease the herbicide's effectiveness as the residues intercept the herbicide and reduce the amount of herbicide that can reach the soil surface and kill germinating seeds. Proper selection of herbicide formulations for application under CA may



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be necessary to increase its efficacy. For example, pre-emergence herbicides applied as granules may provide better weed control than liquid-formations in no-till systems. Some herbicides intercepted by crop residues in CA systems are prone to volatilization, photo-degradation, and other losses. The extent of loss, however, may vary depending upon their chemical properties and formulations. Herbicides with high vapor pressure, e.g. di-nitroaniline herbicides are susceptible to volatilization loss from the soil surface. Climatic conditions and herbicide application methods may also have significant effect on herbicide persistence under CA systems. Crop residues can intercept 15-80% of the applied herbicides and this may result in reduced efficacy of herbicides in CA systems (Chauhan *et al.*, 2012). Choosing an appropriate herbicide and appropriate timing is very critical in CA systems as the weed control under no-till systems varies with weed species and herbicides used.

Several low-dose, high-potency, selective, post-emergence herbicides and mixtures are presently available in India for effectively managing weeds in crops like rice and wheat grown in sequence under CA (Table 4).

**Table 4.** Promising post-emergence herbicides for weed control in rice-wheat cropping system under CA

***(a)* Rice**

| Herbicides | Dose (g/ha) | Time of application | Remarks |
|---|---|---|---|
| Azimsulfuron | 35 | 20 DAS/ DAT | Controls annual grasses and some broad leaved weeds |
| Bispyribac-sodium | 25 | 15-25 DAS/ DAT | Controls annual grasses and broad-leaved weeds |
| Chlorimuron+ metsulfuron | 4 | 15-20 DAS/ DAT | Controls annual broad-leaved weeds and sedges |
| Pyrazosulfuron | 25-30 | 20-25 DAS/ DAT | Controls annual grasses and some broad-leaved weeds |
| Fenoxaprop-p-ethyl | 60-70 | 30-35 DAS/ DAT | Controls annual grasses, especially *Echinochloa* spp. |
| **Herbicides mixture** | | | |
| Fenoxaprop-p-ethyl + 2, 4-D | 60 g + 500 g | 20-25 DAS/ DAT | Controls annual grasses and broad-leaved weeds |
| Fenoxaprop-p-ethyl + almix | 60 g + 20 g | 20-25 DAS/ DAT | Controls annual grasses, broad-leaved weeds and sedges |
| Bensulfuron + pretilachlor | 10 kg/ha | 0-3 DAS/ DAT | Controls annual grasses and broad-leaved weeds |

**(b) Wheat**

| Herbicides | Dose (g/ha) | Time of application | Remarks |
|---|---|---|---|
| Clodinafop propargyl | 60 | 25-30 DAS | Controls annual grasses, especially *Avena* spp. |
| Metribuzin | 175-200 | 30-35 DAS | Controls annual grasses and broad-leaved weeds |
| Sufosulfuron | 25 | 25-30 DAS | Controls annual broad-leaved weeds and grasses |
| **Herbicides mixture** | | | |
| Sufosulfuron + metsulfuron | 32 | 25-30 DAS | Controls annual grasses, broad-leaved weeds and sedges |
| Mesosulfuron + idosulfuron | 12 + 24 | 20-25 DAS | Controls annual grasses, broad-leaved weeds and sedges |



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| | | | |
|---|---|---|---|
| Isoproturon + metsulfuron | 1000 + 4 | 20-25 DAS | Controls annual grasses and broad-leaved weeds |

**Herbicide-tolerant crop cultivars**

Weeds of different types emerge in the field, and therefore, the farmers have to use several types of narrow-spectrum herbicides to control them. This weed control method can be very costly and can harm the environment. Weed management, however, could be simplified by spraying a single broad-spectrum herbicide over the field anytime during the growing season. The important contribution of biotechnology has been the development of herbicide-tolerant crops for effective weed management. Several crops have been genetically modified to be resistant to non-selective herbicides. These transgenic crops contain genes that enable them to degrade the active ingredient in an herbicide, rendering it harmless. Herbicide-tolerant crops (HTCs) offer farmers a vital tool in fighting weeds and are compatible with no-till methods, which help preserve topsoil. They give farmers the flexibility to apply herbicides only when needed, to control total input of herbicides and to use herbicides with preferred environmental characteristics. Farmers can thereby easily control weeds during the entire growing season and have more flexibility in choosing times for spraying. The HTCs of several common crops, viz. soybean, maize, canola and cotton are being used by the growers, and the area under HTCs is rapidly increasing across the globe (Figure 3). Herbicide resistant crops also facilitate low or no tillage cultural practices, which are considered to be more sustainable.

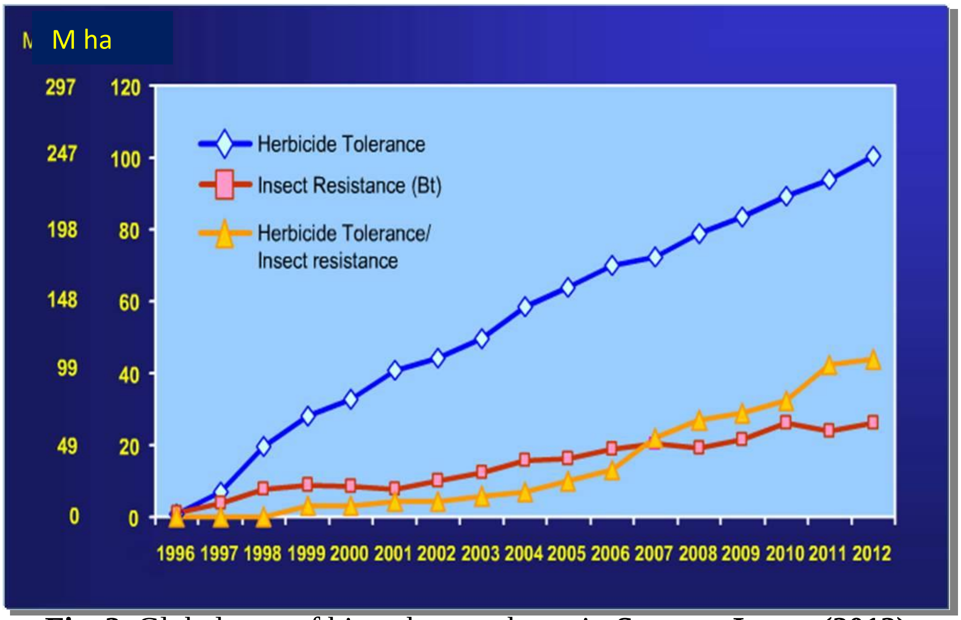


**Fig. 3.** Global area of biotech crops by trait. **Source: James (2012)**

Adoption of HTCs is the fastest growing agro-technologies in several countries of the world, as the area is expanding by 15-20% annually. This is also leading to conservation agriculture-based farming systems, resulting in reduced costs and improved soil health. It is unfortunate that the farmers in some countries are being deprived of such innovations in modern science due to some unfounded apprehensions. Introduction of such approaches will definitely contribute to the livelihood security of farmers and help in bringing about second green revolution in the country. However, herbicide tolerant crop cultivars should not be considered as a stand-alone component of weed management. An integrated weed management strategy should be used to ensure that this important weed management tool remains profitable and environmentally sound over a long period of time.



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**Integrated weed management**

Considering the diversity of weed problems, no single method of weed control, viz. cultural, mechanical or chemical, could provide the desired level of weed control efficiency under CA. Therefore, a combination of different weed management strategies should be evaluated for widening the weed control spectrum and efficacy for sustainable crop production. Integrated weed management system is basically an integration of effective, dependable and workable weed management practices that can be used economically by the producers as a part of sound farm management system. This approach entirely takes into account the need to increase agricultural production, reduce economic losses, risk to human health and potential damage to flora and fauna, besides improving the safety and quality of the environment. Integrated weed management system is not meant for replacing selective, safe and efficient herbicides but is a sound strategy to encourage judicious use of herbicides along with other safe, effective, economical and eco-friendly control measures. The use of clean crop seeds and seeders and field sanitation (weed-free irrigation canals and bunds) should be integrated for effective weed management. Combining good agronomic practices, timeliness of operations, fertilizer and water management, and retaining crop residues on the soil surface improve the weed control efficiency of applied herbicides and competitiveness against weeds. Approaches such as stale seedbed practice, uniform and dense crop establishment, use of cover crops and crop residues as mulch, crop rotations, and practices tor enhanced crop competitiveness with a combination of pre-and post-emergence herbicides could be integrated to develop sustainable and effective weed management strategies under CA systems.

**Payoff-trade off equilibrium in adopting CA systems**

As projected, CA is not a panacea to solve all the agricultural production constraints, but offers potential solutions to scientists and farmers to break productivity barriers and sustain natural resources and environmental health. But, for wider adoption of CA, there is an urgent need for researchers and farmers to change the past mindset and explore these opportunities in a site- and situation-specific manner for local adaptation. The current major barriers in spread of CA systems can be summarized as: (i) lack of trained human resources at ground, (ii) non-availability of suitable machinery other than north-western India and no quality control mechanism in place for CA machinery, (iii) competing use of crop residues in rainfed areas, (iv) weed management strategies, particularly of perennial species, (v) localized insect and disease infestation, and (v) likelihood of lower crop productivity if the site-specific component technologies are not adopted. Several factors including bio-physical, socio-economic and cultural limits the adoption of this promising innovation by the resource-poor small land farmers of south and south-east Asia. Despite several payoffs, there are also many trade-offs to adoption of CA systems (Table 5).

## CONCLUSIONS

It is possible to achieve the same or even higher yield with CA as with conventional tillage. Retention of crop residues on soil surface is essential for success of CA in the long-run. Zero-tillage along with residue has beneficial effects on soil moisture, temperature moderation and weed control. Zero-till systems cause shift in weed flora, and may result in emergence of perennial weeds like *Cyperus rotundus, Cynodon dactylon and Sorgum halepense* in most crops; and others like *Malava parviflora* and *Rumex dentatus* in wheat. Restricting tillage also reduces weed control options and increases reliance on herbicides. Altering tillage practices change weed seed depth in the soil, which play a role in weed species shifts and affect the efficacy of control practices. CA is a machine-, herbicide– and management-driven agriculture for its successful adoption. Integrated weed management involving chemical and non-chemical methods (residue, cover crops, varieties etc.) is essential for success of CA systems in the long-run.



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**Table 5.** Two sides of conservation agriculture

| Payoffs | Trade-offs |
|---|---|
| • Timeliness of operations<br>• Reduces soil erosion<br>• Conserves water<br>• Improves soil health<br>• Reduces fuel and labour costs<br>• Reduces sediment and fertilizer pollution of lakes and streams<br>• Sequesters carbon<br>• Climate smart production practices | • Mindset: transition from conventional farming to no-till farming is difficult<br>• Relatively knowledge intensive<br>• CA equipments are not available locally and adds on cost for transport<br>• Reliance on herbicides and their efficacy<br>• Prevalence of weeds, disease and other pests may shift in unexpected ways<br>• Need to refine nutrient and water management practices |

**Source:** Adopted from Huggins and Reganold (2008); Sharma *et al.* (2012).

**Research needs**

Weed management research is lacking under conditions of CA. Major efforts should be made to get profound understanding of weed, disease and insect responses to NT soil and microclimate conditions on long-term basis. Research should be conducted on soil biological aspects and on rhizosphere environment under contrasting soils and crops and with a special emphasis on optimizing fertilizer management under CA. Because herbicides cannot be eliminated from no-tillage, crop management, degradation pathways, adsorption–desorption and transport processes of herbicides remain important research areas. There is a need to carry out an analysis of factors affecting adoption and acceptance of no-tillage agriculture among farmers. Development of integrated weed, disease or pest control strategies is of paramount importance under conservation agriculture systems.

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# A POTENTIAL WEED CONTROL USING ROBOTIC IMPLEMENTS

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**ABSTRACT** Increasing concerns on food safety and environment have been major factors of driving research in a biological, physical and cultural weed control methods of the rice cultivation in the Republic of Korea. In addition, herbicide resistant weeds, superweed species and weedy(red) rice have recently occurred in rice paddy fields due to continuous specific herbicide use and direct-seeded rice such as a dry seeding as well as wet hill-seeded rice. Weedy rice or feral rice, also known as red rice in the Republic of Korea and some other countries, is conspecific to the crop being extremely hard to distinguish from cultivated rice, especially at early growth stage, weedy rice has particularly associated with its seed that make it a serious agronomic problem in the world. The national research project has been recently conducted to develop a robotic weeder in order to manage a weeds by mechanical and/or physical methods in lowland fields. The objective of our work was to develop a robot that can navigate a paddy in between rows and/or hills which were transplanted by the machine transplanter with equal distance. An initial prototype robotic battery-type weeder was manufactured and tested to navigate and control weeds in rice paddy fields, but a speed was so slow, and thus second engine-type prototype was developed. A working acreage for weed control has been attained at and up to 0.8ha per day. A small and young weed seedlings were uprooted and destroyed by a passive devices in between rows as well as hills. This robot was smoothly navigated in between rows on behalf of the guidance under camera and sensor systems and control weeds with mechanical by the use of implements such as a passive rotary weeders and then weeds would be cut and buried into the soils. Also generated was muddy water during operation which was non penetrated by light for weed germination to occur. We concluded that the robotic was an effective alternative implement to control weeds in lowland rice paddy as long as this tool was systematically introduced into the rice fields at three time intervals, *viz.* 15-20 days, 25-30days, and 35-40days after transplanting of rice seedlings.

***Keywords*****:** Weed control, rice paddy, robotic, transplanting, passive rotary, mechanical

## INTRODUCTION

Rice is the most important staple food crop in the republic of Korea. Food safety has been recently imposed pressure to shift from chemical control using synthetic herbicides of paddy weeds to organic farming due to consumer`s preference. There was a great changes of selective control, reasonable cost, without extensive labor input in comparison of conventional weed control with hand pulling method. Thus, weed science research was mainly focused on herbicide. Most (but surely not all) types of weeds, can now be controlled by herbicides, alone or in combinations (John and Masaru 2011). With the advent of glyphosate and the acetolactate synthase(ALS) inhibitors, there were very few important weed species that could not be well-controlled chemically. Yet, the reliance upon chemical weed control has brought previously unknown technical challenges, such as the advent of resistant



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weeds and adverse environmental impacts (John and Masaru 2011). Among the most troublesome weeds that affect rice worldwide, grasses(especially those belonging to the genera *Echinochloa* and *Leptochloa*) and weedy rice (*O. sativa*) have been a substantial negative impact on rice production. Species of *Echinochloa*, in addition of being ubiquitous to rice fields worldwide, were increasingly evolving resistance to rice herbicides (Heap 2013). Similarly, weedy rice, being the same species as cultivated rice, when present, became the most difficult to control weed in rice (Valverde 2013). Moreover, such challenges have been contributed to the advancement of weed management, including the integration of other biological and physical sciences into the design of weed management programs (John and Masaru 2011). In 21st century, we are facing the mass production of the agricultural crops with organic approaches for the food safety. In conventional way herbicide based weed control method has affected not only weeds which are herbicide resistant, but also the agro-products which are chemically polluted(Nakamura 1994). In order to overcome these side effects, agrochemical-free cultivation has been applied to the rice fields in the world. Recently, it has been widely used based on the animals such as duck, carp, and freshwater snail. However, it has been noted that the cultivation cost and controlling the animal was not the easy solution of these approach so that it was highly needed to find out a new weeding method. For the alternative solution, there were a few attempts based on the robotic weeding devices for the physical weeding(Kim et al 2012, Nagasaka et al 2009 and 2004, Lee et al 1999 and Bell 2000). However, these robots were not available to maneuver the row(inter-row spacing of the rice plant) and column(intra-row spacing of the rice planting) in rice paddy under 3-5cm depth with irrigated moisture condition and its weeding capability was not guaranteed. For the improvement of the row/column maneuver and eco-friendly weeding, this paper was introduced the robotic weeding system containing the weeding wheel device.

## THE ROBOTIC WEEDER : ELACTRICAL MOTOR BASED SYSTEM

First of all, we designed the prototype of the system in order to maneuver row and column with appropriate speed(Fig. 1). For satisfying this condition, we proposed 4 wheel timing belt systems for the driving and steering mechanism with gear placement for the steering.

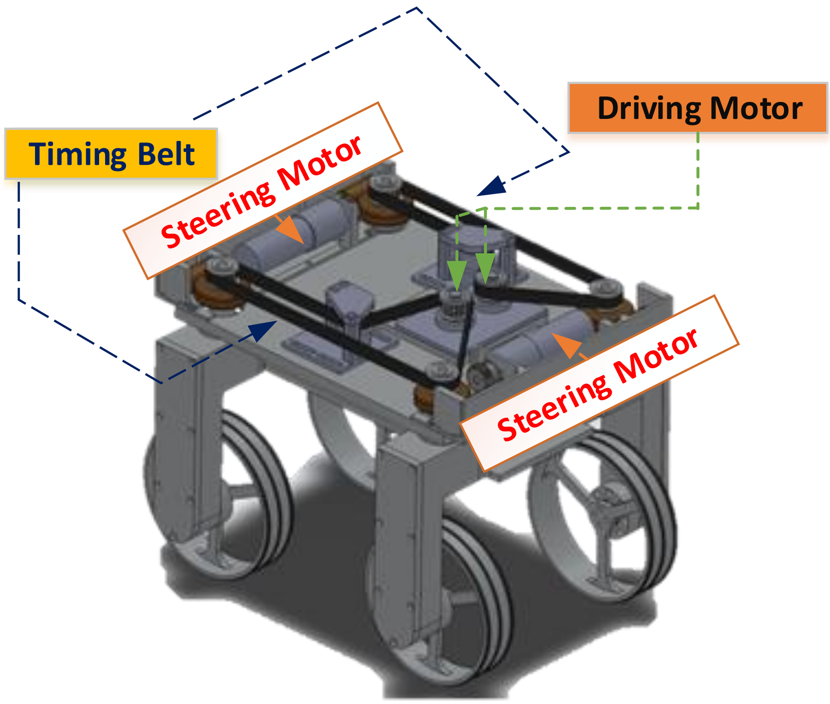


**Fig. 1.** Diagram on the relationship of each mechanical part of the Robotic Weeding System(RWS, Electric Motor based).



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## 1. SYSTEM ARCHITECTURE

*A. 4Wheel Timing Belt Connected Mechanism*

In order to drive robot system in the rice paddy environment, the power of the system should be increased by gear placement. In this reason, the rotational speed of the driving motor was decreased to the worm gear reduction(50:1) so that the driving power was fully enough with 1m/s movement in flat field area. In addition, the robot system was ought to compact enough to fit in 30x15[cm] rice paddy. In order to satisfy this, one single motor was connected to the 4 wheels with timing belt.

*B. Steering Mechanism with Worm Gear*

For the steering issue to move the columns of the rice paddy, 2 electric motors were placed in front and rear part of the wheel. Due to the high torque requirement during the wheel rotation, the worm gear was set to high reduction ratio(10:1).

*C. System Controller*

As far as the controller manipulates the system remotely, the AVR processor was equipped in the robot system. This processor was designed for interacting the remote signal from the operating control unit(OCU) with Zegbee protocol(Fig. 2). Once the signal of the OCU was recognized, the AVR process was generated the PWM signal so that the motors were able to rotate.

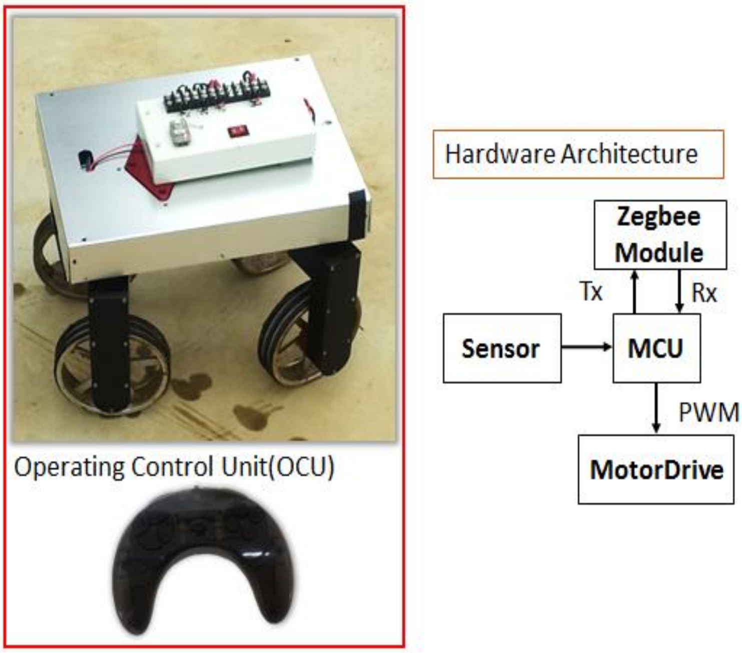


**Fig. 2**. Control system architecture of the Robotic Weeding System(RWS, Electric Motor Based).

## 2. WEEDING WHEEL DESIGN

In order to remove the weeds between column to column, it was necessary to design the proper weeding wheel equipped on the robot system. As far the weeding wheel design requirement, following condition should be satisfied.

- Maximizing Whirlpool Effect
- Maximizing Weed Uprooting

In order to fulfill these conditions, the weeding must be done with designing specialized wheels. As far as the whirlpool effect and weeding uprooting, 4 wheels were designed based



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on triangle-toothed wheel shape(Fig. 3). Since the roots of the weeds on the rice plant were normally 20mm after the rice-planting, the side of the triangle and its included angle were parameterized as $x$ and $\theta$, respectively. When the uprooting the weeds were done with driving, it was expected that the weight of the weeding wheel was to be increased. For minimizing the weight effect of the wheel, the parameters of the wheel was optimized with following equation.

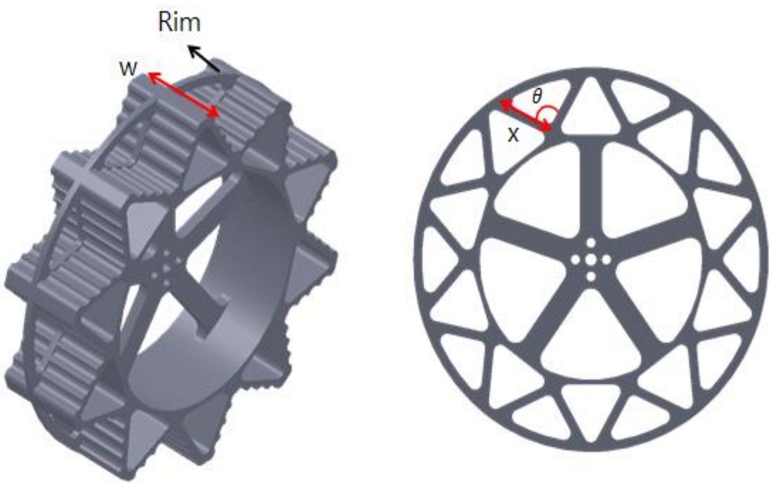


**Fig. 3.** Basic concept design of the weeding wheel for the Robotic Weeding System(RWS).

$$f(x,\theta) = \frac{1}{2}V = \frac{1}{2}x^2 \sin\theta w \qquad (1)$$

In this equation, two parameters, the side ( $x$ ) and the angle of the triangle were assumed to 30mm and 120°, respectively. In addition, the width of the wheel( $w$ ) was set to 40mm due to the size of the robot and the maneuvering space on the rice paddy(300mm). With these facts, it was possible to calculate the mass of the mud jammed in the wheel with assumption that the mud was stuck on the half of the volume of the triangle.

$$m_{increase} = \frac{1}{2}V\rho \qquad (2)$$

( $\rho$ : density of the mud )

Based on the calculated mass of the mud, the simulation of the stress of the wheel was conducted. Assuming that material of the wheel was aluminum(Al 7075), the mass of the

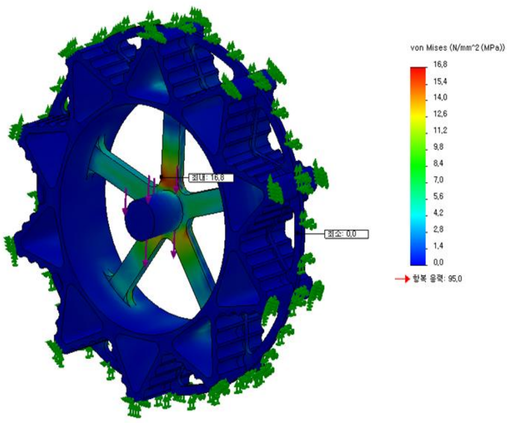


**Fig. 4**. Stress distribution of the weeding wheel.



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wheel was 420g and the yield stress was 95MPa. If the mass of the robot has the mass of 20kg, each wheel is loaded 5kg, respectively. For considering mud stuck in the wheel(safety factor= 4), each wheel is loaded 200N for the stress simulation. The result was shown that the maximum stress of the wheel was 16.85MPa which was much smaller than the yield stress(Fig. 4). With this result, the weeding wheel was manufactured.

## THE WEEDING CAPABILITY OF THE ROBOTIC WEEDER

### 1. TEST ENVIRONMENT

In order to verify the weeding capability of the robotic weeding system, the artificial environment was designed to 2500x2500[mm] size (Fig. 5). For the consideration of real rice paddy in Korea, the space of the row and column of the rice paddy were set to 300mm and 150mm, respectively. As far as the maintaining the size of the rice, the temperature and light were controlled at the PTC(Practical Training Centre) green house in the Korea National College of Agriculture and Fisheries (KNCAF).

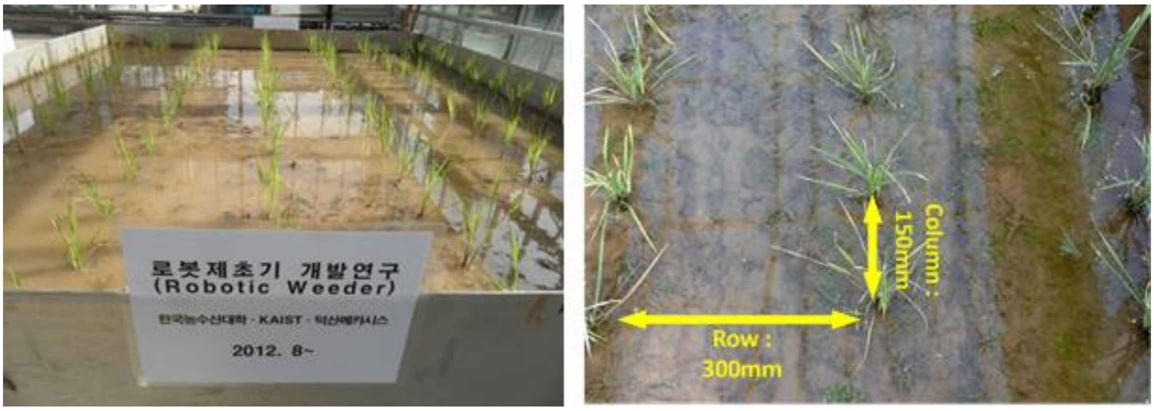


**Fig. 5.** Artificial environment of the rice paddy(2500x2500mm).


**Fig. 6.** Artificial environment of the rice paddy(2500x2500mm).

### 2. RESULT

*A. Column Direction Weeding by the Designed Weeding Wheel*

With the proposed devices, 4 weeding wheels, the weeds on the rice paddy were eliminated. As shown in Fig. 6, there was comparison between the mud condition before weeding wheel use and the condition after the wheel use. Based on the figure, the weeding wheels physically



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were marked their treads so that weeds were uprooted (Fig. 7). In addition, the whirlpool effect was followed during the drive because of the triangle shape on the wheel.

**Fig. 7.** A weed uprooted after operation by Robotic Weeding System

*B. Skid Steering Mechanism for Row Direction Weeding*

After the column weeding is finished, the robot maneuvers itself to the next column position with rotating the wheel. In order to verify this motion, the experiment was conducted after the column weeding. As shown in Fig. 8, the time of the wheel rotation(90°) was made in 22s without touching the rice plants.

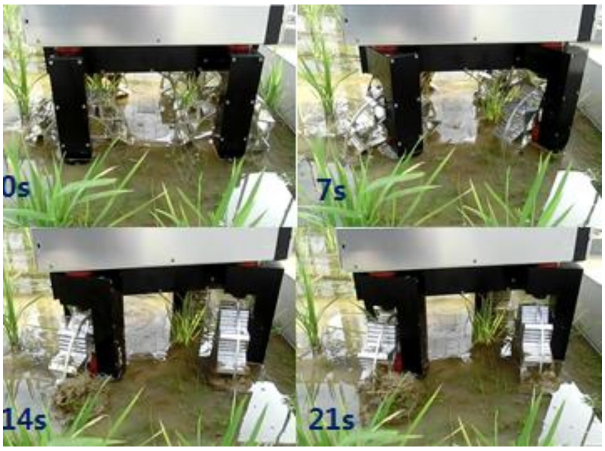


**Fig. 8.** Skid type wheel rotation for the column (intra-row spacing) maneuver.

## VISION SYSTEM FOR WEEDING ROBOT

This section was described the developed vision system for the weeding robot. In order to control weeds using robot wheel system, the robot needs to track a row of rice seedlings. The objective of the vision system was to detect a row and column(inter-row and intra-row spacing) of rice seedlings for autonomous navigation. To prevent damage to rice seedlings, the robot was required to track precisely the row of rice seedlings.

The vision system was composed of a general USB camera and a single board computer. The developed vision system was tested at the plot paddy field of the PTC (Practical Training Centre) green house in the Korea National College of Agriculture and Fisheries(KNCAF) shown in Fig. 9.



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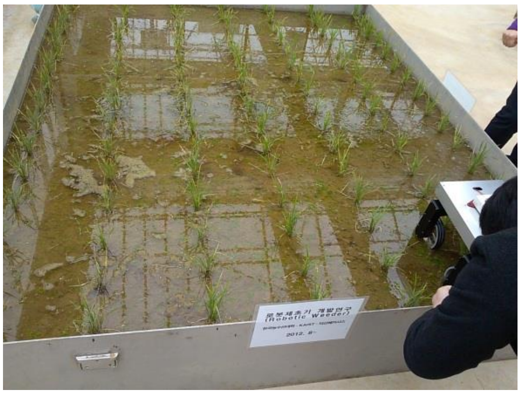

**Fig. 9**. Sample paddy field for vision system test.

Images were acquired by the camera installed on the robot with a rate of 15 frames per second. A sample image acquired by the robot was shown in Fig. 10.

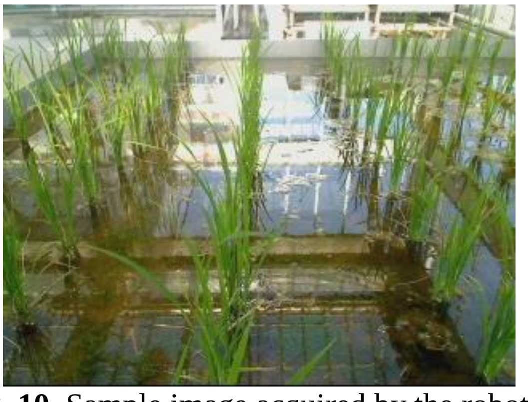

**Fig. 10.** Sample image acquired by the robot (Width: 320 pixels, Height: 240 pixels).

As we shown in Fig. 10, when we used a gray scale image, it would be difficult to classify rice seedlings since surroundings were reflected by water surface of the paddy field. So we decided to adopt color information in classification of rice seedlings.

The goal of the image processing was the calculation of a line which fit onto a row of rice seedlings as red line shown in Fig. 11. Once we could be accurately calculated the line, the robot could be tracked the row of rice seedlings using the slope and x-intercept of the line on the image plane.

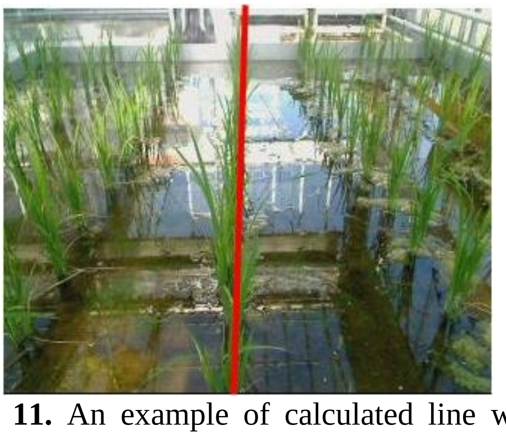

**Fig. 11.** An example of calculated line which fits on a row of rice seedlings.



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Considering the perspective view of the acquired image, ROI (region of interest) was selected as inside of the triangle which was shown in Fig. 12 using yellow lines.

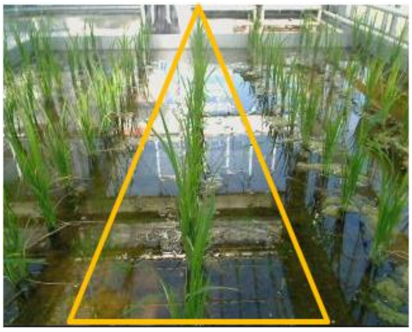

**Fig. 12.** ROI of the acquired image.

Applying classification algorithm to the above image, we could be obtained the image shown in Fig.5 where rice seedlings are appeared as white color region. Using ROI window, the image shown in Fig. 13 can be obtained.

**Fig. 13**. Classification of rice seedlings.

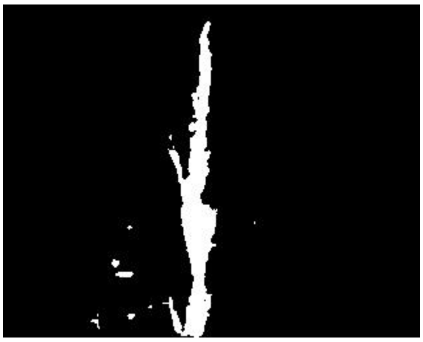

**Fig. 14**. Image after applying ROI.

The line fitted into the white region in Fig. 14 was calculated using least squares method. To avoid an infinite slope value in calculation, the image was rotated by 90 degrees counterclockwise before applying least squares method. After application of least squares method, we could be calculated the red line shown in Fig. 15. By rotating 90 degrees clockwise again, we could finally obtain the line shown in Fig. 15.



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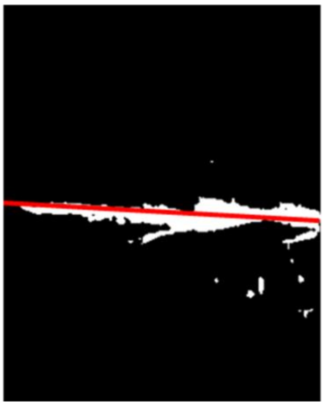

**Fig. 15**. Calculated line(red) after applying least squares method.

## CONCLUSIONS

In this study, we proposed a new type of weeding system for the rice paddy transplanted in equal distance. In order to satisfy the requirement, the system was designed as 4 wheel based skid steering mechanism. With the driving mechanism, the weeding wheel was designed with the optimized functions. Based on the design concepts, the robotic weeding system was applied to the artificial rice paddy environment. In this experiment, the capability of the weeding on the rice field was confirmed with physical uprooting and the whirlpool effect. In the aspect of the column maneuvering, the skid steering was effective to rotate the direction of the wheel to the next column. The vision system for weeding robot was selected by ROI(region of interest) where rice seedlings were appeared as white color region. The line fitted into the white region was calculated using least squares method. The weed control method of robotic weeding system in this study was evulsing, uprooting and/or cutting of weed by tracking over and stamping it flat in between the rice plants. The mud was simultaneously generated as robot's wheels go along the path in an inter-row spacing. It also contributed to the suppression of leaf photosynthesis of the weed plants, as the mud deposit, which might be rest on weed plants in between the rice shoots.

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# MANAGEMENT OF MULTIPLE-HERBICIDE RESISTANT *ECHINOCHLOA* SPP. IN RICE

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**ABSTRACT** *Echinochloa phyllopogon* is a major weed of rice. Populations of this species in California have evolved resistance to multiple herbicides within a system where rice is mostly water seeded, continuously flooded and grown in a mostly monocropping system that is heavily dependent on herbicides. Use of specific enzyme inhibitors, enzyme activity assays following induction by substrate and LCMS/MS suggested multiple resistance was due to enhanced herbicide metabolism via inducible P450s, GSTs and glycosyl transferases. Resistant plants have also insensitivity to ethylene stimulation by quinclorac, mechanisms to detoxify cyanide associated with ethylene biosynthesis, and to mitigate photooxidative stress caused by paraquat. Resistance could relate to enhanced stress tolerance and better adaptation to sub-optimal environments. These could, in turn, pre-select for herbicide resistance evolution in weed populations. Use of synergistic herbicide combinations, alternation of herbicides with different mechanisms of action, alternation of dry and water seeding methods, and use of stale seedbed techniques in combination with no-till are attempts at mitigation of resistance evolution. New options are needed to diversify a system where sustainability is compromised by resistance evolution to multiple herbicides in different weeds.

***Keywords:*** Target site, non-target site resistance, multifactorial, cross-resistance, multiple resistance, *Echinochloa*, *Cyperus*, stale-seedbed, glyphosate, stress tolerance, synergism.

## INTRODUCTION

Irrigated rice is a relevant crop in California, where it occupies approximately 220,000 ha (http:/www.calrice.org). Weeds are a serious problem in California rice, which is mostly grown as a monocrop and only a very small fraction is rotated to other corps. Water-seeding of rice and continuous flooding controls *Echinochloa crus-galli* and *Leptochloa fusca* ssp. *fascicularis* (Adair and Engler 1955). However, therepeated use of this system has resultedin the proliferation of aquatic weeds like *Echinochloa phyllopogon*, *E. oryzoides*, *Cyperus difformis*, *Schoenoplectus mucronatus*, *Sagittaria montevidensis*, and *Ammannia coccinea* (Hill et al. 1994). The aquatic *Echinochloas* are the worst weeds of rice and are poorly suppressed by flooding and farmers must use of herbicides such as thiobencarb, propanil, cyhalofop-butyl, clomazone, penoxsulam, pendimethalin (dry seeded rice) and bispyribac-sodium. The availability of only a few herbicides led to the repeated use over time of herbicides that target the same site of action, or are broken down in plants through similar biochemical pathways, which led to the evolution of herbicide-resistant (R) biotypes in all main weeds of rice. Thus the R *E. phyllopogon* accessions were cross-resistant to three (molinate, thiobencarb, and fenoxaprop-ethyl) of the four major herbicides used for its control (Fischer *et al.* 2000). The same cross-resistance was confirmed in accessions of *E. oryzoides* and *E. crus-galli*. Cross-resistance to bispyribac-sodium was also detected in *E. phyllopogon* accessions of CA (Fischer *et al.* 2000). In addition to these herbicides, *E. phyllopogon* is currently resistant to cyhalofop-butyl, clomazone, penoxsulam, halosulfuron, orthosulfamuron and and quinclorac (Bakkali et al. 2007; Yasuor et al. 2009; Yasuor et al. 2010; and pers. obs). Recently, *E. colona* with resistance to glyphosate was detected near rice fields, in orchard and corn fields subjected to repeated glyphosate treatment (Alarcón-Reverte et al. 2013). The high levels and broad-spectrum of herbicide resistance in *E. phyllopogon* have



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become a major limitation to sustainable rice production in California, where herbicide resistance in grasses and sedges affects the overwhelming majority of rice fields.

## THE EVOLUTION OF RESISTANCE

The evolution of multiple-herbicide resistance in *E. phyllopogon*, as mentioned earlier, appears to be the result of a single founder event by which a preselected biotype was introduced, dispersed and, subsequently, selected through the repeated use of thiocarbamate herbicides (Tsuji et al. 2003). Thus R accessions are genetically, morphologically and physiologically very close. Unlike the more diverse S plants, R *E. phyllopogon* has smaller plants with narrower leaves, thinner culms and lower seed production (Boddy et al. 2012; Tsuji et al. 2003).

Resistance in *Echinochloa oryzoides*, a species that coexists with *E. phyllopogon* in rice fields, has been much less studied but also involves enhanced metabolism of thiocarbamate herbicides, bispyribac-sodium, fenoxaprop-ethyl, and cyhalofop (Fischer et al 2000; Marchesi 2009). Sampled populations exhibit in response to treatment by thiobencarb a continuous gradation from susceptible to highly resistant, with considerable within-population heterogeneity. This suggests, selection for resistance is an on-going process with different levels of progress throughout rice fields (Marchesi 2009). A study of population genetic structure using microsatellites (SSRs) suggests short term dispersal may have contributed to the current patterns of spreads and some populations may have originated from a common R mutant (Osuna et al. 2011). However, the levels and spatial structuring of microsatellite marker variation detected in this study also suggest that resistance originated and evolved independently multiple times in northern California. The San Joaquin Valley in Southern California, remains free of herbicide-resistant *E. oryzoides*; suggesting barriers to dispersal and differences in the selection process (Osuna et al. 2011).

Barnyardgrass (*E. crusgalli*) is not a prevailing weed in water-seeded and continuously flooded rice in California. It is problematic in the limited dry seeded rice areas, or when fields are poorly leveled and poor water control exposes certain fields to aerobic conditions. Therefore, selection pressure for herbicide resistance (resistance patterns are similar to those of *E. oryzoides*) should have been limited, because *E. crus-galli* plants are not massively exposed to herbicides. Samples of this weed collected throughout California rice fields were mostly herbicide-susceptible (Marchesi 2009). Only a reduced number of accessions exhibited resistance when tested against thiobencarb (one of the original selectors for resistance in California) and just a limited number of populations showed the characteristics of a selection process in progress (Marchesi 2009). A microsatellite study involving *E. phyllopogon*, *E. oryzoides* and *E. crusgalli* showed evidences of hybridization between E. *crus galli* and *E. oryzoides*, suggesting gene flow between these two species may have introduced into *E. crus-galli* resistance genes previously selected for in *E. oryzoides* populations of flooded rice areas (Jasieniuk et al 2008, unpublished).

Therefore, selection for herbicide resistance is a complex and widespread on-going process throughout the entire CA rice area involving immigration, seed dispersal, independent mutational events, and possibly inter-species hybridization. Landscape and agricultural practices appear to mitigate resistance evolution in certain areas of Southern California.

## RESISTANCE MECHANISMS

**Target site resistance.** In *Echinochloa* spp. it has only been found in the case of resistance to glyphosate in *E. colona*, which has been selected in orchards, vineyards and in glyphosate resistant corn. This is is worrisome because of the proximity of these fields to rice. The mechanism of resistance involves point mutations in the *EPSPS* gene and presumed enhanced activity of this enzyme (Alarcón-Reverte 2013; García et al. 2013 unpublished). Ohterwise, target-site resistance has not been detected in the polyploid *Echinochloas* growing in California rice fields. Recent studies with Avena fatua and *E. phyllopogon* have postulated



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that polyploid plants with homeologous copies of a target gene may exhibit target-site mutations for resistance but still produce significant amounts of the target protein by the susceptible alleles. Thus a 'dilution effect' in individuals with multiple target site genes could preclude the expression of selectable resistance levels in spite of mutation for target site resistance (Iwakami et al. 2012; Yu et al. 2013).

**Non-target site resistance**. Resistance mechanisms in grass weeds of California rice have been mostly studied in the *Echinochloa* complex, mainly in *E. phyllopogon*, which we will summarize here. Multiple herbicide-resistant *E. phyllopogon* in California rice is the result of the founder introduction of a biotype that presumably came from Asia as rice seed contaminant (Tsuji et al. 2003). This biotype was dispersed throughout rice fields and further selected through the continuous use of thiocarbamate herbicides for more than 40 years. Thus resistance mechanisms in this biotype, which are mostly non-target site, reflect the metabolic detoxification of thiocarbamate herbicides in plants (Devine et al. 1993). Resistance to thiobencarb, bispyribac and fenoxaprop in R *E. phyllopogon* involve different inducible P450 isozymes (Iwakami et al. 2013; Yun et al. 2005). This enhanced monoxidative mechanism also endows resistance to penoxsulam and to clomazone (Yasuor et al. 2009; Yasuor et al. 2010); resistance to cyhalofop-butil resulted from enhanced metabolism of cyhalofop acid to polar metabolites including conjugates of cyhalofop-amide and cyhalofop-diacid, suggesting the involvement of P450 and of phase II conjugation enzymes (Ruiz-Santaella et al. 2006). Resistance to fenoxaprop-ethyl provided further evidence of the involvement of phase II metabolism as a mechanism of resistance in *E. phyllopogon*; thus R plants exhibited greater levels of fenoxaprop acid (the active form of the herbicide) detoxification to glutathione and cysteine conjugates (Bakkali et al 2007).

Resistance to photoactive herbicides like clomazone prompted investigation of the possible involvement of photooxidative protection mechanisms in *E. phyllopogon* resistance to this type of herbicides. Using a herbicide that is not degraded by most plants (paraquat) and supplementing treated plants with glutathione, we uncovered that multiple-herbicide-R *E. phyllopogon* also exhibited a $GR_{50}$-based R/S index to paraquat ~2.0, which could be attributable to glutathione mediated detoxification of reactive oxygen species (Yasuor et al 2008; Yasuor et al 2008, unpublished). However, resistance to paraquat was still more complex and preliminary evidence also suggests the involvement of a sequestration mechanism interfering with accumulation at the target site (Yasuor et al 2008, unpublished).

**Multifactorial and multiple resistance.** Resistance to quinclorac in *E. phyllopogon* is a good example of multifactorial resistance, where different target-site (auxin perception) and non-target-site mechanisms operate together to enable survival of quinclorac-treated R plants. Quinclorac has never been used in California, yet multiple-herbicide resistant *E. phyllopogon* is also R to this herbicide through a complex interplay of target site, hormonal and detoxification mechanisms (Yasuor et al 2011). Thus R plants exhibit low sensitivity to the auxinic stimulus and produce less ethylene in response to quinclorac treatment compared to S plants. Ethylene accumulation involves the concomitant production of cyanide, which is toxic to plants and is the cause of death by this herbicide (Abdallah et al. 2006). However, R plants are able to survive through cyanide detoxification via enhanced β-cyanoalanine synthase (β-CAS) activity. Resistance to quinclorac is associated with high constitutive levels of ethylene production in untreated R plants (Yasuor et al 2011). Ethylene can be produced in response to flooding as a mechanism of survival in plants (Drew et al. 2000). These R *E. phyllopogon* plants also have elevated levels of expression of a *β-CAS* gene, which is induced by flooding in both R and S plants (Alarcón-Reverte and Fischer 2013, unpublished). Flooding also induced greater levels of expression of an ascorbate peroxidase and a catalase gene in R vs. S plants, both genes code for enzymes known to be involved in photoprotection (Alarcón-Reverte and Fischer 2013, unpublished).



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All the resistance patterns and mechanisms discussed in this review for *E. phyllopogon* exist simultaneoulsly in individual plants of the R biotype that prevails as main weed of California rice. Multiple-herbicide resistance in *E. phyllopogon* is an example of multifactorial mechanisms that involve several genes. As suggested by the mechanisms of resistance to quinclorac, these non-target-site and multifactorial resistances may represent enhanced expression of tolerances to diverse environmental stresses. Therefore, intense use of selective herbicides in rice have most likely selected for enhanced levels of pre-existing functions that reflect the adaptive evolution of these *E. phyllopogon* plants to specific environments. This type of resistance that has evolved in weeds of monoculture rice subjected to intensive use of selective herbicides greatly threatens the sustainability of this crop through the upregulation of mechanisms conferring simultaneous resistance to multiple herbicides from different chemical groups and with different modes of action.

## RESISTANCE MITIGATION

**Herbicide use** for resistance management in California rice is based on the classic principle by which repeated use of herbicides with the same mechanism of action is avoided Gressel and Segel 1978). Herbicides representing different mechanisms of action and effective on same weeds are used in mixtures and sequences, which has the objective of delaying the evolution of target site resistance (Gressel and Segel 1990). Herbicide rates greater than needed are avoided to prevent accelerating the evolution of target-site resistance (Gressel & Segel 1978). However, low herbicide rates (such as those resulting from inadequate calibration, late application or diminishing herbicide residues in soil) are avoided. The repeated use of herbicides at low rates can enable the accumulation of minor genes (involved in polygenic trait inheritance or accumulated through gene amplification or sequential allelic mutations) that contribute towards non-target-site or multifactorial resistance ((Gressel et al. 1998; Powles and Neve 2005; Renton et al. 2011). Low herbicide rates are even presumed to induce, through stress, higher than normal mutation rates leading to multifactorial and major gene resistances (Gressel 2010). Therefore, herbicides are used at labeled rates not to favor the evolution of non-target site/multifactorial resistance, and the emphasis is to control plants (or their seed production) that escape control from an initial treatment using a second application with a herbicide (or mixture) of different mechanism of action. Synergism has been a tool for dealing with *E. phyllopogon* resistance to bispyribac-sodium, whereby sublethal rates of thiobencarb added to bispyribac synergistically reduced bispyribac $GR_{50}$ values on R plants by 50% without increasing toxicity to rice (Fischer et al. 2004). These effects could relate to interactions between thiocarbamates and enzymes of Phase I metabolism, resulting in better control at lower rates allowing reducing selection pressure for resistance, weed control costs and the herbicide load on the environment.

However, many weeds have already evolved mechanisms of simultaneous resistance to multiple herbicides through cross- and multiple resistances involving target and non-target site multifactorial mechanisms of broad and unpredictable specificity. Currently, use of herbicides in California rice mostly achieves weed suppression by overwhelming such resistance mechanisms through sequential applications, so that yields can be acceptable in that particular season. But weed survivors abound in fields and resistance problems are on the increase. Mixing and alternating herbicides is no longer a satisfactory solution. Diversifying herbicide packages may even hasten the evolution of non-target site based resistance to multiple herbicides.

**Attempts at diversification.** This is a difficult goal in this crop for which crop rotation is difficult to implement or economically unattractive. Current efforts seek to mitigate the dispersion of R-*Echinochloa* biotypes through a ban on *E. phyllopogon* and *E. oryzoides* contaminant seed in the certified rice seed. Strict monitoring of treated fields seeks to eliminate localized patches of surviving plants using accessory or even non-selective means.



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Field areas heavily infested with resistant weeds are harvested last, whenever possible, and an effort is made to clean machinery that is transported between fields.

A recent effort has sought to further diversify the system by introducing the benefits of crop rotation to a crop that is mostly not rotated with other crops. The technique has two main goals: Firstly, it alternates dry seeding with water seeding to modify the weed recruitment environment shifting from aquatic weeds to dryland adapted weeds (Pittelkow et al. 2012). Many of the aquatic weeds that have evolved resistance to sulfonylurea herbicides are thus disfavored when the system alternates to dry seeding, while water seeding effectively suppresses annual grasses like *E. crusgalli* and *Leptochloa fusca*, which have evolved R biotypes. Secondly, the system introduces the stale seedbed technique by which *Echinochloa* spp are encouraged to germinate using irrigation before seeding rice (Linquist et al. 2008; Pittelkow et al. 2012). The emerged seedlings are controlled with a non-selective herbicide for which resistance has not yet evolved (glyphosate, glufosinate and others still under testing). After this, fields are either flooded or seeded by airplane or drill seeded with no tillage. The objective is to eliminate resistant plants non-selectively before seeding rice, and then proceeding to implant the crop with no further soil movement so as not to bring new seed to the surface (Fischer et al 2004). It is crucial to eliminate most if not all seedlings that can emerge from the top soil; failure to achieve this results in weed seedlings emerging later with the crop, which cannot be effectively controlled due to resistance. For that purpose, a degree-day-based model has been developed to predict germination and emergence of *E. phyllopogon*, such that accumulation of approximately 157 $^{o}$C day results in >90% emergence provided the soil has been kept moist ($\Psi \sim 0$ MPa) (Boddy et al. 2012b). Results so far demonstrate that this alternative system leads to improved R- *E. phyllopogon* control, while remaining viable from an agronomic and economic standpoint in California (Pittelkow etr al. 2012). Other techniques for an integrated management of resistant weeds include the use of competitive cultivars to aid in the suppression of weeds (Perez de Vida et al. 2004).

In spite of these efforts for resistance mitigation, it is realistic to state that the current monocrop practice of California water seeded and continuously flooded rice is clearly not sustainable as far as weed control and management of herbicide resistance is concerned. Except for carfentrazone, all other selective herbicides currently available are affected by resistance. There is an urgent need for new options and for diversification of the system. The more diversified the weed control methods in a production system become, the longer will be the duration of their individual effectiveness.

Herbicide resistance may not just be the result of grower selection by herbicides, but may reflect a broader environmental adaptation. Therefore, intriguing questions can be posed. Can stress tolerance versatility confer weed pre-adaptation to herbicide resistance? Conversely, would herbicide selection for resistance favor highly stress-tolerant and more noxious weeds? Understanding the relationships between resistance to herbicides and gene expression for tolerance to specific stresses should open new crop management opportunities. It is possible that we are currently missing sustainable weed management opportunities due to insufficient knowledge on weed biology (Neve 2007).

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# MOMILACTONE PLAYS A CRUCIAL ROLE IN RICE ALLELOPATHY

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**ABSTRACT** Given the agricultural importance of rice, its allelopathic activity has been extensively studied. Large field screening programs and laboratory experiments in many countries have indicated that some rice varieties are allelopathic and release allelochemical(s) into their environment. A number of compounds such as phenolic acids, fatty acids, phenylalkanoic acids, hydroxamic acids, terpenes and indoles have been identified as potential rice allelochemicals. However, it was demonstrated that the diterpenoid momilactone A and B are the most important rice allelochemicals. Allelopathic rice varieties secrete momilactone A and B into its rhizosphere throughout its entire life cycle. The secretion levels of momilactone A and B increase up to the commencement of flowering activities, and decreases thereafter. The momilactone B secreted from rice seedlings appears to be the major contributor to the allelopathic activity of rice crops at least against barnyard grass. Moreover, the variations in allelopathic activity observed between different cultivars of rice may primarily reflect differences in their level of momilactone B secretion, which then seems to be the major allelochemical in rice. In addition, genetic studies have shown that selective removal of only the momilactone from the complex mixture found in rice root exudates significantly reduces allelopathy, demonstrating that these serve as allelochemicals, and their importance is reflected in the presence of a dedicated momilactone biosynthetic gene cluster in the rice genome.

***Keywords:*** Allelopathy, Allelochemical, Ecosystem, Momilactone, Phenolic acid, *Oryza sativa,* Root exudate.

## INTRODUCTION

The first observation of allelopathy in rice (*Oryza sativa* L.) was made in field examinations in Arkansas, U.S.A. in which about 191 of 5,000 rice accessions inhibited the growth of *Heteranthera limosa* (SW.) Willd./Vahl (Dilday et al., 1989). This finding led to a large field screening program. Since then, more than 16,000 rice accessions from 99 countries in the USDA-ARS germplasm collection have been screened. Of these, 412 accessions inhibited the growth of *H. limosa* and 145 accessions inhibited the growth of *Ammannia coccinea* Rottb. (Dilday et al., 1994; 1998). Similar attempts have been conducted in some other countries, and a large number of rice cultivars were found to inhibit the growth of several plant species when these rice varieties were grown together with these plants under field and/or laboratory conditions (Kim et al., 1999; Olofsdotter et al., 1999; Azmi et al., 2000; Gealy et al., 2003; Seal et al., 2004a; Kim et al., 2005). These findings suggest that rice may produce and secrete allelochemicals into its neighboring environment.

Several phenolic acids were found in rice root exudates of allelopathic and non-allelopathic rice cultivars (Olofsdotter et al., 2002; Seal et al., 2004a). However, allelopathic rice cultivars did not secrete significantly greater amounts of phenolic acids than non-allelopathic cultivars. Furthermore, considering the inhibitory activity of phenolic acids, the secretion level of phenolic acids from rice was insufficient to cause growth inhibition of neighboring plants (Olofsdotter et al., 2002; Seal et al., 2004b).

A number of secondary metabolites, phenylalkanoic acids, hydroxamic acids, fatty acids, terpenes and indoles, were identified in extracts and residues of rice plants as



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candidates for rice allelochemicals (Rimando and Duke, 2003). However, it is not yet clear if these compounds are released from living rice plants. Although most plant tissues contain potential allelochemicals, only compounds released from the plants into the environments are eligible to inhibit the germination and growth of neighboring plant species and, thus, act as allelochemicals in natural ecosystems (Putnam and Tang, 1986).

Dekker and Meggitt (1983) hypothesized that most allelochemicals are released during the early developmental stage when plants are most vulnerable to stress and compete with neighboring plants for resources such as light, nutrients and water. Therefore, in the present study, the allelopathic activity against *E. crus-galli* and secretion level of momilactone A and B into the growth medium were determined in eight rice cultivars at the seedling stage. Based on the interrelation between the cultivar-specific growth inhibitory activity and the secretion level of momilactone A and B, the contribution of momilactone A and B to rice allelopathy is discussed.

## MATERIALS AND METHODS

### Isolation of momilactone in rice culture solution

Rice seeds were sterilized and germinated and uniform germinated rice seedlings were transferred onto a sheet of plastic mesh (9 x 15 cm) that was floated on distilled water (300 mL) in plastic container (12 x 16 x 6 (height) cm), and grown at 25°C with a 12-h photoperiod. The water in the plastic container was kept at the same level by adding distilled water at 24-h intervals. After 14 days, the water in the container was filtered through filter paper. Then, the filtrate was loaded onto a column of synthetic polystyrene adsorbent (Diaion HP20), and eluted with 600 mL of distilled water, and 20 and 80% (v/v) aqueous methanol (300 mL), and with 450 mL of methanol. The biological activity of the fractions was determined using a cress bioassay as described below, and activity was found in fraction obtained by elution with methanol. After evaporation, the residue of the fraction was dissolved in 50% aqueous methanol and loaded onto reverse-phase $C_{18}$ Sep-Pak cartridges (Waters). The cartridge was eluted with 50% aqueous methanol followed by methanol. The activity was found in fraction obtained by elution with 50% aqueous methanol. After evaporation, the active material was purified by HPLC (1.0 x 50 cm, ODS AQ-325; YMC Ltd, Kyoto, Japan; eluted at a flow rate of 2 mL $min^{-1}$ with 70% aqueous methanol, detected at 220 nm), and inhibitory activity was found in a peak fraction eluted between 63 - 65 min, yielding an active component (2.1 mg). The active compound was characterized by high-resolution mass, $^{1}H$ NMR and $^{13}C$ NMR spectra.

### Toxicity of momilactone A and B on rice

Momilatone A and B were dissolved in a small volume of methanol, added to a sheet of filter paper (No. 2) in a 5.5-cm Petri dish and dried. Then, the filter paper in the Petri dishes was moistened with 2 mL of a 0.05% (v/v) aqueous solution of Tween 20. After sterilization, rice seeds were germinated on filter paper in the darkness at 25 °C for three days. Ten germinated seeds were arranged on the filter paper in the Petri dishes, and incubated in the darkness at 25 °C for two days. The length of roots and shoots of rice seedlings were then measured.

### Determination of momilactone A and B

Medium of the bioassay and filter paper in the Petri dishes were extracted with 100 mL of 50% aqueous methanol for two days. The extracts were then loaded onto a reverse-phase $C_{18}$ Sep-Pak cartridge. The cartridge was first eluted with 50% aqueous methanol to remove impurities, and then eluted with methanol to release momilactone A and B. Methanol elates were concentrated to 1 mL and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with the positive-ion mode and nitrogen for the collision gas (10 - 50 µL injection volume). Momilactone A and B, respectively, were detected in the multiple-reaction monitoring mode by the combination of m/z 315 and 217, and the



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combination of m/z 331 and 268. Quantification of momilactone A and B was performed as described by Obara et al. (2002).

**Donor-receiver bioassay**

Eight widely cultivated japonica type cultivars of rice, cvs Kinuhikari, Hinohikari, Nipponbare, Sasanishiki, Yukihikari, Norin 8, Kamenoo and Koshihikari were chosen as donor plants for the donor-receiver bioassay described below. *E. crus-galli* was chosen as a receiver plant because this plant is the most significant biological constrain to rice production (Xuan et al., 2006).

Rice seeds were surface sterilized and germinated on a sheet of moist filter paper (No. 1; Toyo Ltd., Tokyo, Japan) at 25°C with a 12-h photoperiod in a growth chamber. After four days, six rice seedlings per cultivars with uniform root and shoot length, were transferred to 5.5-cm Petri dishes each containing two sheets of filter paper moistened with 3 mL of 1 mM MES buffer (pH 6.0) as described by Weidenhamer et al. (1987), and grown for another three days.

Seeds of *E. crus-galli* were sterilized and germinated on a sheet of moist filter paper at 25°C in the darkness for three days. Ten *E. crus-galli* seedlings with uniform root and shoot length, were then arranged randomly on the filter paper in the Petri dishes and incubated with the 7-day-old rice seedlings at 25°C with a 12-h photoperiod. The medium in the Petri dishes was kept constant by adding the evaporated MES buffer at 12-h interval. After three days, the lengths of the shoots and roots of the *E. crus-galli* seedlings were measured. Control seedlings were incubated in the absence of rice seedlings. Percentage inhibition was determined by the formula: [(control plant length - plant length incubated with rice) / control plant length] x 100. There were three replicated per cultivars and the experiment was repeated seven times with three Petri dishes for each experiment. The liquid growth medium and filter paper in the Petri dishes were collected at the end of the bioassays for determination of momilactone A and B concentration.

## RESULTS AND DISCUSSION

**Discovery of momilactone in rice root exudates**

About 5,000 rice cv. Koshihikari seedlings were hydroponically grown for 14 days in order to isolate the exuded allelochemicals. Bioactivity guided fractionation led to purification of 2.1 mg of phytotoxic compound. The chemical structure of this putative allelochemical was determined from spectral data to be momilactone B (3,20-epoxy-3α-hydroxy-*syn*-pimara-7,15-dien-19,6β-olide, $C_{20}H_{26}O_4$). Secretion of momilactone B was later confirmed for other rice cultivars as well (Kong et al. 2004). In addition, another potential allelochemical, momilactone A (3-oxo-*syn*-pimara-7,15-dien-19,6β-olide, $C_{20}H_{26}O_3$) also was found in the root exudates of rice cv. Koshihikari (Kato-Noguchi et al., 2008b). Momilactone A and B further were found to be secreted by many other rice cultivars (Chung et al., 2006; Mennan et al., 2012).

Momilactone A and B were first isolated from rice husks as plant growth inhibitors (Kato et al., 1973; Takahashi et al., 1976). However, both were later (re)isolated as inducible antibiotics, which are termed phytoalexins (VanEtten et al., 1994), against the fungal rice blast disease pathogen *Magnaporthe oryzae* in rice leaves and straw (Cartwright et al., 1981). Thereafter, the function of momilactone A as a phytoalexin has been extensively studied, and several lines of evidence suggest that momilactone A has a role in rice defense against fungal pathogens (e.g. Jung et al., 2005; Okada et al., 2007; Hasegawa et al., 2010). By contrast, much less has been reported regarding momilactone B function as a phytoalexin.

**The momilactones are not toxic to rice**

Momilactone A and B only inhibited root and shoot growth of rice seedlings at concentrations greater than 100 and 300 μM, respectively. These inhibitory activities are only 1 - 2 % and



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0.6 - 2 % of the effect of the momilactones on the root and shoot growth of barnyard grass and *E. colonum*. Thus, the ability of momilactone A and B to suppress the growth of rice seedlings was much less than their effect on barnyard grass and *E. colonum*, with no visible damage to rice seedlings exerted by momilactone A and B at levels that are cytotoxic to these other plant species (Kato-Noguchi et al., 2008a). Considering the reduced inhibitory activity, and amount of momilactone A and B secreted into the rice rhizopsphere described below, it seems likely that these natural products exert **negligible** effects on rice plants themselves**. The basis for rice resistance is currently unknown, but presumably involves either efflux (e.g. via the same transport mechanism responsible for momilactone secretion), insensitivity of the molecular target (which is currently unknown),** and/or degradation.

**Rice selectively secretes momilactone B**

Rice secretes momilactone A and B into its rhizosphere throughout its entire life cycle (Kato-Noguchi et al., 2008a). The secretion level of momilactone A and B increases until flowering initiates, and decreases thereafter. The secretion rates for momilactone A and B, respectively, at day 80 (around flowering initiation) are 1.1 and 2.3 µg plant$^{-1}$ day$^{-1}$, which are 55- and 58-fold greater than they are at day 30. Interestingly, the endogenous concentrations of momilactone A and B, respectively, in rice are 4.5 and 3 µg/g in the straw (Lee et al., 1999), 4.9 and 2.9 µg/g in the seed husks (Chung et al., 2006), and 140 and 95 µg/g in whole plants, where levels also increased until the initiation of flowering (Kato-Noguchi et al., 2008a). Hence, the ratio of momilactone A to momilactone B within rice plants themselves is 1.5-1.7. By contrast, momilactone B is secreted at higher rate than is momilactone A, which suggests that momilactone B is preferentially secreted into the rhizophere relative to momilactone A. Plants secrete a wide variety of compounds from root cells by plasmalemma-derived exudation, endoplasmic-derived exudation, and proton-pumping mechanisms (Hawes et al., 2000; Bais et al., 2004; Bardi and Vivanco, 2009). However, the mechanism underlying secretion of the momilactones from rice roots is unknown.

**Allelopathic activity of rice cultivars**

Allelopathic activities of rice seedlings of eight cultivars against *E. crus-galli* were determined by a "donor-receiver bioassay". All rice cultivars inhibited the growth of shoots and roots of *E. crus-galli* seedlings, but with a different level of inhibitory activity. Cultivar Koshihikari showed the greatest inhibitory activity on both shoot and root growth of *E. crus-galli*. Previous studies also reported variations in the allelopathic activity among rice cultivars (Dilday et al., 1998; Kim et al., 1999; Olofsdotter et al., 1999; Azmi et al., 2000; Gealy et al., 2003; Seal et al., 2004a; Kim et al., 2005).

The *E. crus-galli* seedlings grew with the rice seedlings without competition for nutrients, because no nutrients were added in the bioassay medium. Competition for light can also be excluded as photosynthesis is considered to be unnecessary during the early developmental stages of these seedlings where most nutrients are withdrawn from seed reserves (Fuerst and Putnam, 1983). In addition, during the period of the bioassay no significant pH changes occurred in the medium (pH 6.0) at all Petri dishes. These results suggest that the inhibitory effect of rice seedlings against *E. crus-galli* may not be due to competitive interference for nutrients and pH changes in the medium but rather an allelopathic effect.

Momilactone A and B were found in the bioassay medium of all eight rice cultivars, but the concentrations in the medium differed. The highest concentrations of momilactone A and B were found in the medium of cv. Koshihikari, the cultivar with the highest allelopathic activity in bioassay. Momilactone A and B, respectively, in the medium of cv. Koshihikari were 6.9- and 5.8-fold greater than those of cv. Kinuhikari, the cultivar showing the least inhibitory activity in bioassay. As in the present bioassay only rice roots were only immersed in the bioassay medium, it is to assume that momilactone A and B were probably secreted by



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the rice roots into the medium in a cultivar-dependent manner. In addition, the concentration of momilactone B was greater than that of momilactone A in the medium of all rice cultivars, although concentration of momilactone A in rice plants was reported to be greater than that of momilactone B over the entire life cycle of rice (Cartwright et al., 1981; Lee et al., 1999; Kato-Noguchi et al., 2008b). Thus, the secretion of momilactone B from rice may be more active than that of momilactone A.

**Inhibitory activities of momilactone A and B**

The biological activities of momilactone A and B isolated from rice husks were determined with *E. crus-galli*. Momilactone A and B, respectively, inhibited the growth of roots and shoots of *E. crus-galli* significantly ($P<0.01$) at concentrations greater than 30 and 1 μM. When inhibition of *E. crus-galli* roots and shoots were plotted against the logarithm of momilactone A and B concentrations as described by Streibig (1998), good logistic functions were obtained. The estimated concentrations required for 50 % growth inhibition ($I_{50}$) of *E. crus-galli* shoots and roots, respectively, were 146 and 91 μM for momilactone A and 6.5 and 6.9 μM for momilactone B calculated from the equations. Thus, the inhibitory activity of momilactone B on *E. crus-galli* shoots and roots, respectively, was 22- and 13-fold greater than that of momilactone A.

**Contribution of momilactone A and B to rice allelopathy**

In order to quantify the contribution of momilactones to overall allelopathy, the potential growth inhibition of *E. crus-galli* caused by secreted levels in the donor-receiver bioassay was estimated using the derived equations of logistic functions and substituting X values by the concentrations found in the growth medium. These estimated inhibition values indicated that momilactone A secretion in bioassay has the potential to inhibit only 0.8 - 2.2% of shoot growth and 0.4 - 0.9% of root growth of *E. crus-galli* depending on the cultivar studied, which suggests that the concentration of momilactone A found in the medium may be far less than those required concentration to cause growth inhibition. However, the values indicated that momilactone B secretion in bioassay has the potential to inhibit 14 - 40% of shoot growth and 11 - 37% of root growth of *E. crus-galli*. Additionally, the synergistic effect of momilactone A and B on the growth inhibition did not find when momilactone A and B were applied to *E. crus-galli* at same time.

The contribution of momilactones A and B to the growth inhibition of *E. crus-galli* by rice seedlings was calculated by the formula: [estimated growth inhibition] / [measured growth inhibition in co-culture] x 100. Momilactone A accounts only for 2.2 - 4.9% and 1.0 - 2.6% of the observed growth inhibition of *E. crus-galli* shoots and roots, respectively, by rice seedlings, and momilactone B accounts for 58.8 - 81.9% and 59.5 - 67.3 % of the observed growth inhibition of *E. crus-galli* shoots and roots, respectively, by rice seedlings. Therefore, the contribution of momilactone B to the growth inhibition may be much greater than that of momilactone A, and momilactone B secreted from rice seedlings into the growth medium may explain a major part of the allelopathic activity of rice seedlings against *E. crus-galli*. Momilactone A secreted from rice seedlings may contribute very limited part of the allelopathic activity of rice seedlings. In addition, the cultivar-specific concentrations of momilactone B in the medium was significantly ($P < 0.01$) correlated with the extent of the growth inhibition of *E. crus-galli* by the eight tested rice cultivars. The regression coefficient ($r^2$) was 0.941 and 0.994 for *E. crus-galli* shoots and roots, respectively. Thus, the differences in the allelopathic activity between cultivars may be primarily caused by the level of momilactone B secretion. This is the first report describing that allelopathic activity of rice may be primarily depend on the secretion level of a specific allelchamical, momilactone B. There has been no information describing such contribution of single allelochemical in other crops to date.



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A total of the estimated contribution of momilactone A and B was 61.4 - 86.6% and 61.3 - 69.9% of the observed growth inhibition of *E. crus-galli* shoots and roots, respectively, by rice seedlings. The remaining 14.4 - 38.6% (shoots) and 30.1 - 38.7% (roots) of allelopathic activity of rice that cannot be explained by secretion levels of momilactone A and B, might be caused by other allelochemicals identified in rice, such as 3-isopropyl-5-acetoxcyclohexene-2-one-1 and 5,7,4'-trihydroxy-3'5'-dimethoxyflavone (Kong et al., 2004). As it is reported that the secretion level of momilactone A and B by rice plants increases until initiation of flowering (Kato-Noguchi et al., 2008b), it may be possible that the contribution of momilactone A and B to rice allelopathy may be also increase until the flowering initiation.

Although mechanisms of the exudation are not well understood, it is suggested that plants are able to secrete a wide variety of compounds from root cells by plasmalemma-derived exudation, endoplasmic-derived exudation, and proton-pumping mechanisms (Hawes et al., 2000; Bais et al., 2004). Through the root exudation of compounds, plants are able to regulate the soil microbial community in their immediate vicinity, change the chemical and physical properties of the soil, and inhibit the growth of competing plant species (MuCully, 1999; Hawes et al., 2000; Bais et al., 2004). Considering the inhibitory activity of momilactone B and the secretion level of momilactone B, momilactone B may play a very important role in rice defense mechanism in the rhizosphere as an allelopathic substance.

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# WEED RISK ASSESSMENT - A REVIEW

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**ABSTRACT** Weeds are known to reduce crop yield, their entry to a country, therefore, must be prevented Weeds are also known as invasive alien plant species. They have bilogical characters that support their behaviour. Those characters specified by many scientists were integrated to identify them. The integrated information is also organized into a Weed Risk Assessment Model to predict plants if they have a potency to be come a weed or an nvasive species. Risk is defined as likelihood of undesired event occuring as a result of behaviour or action. There are 2 WRA models, pre- and post-border ones. The first pre-WRA was developed in Australia, and widely adopted internationally. It consists of 49 questions that must be answered either yes, no or unknow with prescribed score point. Total score point of >6 is rejected to total point of <1 ia accepted, while total score of 1-6 must be rvaluated futher. Despite its wide aceptance internationally, AWRA does not seperate between consequences and likehood, and modified accodingly, modification is also suited to local situation. It is suggested that Indonesia for pre-border WRA adopts HWRA. Post-border WRA is for priorititation of management actions using numerical ratings, and is divided into two main sections: I. Significance of Impact and II. Feasibility of Control or Management. Each section is based on a scale of 100 points. Significance of Impact is further divided into *A*. Current Level of Impact and *B*. Innate Ability of Species to Become a Pest. A species with a combined score of over 50 points for significance of impact to be seriously disruptive and needing appropriate attention. Species receiving high scores for feasibility of control will be easier to control than those receiving lower scores. Many more post border WRA have been developed, and it is suggested that Indonesia adopts post-border WRA that was developed in South Australia.

***Keywords:*** Risk, likehood, consequences, pre-border weed risk assessment, post-border weed risk management.

## INTRODUCTION

### Background

Weeds have been recognized as plant pests capable of inflicting a considerable damage to agricultural production systems, since the beginning of our civiliation. It has been commonly accepted weeds were controlled to avoid crop yield reductions. The control methods progressed following scientific development of agricultural production technology. Ancient agricultural systems in areas between Euphrate and Tigris rivers in the middle east, for example, people weeded their crops by pushing Y-end wooden sticks to steer soil up and killed weed seedlings. The Roman later on, utilized animals to draw the plow and in modern era following industrial revolution in the United Kingdom, fuel replaced human and animal energy. Still further the discovery of herbicides provides other means of controlling weeds, while natural enermies may control weed biologically. Progress in science further drove the development of weed control and attained an advancemence in scientific development to become a weed science, a scientific dicipline in its own. The damaging economic impact of weeds to crops in agroecosystems is recognized internationally, and in WTO ( world trade organization) together with damaging arthropods and pthogens are termed as crop pests. The concept of weed developed and progressed to cover not only as a pest in agroecosystems, but



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also those related to biodiversity in natural ecosystems, as declared during the earth summitt in Rio deJaneiro in 1992. In this occassion a concept of invasive alien species was introduced, through Convention on Biological Diversity (CBD), as stated in 8(h), *the contracting party shall as appropriate as possible to prevent the introduction of, eradicate and control alien species that threaten the ecosystems, habitats and species.* The Indonesian governemnt ratified the convention and put it in the law no 5/1994. Invasive Alien Species includes animals, plants, and microorgaisms. Invasive alien plant species, by definition is a plant growing outside its natural distribution, colonizes, naturalizes, spreads dan damages environment, production systems, and/or human health. Invasive Alien Plant Species such as *Chromolaenaodorata* is also known as a weed. It is soon realized that invasive plants threatens and harms biodiversity considerably and only second to habitat destruction.

The economic growth enjoyed by Indonesia will increase international travel and trade, and will further escalate the extent and frequency of plant species transfer, and exarberate the problems of invasive species. The magnitude of the invasive plant species problem in Indonesia surprized everyone. Indonesia has been an open country for a long period of time, and roughly about 2000 alien species was recorded recently and probably much more than that figure when recent plant importation were included. The real impact was felt by a state forest industry (SFI) when recently a team of assessor presented a set of CARs related to invasive alien species to SFI, and unless SFI able to response satisfactorily she may suffer from suspention of her certificate.

The developmental progresses are not only in the level of concept, but also in the integration of effort at national level in the form of national strategy and actions to overcome problems related to invasive plant species. The national strategy of Invasive alien plant species covers several programs such as *prevention, early detection and rapid response, control and management, capacity buildings, rehabilitation and restoration.*

Biological plant invasion is a function of both plant species and site specific attributes and effected by human activities (Catford, 2009). Any assessment of invasion risk, therefore, must adopt species and site specific perspective and is unlikely to be successful in the absence of detailed information at both species and site specific levels. The development of criteria of invasion process for the species concerned provide a mean by which a quantitative expression of invasion sucsess may be possible. The indentification of invasion criteria must be based upon an indepth knowledge of the life history and environment requirement of the species concerned.

Weeds pose significant primary production, environmental and social impacts worldwide and the number of weed species posing such impacts is increasing annually. Thus, the magnitude of the problem requires prioritization across weed policy, management and legislation. Such prioritization needs have led to the development of mechanisms/processes that assess and account for the risks associated with weed introductions.

**Risk Assessment**

Risk is defined as likelihood of undesired event occuring as a result of behaviour or action (including no action). Risk assessment is the means by which the frequencies and consequences of such events are determined, and should be accompanied by an expression of any uncertainty in the assessment process. The consequences of undesired events in question are usually adverse and are expressed in term of the assessment endpoint. Assessment endpoints are simply an expression of values that one is trying to protect by undertaking the risk assessment procedure, and thus distinguish the environmental risk assessment (ecological risk) from human health risk assessment ( human fatality or human injury endpoint).

The risk associated with invasion of alien plant species can be defined as the likehood of undesired events occuring as a result of these actions. It is important to recognize the interpretation of this definition is entirely dependent upon the endpoint of the assessment. If the endpoint is establishment of an invasive alien plant species in a new locality, then the risk



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is expressed in term of lokelihood of establishment. If the endpoint is environmental damage, then the risk must be defined as the likehood of enviromental damage,arising as a result the introduction and establishment of an alien plant species.

The definition of risk is sensitive to the assessment of endpoint (which in itself simply is an expression of value). If risk is expressed in term of an establishment of an alien plant species –there is thus an implicit assumption that the establishment of an invasive alien plant species in a new localities is an undersired event. This is equivalent to an expression environmental value that wishes to preserve "natural" or existing species assemblages. By contrast, if risk is defined in term of the environmental damage, the establishment of a new invasive alien plant species "per se" does not constitute the undesired event to be avoided it is merely concerned with the subsequent environmental damage that could arise as a result of this. Thus if an assessor could garantee that a particular invasive alien species would have no adverse effect on the environment, then under this definition, there would be no risk.

Quantitatively the definition of risk (an event), *assessed as a mathematical combination (often the product) of the* ***magnitude of the consequence*** *of an event and* ***the likelihood*** *of that event occurring.*

## WEED RISK ASSESSTMENT FOR PRE-BORDER SITUATION

Many weeds in the past was purposely imported as potential new crops (*Acacia nilotica*- gum production) , ornamental plants ( *Eichhornia crassipes, Widelia trilobata,* beautiful flowers *Chromolaena odorata,* white christmas bush in India), or for other purposes (*Mikania micrantha* as medicine). To avoid such unforseen damaging biological impact, regulatory authorities have a statutory responsibility to ensure that all plant taxa proposed to be imported, which are not already prohibited and are not already established, be evaluated for their potential to damage the productive capacity or environment of the importing countries. It is important, therefore, to be able to predict the weediness or the invasive potential of thousand of new potential entry.

Baker (1965) attempted to list characters associated with weeds, that may be utilized to predict a plant as a weed. Progress in identifying the key characteristics of potential weeds was reported in Australia (Scott and Panetta, 1993), New Zealand (Esler *et al*., 1993), South Africa (Richardson *et al*., 1990), Britain (Williamson and Fitter, 1996), and north America (Reichard and Hamilton, 1997), or within closely related groups of taxa including herbs ( Williamson, 1993) or woody seed plants (Rejmanek and Richardson, 1996). Predictions from a non-experimental approach are believed to be reasonably good if based on biological information of the invading species, the bioclimatic features of the recipient and source regions, and the evolutionary history of both ( Richardson *et al*., 1990). A synthesis of this information can lead to general conceptual models of plant invasiveness (Rejmanek, 1995) which in turn can be applied to screening procedures such as decision trees to categorise potential plant imports (Panetta, 1993; Tucker and Richardson, 1995; Reichard, 1996; Reichard and Hamilton, 1997).

Regulatory authorities responsible for biosecurity need a tool that synthesizes much of this fragmented and sometimes theoretical information. An acceptable biosecurity assessment system should satisfy a number of requirements (Hazard, 1988; Panetta, 1993). It should be calibrated and validated against a large number of taxa already present in the recipient country and representing the full spectrum of taxa likely to be encountered as imports into that country. It must effectively discriminate between weeds and non-weeds, such that the majority of weeds are not accepted, nonweeds are not rejected, and the proportion of taxa requiring further evaluation is kept to a minimum. As international trade agreements require that prohibited taxa should fit the definition of a quarantine pest before they can be excluded by quarantine regulations (Anon., 1994, 1995; Walton and Parnell, 1996), the system must be based on explicit assumptions and scientific principles so that the country cannot be accused of applying unjustified non-tariff trade barriers. Ideally the system should be capable of



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identifying which land use system the taxon is likely to invade, to assist in an economic evaluation of its potential impacts. Finally, the system must be cost effective to the prospective importer and the border control authority.

## AUSTRALIAN WRA SYSTEM

The Australian WRA system has received wide recognition and has subsequently been tested extensively in other countries, for example, in New Zealand (Pheloung et al. 1999 ), Hawaii and other Pacific islands ( Daehler etal.2004 ) including the Galapagos and the Japanese Bonin island archipelagos (Kato,et al ,2006) as well as Europe (Gasso et al.2010; Weber and Gut, 2004.) and North America, specifically Florida (Gordon etal.2008. ). This testing has led to some localized adaptations (e.g. to reflect regional climatic and edaphic characteristics (Gordon etal.2008). The system has also been simplified for use by the United Nations Food and Agricultural Organization (UN FAO), and has been made available in English, Spanish and French and applied in many developing countries (Williams,2003). It has also been used as a guide by Pacific Island Ecosystems at Risk (PIER) and Hawaiian Ecosystems At Risk (HEAR) projects.

The Australian WRA system meets the objectives of the World Trade Organization's Sanitary and Phytosanitary (SPS) Agreement guidelines, by providing protection without creating unjustifiable technical barriers to trade (Andersen et al. 2004). In addition, the International Plant Protection Convention (IPPC) has adopted several International Standards on Phytosanitary Measures relating to weed risk analysis (e.g. No. 2 Guidelines for Pest Risk Analysis and No. 11 Pest Risk Analysis for Quarantine Pests, Including Analysis of Environmental Risks and Living Modified Organisms; both of which are available from the IPPC website (www.ippc.int), which are consistent with the WRA system developed to date.

The Australian WRA system is based on a three-tiered process (Walton, 2001]: (1) checking of the target species against exhibited prohibited or permitted lists, (2) a screening process using a series of predetermined questions to evaluate the weedy/invasive potential of species not rejected in tier 1 and (3) further evaluation of those species identified as requiring more information to determine their weedy potential following the assessment process in tier 2. The screening process used in tier 2 of the current Australian WRA system uses 49 questions covering the historical, bio-geographical, biological and ecological aspects of the target species, in which the questions are answered almost entirely using 'Yes'/'No'/ 'Don't know' responses. Scoring is a simple additive process, with values of >6 receiving a 'reject entry' assessment, values ranging from 1 to 6 receiving a 'further evaluate' assessment and < 1 an 'accept entry' assessment. These categories were established based on extensive empirical calibration producing a rejection rate of 10% or less for non-weeds and no more than 30% of the weed species being assessed as 'further evaluate' [Phelong et al, 1999].

Out of 49 questions at least 2 questions of historical, 2 questions from bio-geographical and 6 from biolgical/ecological aspect must be answered.

The questions should be understood and answered accordingly as follows.

### History/Biogeography

#### *1. Domestication / cultivation*

#### 1.01. Is the species highly domesticated? If answer is `no" go to Question 2.01

The taxon must have been cultivated and subjected to substantial human selection for at least 20 generations. Domestication generally reduces the weediness of a species by breeding out noxious characteristics.



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**1.02 Has the species become naturalised where grown?**
Is a domesticated plant, which has introduced from another region, growing, reproducing and maintaining itself in the area in which it is growing. A `yes' answer to question 1.01 will be modified by the response to this question.

**1.03. Does the species have weedy races?**
Only answer this question if the species you are assessing is a sub-species, cultivar or registered variety of a domesticated species. If the taxon is a less weedy subspecies, variety or cultivar, then there must be good evidence that it does not retain the capacity to revert to a weedy form. A `yes' answer to question 1.01 will be modified by the response to this question.

**2.** ***Climate and distribution***

**2.01. Species suited to Australian climates (0-low; 1-intermediate; 2-high)**
This question applies to any one Australian climate type, or more than one. Ideally, base the climate matching on an approved computer prediction system such as CLIMEX , BIOCLIM or Climate. If no computer analysis is carried out then assign the maximum score (2).

**2.02. Quality of climate match data (0-low; 1-intermediate; 2-high)**
The score for this question is an indication of the quality of the data used to generate the climate analysis. Reliable specific data scores 2, general climate references scores 1, broad climate or distribution data scores 0. If a computer analysis was not carried out assign the maximum score of 2.

**2.03. Broad climate suitability (environmental versatility)**
Score `yes' for this question if the species is found to grow in a broad range of climate types. Output from the climate matching program may be used for this question. Otherwise base the response on the natural occurrence of the species in 3 or more distinct climate categories. Use the map of climatic regions provided or one available in a comprehensive atlas.

**2.04. Native or naturalised in regions with extended dry periods**
The species is able to grow in areas with rainfall in the driest quarter less than 25 mm. Plants from this group may potentially grow and survive in arid Australian conditions.

**2.05. Does the species have a history of repeated introductions outside its natural range?**
This history should be well documented. A potential weed must have opportunities to show its potential. A score for Question 2.05 will modify the score for a `no' answer to Question 3.01. Species with repeated introductions that have not established are a lower risk.

***3. Weed Elsewhere***

**3.01. Naturalised beyond native range**
A naturalised species will be cited in floras of localities which are clearly outside of the native range. If the native range is uncertain and the known extent of the naturally growing plants is within the area of uncertainty then the answer is `don't know.'

**3.02. Garden/amenity/disturbance weed**
The plant is generally an intrusive weed of gardens, parklands, roadsides, quarries,etc. This question carries less weight than 3.03 or 3.04. If a plant is listed as a weed in relevant references but the type of weed is uncertain or it is a minor weed – score `yes' for 3.02.

**3.03. Weed of agriculture/horticulture/forestry**
The plant is generally a weed of agriculture/horticulture/forestry and causes productivity losses and/or costs due to control. This question carries more weight than 3.02. If a plant is listed as a weed in relevant references but the type of weed is uncertain or it is a minor weed - score `yes' for 3.02.



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**3.04. Environmental weed**

The plant is documented to alter the structure or normal activity of a natural ecosystem. This question carries more weight than 3.02. If a plant is listed as a weed in relevant references but the type of weed is uncertain or it is a minor weed – score `yes' for 3.02.

**3.05 Congeneric weed**

Documented evidence that one or more species, with similar biology, within the genus of the species being evaluated are weeds.

**Biology/Ecology**

***4 . Undesirable traits***

**4.01. Produces spines, thorns or burrs**

The plant possesses a structure on the plant known to cause fouling, discomfort or pain to animals or man. If the taxon is a thornless subspecies, variety or cultivar, then there must be good evidence that it does not retain the capacity to revert to a thorny form.

**4.02. Allelopathic**

The plant is well documented as a potential suppressor of the growth of other species by chemical (eg. hormonal) means. Such evidence is rare throughout the whole plant kingdom.

**4.03. Parasitic**.

The parasite must have a detrimental effect on the host and the potential hosts must be present in Australia. This question includes wholly and semi-parasitic plants. Such plants are rare.

**4.04. Unpalatable to grazing animals**

Consider the plant with respect to where the plant has the potential to grow and if the herbivores present could keep it under control. This trait may be found at any stage during the lifecycle of the plant and/or over periods of the growing season.

**4.05.Toxic to animals**

There must be a reasonable likelihood that the toxic agent will reach the animal, by grazing or contact. Some species are mildly toxic but very palatable and could cause problems if heavily grazed.

**4.06.Host for recognised pests and pathogens**

The main concerns are plants that are hosts of toxic pathogens and alternate or alternative hosts of crop pests and diseases. Where suitable alternative or alternate hosts are already widespread in cropping or natural systems the answer should be `no' unless the species will affect the current control strategies for the pathogen or pest. Apply a reasonable level of specificity; a pathogen of an entire family, such as takeall, should not be the basis for answering `yes' for an individual species.

**4.07.Causes allergies or is otherwise toxic to humans**

This condition must be well documented and likely to occur under normal circumstances. For example by physical contact or inhalation of pollen from the species.

**4.08. Creates a fire hazard in natural ecosystems**

This question applies to species that have a documented growth habit that leads to the rapid accumulation of fuel for fires when growing in natural or unmanaged ecosystems.

**4.09 Is a shade tolerant plant at some stage of its life cycle**

Shade tolerance can enhance the invasive potential of a species.

**4.10. Grows on infertile soils**

Australian soils are generally very infertile. Species that tolerate low nutrient levels could potentially grow well here. Legumes, tolerant of low soil phosphorus, are a particular concern since they would also modify the soil environment.



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**4.11. Climbing or smothering growth habit**
This trait includes fast growing vines and ivy's that cover and kill or suppress the growth of the supporting vegetation. Plants that rapidly produce large rosettes could also score for this question.

**4.12. Forms dense thickets**
The thickets produced should obstruct passage or access, or exclude other species. Woody perennials are the most likely candidates, but this question may include densely growing grasses.

**5. Plant type**

**5.01. Aquatic**
The question includes any plants normally found growing on rivers, lakes and ponds. These species have the potential to choke waterways and starve the system of light, oxygen and nutrients. Consequently, the score is high (5).

**5.02. Grass**
A large proportion of the grass family (Poaceae/Gramineae) are weeds in some context. As with congeneric weed species, there is a high probability that a species from this family will be a weed.

**5.03. Nitrogen fixing woody plant**
A large proportion of woody legumes (Family Leguminosae/Fabaceae) are weeds, particularly of conservation areas. As with congeneric weed species, there is a high probability that a species from this family will be a weed.

**5.04. Geophyte**
Perennial plants with tubers, corms or bulbs. This question is specifically to deal with plants that have specialised organs and should not include plants merely with rhizomes/stolons (see 6.06). Plants from this group can be particularly difficult to eradicate from a site.

**6. Reproduction**

**6.01. Evidence of substantial reproductive failure in native habitat**
Predators and other factors present (eg. disease) in the native habitat can cause substantial reductions in reproductive capacity. The reproductive output of a species may greatly increase when the plant grows in areas without these factors.

**6.02.Produces viable seed**
If the taxon is a subspecies, variety or cultivar, it must be indisputably sterile. The male plants of a dioecious species are regarded as seed producers.

**6.03. Hybridises naturally**
A `yes' answer for this question requires documented evidence of interspecific hybrids occurring, without assistance, under natural conditions.

**6.04 Self-fertilisation**
Species capable of self seeding, can spread from seed produced by an isolated plant.

**6.05. Requires specialist pollinators**
The invasive potential of the plant is reduced if the species requires specialist pollinating agents that are not present or rare in Australia.

**6.06. Reproduction by vegetative propagation**
The plant must be capable of increasing its numbers by vegetative means. This may include reproduction by: rhizomes, stolons or root fragments, suckers or division.

**6.07. Minimum generative time (years)**
This is the time from germination to production of viable seed, or the time taken for a vegetatively reproduced plant to duplicate itself. The shorter the timespan, the more weedy a



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plant is likely to be. The score for this trait uses the correlation factor (1 year score 1, 2-3 years score 0, greater than or equal to 4 years score -1).

**7 . Dispersal mechanisms**

**7.01.Propagules likely to be dispersed unintentionally**
Propagules (any structure, sexual or asexual, which serves as a means of reproduction), unintentionally dispersed resulting from human activity. An example is plants growing in heavily trafficked areas such as farm paddocks or roadsides.
**7.02. Propagules dispersed intentionally by people**
The plant has properties that make it attractive or desirable, such as an edible fruit, an ornamental or curiosity. The species is readily collected as a cutting or seed. This group includes most horticultural plants.
**7.03. Propagules likely to disperse as contaminants of produce**
Produce is the economic output from any agricultural, forestry or horticultural activity. An example is grain shipments that contain seeds of weed species.
**7.04. Propagules adapted to wind dispersal**
Documented evidence that wind significantly increases the dispersal range of the propagule. An example is an achene with a pappus. This group includes tumbling plants and plants with seeds contained within an explosive capsule or pod.
**7.05. Propagules buoyant**
This question includes any structure containing the propagule that typically becomes detached from the plant and is buoyant. An example is a pod of a legume. This is a limited method of distribution of land plants.
**7.06. Propagules bird dispersed**
Any propagule that may be transported and/or consumed by birds, and will grow after defecation. An example is small red berries with indigestible seeds.
**7.07.Propagules dispersed by other animals (externally)**
The plant has adaptations, such as burrs, and/or grows in situations that make it likely that propagules become temporarily attached to the animal. This can include the spread of plants parts on clothing. This dispersal group includes seeds with an oily or fat-rich outgrowth that aids in ant seed dispersal.
**7.08 Propagules dispersed by other animals (internally)**
The propagules are eaten by animals, dispersed and will grow after defecation.

**8. Persistence attributes**

**8.01. Prolific seed production**
The level of seed production must be met under natural conditions and applies only to viable seed. For grasses and annual species this rate should be (>5000- 10000/m2/yr), for woody annual a rate of (>500/m2/yr) would be considered high. Specific data on this attribute may be unavailable, however, an estimate can be made from the seed/plant and the average size of the plant.
**8.02.Evidence that a persistent propagule bank is formed (>1 yr)**
Greater than 1% of the seed should remain viable after more than one year in the soil. This bank may include both canopy and soil seed banks. Long seed viability increases a plants invasive potential.
**8.03. Well controlled by herbicides**
Documented evidence is required for good chemical control of the plant. This control must be acceptable in the situations in which it is likely to be found. The chemical management should be safe for other desirable plants that are likely to be present. This information will be poorly documented for most non-agricultural plants.



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**8.04.Tolerates or benefits from mutilation, cultivation or fire**

Plants that tolerate or benefit from such disturbance may out-compete other species. This question does not apply to seed banks.

**8.05.Effective natural enemies present in Australia**

A known, effective, natural enemy of the plant may or may not be present in Australia. The answer is 'don't know' unless a specific enemy/enemies are known.

Assessments may be entered manually into Form A and the final score calculated by reference to Form B. in the following pages.

**FORM A**

Questions forming the basis of the Weed Risk Assessment model (WRA)

Weed Risk Assessment system question sheet: Answer yes (y) or no (n), or don't know (leave blank), unless otherwise indicated

| | | |
|---|---|---|
| Botanical name: | | Outcome: |
| Common name: | | Score: |
| Family name | | Your name: |

| | | | | | |
|---|---|---|---|---|---|
| | | | | **History/Biogeography** | |
| A | **1** | *Domestication/ cultivation* | 1.01 | Is the species highly domesticated? If answer is 'no' got to question 2.01 | |
| C | | | 1.02 | Has the species become naturalised where grown? | |
| C | | | 1.03 | Does the species have weedy races? | |
| | **2** | *Climate and Distribution* | 2.01 | Species suited to Australian climates (0–low; 1–intermediate; 2–high) | 2 |
| | | | 2.02 | Quality of climate match data (0–low; 1 intermediate; 2–high) | 2 |
| C | | | 2.03 | Broad climate suitability (environmental versatility) | |
| C | | | 2.04 | Native or naturalised in regions with extended dry periods | |
| | | | 2.05 | Does the species have a history of repeated introductions outside its natural range? | |
| C | **3** | *Weed elsewhere* | 3.01 | Naturalised beyond native range | |
| E | | | 3.02 | Garden/amenity/disturbance weed | |
| A | | | 3.03 | Weed of agriculture/horticulture/forestry | |
| E | | | 3.04 | Environmental weed | |
| | | | 3.05 | Congeneric weed | |
| | | | | **Biology/Ecology** | |
| A | **4** | *Undesirable traits* | 4.01 | Produces spines, thorns or burrs | |
| C | | | 4.02 | Allelopathic | |
| C | | | 4.03 | Parasitic | |
| A | | | 4.04 | Unpalatable to grazing animals | |
| C | | | 4.05 | Toxic to animals | |
| C | | | 4.06 | Host for recognised pests and pathogens | |
| C | | | 4.07 | Causes allergies or is otherwise toxic to humans | |
| E | | | 4.08 | Creates a fire hazard in natural ecosystems | |
| E | | | 4.09 | Is a shade tolerant plant at some stage of its life cycle | |
| E | | | 4.10 | Grows on infertile soils | |
| E | | | 4.11 | Climbing or smothering growth habit | |
| E | | | 4.12 | Forms dense thickets | |
| E | **5** | *Plant type* | 5.01 | Aquatic | |
| C | | | 5.02 | Grass | |
| E | | | 5.03 | Nitrogen fixing woody plant | |
| C | | | 5.04 | Geophyte | |



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| | | | | | |
|---|---|---|---|---|---|
| C | **6** | *Reproduction* | 6.01 | Evidence of substantial reproductive failure in native habitat | |
| C | | | 6.02 | Produces viable seed. | |
| C | | | 6.03 | Hybridises naturally | |
| C | | | 6.04 | Self-fertilisation | |
| C | | | 6.05 | Requires specialist pollinators | |
| C | | | 6.06 | Reproduction by vegetative propagation | |
| C | | | 6.07 | Minimum generative time (years) | 1 |
| A | **7** | *Dispersal mechanisms* | 7.01 | Propagules likely to be dispersed unintentionally | |
| C | | | 7.02 | Propagules dispersed intentionally by people | |
| A | | | 7.03 | Propagules likely to disperse as a produce contaminant | |
| C | | | 7.04 | Propagules adapted to wind dispersal | |
| E | | | 7.05 | Propagules buoyant | |
| E | | | 7.06 | Propagules bird dispersed | |
| C | | | 7.07 | Propagules dispersed by other animals (externally) | |
| C | | | 7.08 | Propagules dispersed by other animals (internally) | |
| C | **8** | *Persistence attributes* | 8.01 | Prolific seed production | |
| A | | | 8.02 | Evidence that a persistent propagule bank is formed (>1 yr) | |
| A | | | 8.03 | Well controlled by herbicides | |
| C | | | 8.04 | Tolerates or benefits from mutilation, cultivation or fire | |
| E | | | 8.05 | Effective natural enemies present in Australia | |

A = agricultural, E = environmental, C = combined.



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# FORM B

**Weed risk assessment scoring sheet for the WRA.**

Form B. Weed Risk Assessment Scoring Sheet

| | a | b | c | d | e |
|---|---|---|---|---|---|
| Section | Question | Response[1] | Score[2] | *N* score | *Y* score |
| A C | 1.01 | | | 0 | -3 |
| C | 1.02 | | | -1 | 1 |
| C | 1.03 | | | -1 | 1 |
| | 2.01 | | | | |
| | 2.02 | | | | |
| C | 2.03 | | | | |
| C | 2.04 | | | | |
| | 2.05 | | | | |
| C | 3.01 | | | | |
| N | 3.02 | | | | |
| A | 3.03 | | | | |
| E | 3.04 | | | | |
| C | 3.05 | | | | |
| B C | 4.01 | | | 0 | 1 |
| C | 4.02 | | | 0 | 1 |
| C | 4.03 | | | 0 | 1 |
| A | 4.04 | | | -1 | 1 |
| C | 4.05 | | | 0 | 1 |
| C | 4.06 | | | 0 | 1 |
| N | 4.07 | | | 0 | 1 |
| E | 4.08 | | | 0 | 1 |
| E | 4.09 | | | 0 | 1 |
| E | 4.10 | | | 0 | 1 |
| E | 4.11 | | | 0 | 1 |
| C | 4.12 | | | 0 | 1 |
| C E | 5.01 | | | 0 | 5 |
| C | 5.02 | | | 0 | 1 |
| E | 5.03 | | | 0 | 1 |
| C | 5.04 | | | 0 | 1 |
| C | 6.01 | | | 0 | 1 |
| C | 6.02 | | | -1 | 1 |
| A | 6.03 | | | -1 | 1 |
| C | 6.04 | | | -1 | 1 |
| C | 6.05 | | | 0 | -1 |
| A | 6.06 | | | -1 | 1 |
| C | 6.07 | | | | |
| A | 7.01 | | | -1 | 1 |
| C | 7.02 | | | -1 | 1 |
| A | 7.03 | | | -1 | 1 |
| C | 7.04 | | | -1 | 1 |
| E | 7.05 | | | -1 | 1 |
| E | 7.06 | | | -1 | 1 |
| C | 7.07 | | | -1 | 1 |
| C | 7.08 | | | -1 | 1 |
| C | 8.01 | | | -1 | 1 |
| C | 8.02 | | | -1 | 1 |
| A | 8.03 | | | 1 | -1 |
| A | 8.04 | | | -1 | 1 |
| C | 8.05 | | | 1 | -1 |
| | **Total score[3]** | | | | |
| | **Outcome[4]** | | | | |
| | Agricultural score[6] | | | | |
| | Environmental[6] | | | | |

These response for these questions is 2 unless a climate analysis is done

Refer to lookup table

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Lookup table for section 3. Locate value of inputs and lookup output for each question | | | | | | | | | | |
| ***Yes*** **to questions 3.01 - 3.05** | | | | | | | | | | **default** |
| Inputs | 2.01 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | **2** |
| | 2.02 | 0 | 1 | 2 | 0 | 1 | 2 | 0 | 1 | **2** |
| Results | 3.01 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | **2** |
| | 3.02 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | **2** |
| | 3.03 | 3 | 2 | 1 | 4 | 3 | 2 | 4 | 4 | **4** |
| | 3.04 | 3 | 2 | 1 | 4 | 3 | 2 | 4 | 4 | **4** |
| | 3.05 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | **2** |
| ***No*** to questions 3.01 - 3.05 | | | | | | | | | | |
| Input | 2.05 | ? | N | Y | | | | | | |
| Results | 3.01 | -1 | 0 | -2 | | | | | | |
| | 3.02-3.05 | 0 | 0 | 0 | | | | | | |

**Procedure**

1. Record appropriate responses in column b.
2. Look up score in columns d & e and record result in column c.
3. Calculate total score.
4. Lookup and record recommendation.
5. Verify that minimum number of questions from each section are answered.
6. Compute Agricultural (A&C) and Environmental (E&C) scores: if either score is less than 1, the outcome pertains to the other sector.

| Lookup table for 6.07 | | | |
|---|---|---|---|
| years | 1 | 2 | 4 |
| score | 1 | 0 | -1 |

| Score | Outcome |
|---|---|
| < 1 | Accept |
| 1-6 | Evaluate |
| > 6 | Reject |
| Section | Minimum # questions[5] |
| A | 2 |
| B | 2 |
| C | 6 |
| Total | 10 |

As mentioned before Australian WRA (A-WRA) has been modified by Daehler et al (2004) into Hawaian WRA (H-WRA). The questions, 2.01, 2.04, 2.10 and 8.05. have been modified by Daehler et al. (2004), to suit Hawai and the Pacific islands condition, each by "tropical and subtropical climate, native or naturalized in tropical and subtropical climate, tolerate limestone or a wide range of soil, and Effective natural enemies presents in Hawaii and Pacific islands". Climate matching is an essential component of the H-WRA and whilst the use of climate matching software has been advocated (Pheloung et al., 1999), other



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proxies or default scores have generally been used (Gordon et al., 2008a). They generated systematic scores for the climate match questions based on information on the species native latitudinal range. For question 2.01 (species suitable to tropicalor subtropical climates), species with a latitudinal range midpoint between $20^0$ North and $20^0$ South (i.e. centred on the tropics), were given a score of two; those with midpoints between $20^0$ and $30^0$ North or South (corresponding to 'subtropical') were given a score of one, and species with midpoints >$30^0$ North or South (i.e. 'temperate') were scored as zero. To address question 2.02
(quality of climate match data), a score of two was given for species that had a published latitudinal range, a score of one if the range was described but latitudes had to be obtained from atlases, and if the range was uncertain, the score was zero. Standard protocols for answering all other questions were followed across all species (Gordon et al., 2008b).

A more significant modification was the addition of second screening, where species which fell in between 1-6 were subjected to second screening in the form of decission tree as in Fig. 1

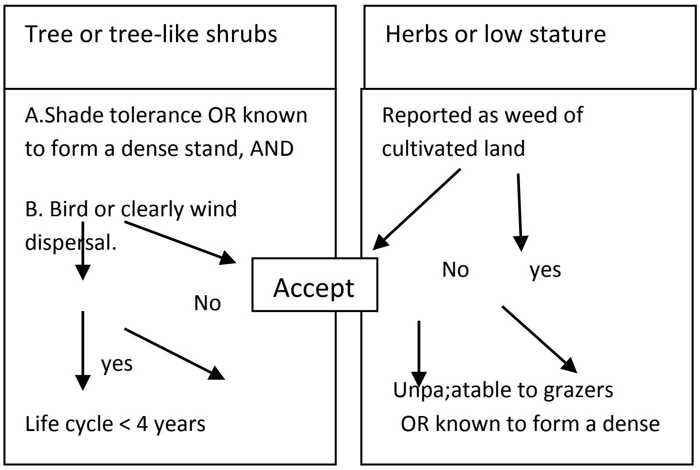


**Fig. 1**. Decission tree used to screen harmful plants having score between 1 – 6, Reject indicates predicted pests, while accept indicates likely nonpest (Daehler et al 2004) .

This additional second screening H-WRA successfully identified 85% nonpest correct compare to 65% without second screening. Without second screening H-WRA failed to identify 24% of the species tested, although second screening can not reached 100% but leaving only 8% unidentified. It seems quite good able to increase the capacity of WRA through HWA to predict correct decission. The decission tree utilized in the second screening was claimed by the inventor (Daehler et al 2004) developed purely based on logical reasoning not on empirical data, therefore, it is possible there are other alternatives of the decission tree.

The much discussed criterium to predict a plant to be weed or to be invasive if it has been a weed or invasive species somewhere has raised a considerable questions . Many experts discovered that there were many plants which did not have historical record to be weed or invasive species anywhere and then become a weed or invasive species, and they critizised that the criterium of weed somwhere was not a good criterium of predicting an invasive species or a weed. However, that criterium has predicted correctly in many ocassion, and if those species which do not have historical record being an invasive species somewhere probably they are not in close contact with human activities, or in other word those species which did not have historical record when they become invasive species it is their first record.

The criterium of "weed somewhere" does not satisfy the scientific mind of experts and a stronger analytical mind is explored such as what is the consequence of any invasion and what is the likelihood of that event. This is presented in Table 1.



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**Table 1.** WRA systems, highlighting the type of risk addressed by each and the measures used to assess the risk.

| Risk Assessment system | Risk (event) | Consequence[1)] (assessment based on) | Likelihood[2)] (assessment based on) |
|---|---|---|---|
| Pre- border (prevention) | Importation of a new weed species | (1) The type of impact a weed species may have (i.e. potent ial impact) to agriculture, biodi versity and/or human health and (2) where the impact could occur (i.e. the potential distribu tion), if it were to be introduced[3)] | A prediction of a plant species potential to become weedy, using attributes associated with its ability to (1) establish, reproduce and, disperse and (2) pose a threat, if it were to be introduced [3)] |
| Post-border (generally eradication & containment ) | The invasion of a newly established weed species or expansion of an existing weed species | (1) The type of impact a weed species may have (i.e. potential impact) to agriculture, biodiversity and/or human health and (2) where the impact could occur (i.e. the potential distribution). | (1) The ability to prevent the spread of a weed species based on the control options available, (2) degree of coordi nation available, (3) the invasiveness of the weed species (based on the ability to establish, reproduce and disperse) and (4) the threat posed |
| Protection of environmental assets[4)] | The extinction or extirpation of native species due to the invasion of an existing weed species | (1) The degree of impact posed by a weed species to native species and (2) where the impact currently occurs relative to the native species at risk and (1) above | (1) The ability to achieve a conser vation outcome (i.e.recoverability of the species).(2) feasibility of weed control and (3) the degree ofurgency for manage ment based on the impact posed by the weed species |

**Source:** Downey et al (2010).

**Note** : [1)] Impact is defined as the effect that a weed has on native species . [2)] Threat is defined as the possible danger posed to a native species as a result of a weed, i.e. there is the possibility of an impact, rather than an actual current impact . [3)]The Australian WRA system does not separate consequence and likelihood. Based on the risk measures used, we have separated them here for comparative purposes only.

Despite being a formalized risk assessment process, the Australian WRA system does not adequately separate the consequence from the likelihood; rather the outcome conveys the risk as a single score based on the answers to 49 questions. Daehler and Virtue [2010] examined the questions and identified the ones that most closely reflect the likelihood (i.e. of spread) and consequences (i.e. impacts) to establish if this lack of separation resulted in assessment problems. The authors found that there was a slight improvement in identifying weeds compared to the original WRA, with no change for non-weeds, if groupings of likelihood and consequence questions were considered independently. To enable comparisons across the weed management spectrum, it is tried to separate consequence and likelihood in the WRA (see Table 1). The degree of likehood and consequences will have to be scored to obtain a scale of risk.

Given that many countries have examined the use of a modified version of the Australian WRA system, Indonesia may also adopt this system with some modifications to



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accomodate the country's interest. Some invasive alien plant species have been utilized as livelihood by local people and prohibitation of their use must be carried out with care and by providing alternatives. Beside that there is a growing need to develop better transfer of assessment data to enable comparisons (Onderdonk et al. 2007.), if the system is adopted widely by quarantine agencies. It was noticed during a national discussion that understanding the questions and therefore how to answer them was not easy, and creating wrong answers, it is important, therefore, to standardize how to respond to questions correctly would increase the efficiency of WRAs (Walton 2001).

A key advance in this regard is the recent international collaboration to provide guidance on how to answer the questions in the Australian WRA system (Gordon et al.2008). They believe the current WRA system has been tested sufficiently to warrant broader adoption. Given the negative impact of not having a WRA system and the relative success of the Australian WRA systems, a broader adoption would have significant benefits. Challenges for the future development of WRA systems include: (i) international agreement on the questions used; (ii) the need and further development of a secondary screening process; (iii) consistency across the different systems developed to date; (iv) broader adoption in other countries; (v) better transfer of WRA output assessments (i.e. for individual species) between different users and systems; and (vi) further examination of the use of consequence and likelihood (Downey et al, 2010) .

## WEED RISK MANAGEMENT FOR POST-BORDER SITUATION

In contrast to pre-border WRA systems which aim to prevent the risk of new weed incursions, post-border WRM systems aim to prioritize the management of invasive alien plants species which are present in a country or region, based on the level of risk they pose. The term risk management (i.e. *whether to avoid, mitigate,or tolerate the risk*) has been adopted to be aligned with that used in generic standard for risk management. This standard, which has now been accepted as an International Organization for Standardization (ISO) standard, considers risk management as the overall process of identifying, assessing and treating risks. It is, however, different from the International Plant Protection Convention (IPPC) international standards on phytosanitary measures, which considers *risk analysis as the overall process and equates risk management to risk treatment.*

## HIEBERT AND STUBBENDIECK METHOD

Hiebert and Stubbendieck (1993) developeda ranking system for resource managers to sort exotic plants within a park according to the species level of impact and its innate ability to become a pest. This information was then weighed against the perceived feasibility or ease of control. This Exotic Species Ranking System was designed to first separate the innocuous species from the disruptive species. The separation allowed park managers to then concentrate further efforts on species in the disruptive category. The system was also designed to identify those species that were not presently a serious threat but have the potential to become a threat and, thus, should be monitored closely. Finally, the system asked the park manager and the ecologist to consider the cost of delaying any action.

The Exotic Species Ranking System uses numerical ratings, is written in outline format, and is divided into two main sections: I. Significance of Impact and II. Feasibility of Control or Management. Each section is based on a scale of 100 points. Significance of Impact is further divided into *A*. Current Level of Impact and *B*. Innate Ability of Species to Become a Pest. Stubbendieck et al (1992) considered a species with a combined score of over 50 points for significance of impact to be seriously disruptive and needing appropriate attention. Species receiving high scores for feasibility of control will be easier to control than those receiving lower scores.



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**Table 2. Exotic Species Ranking System**

| | |
|---|---|
| **I. Significance of Impact** | |
| ***A. Current Level of Impact*** | |
| 1. Distribution relative to disturbance regime | |
| a. found only within sites disturbed within the last 3 years of sites regularly disturbed | -10 |
| b. found in sites disturbed within the last 10 years | 1 |
| c. found in midsuccessional sites disturbed 11-50 years before present (BP) | 2 |
| d. found in late-successional sites disturbed 51-100 years BP | 5 |
| e. found in high-quality natural areas with no known major disturbance for 100 years | 10 |
| 2. Abundance | |
| a. number of populations (stands) | |
| (1) few; scattered (<5) | 1 |
| (2) intermediate number; patchy (6-10) | 3 |
| (3) several; widespread and dense (>10) | 5 |
| b. areal extent of populations | |
| (1) <5 ha | 1 |
| (2) 5-10 ha | 2 |
| (3) 11-50 ha | 3 |
| (4) >50 ha | 5 |
| *3. Effect on natural processes and character* | |
| a. plant species having little or no effect | 0 |
| b. delays establishment of native species in disturbed sites up to 10 years | 3 |
| c. long-term (more than 10 years) modification or retardation of succession | 7 |
| d. invades and modifies existing native communities | 10 |
| e. invades and replaces native communities | 15 |
| *4. Significance of threat to park resources* | |
| a. threat to secondary resources negligible | 0 |
| b. threat to areas' secondary (successional) resources | 2 |
| c. endangerment to areas' secondary (successional) resources | 4 |
| d. threat to areas' primary resources | 8 |
| e. endangerment to areas' primary resources | 10 |
| *5. Level of visual impact to an ecologist* | |
| a. little or no visual impact on landscape | 0 |
| b. minor visual impact on natural landscape | 2 |
| c. significant visual impact on natural landscape | 4 |
| d. major visual impact on natural landscape | 5 |
| **Total Possible** | 50 |
| ***B. Innate Ability of Species to Become a Pest*** | |
| 1. Ability to complete reproductive cycle in area of concern | |
| a. not observed to complete reproductive cycle | 0 |
| b. observed to complete reproductive cycle | 5 |
| 2. Mode of reproduction | |
| a. reproduces almost entirely by vegetative means | 1 |
| b. reproduces only by seeds | 3 |
| c. reproduces vegetatively and by seed | 5 |
| 3. Vegetative reproduction | |
| a. no vegetative reproduction | 0 |
| b. vegetative reproduction rate maintains population | 1 |
| c. vegetative reproduction rate results in moderate increase in population size | 3 |



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| | |
|---|---|
| d. vegetative reproduction rate results in rapid increase in population size | 5 |
| 4. Frequency of sexual reproduction for mature plant | |
| a. almost never reproduces sexually in area | 0 |
| b. once every five or more years | 1 |
| c. every other year | 3 |
| d. one or more times a year | 5 |
| 5. Number of seeds per plant | |
| a. few (0-10) | 1 |
| b. moderate (11-1,000) | 3 |
| c. many-seeded (>1,000) | 5 |
| 6. Dispersal ability | |
| a. little potential for long-distance dispersal | 0 |
| b. great potential for long-distance dispersal | 5 |
| 7. Germination requirements | |
| a. requires open soil and disturbance to germinate | 0 |
| b. can germinate in vegetated areas but in a narrow range or in special conditions | 3 |
| c. can germinate in existing vegetation in a wide range of conditions | 5 |
| 8. Competitive ability | |
| a. poor competitor for limiting factors | 0 |
| b. moderately competitive for limiting factors | 3 |
| c. highly competitive for limiting factors | 5 |
| 9. Known level of impact in natural areas | |
| a. not known to cause impacts in any other natural area | 0 |
| b. known to cause impacts in natural areas, but in other habitats and different climate zones | 1 |
| c. known to cause low impact in natural areas in similar habitats and climate zones | 3 |
| d. known to cause moderate impact in natural areas in similar habitats and climate zones | 5 |
| e. known to cause high impact in natural areas in similar habitats and climate zones | 10 |
| **Total Possible** | 50 |

**II. Feasibility of Control or Management**

***A. Abundance Within Park***

| | |
|---|---|
| 1. Number of populations (stands) | |
| a. several; widespread and dense | 1 |
| b. intermediate number; patchy | 3 |
| c. few; scattered | 5 |
| 2. Areal extent of populations | |
| a. > 50 | 1 |
| b. 11-50 ha | 2 |
| c. 5-10 | 3 |
| d. < 5 ha | 5 |

***B. Ease of Control***

| | |
|---|---|
| 1. Seed banks | |
| a. seeds remain viable in the soil for at least 3 years | 0 |
| b. seeds remain viable in the soil for 2-3 years | 5 |
| c. seeds viable in the soil for 1 year or less | 15 |
| 2. Vegetative regeneration | |
| a. any plant part is a viable propagule | 0 |
| b. sprouts from roots or stumps | 5 |
| c. no resprouting following removal of aboveground growth | 10 |
| 3. Level of effort required | |



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| | |
|---|---|
| a. repeated chemical or mechanical control measures required | 1 |
| b. one or two chemical or mechanical treatments required | 5 |
| c. can be controlled with one chemical treatment | 10 |
| d. effective control can be achieved with mechanical treatment | 15 |
| 4. Abundance and proximity of propagules near park | |
| a. many sources of propagules near park | 0 |
| b. few sources of propagules near park, but these are readily dispersed | 5 |
| c. few sources of propagules near park, but these are not readily dispersed | 10 |
| d. no sources of propagules are in dose proximity | 15 |
| C. Side Effects of Chemical/Mechanical Control Measures | |
| 1. control measures will cause major impacts to community | 0 |
| 2. control measures will cause moderate impacts to community | 5 |
| 3. control measures will have little or no impact on community | 15 |
| D. Effectiveness of Community Management | |
| 1. the following options are not effective | 0 |
| 2. cultural techniques (burning, flooding) can be used to control target species | 5 |
| 3. routine management of community or restoration or preservation practices (e.g., prescribed burning, flooding, controlled disturbance) effectively controls target species | 10 |
| E. Biological Control | |
| 1. biological control not feasible (not practical possible, or probable) | 0 |
| 2. potential may exist for biological control | 5 |
| 3. biological control feasible | 10 |
| Total Possible = 100 | |

Urgency

| | |
|---|---|
| Delay in action will result in large increase in effort required for successful control. | **High** |
| Delay in action will result in moderate increase in effort required for successful control. | **Medium** |
| Delay in action will result in little increase in effort required for successful control. | **Low** |

**The description of scoring apropriation are as the following**

***I. Significance of Impact***

*A. Current Level of Impact*: This section concentrates on ranking the species based on the present degree and extent of impact caused by the exotic species. Element 1 addresses where the species is found along a disturbance regime. If the species is found in only sites that are recently or frequently disturbed, the species is not considered a serious threat. If the species is found in mature undisturbed natural communities, the species is considered a serious threat. Element 2 addresses how many populations (stands) are found in the park and the size of the populations. Element 3 rates a species based on its effects on the ecological processes and structure of native communities. Element 4 addresses which park resources are threatened. Finally, element 5 addresses the visual impact as seen by an ecologist.

*B. Innate Ability of Species to Become a Pest*: This section ranks a species based on the life history traits that preadapt it to become a problem and its known impacts in other areas. Important life history characteristics include potential rate of increase, adaptations for long-distance dispersal, and the breadth of habitats in which the species can colonize and thrive. Element 1 is essentially a screening device. If the species cannot reproduce in the area, the species most likely will not pose much of a threat. Likely species that will not reproduce in an



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area are horticultural species transferred from areas with different environmental conditions. Element 2 addresses how a species reproduces. The assumption is that vegetative reproduction allows an adapted ecotype to be maintained, resulting in local spread. Sexual reproduction allows for the maintenance of genetic variation and propagules for long-distance dispersal and the possibility of forming highly adapted gene combinations. If the species can reproduce both vegetatively and sexually, that species has the best of both worlds.
Elements 3, 4, and 5 address the factors that determine the intrinsic rate of increase of a species—how many seeds are produced how often. Element 6 deals with the species ability to disperse. This factor can usually be rated based on the presence or absence of special adaptations for seed or fruit dispersal, such as wings and pappi for wind dispersal, bladders for water dispersal, or bristles for animal dispersal. Element 7 asks if the species needs bare soil (disturbed) to germinate or if the species can germinate in a relatively closed (undisturbed) community. Element 8 looks at what the species can do once the species has colonized an area. Is the species able to outcompete native species for light, water, etc.? Finally, scientists should not ignore what the effects of the species have been in other natural areas.

**II. Feasibility of Control or Management**

Less is known about the feasibility of managing exotic plants in natural areas than what impacts they have on the natural systems. Most research efforts in controlling plants have been in agriculture where the goal is to control all but one species while not harming the single-crop species. In natural areas, the goal is to control one or a few species while not harming diverse assemblages of native species. However, many factors will affect the funds and effort required for control and the probability of success.
*A. Abundance Within Park*: No explanation is needed here. The larger the populations and the larger the number of populations, the larger the funds and effort required to manage the species.
*B. Ease of Control*: This section not only deals with life history characteristics that impact the level of effort that will be needed to control the species, but also the probability of success if unlimited funds and personnel are used. Element 1 addresses the seed bank which directly influences the needed duration of a control program. Information on the longevity of viable seeds in soil is not available for many species, therefore making this element hard to score. However, a best estimate should be made based on the information that is available. Element 2 addresses the vegetative reproduction of the species, which influences the number and kinds of treatments required to control the species, whether the underground parts of the plant must be removed, and also dictates the protocol for disposal of plant material. Element 3 not only addresses the level of effort required, but also the kind(s) of control measures required. Integrated Pest Management Program in that mechanical treatment is preferred over chemical treatment. Element 4 deals with the presence or absence of propagules adjacent to the park and the probability of propagules being dispersed into the park. Consideration should be given to the park's ability to control the species outside its boundaries through cooperative control programs.
*C. Side Effects of Chemical/Mechanical Control Measures*: As stated earlier, researchers must consider what effects eradication or control measures will have on the system being restored or preserved. Will the treatment open up areas for the same species to recolonize or be invaded by other equally or more impacting exotics? In some cases, the lesser of two unsatisfactory options may be not taking any action.
*D. Effectiveness of Community Management*: Controlling exotic species through sound management of the system based on ecological study is by far the preferred control method. In some cases, controlling trampling by visitors, restoring historical fire regimes, or restoring shoreline processes or natural hydrological regimes will shift the competitive edge to the desired native species.



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*E. Biological Control*: Biological control is ecologically feasible for many exotic species. However, due to the high costs to develop well-tested biological control agents, it is only economically feasible for exotic species that its biocontrol agent has been tested and found effective anywhere.

**Urgency**

After the species are ranked according to their level of impact and feasibility of control or management, the exotic species that demands the most attention should be addressed first. The cost of delaying an action either financially or in impact to the natural resources of the park is a good criterion to use in making this often difficult decision.

Work should be conducted both in the field and in the library. The first step is to inventory the exotic plant species. Names of plant species should be assembled from (1) species lists and research reports for the park, (2) the catalog of specimens from the park herbarium, and (3) a preliminary field survey of the vegetation. Each species on the completed list should be checked, especially the flora for the area, to determine if a species is native or exotic. The second step is to conduct an intensive survey of the park. The survey should include the location and extent of populations of each exotic species. The information obtained in this survey will be used to complete Current Level of Impact (I.A.), a portion of Innate Ability of Species to Become a Pest (I.B.), and Abundance within Park (II.A.) . Survey should be conducted toward the end of wet season, species are flowering, and not during dry season. The map will be important for managers to locate exotic species for continued monitoring and future control. The third step is a comprehensive search of the literature for information on the ecology, biology, and control methods for each exotic species. Information from this part of the process will be used for a portion of Innate Ability of Species to Become a Pest (I.B.) and the majority of II. Feasibility of Control or Management. Occasionally, ranking an individual species may be difficult because not enough information can be located. For example, no reference may be available that contains few articles on less abundant exotic species. Occasionally, ranking an individual species may address the length of time seeds remain viable in the soil. The person ranking the species may then need to investigate seed bank ecology of other species within the genus or make a decision based on seed morphology. The next step of the process is to complete the Exotic Species Ranking System Data Summary Form for each species by bringing together all of the information that has been gathered in the previous three steps. The person conducting the ranking should read each step of the Exotic Species Ranking System outline in Table 1 and, based on information gathered, select the appropriate numerical value That value is placed on the Data Summary Form.

**Applying Results to Management Action**

The logical species to give the highest priority are those that seriously threaten natural resources yet appear to be easy to control. The lowest priority should be given to those species that pose little threat and would be difficult to control. An easy way to categorize the ranked exotics is to plot the level of impact against the feasibility of control.

As shown in Fig. 2. there was no species in the category of having serious threat and easy to control. This is a norm, and in the field it is not easy to select which species to be tackled first. However with the above works we are dealing with much smaller number of species, and would be much easier than before. Tjitrosoedirdjo et al ( 2011) utilized the technique on invasive alien plant species in Baluran National Park and come out that the first priority to be controlled was *Acacia nilotica*. This was relatively easy to decide because beside being impacting seriously to the park, by converting savanna into bushland, a considerable effort have been spent on this invasive species, therefore, deciding to continue previous partly successful effort was justifyably acceptable.



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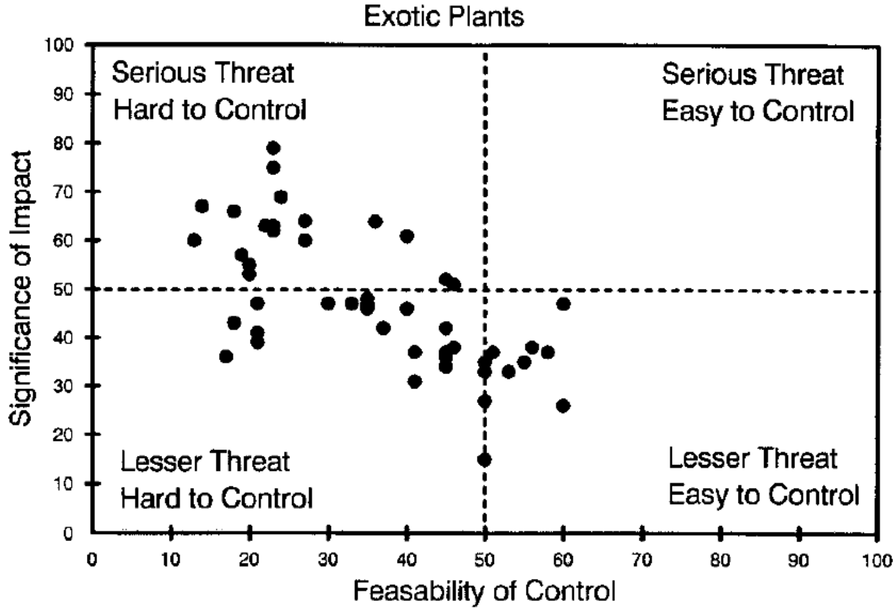


**Fig. 2**. Example of plot between significant of Impact and Feasability of Control (adapted from Hiebert & Stubbendieck, 1993).

## AUSTRALIAN POST BORDER WEED RISK MANAGEMENT

Since the publication of Exotic Species Ranking System by Hiebert & Stubbendieck (1993) many context-specific risk management systems have been developed for weeds around the world (e.g. Reichard and Hamilton, 1997; Virtue, 2010) . WRM is currently, based on the broader risk management standard and the term **consequence** refers to the **type of impact** a weed could have as well as where that impact could occur (i.e. the potential distribution), the **event (risk)** refers to the invasion of a new weed and the **likelihood** refers to the invasiveness of the weed species ( Table 1).

WRM protocol aims to provide guiding principles that identify: (i) species for inclusion in (or removal from noxious (legislative) weed lists; (ii) priorities for eradication or containment programmes (Panetta, 2009 ); (iii) priorities for prevention of and early intervention against new weed incursions; (iv) plant species with existing or potential commercial uses that pose a weed risk and require active management to limit their spread from plantings; and (v) priorities for investment into research and extension leading to improved weed management .

There are six stages in the Australian WRM protocol (Figure 3). Stage 1 is based on establishing a context within which a WRM system will operate and the methodologies for later stages and outcomes. Stage 2 is associated with the identification of weed candidates both existing and emerging. Stage 3 is associated with an analysis and evaluation of the weed risk, based on the control options and three key criteria: invasiveness (i.e.ability to establish, reproduce and disperse), impacts (e.g.to the environment, agriculture or human health) and potential distribution. Stage 4 focuses on an analysis and evaluation of the feasibility of coordinated control based on three key criteria, being: current distribution, control costs and duration. Stage 5 determines the weed management priorities, by comparing the weed risk and feasibility of coordinated control for different weed species. Finally, stage 6 is associated with the implementation of the weed management actions as determined from stages 1 to 5, being a transition from strategic planning to on-ground actions [Anon, 2006.]. Management actions include preventing entry, eradication, containment and improving targeted management techniques. Communication and consultation,and monitoring and review are key elements at each stage of the WRM process.



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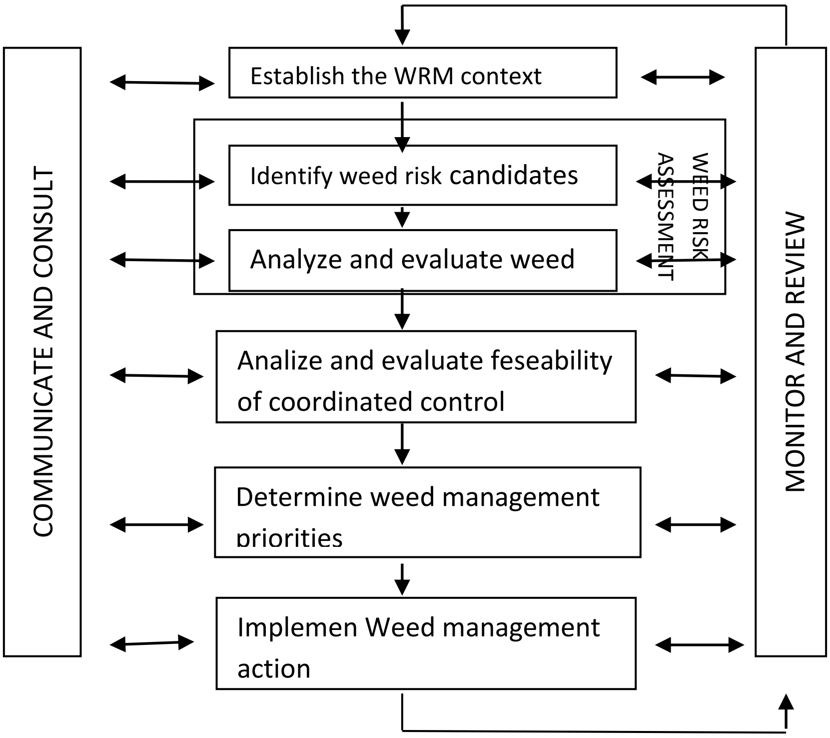


**Fig. 3**. The six stages of the Australian WRM process (Anon, 2006)

Using a question-based scoring system for stages 3 and 4, the results are put into a decision matrix (WRM matrix) in which the ranked weed risk (stage 3) is assessed against the ranked feasibility of coordinated control (stage 4). The ranked values of each are assigned a category, for example, negligible, low, medium or high. The combination of each criterion is given a management objective that is applied to the respective cells of the matrix (Figure 4).

| | | FEASIBILITY OF COORDINATED CONTROL | | | |
|---|---|---|---|---|---|
| | | **Negligible** | **Low** | **Medium** | **High** |
| **WEED RISK** | **Negligible** | No action | No action | No action | No action |
| | **Low** | Improve general weed management | Improve general weed management | Targeted control<br><br>Improve general weed management | Monitor<br><br>Protect priority sites |
| | **Medium** | Targeted control | Targeted control | Protect priority sites | Prevent entry<br><br>Contain regional spread |
| | **High** | Targeted control (incl. biocontrol) | Targeted control (incl. biocontrol)<br><br>Protect priority sites | Prevent entry<br><br>Contain regional spread | Prevent entry<br><br>Regional eradication |

**Fig. 4**. An example of a WRM matrix (Anon, 2006)

## FAO WEED RISK MANAGEMENT

Based on the above concept FAO (2011) developed WRM with slight modification. FAO model emphisizes more on the weed risk side and less on the feasability of coordinated control. Weed Risk side dwells upon three critarion ,i.e. invasiveness, Impact and potential distribution instead of on only likehood x consequences



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**Risk = invasiveness x Impact x potential distribution**

*Invasiveness criterion* gives a relative index of the rate of spread of a weed. There are three factors to be considered: (1).Establishment ability of the plant to establish amongst a dense vegetation, such as an advanced crop or closed forest, and considered as a higher weed risk. However, those that mainly establish after significant vegetation disturbance events, such as fire, cultivation, drought or extreme grazing pressure, are considered a lower weed risk.
(2). Reproductive ability which depends on the rate of reproduction. Highly invasive species are those which mature early, have high seed or other propagules (e.g., bulbs, tubers, root suckers, rhizomes, stolons) production. (3). Dispersal ability. Species with propagules (seed and/or vegetative) that are regularly moved long distances from parent plants pose a higher weed risk. Dispersal ability depends on the number of dispersal modes for a weed, their frequency of occurrence and the distance moved. The dispersal modes that should be considered are wind, water, flying animals, ground animals (including native, pests and livestock), deliberate human dispersal (i.e., species grown as crops, pastures and/or ornamentals), accidental human dispersal (e.g., attachment to clothing), vehicles (e.g., cars, farm machinery, boats) and produce contaminants (e.g., hay, grains, gravel).

*The Impacts criterion*considers the economic, environmental and social effects of weeds, these being the basis for such plant species being called ‘weeds’. It is difficult to value such impacts in monetary terms, due to the limited availability of data for many weeds and due to difficulties in economic valuation where natural ecosystems are concerned. Hence it is simpler to focus on the types of impacts a weed can have, and the magnitude of these. Impacts in this criterion are considered on a per unit area basis, and the magnitude of these impacts will often be related to the weed’s density or abundance. Total potential impacts are then a function of Impacts and Potential Distribution (at its simplest; impacts per unit area × total area). Six key factors are: (1). Competitive exclusion of other plants. Weeds that, through competition or allelopathy, significantly reduce establishment of desired plants (i.e. crops, pastures, indigenous vegetation) are a greater risk. In extreme cases certain weed species can form monocultures. (2). Reduction in yield/biomass of other plants. This considers the weed’s competitive effects on sizes of desired plants at harvest or maturity. This may be a reduction in grain, pasture, fruit or timber yields, or a reduction in biomass of native vegetation.(3). Reduction in quality of products/services. Examples of this impact include tainting of meat or milk, colouration of drinking water, weed seed contamination of grains, hay or wool, and structural damage to roads and buildings. For natural ecosystems the main impact of concern is a decline in indigenous plant species diversity, reducing nature conservation, recreational and tourism values. (4). Restriction of physical movement. This could include restrictions on movement of water (in natural and man-made systems), of people (e.g., walking or using vehicles, machinery, boats) and animals (e.g., livestock access to pasture and water, native animal access to breeding sites). Weeds that form tall, dense, spiny thickets rate highly for this risk factor. (5). Human and/or animal health. This considers the likelihood of poisoning, allergic reactions and/or physical injuries from thorns or spines. (6). Altered ecosystem processes. Ecosystem processes that may be significantly changed by high weed densities include fire regimes (through various effects upon fire frequency and intensity), levels of nitrogen fixation, water supply and use, soil sedimentation or erosion and salt accumulation. In addition, weeds may provide habitats and/or food sources for pest animals or act as alternate hosts for plant pests and diseases.

*The Potential Distribution* criterion considers the total area that a weed could occupy if it were to spread uncontrolled. The greater the Potential Distribution the greater the weed risk. It may be described in terms of area at risk (e.g., hectares), proportion of a region at risk (%) or proportion of a land use at risk (%). Potential distribution is ideally predicted using climate modelling overlaid with soil and land use tolerances in a Geographic Information System (GIS) framework: (1). Climate matching. Climate modelling software such as



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CLIMEX can give good estimates of areas favourable for a weed, provided input data is based on a representative set of point locations of the current occurrence of the species in both its native and naturalised world range. At a minimum, potential distribution can be ranked by visually matching known overseas distribution to similar climatic zones within the management area using maps (FAO 1999). Climate modelling can be quite variable in the accuracy of predictions, due to limits of distribution data, the models themselves and whether factors other than general climate place significant limits on a species distribution (e.g.,plant competition, pests and diseases). Potential distributions can also be significantly overestimated for species that are normally restricted to areas that remain damp, such as riparian and swamp habitats. However, this method is not possible to be used in several developing countries due to the lack of necessary equipment and data. At this point the best solution is to retrieve available information from the literature about the influence of climatic condition on the behaviour of the plant. (2). - Soil tolerance. Overlaying soil tolerance with climate-based predictions can significantly refine weed potential distributions. However, this is dependent on the availability of soil maps for the region of interest, and on knowledge of weeds' soil tolerance. (3). Susceptible land uses. Different weeds invade and impact in different land uses/ecosystems, due to differences in resource availability and disturbance regimes. When maps of these land uses are available then these can be overlaid with the climatic and soil tolerance to further refine potential distribution.

For aquatic weeds rainfall is generally irrelevant in predicting potential distribution, so only temperature parameters should be used in climate analysis although plants believed to be tropical and subtropical are now found in temperate areas. Soil tolerance are similarly mostly irrelevant for water weeds.

A **weed risk category is calculated** by combining the above three criteria into a decision framework, usually a semi-quantitative weed risk analysis system that calculates a relative score. The application of the scores should be based on the available information and with minimal subjectivity to ensure repeatable results. For example, each critarion can be scored as follows:

1- Low
2- Medium
3- High

Multiplying the scores of evaluated criteria will give products of 1-2-3-4-6, 8, 9, 12, 18 and 27. Just for giving an idea, some examples are given below:

Invasiveness (1) X Impacts (1) X Potential distribution (1) = 1
Invasiveness (3) X Impacts (2) X Potential distribution (1) = 6

The first three values should be assumed as C (see categories described below), for 4-6 it will be B, and for 9 or higher is of high risk (A):

*A- (High)* Of high dispersal and serious impacts to the ecosystem.
*B- (Medium)* Problems as above but limited to specific areas of the country or the region, e.g. a plant coming from a region of temperate climate unable to grow well in hot climate conditions but able to establish and reproduce in hilly areas where the temperatures are soft and much lower than in plain areas.
*C- (Low)* The presence of the plant does not pose any particular

**Technically-feasible coordinated control**

The risk of the plant and its actual spread in the field will give the proper advice for the coordinated control action to be implemented. When the plant is scarcely spread eradication is the ideal option, but if it is spread to different sites, some of them with problems of accessibility, the best approach is to contain its further spread. To this end, the **Current distribution criterion** is useful, as it describes the total known extent of spread of the weed. Mapping of a weed's present distribution in the management area is needed to accurately address this criterion. The smaller the size and number of infestations of a weed species the



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easier it is to achieve a coordinated control. Three key factors are: (1). *Total area infested*. This is the area bounded by all known plants, summed for all known infestations. It includes all land uses in which the weed occurs within the region of interest. It also includes areas where it may be deliberately grown in gardens or on farms, with mass plantings for commercial or amenity use adding considerably to total area infested. Infested area may be described in terms of actual area (e.g., hectares), proportion of the region occupied (%) or proportion of the land use occupied (%). (2). *Number of infestations*. This is the number of distinct infestation sites that need to be independently searched and treated. Infestations may be separated by distance, barriers (e.g., a river), property/jurisdictional boundaries or different land types. Work effort increases with the number of infestations (e.g., frequent packing up of equipment, greater liaison effort with landholders). When the new plant is detected the likely area at risk should be determined for immediate monitoring. This observation may also compel the monitoring of other new areas depending on the spread of the plant. Usually new plants are not detected once they enter into the new territories and for this reason monitoring is a compulsory action.

(3) *Accessibility of infestations*. This relates to travelling times to and movement within infestations, for searching and control activities. Two sub factors are the maximum distance between infestations

and the ease of movement within infestations (e.g., limits due to slope, rockiness, dense vegetation and/or presence of water).

In all cases, no matter whether eradication or containment is to be implemented, efforts should be made to prevent the reproduction of the plant and the build up of seeds or other propagules in soil. The feasibility of the coordinated control will also depend on the treatment(s) to be used. In this stage the control action is chosen based on what is advised to be done. Table 3 gives an example of possible control actions according to weed risk/spread of the plant. Final decisions should be discussed and agreed upon by the steering committee in close consultation with all stakeholders.

**Table 3.** Examples of weed risks and likely control actions to be implemented

| Weed risk/ Required control action | Scarce presence of the plant, found in a few sites | Plant found in several sites but with low abundance | Plant widely spread in specific climate conditions |
|---|---|---|---|
| C- Low | Eradication | Eradication | Eradication/containment |
| B-medium | Eradication | Eradication Control | Control |
| A-High | Containment | Control* | Control |

Note: *Control means : the implementation of traditional strategies for the suppression of native or naturalised weeds.

The score appropriation of 1 to 3 being, low, medium, and high to risk critaria was not the only appropriation of scores, Labrada (2011) adopting WRM from South Australia utilized scoring from 0 – 10 for all criteria to calculate index of risk, instead of score 1-3. Labrada also adopted utilized scores of 0 – 10 for feasibility of control, and produced the following digram of management

| Weed Risk | Feasibility of coordinated control | | |
|---|---|---|---|
| | Low | Medium | HIgh |
| Low | No Action | No Action | Monitoring |
| Medium | General Improve ment of Weed | General Improve ment of Weed | Prevent the entry and Regional |



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| | | | |
|---|---|---|---|
| | Management | Management and local containment | containment |
| High | Direct manage ment including biological control and local containment | Direct manage ment. Regional containment and local eradication | Prevent entry and regional eradication |

**Fig. 5.** The diagram of actions appropriation as dictated by weed risk management (after Labrada, 2011).

**Conservation Risk Assessment for Weed Management (CRAWM) or Asset-Protection Triage**

As outlined above, many of the risk assessment systems for weeds developed to date have had prevention of entry, eradication of new weeds or containment of existing weeds as the assessment endpoints or objectives. Given that relatively few weed introductions have been successfully eradicated, especially on continents (e.g. [Myers, 2000; Mack and Lonsdale, 2002]) and many weed eradication programmes underestimate the task (e.g. Jupp, et al, 2002); and that the larger the infestation the less likely it is that eradication can be achieved (Rejma´nek and Pitcairn ,2002; Parkes and Panetta, 2006), there is also an important need to evaluate and prioritize the risk of established weed species to assets (e.g. native species Downey et al,2010). Thus, for widespread weeds the emphasis needs to move from prevention to mitigation of the consequence (e.g. [Perring, 2005). In this section, we focus specifically on the risk associated with the decline in native species (or protection of biological assets) given a current impact from widespread weeds, mainly because systems have not been as extensively developed for other areas or types of assets. In a recent review of the approaches used to assess the status of weeds species currently occurring in natural areas, Fox and Gordon (2009) classified four types of assessments: (1) the adoption of pre-existing lists; (2) systems that are easy to use and quick to develop and implement; (3) systems that use more robust approach (i.e. with yes/no answers); and (4) systems that use more rigorous evaluations (i.e. multiple-choice and low, medium and high answers). The authors also found a range of problems: specifically that few systems identified if or how the systems had been tested, and that there were many inconsistencies across systems examined. This review highlights the need for greater development around a uniformed prioritization/risk assessment system for natural areas. In addition, the authors state that the identification of weeds posing an impact (ecological or economic), using accepted assessment processes/ methods, should increase the effectiveness of management efforts.

The criteria used to assess the ‘ecological or biological’ impacts in the pre- and post-border systems developed todate tend to be generic in nature, in part because specific information on the exact impact is unavailable for many weed species (Reid et.al. 2009) and that assessments need to occur within a limited time frame (e.g. in 1–2 days for the Australian WRA system [Pheloung, 2001]). While improving such assessment criteria to reflect better the impact of weeds on biodiversity will lead to a more robust risk assessment framework for pre- and post-border assessments in the future, it will not address the current impact of established widespread weeds on biodiversity. While Parker et al. (1999) outlined a framework for determining the impact from invasive species, it remains untested and is yet to be adopted despite being proposed over a decade ago, in part because, as the authors state, there are difficulties applying it. Thus, an alternative to Parker et al.’s [80] impact measure is needed. The concept of managing current weeds for both prevention and mitigation under one strategy has been outlined previously (e.g. in New Zealand through the Department of Conservation’s species- and site-led approach ( Owen, 1998]). The species-led approach aims to eradicate or contain newly established weeds where they occur, based on an assessment of



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specific weed species,while the site-led approach aims to manage the threat of weeds (generally) to protected natural areas, by prioritizing such areas for targeted control, based on the value of the biodiversity present and the urgency of control relative to the extinction risk. However, this system, specifically the site-led approach, has not been used or evaluated more broadly to help manage the risk posed by widespread weeds to native species (notable exceptions include (NISC,2005) and (Platt et al. 2005) as a pilot approach in Australia).

One reason for this lack of adoption is a misinterpretation of the meaning of the terms species-led and site-led, based on the assumption (incorrectly) that any species-specific programme is species-led (irrespective of whether it is aimed solely at eradication or containment of newly established) and management of specific sites (irrespective of whether it is aimed solely at protecting biodiversity) is site-led (Downey and Sheppard, 2005). Recently, Downey et al. (2010) outlined an approach, using a triage system, based on an assessment of the native species/biodiversity at risk from a weed species and the probability of achieving a conservation outcome at invaded sites. While the idea of triage has been used for conservation previously (e.g. (Hobbs and Kristjanson,2003) and the site-led approach in New Zealand assesses conservation priorities (see above), the approach outlined by Downey et al. (2010) includes measures to assess the recoverability of the biodiversity following weed control and thus the likelihood of management success.

This triage system uses three key assessment criteria for determining the species or biodiversity at risk from a weed species, being: the (1) threat to the species or susceptibility of the habitat to invasion, (2) ability of the species to respond following control and (3) degree of match between the native and weed species' distribution. It also uses three key assessment criteria for determining the sites for control, based on the: (i) actual impact present, as the impact is not equal across all locations of each species at risk, (ii) ability to achieve effective control based on site accessibility and availability of control measures with limited off-target damage and (iii) the value of the site to the species survival. Thus, this triage approach reduces the risk of directing resources to areas where protection is unachievable, or where the impact on biodiversity is low, or where the site has a low biodiversity value (Figure 6). A strength of this approach is that data can be collated from a wide range of sources to assess the sites and biodiversity at risk.

This triage system which also establishes a CRAWM system assesses the risk from established weeds to biodiversity and sites for control. It also fills a significant gap in assessing the risk from weeds across the spectrum of weed management. In this instance, the focus of ris management moves from preventing new weed introductions or mitigating the potential impacts of newly established weeds, to preventing the extinction or extirpation of native species from the large number of established widespread weeds that are currently posing a significant impact. The assessment system thus uses a triage approach to identify the priorities based on the degree of urgency required to protect the native species from the impact of weeds.

While this triage system also encompasses risk analysis associated with the magnitude of the consequence of an event and the likelihood of that event occurring (Anon. 2004), the emphasis shifts from the weed to the asset as follows, *the consequence refers to the degree of impact a weed poses to biodiversity and where that impact occurs, the event refers to the decline of an environmental asset associated with the invasion of a weed and the likelihood refers to the ability to achieve a conservation outcome from weed management activities, relative to the degree of urgency associated with a management activity (also see Table 1).*

The risk of inaction is rarely accommodated in the management of widespread weed species. While the economics on inaction can be assessed, for example, taking no action (e.g. Jupp et al.2002. ) or delaying action (e.g. Myers et al, 2000) can increase the costs of control and decrease the changes of success (e.g. (Timmins and Owen, 2002), the same level of assessment of the effects of inaction on the protection of biodiversity has not occurred. However, a recent study provides some insights based on the observation of a weed density



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threshold effect leading to the decline of native species( Gooden et al, 2009). The concept of management thresholds for weeds has been applied to protected areas management (including biodiversity conservation) in South Africa (Foxcroft and Downey 2008). While this triage approach has enabled the prioritization of native species (assets) at risk from weeds and sites for control, based on achieving the greatest conservation outcome, an evaluation of the outcome is yet to be completed. However, the interim results are promising, irrespective of which, this approach needs to be backed up with effective monitoring (Blossey 1999) which

| | | Probability of protecting biodiversity at specific sites | | |
|---|---|---|---|---|
| | | **High** | **Medium** | **Low** |
| **Level of threat to biodiversity** | **High** | **A** - Weed management is critical, immediate, targeted and long-term | **B** - Targeted management action needs to occur promptly and long-term | **C** - Broad management (i.e. of multiple threats simultaneously) |
| | **Medium** | **D** - Targeted management action needs to occur promptly and long-term | **E** - General management to reduce the impact of weed populations | **F** - General low level management to reduce the threat |
| | **Low** | **G** - Actions to minimise the threat and prevent further elevation of the problem | **H** - Low level of management only | **I** - No immediate action, management action required only after completion of higher priorities |

**Fig. 6**. An example of a WRM matrix (Anon 2006)

assesses not only the response of the weed to control, but importantly the species at risk as well. Such monitoring has rarely been undertaken or reported (Reid et al, 2009), despite conservation being a significant aim of many weed programmes (e.g. King and Downey, 2008) and it being a decade since Blossey (1999) outlined the failures and implications associated with the absence of weed monitoring programmes. To overcome this issue, standard monitoring protocols have been developed to support the implementation of the triage system described above (Hughes et.al.2009)

While the above review provides guidance, the inclusion of asset protection systems for weeds using risk assessment procedures is relatively new. Challenges for the future development of any CRAWM system include:

(i) a broad agreement on a specific system and its requirements/components;

(ii) examination and testing with respect to the applicability of the systems outlined above to other areas and regions/countries;



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(iii) balancing the conflicts of both positive and negative impacts;
(iv) establishing processes to integrate or link with the WRM system and other post-border systems; and
(v) identification of suitable data and collation processes to make assessments; lack of data in this regard is no excuse for inaction (Simberloff, 2003)

With the above WRM protocol progression a detail WRM is given below and it seems well standardised in term of risk analysis and may be applicable to Indonesia condition with the necessary adjustment.

## WEED RISK MANAGEMENT GUIDE OF SOUTH AUSTRALIA

The SA Weed Risk Management System was developed by the Animal and Plant Control Commission, in cooperation with Animal and Plant Control Boards, to help in prioritising weeds for control programs. A series of questions are answered to compare the relative risk and feasibility of control of different weeds. Weeds are assessed separately for various ***land uses***, so that the most important weeds of different land uses can be identified.

The questions can apply to any type of weed in any land use. There may be **questions where you don't know the answer** for a certain weed, especially if it is not present in your area. In such cases choose the "don't know" option, and seek opinions from others (e.g. landholders, advisers, other Boards, researchers). "Don't know" is treated as a "0" for the Comparative Weed Risk scoring and gets a maximum score for the Feasibility of Containment scoring. This avoids bias against weeds which have a score for all questions. However, weeds which have one or more questions answered as "don't know" are indicated as such at their final score. Sharing information and scores is the key to building up knowledge and getting the most out of the SA Weed Risk Management System. Answering questions as a group is better than individually. It"s particularly important to get consensus on assumptions about typical weed control in the land use.

This scoring system is a tool to help in making standard, informed decisions on weed control. The protocol was developed by Dr John Virtue, Weed Ecologist, Animal and Plant Control Group Department of Water, Land & Biodiversity Conservation. GPO Box 2834, Adelaide SA 5001. *Phone:* 08 8303 9502 , *Email:* virtue.john@saugov.sa.gov.au

## COMPARATIVE WEED RISK

The weed risk questions are divided into three main criteria; invasiveness, impacts and potential distribution. ***Invasiveness*** looks at the weed's rate of spread, faster spreading weeds being a higher priority for control. ***Impacts*** are the economic, environmental and social effects the weed has. ***Potential distribution*** indicates what total area the weed could spread to. Scores for each of these criteria are multiplied (each ranging between 0 and 10), to give a weed risk score out of 1000.

## INVASIVENESS

This section indicates how fast the weed can spread within a particular land use. It takes account of how well the weed can establish, reproduce and disperse. Answer all questions with the land use in mind, except for question 5(a).



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| **1. What is the weed's ability to establish amongst existing plants?** | | SCORE |
|---|---|---|
| ☐ **very high** | "Seedlings" readily establish within dense vegetation, or amongst thick infestations of other weeds. | 3 |
| ☐ **high** | "Seedlings" readily establish within more open vegetation, or amongst average infestations of other weeds. | 2 |
| ☐ **medium** | "Seedlings" mainly establish when there has been moderate disturbance to existing vegetation, which substantially reduces competition. This could include intensive grazing, mowing, raking, clearing of trees, temporary floods or summer droughts. | 1 |
| ☐ **low** | "Seedlings" mainly need bare ground to establish, including removal of stubble/leaf litter. This will occur after major disturbances such as cultivation, overgrazing, hot fires, grading, long-term floods or long droughts. | 0 |
| ☐ **don't know** | | ? |

Ignore any weed control practices for this question. Depending on the land use, "vegetation" may be crops, pastures, lawns and/or native vegetation. Weeds that invade well-managed land uses (where a dense vegetative cover over soil is maintained) are assumed to be more important. High scoring weeds would include wild radish, bridal creeper and dodder.

Assume the plant has just arrived. "Seedlings" includes growth from dispersed vegetative propagules (e.g. broken fragments of couchgrass stems or silverleaf nightshade roots) and spores, in addition to seeds. "Seedlings" does not include new vegetative growth whilst still attached to the parent plant (e.g. by stolons, rhizomes or lateral roots). This feature is accounted for in question 3(c).

Features which can help a weed establish amongst existing plants include:
the ability to germinate under the canopy of other plants (e.g. weeds that have staggered germination in crops) large seeds or vegetative propagules (e.g. bulbs, root fragments, tubers) provide more reserves to help the weed establish in competition with other plants the ability to tolerate or avoid competitive stresses (e.g. by rapid root growth, fixing own nitrogen, or rapid vertical shoot growth)

| **2. What is the weed's tolerance to average weed management practices in the land use?** | | SCORE |
|---|---|---|
| ☐ **very high** | Over 95% of weeds survive commonly used weed management practices. | 3 |
| ☐ **high** | More than 50% of weeds survive. | 2 |
| ☐ **medium** | Less than 50% of weeds survive. | 1 |
| ☐ **low** | Less than 5% of weeds survive. | 0 |
| ☐ **don't know** | | ? |

Assume the weed is new to an area. This question looks at whether the new weed is killed by the weed management practices which are commonly used across the land use. If most are killed then there will be few plants to reproduce and spread. If few are killed then changes to weed management practices will eventually be needed. Weed management practices include herbicides, cultivation, cutting/slashing, grazing, and fire. The types and timing of these practices may vary within land uses (e.g. for cereals and broadleaf crops, or vineyards and citrus), but average these. If a weed grows and seeds when there is normally no weed management (e.g. summer) then it is highly tolerant of the common weed management practices. Weeds with high tolerance to routine weed management would include silverleaf nightshade (difficult to kill), caltrop (quick to seed), and broomrape. In native vegetation there may be no commonly used weed management practices at a regional level - if so then include this in your assumptions about the land use.



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| 3. What is the reproductive ability of the weed in the land use? | | | | Total (a+b+c) | SCORE |
|---|---|---|---|---|---|
| (a) Time to seeding | (b) Seed set | (c) Vegetative reproduction | | | |
| ☐ 1 year 2 | ☐ high 2 | ☐ fast 2 | ☐ high | 5 or 6 | 3 |
| ☐ 2-3 yrs 1 | ☐ low 1 | ☐ slow 1 | ☐ medium-high | 3 or 4 | 2 |
| ☐ >3 yrs/never 0 | ☐ none 0 | ☐ none 0 | ☐ medium-low | 1 or 2 | 1 |
| ☐ don't know ? | ☐ don't know ? | ☐ don't know ? | ☐ low | 0 | 0 |
| | | | ☐ don't know | | ? |

This question looks at how well the weed can reproduce, to rapidly build up its numbers at a site, and to spread quickly to other sites. If a weed never gets to reproduce in a land use then it will score 0. Three factors are considered in scoring the weed:

(a) Time to seeding is the time from establishment (from seed or vegetative propagule) to seed production.

(b) Consider seed set as the average number of viable seed produced per square metre of ground per year, in a patch of the weed. This may be from one large weed (e.g. a tree) or many small weeds (e.g. grasses). High would be >1000 seeds per m2. Your answer to question 2 may influence seed set.

(c) Consider vegetative reproduction as the average number of new plants produced each year by such means as bulbs, bulbils, corms, tubers, rhizomes, stolons, root suckers, root fragments and shoot fragments. High would be >10 new plants per year from a mature parent plant. In certain land uses cultivation may increase vegetative reproduction (e.g. Lincoln weed). "New plants" are defined as shoots with their own root system. There may still be some connection to the parent plant (e.g. couchgrass).

| 4. How likely is long-distance dispersal (>100m) by natural means? | | Total (a+b+c+d) | SCORE |
|---|---|---|---|
| (a) Flying birds | (b) Other wild animals | | |
| ☐ common 2 | ☐ common 2 | 6, 7 or 8 | 3 |
| ☐ occasional 1 | ☐ occasional 1 | 3, 4 or 5 | 2 |
| ☐ unlikely 0 | ☐ unlikely 0 | 1 or 2 | 1 |
| ☐ don't know ? | ☐ don't know ? | 0 | 0 |
| | | | ? |
| (c) Water | (d) Wind | | |
| ☐ common 2 | ☐ common 2 | | |
| ☐ occasional 1 | ☐ occasional 1 | | |
| ☐ unlikely 0 | ☐ unlikely 0 | | |
| ☐ don't know ? | ☐ don't know ? | | |

This question looks at how well the weed can spread its propagules (seed or vegetative) by natural means, to start new weed outbreaks a long distance from the original outbreak. Weeds which have more means of dispersal tend to spread faster. Consider if a weed is adapted for long-distance dispersal by any of the above means, and how regularly these means of dispersal occur. How often do you see new outbreaks starting at least 100 metres away from an original infestation?

Features favouring long-distance dispersal by flying birds and other wild animals (e.g. foxes, kangaroos, rabbits, emus) are:
whole fruits are eaten, and viable seeds are then defecated or regurgitated (e.g. olives, sweet briar) propagules have hooks, barbs or sticky substances that attach to feathers, hairs or skin (e.g. horehound, brome grass) very small seeds which can lodge within feathers, hairs or feet (e.g. nutgrass)

Features favouring long-distance water dispersal are: propagules which float (consider wind-assisted movement as water dispersal) weeds located in or near to moving water or frequent floods



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Mainly aquatic weeds such as salvinia and seeding willows would be commonly dispersed over 100m by water movement.

Research has shown that seeds of most wind dispersed weeds actually land close to the parent plants. Long-distance dispersal is more likely to be common for tall trees with light seeds (with wings, plumes or hairs) which are subject to frequent strong winds, and for weeds which snap off after fruiting and roll across sparsely-vegetated ground (e.g. wild turnip, serrated tussock).

| **5. How likely is long-distance dispersal (>100m) by human means?** | | | | Total (a+b+c+d) | *SCORE* |
|---|---|---|---|---|---|
| ***(a) Deliberate spread by people*** | | ***(b) Accidentally by people and vehicles*** | | 6, 7 or 8 | *3* |
| ☐ common | *2* | ☐ common | *2* | 3, 4 or 5 | *2* |
| ☐ occasional | *1* | ☐ occasional | *1* | 1 or 2 | *1* |
| ☐ unlikely | *0* | ☐ unlikely | *0* | 0 | *0* |
| ☐ don't know | *?* | ☐ don't know | *?* | | *?* |
| ***(c) Contaminated produce*** | | ***(d) Domestic/farm animals*** | | | |
| ☐ common | *2* | ☐ common | *2* | | |
| ☐ occasional | *1* | ☐ occasional | *1* | | |
| ☐ unlikely | *0* | ☐ unlikely | *0* | | |
| ☐ don't know | *?* | ☐ don't know | *?* | | |

This question looks at how well the weed can spread its propagules (seed or vegetative) by human-influenced means, to start new weed outbreaks a long distance from the original outbreak. Weeds which have more means of dispersal tend to spread faster. Consider if a weed is adapted for long-distance dispersal by any of the above means, and how regularly these means of dispersal occur. How often do you see new outbreaks starting at least 100 metres away from an original infestation?

Deliberate human spread includes weeds which have been planted for use in agriculture, forestry, horticulture, amenity, windbreaks and/or soil protection. Those which are or have been widely planted have greater potential for dispersal due to many introduction points. **Ignore the land use for this question.** Examples include olives, African lovegrass and Aleppo pine. Deliberate human spread also includes weeds with attractive flowers which are picked and then discarded (e.g. Calomba daisy, cape tulip). A weed may be legally restricted from sale, but is it still planted?

Features favouring accidental people and vehicle dispersal are: weeds which grow in heavily trafficked areas, such that transport by footwear, clothing or vehicles (including farm machinery and boats) may occur weeds which are dragged by farm machinery (e.g. silverleaf nightshade) propagules have hooks, barbs, or sticky substances to attach to objects (e.g. caltrop) very small propagules which can lodge in cracks in footwear, clothing or vehicles (e.g. Lincoln weed)

For contaminated produce consider crop seed, pasture seed, hay, soil, gravel, fertilisers, manures, and/or mulch. Examples of weeds which may be commonly spread by such means include bifora, salvation Jane, and soursob. Do not consider wool as this relates to the sale of farm animals between properties, which is covered in (d). Features favouring dispersal by domestic/farm animals (e.g. sheep, cattle, horses, dogs) are: whole fruits are eaten, and viable seeds are then defecated or regurgitated (e.g. cutleaf mignonette, charlock) propagules have hooks, barbs or sticky substances that attach to feathers, hairs or skin (e.g. horehound, brome grass) very small seeds which can lodge within feathers, hairs or feet (e.g. nutgrass)

## IMPACTS

This section indicates the **potential impacts** the weed has. Each question is answered with a land use in mind. Assume that the **weed has spread** across a whole paddock, orchard,



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plantation, nature reserve or water body, and that **commonly-used weed management practices have not been changed to specifically target the weed**. If the weed is well-controlled by these common practices then it will occur at a low density and will have minimal impacts. Alternatively, if the weed is poorly controlled by these common practices then it may get to a high density and have substantial impacts. If the weed has an effective biocontrol agent established which substantially reduces its growth then the weed's impacts will be reduced. **Decide if the weed is likely to reach a low, medium or high density in the land use.**

| **1. Does the weed reduce the establishment of desired plants?** | | SCORE |
|---|---|---|
| ☐ **>50% reduction** | The weed stops the establishment of more than 50% of desired plants (e.g. regenerating pasture, sown crops, planted trees, regenerating native vegetation), by preventing germination and/or killing seedlings. | 3 |
| ☐ **10-50% reduction** | The weed stops the establishment of between 10% and 50% of desired plants. | 2 |
| ☐ **<10% reduction** | The weed stops the establishment of less than 10% of desired plants. | 1 |
| ☐ **none** | The weed does not affect the germination and seedling survival of desired plants. | 0 |
| ☐ **don't know** | | ? |

This question looks at whether the weed prevents the establishment of desired plants, so the density of these plants is reduced. The weed may prevent germination by dense shading, or by forming physical barriers to water movement into the soil. The weed may kill seedlings by denying them access to soil moisture, sunlight and nutrients.

Note that the desired plants may mainly establish after a major disturbance (e.g. cultivation prior to planting, bushfire), so the weed itself may also be establishing. In these cases does the weed actually have a major effect?

Weeds which are likely to cause over 50% reductions in establishment are gorse and early-germinating (and unsprayed) salvation Jane in pastures, and phlaris and watsonia in native vegetation.

| **2. Does the weed reduce the yield or amount of desired vegetation?** | | SCORE |
|---|---|---|
| ☐ **>50% reduction** | The weed reduces crop, pasture or forestry yield, or the amount of mature native vegetation by over 50%. | 4 |
| ☐ **25-50% reduction** | The weed reduces yield or amount of desired vegetation by between 25% and 50%. | 3 |
| ☐ **10-25% reduction** | The weed reduces yield or amount of desired vegetation by between 10% and 25%. | 2 |
| ☐ **<10% reduction** | The weed reduces yield or amount of desired vegetation by up to 10%. | 1 |
| ☐ **none** | The weed has no effect on growth of the desired vegetation. Or the weed may become desirable vegetation at certain times of year (e.g. providing useful summer feed), which balances out its reduction in the growth of other desirable plants. | 0 |
| ☐ **don't know** | | ? |

This question looks at the degree of yield loss (in crops, pastures, forestry) or suppression (in mature native vegetation) caused by the weed. It follows on from question 1, and looks at the growth achieved by plants which did establish despite the weed. The question is answered on a per hectare basis, in comparison to similar vegetation which is free of the weed. For native vegetation it may be useful to think in terms of percentage cover.

Weeds will reduce growth of other plants by competing for sunlight, water and nutrients. Competition is greater where a weed is larger (e.g. tall with a dense leaf canopy and an extensive root system) and grows at the same time as the desirable plants. Some weeds also



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compete by forming physical barriers which stop plants growing to reach light, water and/or nutrients (e.g. tuber mat of bridal creeper). A special case are parasitic weeds which directly attack other plants. Weeds which could cause >50% reductions in the yield/amount of desired vegetation would include Allepo pines, serrated tussock and branched broomrape.

Some weeds may increase the amount of useful vegetation in a land use. For example, does a perennial weed of grazing land provide nutritious summer feed, thus increasing total pasture available throughout the year?

| **3. Does the weed reduce the quality of products or services obtained from the land use?** | | *SCORE* |
|---|---|---|
| ☐ **high** | The weed severely reduces product quality such that it cannot be sold. This may be due to severe contamination, toxicity, tainting and/or abnormalities (chemical and/or physical). For ***native vegetation***, the weed severely reduces biodiversity (plants and animals) such that it is not suitable for nature conservation and/or nature-based tourism. For ***urban*** areas, the weed causes severe structural damage to physical infrastructure such as buildings, roads and footpaths. | *3* |
| ☐ **medium** | The weed substantially reduces product quality such that it is sold at a much lower price for a low grade use. For ***native vegetation***, the weed substantially reduces biodiversity such that it is given lower priority for nature conservation and/or nature-based tourism. For ***urban*** areas, the weed causes some structural damage to physical infrastructure such as buildings, roads and footpaths. | *2* |
| ☐ **low** | The weed slightly reduces product quality, lowering its price but still passing as first grade product. For ***native vegetation***, the weed has only marginal effects on biodiversity but is visually obvious and degrades the natural appearance of the landscape. For ***urban*** areas, the weed causes negligible structural damage, but reduces the aesthetics of an area through untidy visual appearance and/or unpleasant odour. | *1* |
| ☐ **none** | The weed does not effect the quality of products or services. | *0* |
| ☐ **don't know** | | *?* |

This question looks at whether the weed effects the quality of products or services obtained from a land use. Products affected by the weed may include meat, grain/seed, milk, wool, timber, fruit, hay, and/or water. For native vegetation, consider services such as nature conservation and tourism. An example of a high effect on quality is dodder preventing the sale of seed crops. Reduction in stock condition/liveweight should not be considered here - this is due to either a reduction in available feed (question 2) or animal health effects caused by eating the weed (question 5).

For this question, ignore a weed's proclamation status with regard to moving contaminated produce in South Australia, but do consider noxious weed lists and seed quality standards of other states or countries. This prevents bias against non-proclaimed weeds when comparing them to existing proclaimed plants.

| **4. Does the weed restrict the physical movement of people, animals, vehicles, machinery and/or water?** | | *SCORE* |
|---|---|---|
| ☐ **high** | Weed infestations are impenetrable throughout the year, preventing the physical movement of people, animals, vehicles, machinery and/or water. | *3* |
| ☐ **medium** | Weed infestations are rarely impenetrable, but do significantly slow the physical movement of people, animals, vehicles, machinery and/or water throughout the year. | *2* |
| ☐ **low** | Weed infestations are never impenetrable, but do significantly slow the physical movement of people, animals, vehicles, machinery and/or water at certain times of the year **or** provide a minor obstruction throughout the year. | *1* |
| ☐ **none** | The weed has no effect on physical movement. | *0* |
| ☐ **don't know** | | *?* |

This question looks at the degree to which a dense infestation of the weed physically restricts movement. Weeds may restrict movement by being tall, thorny, tangled and/or dense.



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For this question, ignore any deliberate restrictions on movement aimed solely at limiting the spread of weed propagules.

Examples of weed limits on movement include:
slowing of stock mustering
blockages of farm machinery at crop sowing and/or harvesting
tyre punctures
slowing of water flow in irrigation systems
interference with boat access
interference with thinning operations in forestry
preventing stock access to pasture and/or water
preventing animal access to nesting sites

Weeds which would score highly include blackberry and gorse at high densities, forming impenetrable thickets.

| **5. Does the weed affect the health of animals and/or people?** | | *SCORE* |
|---|---|---|
| ☐ **high** | The weed is highly toxic and frequently causes death and/or severe illness in people, stock, and/or native animals. | *3* |
| ☐ **medium** | The weed occasionally causes significant physical injuries (due to spines or barbs) and/or significant illness (chronic poisoning, strong allergies) in people, stock, and/or native animals, occasionally resulting in death. | *2* |
| ☐ **low** | The weed can cause slight physical injuries or mild illness in people, stock, and/or native animals, with no lasting effects. | *1* |
| ☐ **none** | The weed does not affect the health of animals or people. | *0* |
| ☐ **don't know** | | *?* |

This question looks at how the weed affects the health of animals (domestic stock and native) and people. Note that if a weed is toxic but is not palatable then it may not actually be grazed. Ignore any starvation effects from reduced growth of pasture or reduced access to pasture, as these have been covered in questions 2 and 4. A weed with high effects on health would be poison ivy.

| **6. Does the weed have major, positive or negative effects on environmental health?** | | | | |
|---|---|---|---|---|
| | ☐ **major positive effect** | ☐ **major negative effect** | ☐ **minor or no effect** | ☐ **don't know** |
| scoring for (a) - (f): | *−1* | *1* | *0* | *?* |
| **(a) food/shelter ?** | *Examples of negative effects are blackberry harbouring rabbits and grass weeds hosting wheat root diseases. An example positive effect is boxthorn providing stock shelter. Ignore pasture for livestock as this was covered in question 2.* | | | |
| **(b) fire regime?** | *This includes changes to the normal frequency, intensity, and/or timing of fires. Examples of weeds having major effects include exotic grasses invading shrubby native vegetation.* | | | |
| **(c) increase nutrient levels?** | *For example, legumes can increase soil nitrogen. This may make native vegetation more prone to invasion by other weeds, but would be beneficial in agriculture. Ignore competition for nutrients (decreased nutrient levels) as this was covered indirectly in question 2.* | | | |
| **(d) soil salinity?** | *Are the leaves of the weed high in salt? Leaf decomposition may increase salinity at the soil surface. Example plants are iceplant and tamarix.* | | | |
| **(e) soil stability?** | *Does the weed increase soil erosion, or silting of waterways?* | | | |
| **(f) soil water table?** | *Does the weed substantially raise or lower the soil water table compared to other plants present? Is this positive or negative? Ignore competition for water as this was covered in question 2.* | | | |
| Total (a + b + c + d + e + f) | >3 | 2 or 3 | 1 | 0 or less |
| *SCORE FOR 6.* | *3* | *2* | *1* | *0* |

This question looks at whether the weed has major, long-term effects on a land use's environment. These effects may be beneficial or detrimental. Effects are more likely where



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the weed substantially changes the vegetation structure, such as woody weed invasion of grassland. Decisions on major effects should be well-known (e.g. backed up by scientific studies or expert opinion).

## POTENTIAL DISTRIBUTION

This section looks at what proportion of a land use is at risk from the weed in question. This will depend on the **climate and soil preferences of the weed**. For example, some weeds may only be suited to higher rainfall areas of a Board, or only be a problem on alkaline soils. **Differences within the land use also need to be considered.** For example in the perennial horticulture land use, a weed may be a problem in citrus but not occur in vineyards. This score should also be based on where the weed will grow at the density you assumed in scoring Impacts. That is, if you assumed a high density in scoring impacts then ignore areas where the weed would only persist at a low density when determining potential distribution

This question is best answered with topographic, land use and soil maps for the Board area. These can be analysed electronically using a GIS system such as ArcView, or done on paper maps. Data and maps can be obtained from PIRSA. If using maps the following steps will help in estimating the percentage area of a land use that is suitable for the weed:

1). Map the land use in your Board. If you do not have a land use map, you couldshade areas on clear plastic laid over topographic maps.

2). Consider the climatic and soil preferences of the weed, and the vegetation/crop/pasture types within the land use to which the weed is suited. Lay a sheet of plastic over the land use map, and shade the areas of the land use which are suitable for the weed.

3). Compare the weed's map to the land use map to estimate the percentage of the land use which is suitable for the weed. Answer as follows:

| **In the Board, what percentage area of the land use is suitable for the weed?** | | *SCORE* |
|---|---|---|
| ☐ **> 80% of land use** | The weed has a potential to spread to more than 80% of the land use in the Board. | *10* |
| ☐ **60-80% of land use** | The weed has a potential to spread to between 60% and 80% of the land use in the Board. | *8* |
| ☐ **40-60% of land use** | The weed has a potential to spread to between 40% and 60% of the land use in the Board. | *6* |
| ☐ **20-40% of land use** | The weed has a potential to spread to between 20% and 40% of the land use in the Board. | *4* |
| ☐ **10-20% of land use** | The weed has a potential to spread to between 10% and 20% of the land use in the Board. | *2* |
| ☐ **5-10% of land use** | The weed has a potential to spread to between 5% and 10% of the land use in the Board. | *1* |
| ☐ **1-5% of land use** | The weed has a potential to spread to between 1% and 5% of the land use in the Board. | *0.5* |
| ☐ **unsuited to land use** | The weed is not suited to growing in any part of the land use in the Board. | *0* |
| ☐ **don't know** | | *?* |

## COMPARATIVE WEED RISK SCORE

The score for weed risk is calculated by adjusting the invasiveness, impacts and potential distribution scores to range from 0 to 10, and then multiplying these. Weed risk will have a maximum of 1000, and a minimum of 0. The spreadsheet does this for you.



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**To calculate manually, adjust the raw scores as follows:**

| | |
|---|---|
| Invasiveness: | Divide by 15 and multiply by 10. Round off to one decimal place. |
| Impacts: | Divide by 19, and multiply by 10. Round off to one decimal place. |
| Potential distribution: | Leave unchanged. |

**Comparative Weed Risk = Invasiveness × Impacts × Potential distribution**

Splitting up these possible scores into bands of 20% gives cutoffs for classes of weed risk:

| Frequency Band | | Weed Risk Score | Weed Risk |
|---|---|---|---|
| 80 - 100% | (top 20% of possible scores) | 192+ | *Very high* |
| 60 - 80% | | < 192 | *High* |
| 40 - 60% | | < 101 | *Medium* |
| 20 - 40% | | < 39 | *Low* |
| 0 - 20% | (bottom 20% of possible scores) | < 13 | *Negligible* |

**Do not compare scores between land uses.** Land uses differ in their value and this is hard to measure. Also, average weed risk scores may be lower in agricultural land uses compared to other land uses. This is simply because of the greater level of weed management in agriculture. It does not mean that agricultural weeds are less important

**Why multiply the invasiveness, impacts and potential distribution scores?**

- Multiplying gives a greater spread in the scores than adding (i.e. range from 0-1000 compared to 0-30).
- Multiplying is logical, as it recognises the interactions between the criteria. Say the impacts of a weed can be measured in dollars per hectare per year, the potential distribution is known in hectares, and the invasiveness (i.e. rate of spread) is measured in terms of the increase in hectares compared to the previous year:

| Impact | × | Potential Distribution | × | Invasiveness |
|---|---|---|---|---|
| *$ / hectares / year* | | *hectares* | | *hectares(current year) / hectares (previous year)* |

*When multiplying, all of the hectares units cancel so that weed importance is measured in total dollars per year. In multiplying the invasiveness, impacts and potential distribution criteria scores, we are mimicking the above calculation, without having the actual dollar and hectare figures.*

## FEASIBILITY OF CONTAINMENT

The feasibility of containment questions are divided into three main criteria; control costs, current distribution and persistence. ***Control costs*** considers the weed management costs of detection, on-ground control and enforcement/education needs. ***Current distribution*** considers how widespread the weed is. ***Persistence*** refers to the expected duration of control works. Scores for each of these criteria are multiplied (each ranging between 0 and 10), to



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give a feasibility score out of 1000. Assess feasibility for the **land use at risk**, so that its score can be directly compared to the weed risk score from the same land use to set control priorities.

In the following questions higher scores indicate lower feasibility of containment.

## CONTROL COSTS

This section indicates the control cost per hectare in the **first year of targeted control**, for an infestation of the weed that has reached its **maximum density in the land use at risk**. The four main cost factors associated with coordinated control programs are searching for the weed, accessing and treating infestations, and achieving landholder commitment

| **1. How detectable is the weed?** | | | | Total (a+b+c+d) | *SCORE* |
|---|---|---|---|---|---|
| *(a) Height at maturity* | | *(b) Shoot growth present* | | 7 or 8 | *3* |
| ☐ <0.5 m | *2* | ☐ <4 months | *2* | 5 or 6 | *2* |
| ☐ 0.5-2 m | *1* | ☐ 4-8 months | *1* | 3 or 4 | *1* |
| ☐ >2 m | *0* | ☐ >8 months | *0* | 0, 1 or 2 | *0* |
| ☐ don't know | *?* | ☐ don't know | *?* | | *?* |
| *(c) Distinguishing features* | | *(d) Pre-reproductive height in relation to other vegetation* | | | |
| ☐ non-descript | *2* | ☐ below canopy | *2* | | |
| ☐ sometimes distinct | *1* | ☐ similar height | *1* | | |
| ☐ always distinct | *0* | ☐ above canopy | *0* | | |
| ☐ don't know | *?* | ☐ don't know | *?* | | |

*This question indicates the cost of finding infestations of the weed. Parts (a), (b) and (c) relate to finding new infestations. Part (d) relates to finding and treating plants prior to reproduction.*
*(a) Taller plants can be spotted from greater distances.*
*(b) Shoot growth considers when shoots are visible (live or dead). Annuals and some perennials (e.g., bridal creeper, bulbil watsonia) have shoots present for a limited period of the year.*
*(c) Distinguishing features include appearance and smell of foliage, flowers and fruits. This indicates how conspicuous the weed is amongst other vegetation. For example, the shape and foliage of a pine tree is quite obvious amongst native vegetation.*
*(d) Pre-reproductive height refers to locating the weed for control prior to seed set or bulb formation. Control must occur before reproduction if local eradication is to occur. The pre-reproductive height will mostly be less than at maturity and the weed will also probably be growing amongst other vegetation. Hence the weed's height is described relative to the canopy height of this other vegetation. For example, if considering a weed of the Crop/Pasture Rotation land use then the canopy will be the height of the crop.*

| **2. What is general accessibility of known infestations?** | | *SCORE* |
|---|---|---|
| ☐ low | Most infestation sites difficult to access | *2* |
| ☐ medium | Most infestation sites readily accessible | *1* |
| ☐ high | All infestation sites readily accessible | *0* |
| ☐ not present | Not known to be present in Board | *0* |
| ☐ don't know | | *?* |

*Sites may be difficult to traverse due to slope, rockiness, dense vegetation and/or surface water. This will slow down searching and control activities. There may be seasonal differences in accessibility (e.g. winter waterlogging), but answer in terms of the optimal search and control times for the weed.*



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| **3. How expensive is control of the weed, using techniques which both maximise efficacy and minimise off-target damage?** | | | | | ***SCORE*** |
|---|---|---|---|---|---|
| ***(a) Chemicals, fuel and equipment operating costs*** | | ***(b) Labour costs*** | | Total (a+b) | Range between 0 and 8 |
| ☐ very high | *4* | ☐ very high | *4* | | |
| ☐ high | *3* | ☐ high | *3* | ☐ don't know | *?* |
| ☐ medium | *2* | ☐ medium | *2* | | |
| ☐ low | *1* | ☐ low | *1* | | |
| ☐ not applicable | *0* | ☐ not applicable | *0* | | |
| ☐ don't know | *?* | ☐ don't know | *?* | | |

*Select a cost category (A, B or C) for the land use being considered. This allows for more realistic control cost estimates.*

| | ***Cost Categories*** | | | |
|---|---|---|---|---|
| | **A** | **B** | **C** | ***SCORE*** |
| ***Very high*** | *>$1000/ha* | *>$500/ha* | *>$100/ha* | *4* |
| ***High*** | *$500-1000/ha* | *$250-500/ha* | *$50-100/ha* | *3* |
| ***Medium*** | *$250-500/ha* | *$100-250/ha* | *$25-50/ha* | *2* |
| ***Low*** | *<$250/ha* | *<$100/ha* | *<$25/ha* | *1* |

*Herbicides are the main means by which weeds are controlled. Physical control methods may be cultivation, cutting/slashing stems or extraction (e.g., boxthorn plucker). Do not consider capital costs for purchasing application equipment in this question.*

| **4. What is the likely level of cooperation from landholders within the land use at risk?** | | ***SCORE*** |
|---|---|---|
| ☐ low | Weed control is rarely undertaken in the land use. Cost of control is beyond the financial and technical capacity of landholders. | *2* |
| ☐ medium | Control of the weed will require a significant change in existing weed management practices, but this will be within the financial and technical capacity of landholders. | *1* |
| ☐ high | Control of the weed will require minimal change in existing weed management practices. | *0* |
| ☐ don't know | | *?* |

*Aside from the "on-ground" costs of searching and control, a coordinated control program will have overarching costs of extension/education, enforcement, project management and administration. The ease of motivating and coordinating landholders in an ongoing program will vary between land uses, particularly in relation to their financial capacity to support a control program.*

## CURRENT DISTRIBUTION

This section indicates how widespread the weed currently is within the land use. It considers the proportion of the land use infested, and the overall pattern of infestations.



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| **1. What percentage area of the <u>land use</u> is currently infested by the weed?** | | *SCORE* |
|---|---|---|
| ☐ **>80% of land use** | The weed infests more than 80% of the land use in the Board. | *10* |
| ☐ **60-80% of land use** | The weed infests between 60% and 80% of the land use. | *8* |
| ☐ **40-60% of land use** | The weed infests between 40% and 60% of the land use. | *6* |
| ☐ **20-40% of land use** | The weed infests between 20% and 40% of the land use. | *4* |
| ☐ **10-20% of land use** | The weed infests between 10% and 20% of the land use. | *2* |
| ☐ **5-10% of land use** | The weed infests between 5% and 10% of the land use. | *1* |
| ☐ **1-5% of land use** | The weed infests between 1% and 5% of the land use. | *0.5* |
| ☐ **<1% of land use** | The weed is present in the land use but infests less than 1%. | *0.1* |
| ☐ **0% of land use but in 20-40% of Board** | The weed is not known to be present in the land use but does infest between 20% and 40% of the Board area. | *2* |
| ☐ **0% of land use but in 10-20% of Board** | The weed is not known to be present in the land use but does infest between 10% and 20% of the Board area. | *1* |
| ☐ **0% of land use but in 5-10% Board** | The weed is not known to be present in the land use, but does infest between 5% and 10% of the Board. | *0.5* |
| ☐ **0% of land use but in 1-5% Board** | The weed is not known to be present in the land use, but does infest 1-5% of Board. | *0.1* |
| ☐ **0% of land use but <1% of Board** | The weed is not known to be present in the land use, but does infest <1% of Board. Or the species is not naturalised in the Board but is cultivated (e.g. olives). | *0.05* |
| ☐ **0% of Board** | The species is not known to be present in the Board. | *0* |
| ☐ **don't know** | | *?* |

*The aim of containment is to prevent weed spread to a susceptible land use. The greater the area of land use that is already occupied, then the less feasible is containment. In the above table it is assumed to be highly unlikely that a weed could infest >40% of the Board area and not also be present in the land use.*

| **2. What is the pattern of the weed's distribution across the Board area?** | | *SCORE* |
|---|---|---|
| ☐ **widespread** | The weed occurs in large and small infestations across most of the Board area. | *2* |
| ☐ **evenly scattered** | The weed occurs as discrete, mainly small infestations across much of the Board area. | *1* |
| ☐ **restricted** | The weed is localised to 1-2 hundreds of the Board area. Or the weed is not known to be naturalised in the Board area. | *0* |
| ☐ **not present** | The species is not known to be present in the Board. | *0* |
| ☐ **don't know** | | *?* |

*A weed which is widespread will be more difficult to contain than one which is restricted to a small section of the Board. The former will have more landholders potentially exposed to spread of the weed.*

## PERSISTENCE

This section indicates how long it takes to eradicate the weed. It considers the efficacy of targeted control treatments, reproductive age, seedbank longevity and the likelihood of ongoing dispersal.



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| 1. How effective are targeted control treatments applied to infestations of the weed? | | SCORE |
|---|---|---|
| ☐ low | More than 25% of weeds survive annual targeted treatment/s. | *3* |
| ☐ medium | Up to 25% of weeds survive annual targeted treatment/s. | *2* |
| ☐ high | Up to 5% of weeds survive annual targeted treatment/s. | *1* |
| ☐ very high | Up to 1% of weeds survive annual targeted treatment/s. | *0* |
| ☐ don't know | | *?* |

*Do the herbicide and physical control treatments costed above kill all plants in an infestation? Efficacy can be reduced due to:*

*(i) tolerance to or recovery from treatment*

*(ii) incomplete application of a treatment (e.g., some plants receive a sub-lethal dose of herbicide, missed plants)*

*(iii) vegetative regeneration (e.g. silverleaf nightshade)*

*"out of season" growth (e.g. early or late germination of annuals)*

| 2. What is the minimum time period for reproduction of sexual or vegetative propagules? | | SCORE |
|---|---|---|
| ☐ <1 month | Minimum generation time <1 month. | *3* |
| ☐ <1 year | Minimum generation time 1-12 months. | *2* |
| ☐ <2 years | Minimum generation time 12-24 months. | *1* |
| ☐ >2 years | Minimum generation time >24 months. | *0* |
| ☐ don't know | | *?* |

*The shorter the time period to reproduction, the greater the frequency of control treatments required and the greater the chance of plants being missed prior to reproduction. Aquatic plants such as salvinia can have rapid vegetative reproduction.*

| 3. What is the maximum longevity of sexual or vegetative propagules? | | SCORE |
|---|---|---|
| ☐ >5 years | Sexual or vegetative propagules can remain dormant for at least 5 years. | *2* |
| ☐ 2-5 years | Sexual or vegetative propagules can remain dormant for 2-5 years. | *1* |
| ☐ <2 years | Sexual or vegetative propagules remain dormant for less than 2 years. | *0* |
| ☐ don't know | | *?* |

*Soil seedbank longevity is the primary determinant of how long an infestation must be treated to achieve eradication.*

| 4. How likely are new propagules to continue to arrive at control sites, or start new infestations? | | | | Total (a+b) | SCORE |
|---|---|---|---|---|---|
| ***(a) Long-distance dispersal by natural means*** | | ***(b) Grown*** | | 4 | 3 |
| ☐ frequent | *2* | ☐ commonly planted | *2* | 2-3 | 2 |
| ☐ occasional | *1* | ☐ occasionally planted | *1* | 1 | 1 |
| ☐ rare | *0* | ☐ not planted | *0* | 0 | 0 |
| ☐ don't know | *?* | ☐ don't know | *?* | ☐ don't know | *?* |

## FEASIBILITY OF CONTAINMENT SCORE

The score for feasibility of containment is calculated by adjusting the control costs, current distribution and persistence scores to range from 0 to 10, and then multiplying these. Feasibility of containment will have a maximum of 1000, and a minimum of 0. The spreadsheet does this for you.



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**To calculate manually, adjust the raw scores as follows:**

Control costs: Divide by 15 and multiply by 10. Round off to one decimal place.

Current distribution: Divide by 12, and multiply by 10. Round off to one decimal place.

Persistence: Divide by 11, and multiply by 10. Round off to one decimal place.

**Feasibility of Containment = Control Costs × Current Distribution × Persistence**

Splitting up these possible scores into bands of 20% gives cutoffs for classes of feasibility of containment:

| Frequency Band | | Feasibility Score | Feasibility of Containment |
|---|---|---|---|
| 80 - 100% | (top 20% of possible scores) | 113+ | *Negligible* |
| 60 - 80% | | < 113 | *Low* |
| 40 - 60% | | < 56 | *Medium* |
| 20 - 40% | | < 31 | *High* |
| 0 - 20% | (bottom 20% of possible scores) | < 14 | *Very High* |

**Why multiply the Control Costs, Current Distribution and Duration of Control scores?**

- Multiplying gives a greater spread in the scores than adding (i.e. range from 0-1000 compared to 0-30).
- Multiplying is logical, as it recognises the interactions between the criteria. Say the control costs of a weed can be measured in dollars per hectare per year, the current distribution is known in hectares, and the duration of control is known in years:

| Control Costs | × | Current Distribution | × | Duration of Control |
|---|---|---|---|---|
| *$ / hectares / year* | | *hectares* | | *years* |

*When multiplying, all of the hectares units cancel so that feasibility of control is measured in total dollars. In multiplying the control costs, current distribution and duration of control criteria scores, we are mimicking the above calculation, without having the actual dollar and hectare figures.*

## DETERMINING PRIORITIES

The following matrix gives guidance on appropriate strategic, weed management actions. Different weed species will appear in different positions on the matrix, based on their risk and feasibility of containment scoring. Each land use will have a separate matrix.



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| WEED RISK | FEASIBILITY OF CONTAINMENT | | | | | |
|---|---|---|---|---|---|---|
| | *Negligible >113* | *Low >56* | *Medium >31* | *High >14* | *Very High <14* | |
| *Negligible <13* | LIMITED ACTION | LIMITED ACTION | LIMITED ACTION | LIMITED ACTION | MONITOR | |
| *Low <39* | LIMITED ACTION | LIMITED ACTION | LIMITED ACTION | MONITOR | MONITOR | |
| *Medium <101* | MANAGE SITES | MANAGE SITES | MANAGE SITES | PROTECT SITES | CONTAIN SPREAD | ALERT |
| *High <192* | MANAGE WEED | MANAGE WEED | PROTECT SITES | CONTAIN SPREAD | DESTROY INFESTATIONS | |
| *Very High >192* | MANAGE WEED | PROTECT SITES & MANAGE WEED | CONTAIN SPREAD | DESTROY INFESTATIONS | ERADICATE | |

The following are guiding principles for each of the management categories in the matrix. At a landscape scale these principles need to be interpreted in terms of different outcomes per land use for different weeds. For example, a weed may rank as "destroy infestations" in one land use and "limited action" in others. In this case coordinated control may still be required in the latter land uses to enable protection of the former land use.
The term "management area" can be used below to apply to a range of spatial scales (e.g. NRM Board, sub-regional, land use)

**ALERT**
Species that are not known to be present in the management area and which represent a significant threat. Such species would score "0" in Feasibility of Containment due to their absence.
**Aims to prevent the species arriving and establishing in the management area**
Prevention of entry to management area
Ongoing surveillance for incursions of the species (e.g. nursery inspections)
Training and awareness activities for the community to enable early detection

**ERADICATE**
**Aims to remove the weed species from the management area**
Detailed surveillance and mapping to locate all infestations
Destruction of all infestations including seedbanks
Prevention of entry to management area and movement and sale within
Must not grow and all cultivated plants to be removed
Monitor progress towards eradication

**DESTROY INFESTATIONS**
**Aims to significantly reduce the extent of the weed species in the management area**
Detailed surveillance and mapping to locate all infestations
Destruction of all infestations, aiming for local eradication at feasible sites
Prevention of entry to management area and movement and sale within
Must not grow


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Monitor progress towards reduction

**CONTAIN SPREAD**

**Aims to prevent the ongoing spread of the weed species in the management area**

Surveillance and mapping to locate all infested properties

Control of all infestations, aiming for a significant reduction in weed density

Prevention of entry to management area and movement and sale within

Must not allow to spread from cultivated plants (if grown)

Monitor change in current distribution

**PROTECT SITES**

**Aims to prevent spread of the weed species to key sites/assets of high economic, environmental and/or social value**

Weed may be of limited current distribution but only threatens limited industries/habitats (lower weed risk). Or the weed may be more widespread but is yet to invade/impact upon many key industries/habitats (higher weed risk).

Surveillance and mapping to locate all infested areas

Identification of key sites/assets in the management area

Control of infestations in close proximity to key sites/assets, aiming for a significant reduction in weed density

Limits on movement and sale of species within management area

Must not allow to spread from cultivated plants (if grown) in close proximity to key sites/assets

Monitor change in current distribution within and in close proximity to key sites/assets

**MANAGE WEED**

**Aims to reduce the overall economic, environmental and/or social impacts of the weed species through targeted management**

Research and develop integrated weed management (IWM) packages for the species, including herbicides and biological control where feasible

Promote IWM packages to landholders

Monitor decrease in weed impacts with improved management

Identify key sites/assets in the management area and ensure adequate resourcing to manage the weed species

**MANAGE SITES**

**Aims to maintain the overall economic, environmental and/or social value of key sites/assets through improved general weed management**

Promote general IWM principles to landholders, including the range of control techniques, maintaining competitive vegetation/crops/pastures, hygiene and property management plans.

Identify key sites/assets in the management area and ensure adequate resourcing to manage these to maintain their values

Broaden focus beyond weeds to all threatening processes

**MONITOR**

**Aims to detect any significant changes in the species' weed risk**

Monitor the spread of the species and review any perceived changes in weediness

**LIMITED ACTION**

**The weed species would only be targeted for coordinated control in the management area if its local presence makes it likely to spread to land uses where it ranks as a higher priority.**



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Undertake control measures if required for the benefit of other land uses at risk
Otherwise limited advice to land managers if required

## DISCUSSION

Weeds have been considered troublesome since the very early establishment of cultivation up to current practices of production systems. During the earth summit in 1992 Rio de Janeiro, Brazile, invasive alien species were recognized threatening habitat, ecosystems, and local species and an international convention on biodiversity instructed to prevent the introduction of, eradicate and confine the invasive alien species. Soon the problems were recognized to be enormous threatening the integrity of our fragile ecosystems. The modern life seems to exarberate the problems of invasive alien species, including invasive alien plant species. Weeds together with invasive plants pose significant primary production, environmental and social impacts worldwide and the their number posing such impacts is increasing annually. Thus, the magnitude of the problem requires prioritization across weed or invasive alien plant species policy, management and legislation. Such prioritization needs have led to the development of mechanisms/processes that assess and account for the risks associated with their introduction and management.

Weed Risk Assessment (WRA) is a system designed to screen out imported plants that may in the future become a pest. The protocol of Australian WRA works by allocating scores on answers of questions based on the knowledge of biology, biogeography, history of plants and empirically tested to produce 3 differents three decission, (1) reject when score >6 , (2) accept when the score <1, and (3) scores in between 1-6 shall be evaluated further. The performance of Australian WRA has been considered good and has been adopted by FAO and IPPC. However some of the criteria utilised in the system have recently been critised.

The protocol is less effective at identifying risks to tropical forests than open habitats and does not adequately characterise the risk posed by palms to these ecosystems. One possibility would be to weigh question 4.09 ‘‘Is a shade tolerant plant at some stage of its life cycle” more heavily in the overall scoring. However, it is not entirely clear at which stage of the life cycle, if any, shade tolerance is likely to facilitate invasion of forests.Modification of the shade tolerance question score would require support from sound experimental work testing the importance of shade tolerance in facilitating invasions into less disturbed vegetation such as forest. In addition, undesirable traits should be less focused on agricultural perspectives (e.g. palatability to grazing animals; host for pathogens and allergenicity) and more on impacts on biodiversity, hydrology and soil erosion.

A more serious criticism was that WRA did not seperate consequence from likelihood, as requested by risk analysis procedure. Although the supporter of WRA would answer both are considered and applied in one.

Weed management as shown by Hiebert and Subbendieck (1993), requires risk assessment to prioritize weeds based on their impact either current of potential impact and based upon which management options are designed. The protocol developed by Hiebert and Stubbendieck was very impresive, however, the protocol did not differentiate the consequent from likehood.

The process of weed Risk management developed by the Australian (Downey *et al.* 2010) involves 6 steps, at the 2nd and 3rd requires risk analysis, and many people are still using WRA, a protocol designed to screen out imported weeds, based upon the threat of the imported plants to production systems, the environments and human health. Downey et al (2010) emphasized the differential problems of threat and impact, although Hiebert and Subbendieck (1993) overcome the problems by recognizing the two as similar, as threat was only a potential impact but the most crucial one is the definition of risk assessment, whether the endpoint is establishment of invasive alien plant species or in term of environmental damage.



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The concept of managing current weeds for both prevention and mitigation under one strategy has been outlined previously (species- and site-led approach (Timmins and Owen, 2001). The species-led approach aims to eradicate or contain newly established weeds where they occur, based on an assessment of specific weed species, while the site-led approach aims to manage the threat of weeds (generally) to protected natural areas, by prioritizing such areas for targeted control, based on the value of the biodiversity present and the urgency of control relative to the extinction risk. However, this system, specifically the site-led approach, has not been used or evaluated more broadly to help manage the risk posed by widespread weeds to native species. One reason for this lack of adoption is a misinterpretation of the meaning of the terms species-led and site-led, based on the assumption (incorrectly) that any species-specific programme is species-led (irrespective of whether it is aimed solely at eradication or containment of newly established) and management of specific sites (irrespective of whether it is aimed solely at protecting biodiversity) is site-led [Downey and Shepper, 2004).

The risk of inaction is rarely accommodated in the management of widespread weed species. While the economics on inaction can be assessed, for example, taking no action (Jupp et al.2002) or delaying action (Myers et al 2000). can increase the costs of control and decrease the changes of success (Timmins and Owen, 2001]), the same level of assessment of the effects of inaction on the protection of biodiversity has not occurred. However, a recent study provides some insights based on the observation of a weed density threshold effect leading to the decline of native species (Gooden et al, 2009). The concept of management thresholds for weeds has been applied to protected areas management (including biodiversity conservation) in South Africa (Foxcroft and Downey , 2008).

The above review reveals that WRA has developed far, but still evolving, such as the application of formal terminology of risk management ( consequences and likelihood), the definition of risk management being sensitive to the endpoint, and beside the inclusion of asset protection systems for weeds using risk assessment procedures is relatively new. It is also realised that Indonesia does not have any protocol of pre-border weed risk assessment nor post border weed risk management yet, in light of the above review we suggest to utilize modified AWRA or HWRA for pre-border analysis and post-border weed risk management protocol developed by Virtue (2010) , for post border analysis, although the authors utilised that developed by Hiebert and Stubbendieck (1993), in the past.

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# CHALLENGES AND PROBLEMS IN MANAGING WEEDS IN RICE: PRESENT AND FUTURE SOLUTIONS

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**ABSTRACT** Although the Green Revolution has increased rice production by 130% in the past 40 years, there is a need to produce 35% more ricewith less water, land, labor, and chemicals in the next 20 years. To meet this challenge, rice scientists have developed water-saving production technologies without flooding and transplanting, two basic practices that suppress weed growth. Weed management perspectives are changing and bringing into greater focus the critical role of managing "millennial" weeds in rice in the 2000s. Flooding to suppress weed growth without injury to wet-seeded rice remains a challenge to researchers faced with the water-weed-rice dilemma of obtaining good rice germination and good weed control. Answers to this challenge include studying rice and weed adaptation mechanisms during germination under hypoxia in the search for flood-tolerant rice varieties. Increasing evolution of herbicide-resistant weeds requires herbicides with new modes of action and innovative non-chemical control options. Deeper understanding of multiple resistance mechanisms is needed to manage *Echinochloa crusgalli*, *Sagittaria* spp and *Cyperus* spp, weeds which have evolved multiple resistance to herbiicdes. Use of imidazolinone-resistant rice provides effective control of weedy rice but should include measures to avoid evolution of herbicide resistant weeds. Potential future technologies being explored are physiological approaches to develop allelopathic rice and $C_4$ rice and biotechnological approaches to enhance rice competitiveness or reduce weed competitiveness.

***Keywords*:** Aerobic rice, direct-seeded rice, herbicide resistant weeds, herbicide-resistant rice

## INTRODUCTION

Rice yields have increased by 130% from 276 M tons in 1966 to 600 M tons in the 2000s due to the Green Revolution in the 1960s and introduction of direct-seeding in the 1970s (Maclean *et al*, 2002). However, by 2025 to 2030, farmers still need to produce 38% more rice to feed 5 B more rice eaters with less land, less water, less labor and less chemical inputs (Khush, 2004). Of 154 M ha planted to rice in 114 countries, 79 M ha are grown to irrigated rice and 54 M ha are grown to rainfed rice, mostly in the humid tropics and subtropics of south, southeast, and east Asia (Maclean *et al*, 2002). Irrigated lowland rice ecosystems are threatened by water shortage due to decreasing water resources and competition from urban and industrial sectors (Postel, 1997). To meet the water shortage challenge, rice scientists have developed production practices that require less water than transplanted rice, ranging from water-seeding, wet-seeding, dry-seeding, alternate wetting and drying (AWD) and aerobic rice. The trade-offs to these water-saving technologies is the removal of factors or processes that facilitate the control of weeds - short-statured high-yielding but less weed competitive rice cultivars, direct-seeding which eliminates the headstart over weeds that transplanted rice has, and water-saving technologies that eliminate flooding as a means to suppress weed growth.

## DISCUSSIONS

Yield-enhancing and water-saving production practices have made significant changes in weed management perspectives and emphasizing the critical role of weeds in rice production. With the evolution of herbicide-resistant weeds and other "superweeds" or "millennial"



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weeds, rice weed scientists are faced with several challenges in the 2000s: 1) managing weeds using production practices that involve less water; 2) making wet-seeded rice as competitive as transplanted rice; 3) search for innovative cultural and non-chemical methods; 4) search for herbicides with new target sites; 5) managing herbicide-resistant weeds, multiple resistance, and emerging weed problems; and 6) developing physiological and biotechnological strategies using less chemicals such as allelopathic rice, C4 rice and possibly, a "weed-resistant" rice.

**Making wet-seeded rice competitive: the water-weed-rice conflict**

In wet-seeded rice, after sowing seeds on puddled soil, the soil remains saturated until 7 DAS to allow rapid germination and emergence of rice seedlings. Flooding early suppresses weed germination but also suppresses rice germination, resulting in poor root anchorage and reduced stand (De Datta, 1986). Flooding late enhances rice germination but also enables weeds, especially grasses which prefer saturated soil, to grow profusely, easily outgrowing rice seedlings germinating at the same time. In their studies on direct-seeded rice, Kim and Moody (1989) have shown that grasses such as barnyardgrass produce their first true leaves with chlorophyll ahead of the rice seedlings, with a 2-3 day advantage. The challenge is how to use flooding to suppress weeds yet allow rice seedlings to germinate and emerge ahead of the weed seedlings in wet-seeded rice.

Research efforts are focused on how early should flooding be introduced to achieve the combined effects of weed growth suppression and excellent rice stand. In their studies on wet-seeded rice, Tuong *et al* (2000) observed that flooding at 3 DAS reduced weed growth by 73-88% compared with the farmers' practice of flooding at 7 to 10 DAS, where *E. glabrescens* dominated direct-seeded rice fields. To enable early flooding, flood-tolerant rice varieties which can germinate and grow fast even in submergence are being developed. Flooding adaptation mechanisms being determined to develop flood-tolerant rice include the ability to produce long coleoptiles and ability to break down and mobilize carbohydrate reserves into soluble sugars as substrates in anaerobic respiration during germination under hypoxia (Ismail *et al*, 2009; 2012). Normal emergence of coleoptiles and radicles of cv. IR42 and a flood-tolerant rice genotype *Khao Hlan On* in 10 cm floodwater within 0 to 4 days of seeding indicate their possible competitive advantage over *Echinochloa* sp even in early flooding (Estioko *et al*, 2010).

Another practice to allow delay in flooding of up to 7 DAS is to combine flooding with a preemergence herbicide at full or half the recommended rate. Safened pretilachlor combined with early flooding reduced weed growth by 75% to 90% even at only half the recommended rate (Janiya and Johnson, 2005; Pablico and Moody 1993). During times of shallow floodwater because of limited rainfall, application of a preemergence herbicide can improve weed control.

**Producing more rice with less water: Aerobic rice**

In the 2000s, IRRI started activities to develop "aerobic rice" for the Asian tropics(Bouman, 2001). A new approach to water-saving technology, it is similar to growing upland rice but unlike traditional upland rice, it aims at high yields with optimum inputs similar to those used in irrigated rice (Tuong and Bouman, 2003). Aerobic rice is grown in non-puddled, non-flooded aerobic soil using rainfall and flush irrigation or alternate wetting and drying (AWD) as the water source. It reduces water use by 40 to 60% compared with transplanted rice (Bouman *et al*, 2005). However, dry tillage and alternate wetting and drying to maintain aerobic soil conditions enhance growth of weeds, which easily outgrow rice and reduce yields by 50 to 91% (Rao *et al*, 2007). In India, yield losses ranged from 80 to 90% in aerobic rice, whether grown on raised beds or on flat lands, compared to lower yield losses (38 to 58%) in transplanted rice (Singh *et al*, 2008). Most upland and aerobic rice growers in Asia mechanically weed their crops two or three times per season, investing up to 190 person-



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days/ha in handweeding. Since the concept of aerobic rice is new, growing rice under aerobic conditions on raised beds or on flat lands would require development of new improved cultural and chemical methods.

**Cultural control: greater focus on land leveling and land preparation**

Properly timedhandweeding or rotary weeding combined with use of herbicides are still the most common cultural control methods in small tropical farms. Two cultural practices which are often not given adequate attention in most small farms in tropical Asia are thorough land preparation and precise land leveling. Land leveling should be an integral part of tillage operations because it is extremely important for good drainage in lowland rice ecosystems. An uneven field results in poor rice emergence in low spots and enhanced weed growth in high spots. In the U.S., about 90 to 100% of rice fields are precision-leveled by laser-guided equipment, and increased yield is considered to be not only due to the introduction of HYVs but also to the use of precision land leveling (Maclean *et al*, 2002). Most Asian rice farmers still level their fields with wooden planks or comb harrows, or dig trenches around the fields (Takashima, 1984). But laser leveling and similar equipment to level fields are also now becoming popular in Asia and other rice-growing countries. Developing affordable land leveling equipment suited to small farm conditions in tropical Asia is an important challenge for rice researchers.

**Chemical control: need for herbicides with new modes of action**

With increasing costs and scarcity of labor for weeding, herbicides remain the most cost-effective option in rice weed control particularly in large farms. There are about 85 active ingredients in 32 chemical families and at least 200 herbicide products used for weed control in rice (Tomlin, 2000). From 1940s to 1980s, herbicides widely used in rice are chloroacetamides thiocarbamates, aryloxyphenoxys for grass control and phenoxys, sulfonylureas for broadleaf and sedge control. New herbicides in the 1990s and 2000s have broader weed spectrum and wider application windows and include the pyrimidinyloxybenzoates, triazolopyrimidines, triazinones, oxazolidinediones, phenylthalimides, pyrazoles, pyridinecarboxamides, and isoxazolidinones. New herbicides with modes of action still unclear include benzobicyclon, ipfencarbazone, terfuryltrione, bromobutide, oxaziclomazone, and indanofan.

According to Gressel (2002), there are about 30 plant biochemical processes which can serve as herbicide target sites. Existing commercial herbicides have the following target sites: fatty acid synthesis (acetylcoA carboxylase) for aryloxyphenoxys, amino acid synthesis (ALS, AHAS, EPSP synthase) for sulfonylureas, imidazolinones, and glyphosate, glutamine synthesis (glutamine synthethase) for glufosinate and bialaphos. The recently developed pigment synthesis inhibitors affect either one of these three enzymes: protoporphyrinogen oxidase, 4-p-hydroxyphenyl pyruvate dioxygenae, and phytoene desaturase. Future potential herbicide target sites include enzymes of purine nucleotide biosynthesis, sterol biosynthesis, terpenoid biosynthesis, and folic acid biosynthesis (Gressel, 2002; Wakabayashi and Boger, 2004). Potential new modes of action may also be patterned from the modes of action of natural compound inihibitors such as hydantocidin, mollicelin D, fumonisins, tentoxin, B-rhizobitoxine, fosmidomycin, pseudomonic acid, gabaculine and phaseolotoxin (Gressel, 2002).

**Evolution and management of herbicide-resistant weeds**

The first herbicide resistant weeds in rice were reported in the late 1980s and early 1990s, consisting of 9 weed species in 8 countries that have developed resistance to 4 herbicides (Gressel and Baltazar, 1996). This increased to 30 species in 2001, then to 40 species resistant to 34 herbicides in 26 countries in 2012, with a total of 222 resistant biotypes (Valverde and Itoh, 2001; Uchino, 2011; Heap, 2013). Not surprisingly, Asia, where 90% of rice is grown



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has the highest incidence, with 9 countries having a total of 110 resistant biotypes (47%), north and south America with 12 countries have 79 biotypes (37%) and Europe with 5 countries have 34 biotypes (16%) (Heap, 2013; Uchino 2011).

Most of the resistant species have multiple resistance, with *Echinochloa crusgalli* resistant to a total of 16 herbicides, *Cyperus difformis* resistant to 11 herbicides, and *E. colona*, *S. pygmaea* and *S. montevidensis* each resistant to a total of 9 herbicides. The rest (35 species) are resistant to eight herbicides or less.Of the 34 herbicides to which the 40 weeds have evolved resistance, 18 are ALS inhibitors, 6 ACCase inhibitors, 4 cell division inhibitors, 2 photosynthesis inhibitors, 2 growth regulators, 1 pigment synthesis inhibitor and 1 EPSP synthase inhibitor. Bensulfuron has the highest number of resistant weeds, with 28 species, followed by pyrazosulfuron with 14 species.

Managing (delaying or avoiding) herbicide resistance requires planning a broad-based approach involving long-term management strategies, the first of which involves recognizing or detecting herbicide resistance, knowing how resistance evolved in a particular farm (cropping and herbicide history is important), knowing herbicides and their modes of action as well as weed characteristics that contribute to evolution of resistance, how to manage, prevent or delay evolution of resistance and how to control herbicide-resistant weeds.

Most farmers in small tropical Asian farms may not be capable of differentiating if weed control failure is due to incorrect herbicide application or due to evolution of resistance. Certain criteria set by the Herbicide Resistance Action Committee (HRAC) should be met in identifying herbicide-resistant species. Colorimetric methods and rapid tests to detect resistance to sulfonylureas, propanil, fenoxaprop and other herbicides in various weeds have been developed (Gerwick *et al*, 1993; Kim *et al*, 2000; Kuk *et al*, 2003). New cases are reported online at the website of the International Survey of Herbicide Resistant Weeds (Heap, International Survey of Herbicide Resistant Weeds, http://www.weedscience.org). This website was launched in 1993 with Ian Heap currently in charge of the survey.

The single biggest reason for resistance to evolve in a particular weed is high selection pressure due to continuous use of the same herbicide or different herbicides with the same mode of action. Avoiding crop monoculture, rotating crops and rotating herbicides with different modes of action will help reduce selection pressure. There should be increased farmer awareness on the importance of rotating herbicides and how to recognize herbicides with different modes of action. The WSSA and HRAC have developed a system of classifying and grouping herbicides according to their modes of action (Schmidt, 1997; Mallory-Smith, 2003). Pesticide regulatory agencies in some countries now require inclusion of information on herbicide mode of action in herbicide container labels. However, a more farmer-friendly system of classification is needed to enable small farmers in the tropics to easily recognize and differentiate the various herbicides and their modes of action.

Reducing selection pressure also means not only avoiding reliance on a single herbicide or a single mode of action but also avoiding sole reliance on chemical control. There should be an integrated approach to include non-chemical control methods. In small farms in southeast Asia, follow-up handweeding after herbicide spraying removes both susceptible and resistant biotypes and is believed to delay or prevent the development of resistance. Combining cultural methods with minimum herbicide use in south and southeast Asia compared to high herbicide use in the Americas and Europe is probably one reason for the slower development of resistance in small farms in southeast Asia than in large farms in the Americas, Australia and Europe (Gressel and Baltazar, 1996). However, recent developments show increasing herbicide use in east Asia and southeast Asia such as South Korea, Japan, China, Malaysia, Thailand, Vietnam and Philippines.



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**Managing major weed problems (herbicide-resistant weeds and emerging weed problems)**

*Echinochloa* spp.

Eight species of *Echinochloa* (*E. crusgalli*, *E. colona*, *E. hispidula*, *E. oryzoides*, *E. phyllopogon*, *E. cruspavonis*, *E. oryzoides* and *E. erecta*)have evolved multiple resistance to 9 herbicides, with 42 resistant biotypes (Heap, 2013). *E. crusgalli* has the most number of resistant biotypes and has developed multiple resistance to 16 out of 25 herbicides developed for grass control since the 1960s. Propanil-resistant barnyardgrass can be controlled by mixing propanil with certain herbicides as synergists such as anilofos and piperophos, which are potent inhibitors of arylacylamidase, the propanil-degrading enzyme (Moss, 2002; Hirase and Hoagland, 2006). Thiobencarb has been reported to enhance the activity of bispyribac-sodium against resistant and susceptible E. phyllopogon (Fischer *et al*, 2004). Pyriftalid, a new herbicide developed for control of barnyardgrass (Kobayashi and Tsunekawa, 2010), and the relatively new amide herbicides thenylchlor (Kobayashi *et al*, 2004) and cafenstrole (Takahashi *et al*, 2000) can also be used for control of herbicide-resistant barnyardgrass.

*Weedy rice or red rice*

Having the same genus and species as commercial white rice, no selective chemicals are available to control weedy rice. The most recent strategy developed to obtain selective control of weedy rice in commercial rice is use of herbicide-resistant rice. The most common herbicide-resistant rice being grown since the 2000s is imidazolinone-resistant (IMR) rice. Use of IMR rice allows use of imazethapyr, imazamox, imazapic, or imazaquin to control red rice without injuring rice. These herbicides control barnyardgrass as well as other grasses, broadleaves and sedges infesting rice fields. IMR rice was introduced in the U.S. (Mississippi, Arkansas, Louisiana) in 2002, in Costa Rica and Colombia in 2003, Brazil and Argentina in 2004, Italy in 2009, Malaysia in 2010 and Spain in 2011 (Anonymous, BASF Leaflet). Starting in 2006, imidazolinone-resistant red rice, *Ischaemum rugosum* and *Ecrusgalli* have been reported in the US, Brazil and Italy (Heap, 2013; Burgos *et al*, 2012). Management strategies to prevent or minimize outcrossing of IMR with red rice such as crop rotation and manual removal are being done but new technologies that can provide red rice control in rotation or continuous rice systems are needed (Burgos *et al*, 2008).

*Leptochloa chinensis, Ischaemum rogusum*

Fenoxaprop controlled *Leptochloa chinensis* until it developed resistance to fenoxaprop, quizalofop, profoxydim in 2002 in Thailand and to cyhalofop in 2011 in China (Maneechote, 2005). In Thailand, *L. chinensis* resistant to fenoxaprop and other ACCase inhibiting herbicides are controlled with herbicides having other modes of action such as the cell division inhibitors butachlor, dimethanamid, flufenacet, thiobencarb, and pigment synthesis inhibitors such as clomazone, diflufenican, oxadiargyl and oxadiazon, and the PSII inhibitor propanil (Maneechote, 2007).*Ischaemum rugosum* is reported to have evolved multiple resistance to ACCase and ALS inhibitors fenoxaprop, profoxydim, and bispyribac in some countries. Herbicides with other modes of action can be used to control biotypes that have evolved resistance to ACCase and ALS inhibitors.

*Broadleaves and sedges with multiple resistance to ALS inhibitors*

There are 96 resistant biotypes from 21 broadleaves and 8 sedges, all resistant to at least one, and at most, 10 ALS inhibitors, mostly sulfonylureas (Heap, 2013). Those with highest number of resistant biotypes are *Cyperusdifformis* (10), *Sagittaria montevidensis* and *Sagittaria pygmaea* (9 each), and *Lindernia dubia* (8). The 25 other species have at least one, to at most seven, resistant biotypes. New herbicides with new modes of action, mostly pigment synthesis inhibitors are used to control the ALS-resistant biotypes. In Japan, two new HPPD (pigment synthesis) inhibitors, tefuryltrione and mesotrione introduced for rice weed



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control in 2010 can be used for control of sulfonylurea-resistant weeds (Watanabe, 2011). Pentoxazone, a new oxazolidinedione herbicide, is effective against ALS resistant *Lindernia dubia* (Watanabe, *et al* 1998). Bromobutide, benzobicyclon or clomeprop are used against bensulfuron-resistant *S. juncoides* and *M vaginalis* var *plantaginea*, clomeprop or pentoxazone for resistant *Lindernia* spp., benfuresate for resistant *S. trifolia* and pyraclonil for resistant *M. vaginalis* var *plantaginea* (Watanabe, 2011). In Australia, the pyrazole benzofenap is used as alternative against ALS-resistant weeds, while in California, carfentrazone-ethyl is used against ALS resistant *S. montevidensis* and *S. mucronatus* (Valverde and Itoh, 2001). New ALS inhibitors developed in the 2000s which control sulfonylurea-resistant weeds include pyrimisulfan (sulfonanilide), penoxsulam (triazolopyrimidine), and the new sulfonylureas with fused heterocyclic moiety, propyrisulfuron, flucetosulfuron and metazosulfuron (Suzuki *et al*, 2004, Tanaka *et al*, 2006, Hamamura 2011; Ikeda *et al*, 2011).

However, researchers agree that the strategy of using alternative herbicides to control multiple resistant weeds will only lead to more complicated patterns of multiple herbicide resistance and a diversity of resistance mechanisms (Mallory-Smith, 2001; Powles and Preston, 1995). Eventually, these species will be very difficult to control with alternative herbcides with different modes of action. Development of integrated management strategies involving non-chemical control methods which will reduce selection pressure on multiple resistant species should be an important priority and should be a major challenge for weed researchers in rice as well as in other important agricultural crops. More studies on genetics and physiology of weeds and on mechanisms of multiple resistance are needed to minimize multiple resistance.

**Managing emerging weed problems: Lowland purple nutsedge**

*Cyperus rotundus*, the most troublesome weed in upland crops, is also now emerging as a problem weed in rice-based cropping systems where lowland rice is rotated with upland crops (Baltazar *et al*, 2006). Over a span of 30 years, a lowland ecotype has evolved mechanisms to adapt and survive oxygen-deficient environment in flooded soils. These mechanisms include having bigger tubers with higher carbohydrate and soluble sugar content and the ability to use these carbohydrate reserves to generate energy through anaerobic respiration more efficiently than the upland ecotype (Pena-Fronteras *et al*, 2009; Fuentes *et al*, 2010). Bentazon, 2,4-D, MCPA, bensulfuron can control lowland and upland *C rotundus* as well as reduce tuber populations in the soil by 60% during the rice rotation (Islam *et al*, 2001; Ramos *et al*, 2011). The challenge is how to control *C rotundus* selectively during the upland crop rotation because no selective herbicides can be used when it is growing with upland crops. Cultural methods such as the stale-seedbed technique (two sequential harrowings after the last tillage operation before planting) can also reduce tuber populations by 80 to 90% and can be used during both lowland rice and upland crop rotations (Islam *et al,* 2001).

**Future weed management strategies**

*Physiological approaches: C4 rice to compete with C4 weeds*

In the 1960s, high-yielding cultivars were developed through conventional breeding during the Green Revolution, and rice culture was never the same since then. Today, in the 2000s, rice scientists are aiming for a second Green Revolution by developing a $C_4$ rice through traditional breeding or transgenic methods. $C_3$ plants, which includes rice, are less efficient and less competitive than $C_4$ plants, which includes most tropical weeds like *E. crusgalli, Rcochinchinensis* and *C. rotundus*. A $C_4$ rice will be more competitive against weeds, more efficient in photosynthesis, have high water use efficiency, and will yield high (10-15 t/ha) even with less water, since water requirement of $C_4$ plants is much lower than that of $C_3$ plants. A research program at IRRI is now underway to develop $C_4$ rice (Gunawardana, 2008). Studying genomes of $C_4$ weeds like *E crusgalli* and *C rotundus* will allow comparison of the



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genetic variations between $C_3$ rice and $C_4$ weeds and possibly obtain information on how to increase competitiveness of rice against weeds.

*Physiological approaches: Developing allelopathic rice*

Some rice lines or wild rice species as well as several other plant species have been shown by research to be allelopathic and can inhibit the growth of some weeds like barnyardgrass and broadleaf weeds (Fujii, 1992; Olofsdotter *et al*., 1995). Researchers are working at identifying allelochemicals and the genes responsible for their production in plants then aim to develop allelopathic rice cultivars through conventional breeding or transgenic methods (Kim and Shin, 2005). For example, some tropical japonica rice cultivars are allelopathic to barnyardgrass. If allelopathic rice cultivars are used, they can be more competitive against weeds, thus reducing the use of herbicides or handweeding.

*Biotechnological approaches: "harmless" weeds or "weed-resistant" rice*

In his book, Molecular Biology of Weeds, Gressel (2002), has proposed future novel approaches of controlling weeds with less chemicals by enhancing crop competitiveness or reducing weed competitiveness through molecular biology techniques. He suggests that weeds can be "forced" to revert to being "innocuous" wild species by inserting: 1) genes inhibiting degradation of herbicides, mimic herbicide action or cause accumulation of lethal metabolites; 2) genes that suppress weed germination or enhance dormancy; 3) genes that modulate hormone levels or suppress auxin biosynthesis or stimulate cytokinin synthesis. Conversely, weed-competitive cultivars can be developed by: 1) use of improved transporter genes to take up and absorb nutrients more efficiently than weeds; 2) improved tolerance to drought or oxidant stress; 3) leaf-modifying genes which can give different canopy types in a single variety. However, because traits that confer competitiveness like taller plants and bigger canopies are traits that result in low yields, the big challenge is how to combine "weed competitiveness" and "high-yielding" traits in a single variety.All of these techniques will require deeper understanding of the mechanisms of physiological and biochemical processes in weeds. Physiology, biochemistry and genomes of most crops are already known but there is very limited information on weed genomes and physiology, particularly on dormancy and germination of weed seeds.

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# CAN WE SUCCESSFULLY MANAGE WEEDS BY MANIPULATING THE WEED SEED BANK?

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**ABSTRACT** The soil weed seed bank determines the density and diversity of the weed problems growers are likely to face in their crops. However, the weed seed bank is also arguably the most resilient phase of the life cycle of weeds. Undermining weeds by successfully manipulating the soil weed seed bank requires a well-planned, systematic and long-term approach. This begins by being able to determine with some degree of certainty the content and size of the seed bank. Of the two classical methods, seed enumeration or seed germination, the germination method is simpler and provides adequate estimation of species and numbers. The next requirement is for an understanding of some key ecological traits for each of the weeds present. These include; depth from which the seed can emerge, longevity in the seed bank, seasonality of emergence, local weather data and germination triggers. Inherent to their successful management is a thorough understanding of the impacts of various agricultural practices employed by the growers. These may include cultivation practices, management of emerged weeds and of natural predators. By carefully melding the basic ecological data with the prevalent farm management system being practiced, programmes can be developed to effectively target the soil seed bank phase of the weeds life cycle rather than continually deal with the resultant weed infestation. For example larger seeds usually emerge from greater depths than small seeds, rendering soil inversion less effective. Further if seeds are long lived in the soil then burying them effectively preserves them for emergence when they are returned to the surface by subsequent soil inversions. Information on timing of major flushes of specific weeds could be helpful for determining most appropriate cultural or chemical control measures. Although difficult to achieve in the short term, a programmed approach towards a continual reduction of the soil weed seed bank would be a strategy for long term success and sustainability.

***Keywords:*** Weed seeds, soil seed bank, seed burial, seed persistence, germination.

## INTRODUCTION

### Managing weeds - It starts with the seeds

Despite advances in weed control methods and development of new herbicides, weeds remain the major factor limiting crop yields through competition and interference with harvest (Gallandt and Weiner 2007). Control measures are also an added cost to production which could possibly be avoided (Auld et al. 1987). Most of the weeds growers are forced to manage in their crops are annuals that are dependent on regeneration from the soil weed seed bank. Effective and sustainable weed control ultimately requires the reduction of this seed bank (Buhler et al. 1997; Davis 2006). Therefore, some knowledge of the seed bank would be a good starting point for an integrated weed management programme (Forcella 1993; Cardina and Sparrow 1996). It has been suggested that estimates of weed seed bank populations in the soil have potential for predicting future weed populations. Such information would be of great value in planning crop rotations, herbicide usage or alternate control measures. For example, the weeds could be managed better by adjusting sowing dates to avoid weed emergence peaks, by adjusting sowing rates and spacing to provide maximum competition, by using different cultivars or by planting another crop in which there are more options to control particular weeds.



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Replenishment of the soil weed seed bank is relatively straight forward with changes in seed bank size being represented by the following equation:

soil weed seed bank = residual seed + new seed – seed death/predation

It is easy to generate a model predicting weed emergence (Roberts and Ricketts 1979), which could be:

weed emergence = soil weed seed bank x germination fraction

As simple as this model appears however, estimating the size of the seed bank of arable weeds and predicting the emergence of different weed species is very difficult (Grundy *et* al., 1999; Forcella et al. 1992). To assist growers in attaining more sustainable weed management, researchers need to make significant inroads into converting this model into a useful, functional tool. Some of the information needed for this model is readily measurable. The reason for its lack of practical application appears to be the complexity and unpredictability of the dynamics of the soil weed seed bank and the inability to adequately manage the input parameters.

**Residual seed.** This is the number of weed seeds in the soil weed seed bank at any time. Unfortunately the small size of seeds and their location in the soil makes estimation of the weed seed bank very difficult. To address this problem, many studies have been conducted in recent years to optimize both seed bank sampling and the enumeration procedures. The two main methods for this are seed removal/washing or seed germination. These two methods require different levels of technical resources and sometimes give different results (Rahman et al. 1995).

**New seed.** This is the amount of fresh seed falling to the ground plus seed that may be introduced from further afield by human or other activity. Fresh seed falling to the ground (seed rain) can be measured using seed traps but seed introduced from outside is more difficult to quantify. However, extraneous sources of seed are often small: the main concern is the introduction of entirely new weed species. This new seed variable is the one that growers can influence the most, giving them some ability to manage outcomes from the model (Gallandt 2007).

**Seed death/predation.** This unfortunately, is a very difficult parameter to quantify. However, with sufficient experimentation researchers should be able to generate useful data which can be used in the model. It is also an area where management practices may be used to increase the death or predation of seed in the soil (Mulugeta and Stoltenberg 1997).

**Germination fraction.** Although this is possibly the easiest of the four parameters to quantify, it needs to be done in the context of the seeds' environment otherwise there could be greater variability and less predictability. Many management practices impact on this variable and for the thousands of years before herbicides were invented, cultivation was the principle way of managing weed emergence (Timmons 1970).

This paper looks at all four input parameters to the model, discussing their implications and looking at some management practices which may aid in reducing the weed seed bank.

## RESIDUAL SEED

This is the number of weed seeds in the soil weed seed bank at any time and although the ability to accurately measure it has no direct influence on its on-going content, it is an



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important measure in determining the success or otherwise of applied weed management strategies.

**Importance of the weed seed bank**

The soil seed bank acts as a reservoir of weed seeds and largely determines the potential density and species composition of weeds that subsequently interfere with crops during the growing season (Forcella 1993). Weed seed banks have received much attention in both applied and fundamental research during recent decades. These have been studied for their persistence and timing of germination (Forcella et al. 2000) as well as for their regeneration potential in different communities (Bekker et al. 2003). Buried seeds should be considered an essential part of the community species pool (Zobel et al. 2000). This implies that species absent from the above ground vegetation but present in the soil seed bank must be taken into account for future weed infestations. Some bioeconomic weed management models now use seed bank estimates to predict weed population dynamics and competitiveness (Wiles and Schweizer 1999).

**Variability of the weed seed bank (***Vertical/horizontal/location within the field)*

Most studies on weed seed banks have demonstrated large variability in both density and composition between samples taken from the same site (Benoit et al. 1992; Rahman et al. 1996). This is directly related to the problematic nature of seed banks in cropping systems, such as clustered seed patterns and skewed frequency distribution of most species (Mickelson and Stougaard 2003; Wilson and Aebischer 1995). This patch distribution within a field, in conjunction with various cultural factors results in seed banks that are spatially heterogeneous (Rew and Cussans 1997). Different types of cultivation also influence seed distribution among soil aggregates and in the soil profile.

A detailed study on the vertical variability in distribution of weed seeds showed a linear decline with depth in the mean seed numbers for *Chenopodium album* ($P<0.01$), a weed with medium-sized seed. The decline had the effect of approximately halving the number of seeds for every 5 cm increase in depth (Rahman et al. 1999). Rahman et al. (2000) recorded similar trends for many other weed species in a later study. As the trend was very similar for viable, non-viable and empty seeds, it would appear that the distribution was not affected significantly by germination or predation. Investigations of horizontal variability showed some indication of localized clumping, which may have been associated with the seed fall from a large plant or a small clump of plants. However, samples taken at least 1 m apart were found to be statistically independent and not affected by the adjacent sample (Rahman et al. 1999). Also, casual observations suggest and our studies have confirmed that the edges and corners of arable fields tend to be areas of greater botanical diversity and weediness (Rahman et al. 1996), which has implications for sampling for weed seed bank estimates.

**Soil sampling for estimating the seed bank** *(Spatial variability of soil seed bank)*

Many weed seed bank studies often suffer from methodological inadequacies such as absence of appropriate statistical data analysis or low sampling intensity. Soil sampling is time and labour intensive and hence knowledge of the effort required versus the level of precision achieved is extremely important. Two obvious questions arise in relation to sampling a weed seed bank: how many and what size samples, are required to reliably estimate the seed bank? Many researchers have reported investigations aimed at optimizing sampling protocols (e.g. Ambrosio et al. 1997; Bigwood and Inouye 1988; Dessaint et al. 1996; Mickelson and Stouggard 2003) and the attention has typically focussed on the relationship between the number of sampling units, their size and the desired statistical precision of estimates.

Our results based on samplings from a large number of arable sites have shown considerable within-site variation. For predicting a given seed population with some accuracy, the species has to be present in large numbers ($>1000/m^2$) and/or large numbers of soil cores



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need to be taken (Rahman et al. 1997; 2001b). Comparison of results from 25 mm and 75 mm diameter cores showed that collecting the same quantity of soil with a greater number of smaller cores would provide a more accurate estimate. Taking smaller cores allows for a better coverage of the field and reduces problems caused by spatial variability.

**Methods for enumerating weed seed banks**

Determination of the density of viable weed seeds in a soil sample is a tedious and slow process. Typically this is estimated either by physical extraction of seeds or by greenhouse germination and enumeration of seedlings. However, there are advantages and disadvantages with both of these methods (Gross 1990; Forcella 1993; Rahman et al. 1995; Mesgaran et al. 2007). We have determined the seed bank species composition and density in a large number of soil samples collected over more than 10 years using two methods: (i) seed extraction by washing and dry sieving; and (ii) seedling enumeration in trays placed in the glasshouse. Details of these methods are provided by Rahman et al. (1995; 1998).

We found the weed seed extraction method fast and efficient, but it requires specialized expertise and equipment. The seedling emergence method on the other hand, needs more time and specific growing conditions for maximum emergence of some species. Variations in environmental conditions as well as growing conditions such as soil depth, soil amelioration and watering method have all been found to affect the degree of emergence (Hartley and Rahman 1995). In most cases 80% of the weed seedlings emerged within the first incubation and 95% within seven incubations (Rahman et al. 1998). Comparing the number of our 15 common cropping weeds, we found that both methods gave similar estimates for grass weeds but the seed extraction method generally found more broadleaf weed seeds (Rahman et al. 1998). Nevertheless, there was a very strong correlation ($r^2 = 0.92$) between the two methods (Rahman et al. 1996; 2001b).

**Correlating the seed bank with weed populations in the field**

Weed species vary in the fraction of their seed banks emerging as seedlings because of species-specific dormancy and germination characteristics (Egley 1986; Forcella 1993; Grundy et al. 1999). Also, many species are capable of extended flushes of emergence over several weeks under certain environmental conditions (Forcella et al. 2000).

In a 3-year study we investigated the relationship between laboratory enumeration of the soil seed bank and field populations of various weed species in 30 maize fields (Rahman et al. 2003; 2004; 2006). Results showed that for the most abundant weed species, on average 2.1 – 8.2% of the seeds of the broadleaf species and 6.2 – 11.9% of the seeds of grass weeds in the soil seed bank emerged in any one year. Overall, the results showed a strong linear relationship between the seed numbers in the soil and the seedling numbers in the field for all the grasses and for most broadleaf weeds. Thus an estimate of the soil seed bank combined with knowledge of the germination and behaviour of specific weed species would have a good potential for predicting future weed infestations in arable fields.

**Persistence of soil weed seed banks**

In addition to estimating the weed seed populations in the soil, some knowledge about the persistence of the seed bank is important for developing long term weed management strategies. Normally in the absence of fresh seed input, both the weed seed bank and the number of seedlings emerging annually from regularly disturbed soil declines exponentially for most weed species (Wilson and Lawson 1992; Popay et al. 1994; James et al. 2010).

Over a 4 year period, in three different regions of New Zealand, we measured the rate of seed loss of certain weed species from the seed bank. This was in the absence of seed input and when the soil was cultivated monthly to a depth of 100 mm. Results showed that in the first year the size of the weed seed bank dropped to 39-56% of its initial size. By the end of the fourth year of the experiment, the numbers had declined to 1-2% of the original seed bank



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for each of the four most abundant species present. Despite this impressive reduction, there still remained between 0.2-6.4 million seeds per hectare of these species. This illustrates the size of the problem of reducing the weed seed bank and the length of time it may take (Rahman et al 1998; 2001c).

## NEW SEED

Without the addition of new seed, the weed seed bank would soon be depleted (Renton et al. 2008) so eliminating this new seed is the obvious answer. However, for the last 60 years herbicides have in many cases taken over as the primary means of weeds control and although they are arguably very effective, in most cases they have not managed to significantly impact the size of soil weed seed bank although they may have altered the composition to species with long-lived and numerous seeds (Rahman et al. 2001b; Bastiaans et al. 2008). This is evidenced by the use of herbicides continually increasing rather than the other way around. In many cases where herbicides have been used excessively, possibly in an attempt to eliminate seed rain, weeds have evolved resistance to herbicides, thus exacerbating the problem (Heap 2013). New Zealand has not been immune to this. Currently there are 11 herbicide-resistant weeds including two resistant to glyphosate (Rahman et al. 2001a; Ghanizedah et al. 2013). This increasing trend in resistance occurrences demonstrates that total reliance on herbicides for weed control in crops is unsustainable (Harker et al. 2012). Clearly growers require new weed management systems that not only eliminate crop competition but also reduce the seed rain.

If weeds cannot be adequately controlled within the crops then both concurrent and post-harvest weed seed management options need to be considered. In Western Australia this is a critical issue, so harvest residue is often collected and dumped or wind-rowed and burnt (Walsh and Newman 2007). Another development is the Harrington Seed Destructor which destroys >95% of all weed seeds exiting a combine harvester (Harrington and Powles 2012).

## SEED DEATH/PREDATION

Weed seeds shed by the maturing plants either remain on the soil surface or are incorporated into the soil by natural or artificial means. However, the fate of weed seeds in the soil is determined by both internal physiological conditions and the environmental conditions encountered in the soils (Murdoch and Ellis 1992). These influences, combined with dormancy, natural seed loss and mortality (James et al. 1998) result in only about 3 to 6% of the seed bank establishing as seedlings each year (Wilson and Lawson 1992).

### Seed death

Most seeds are protected by a seed coat of varying thickness and hardness. Most grasses have a thin, soft coat while those of broadleaf weeds are generally thicker and harder. The seed coat provides an important defence from decay. However, if the seed coat is damaged in such a way as to let in water, the seed will either grow (if the conditions are right) or decay (if they are not). Cultivation brings seeds to the surface, where they are exposed to light and suitable temperatures, and can abrade the seed coat. All this gives rise to a flush of germination within days of the event. Timing of a cultivation event so that the weeds can be controlled before the crop is planted is a frequently used method (stale seed bed) for reducing weed pressure. Other strategic timings of cultivation may also be useful, especially a post-harvest cultivation (Ball 1992; Pekrun et al. 1998).

The role of allelopathic chemicals resulting in seed death is poorly understood but has some potential in reducing seed numbers (Putnam et al. 1983; Jefferson and Pennacchio 2003). There are many reports on the effects of allelopathic plants and extracts on weeds but unfortunately few addressing the impacts on the soil weed seed bank.



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**Seed predation**

Weed seed predation by both vertebrates and invertebrates can be a major contributor to reductions in the weed seed bank (Menalled et al. 2000). In a study within an organically grown oat crop, Westerman et al. (2003) showed that vertebrates, presumably mice, accounted for the larger part of weed seed consumption (30–88%) while invertebrates, probably granivorous ground beetles, accounted for a smaller part of seed consumption (4–38%). Many factors influence the level of predation, including crop type and season. O'Rourke et al. (2006) found that invertebrates consumed less than 30% of seeds in early summer and autumn but up to 80–90% of seeds in mid- to late summer. They also found that seed predation was higher in maize and soybean crops compared to triticale and alfalfa.

While predation by vertebrates was less variable than by invertebrates, it is probably not an aspect that should be promoted as vertebrates such as mice and birds also consume crop grains. However, granivorous invertebrates can significantly reduce weed seed numbers and more could be done to identify, encourage and protect these as an additional means of reducing the weed seed bank. The role of micro-organisms in seed death has been largely overlooked, probably as the chance of finding an agent which selectively targets weed seeds and leaves crop seed untouched is slight. However, micro-organisms are involved in the dynamics of the soil weed seed bank and should not be ignored (Kremer 1993).

Tillage systems also need to be evaluated in terms of soil weed seed bank management objectives. Timing and cultivation method, or the lack of it, is an increasing important management option with respect to environmental and conservation concerns (Holland 2004). An understanding of the impacts of these practices on the soil weed seed bank is very important. Within the allowances of the environmental concerns, there may be some leeway to choose better options which have more favourable impacts on the weed seeds in the soil. On one hand, cultivation may bury seed and protect it from predation, but on the other hand it could abrade it causing it to decay (Balleré et al. 2008). Our work has shown that cultivation with power harrows and rotary hoes does not significantly alter the weed seed distribution down the soil profile compared to the distribution in non-cultivated plots (Rahman et al. 2000). Ploughing resulted in re-distribution of weed seeds but even after this cultivation method, more than 50% of the weed seed remained near the top 100 mm of soil. The significance of distribution of seed in the profile, or burial depth, depends on the size of the seed. Large seed like that of *Panicum miliaceum* can emerge from depths of 120 mm or more in many soil types (James et al. 2011) while very small seed such as *Juncus bufonius* and *Gamochaeta* spp. will only emerge from the top 5 mm or less of soil (James et al. 2002).

## GERMINATION FRACTION

Weed species vary in the fraction of their seed banks emerging as seedlings because of species-specific dormancy and germination characteristics and requirements (Egley 1986; Rahman and James 1993). Sometimes the germination characteristics can be used to decrease the germination fraction, such as planting early so that those weeds requiring more thermal units will not germinate (Gower et al. 2002).

**Effect of burial depth**

The distribution of weed seeds in the seed bank in relation to soil depth has a major influence on seedling recruitment. Factors such as weed species, soil type, tillage, seeding practice and soil-applied herbicides all have a strong bearing on the depth of seedling recruitment (Buhler and Mester 1991; du Croix Sissons et al. 2000). Many recruitment experiments have been conducted by placing weed seeds at specific depths in the soil (e.g. Grundy et al. 1999). However, only a few studies have been conducted to determine recruitment depth from natural weed seed banks (Buhler and Mester 1991; Chancellor 1964; du Croix Sissons et al. 2000) and these have shown the direct influence of depth and the tillage system. This



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information will advance the development of a robust model for predicting weed emergence based on the size of the weed seed bank in arable soils.

**Seed dormancy**

Seed dormancy is a protection mechanism within the seed to ensure that it does not germinate prematurely in the wrong season. However, for some species dormancy is extended to cover many seasons and is part of the plants' overall survival mechanism (James et al. 2010). These 'long-lived' seeds also need to be taken into consideration when estimating the germination fraction of the weed seed bank.

Far a few weed species, nearly the entire seed bank germinates annually. However, for most arable weed species only a small proportion of buried seeds germinate and emerge each year (Dowsett and James 2012). Moreover, the magnitude of this proportion varies considerably from year to year for these latter species (Forcella 1993). Understanding this dormancy process is fundamental to improving weed emergence models and subsequent management decisions, as reviewed by Benech-Arnold et al. (2000). Bradford (2002) suggested the possibilities of using hydrothermal time to predict dormancy relief, however, the actual physiological processes by which seeds actually perceive temperature is still not well understood. One proposal is that it is associated with biochemical changes in membrane cellular properties and Vleeshouwers and Kropff (2000) have suggested that ultimately the most important progress towards the development of dormancy submodels may come from research at the molecular physiological level.

There could be many explanations for the annual variations in germination percentages of weed species, Forcella (1993) suggests that the most probable explanation is differential induction of non-dormant seeds into states of secondary dormancy caused by weather related events. For summer annual weed species, two such events are abnormally high soil temperature and anoxia caused by soil flooding. Laboratory simulations of both situations are known to induce non-dormant seeds into secondary dormancy (Simpson 1990). Field studies have also suggested that these two factors are important in lowering the percentage of buried seeds that germinate and emerge in any given year. Examples of contemporary methods and opportunities for understanding dormancy are described by Chao (2002).

## DISCUSSION

A farm-wide systems approach to reducing seed rain and increase seed losses would involve the co-ordination of truly integrated weed management strategies with crop rotations and specific weed seed management techniques. It would need to take into account the dynamics of the weed seed bank as discussed above. Within this system, specific objectives would be set and these would be the basis for farming decisions (Gallandt 2006). An over-arching objective may be to increase productivity or profitability by a set percentage. Under that would be the objectives or milestones which would lead to that end point. These lower level objectives or milestones would have to be based on sound and proven scientific knowledge. As an example, an objective may be to reduce seed rain each year by 50%, knowing from good research that after a certain number of years this would start to impact on the soil weed seed bank (Forcella et al. 2000; Grundy 2003). The overarching objective would then begin to be achieved as less money and effort may need to be devoted to control of weeds.

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# ISOLATION AND IDENTIFICATION OF ALLELOCHEMICALS FROM TRADITIONAL CROPS AND THEIR UTILIZATION FOR AGRICULTURE

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**ABSTRACT** We have engaged in the project to screen, isolate and identify allelochemicals, from allelopathic plants, and seek their potential practical use for weed control in the field. Useful annual cover crops and their allelochemicals are 1) velvetbean (*Mucuna pruriens*): L-DOPA [1], 2) hairy vetch (*Vicia villosa*): cyanamide [2], 3) buckwheat (*Fagopyrum esculentum*): rutin [3]. These crops are of practical use and useful resources as food. Potential perennial cover plants are 4) higan-bana or red spider lily (*Lycoris radiate*): lycorine [4], 5) jyano-hige or dwarf mondo grass (*Ophiopogon japonicas*): salicylic acid [5], 6) shi-ran or hyacinth orchid (*Bletilla striata*): militarine [6], and 7) yuki-yanagi or Thunberg spiraea (*Spiraea thunbergii*): *cis*-cinnamic acid [7]. Arguably, these plants have potential as emergency food or biomass resources.

***Keywords:*** Allelopathy, allelochemicals, cover crops, weed suppression.

## INTRODUCTION

Allelopathy is a phenomenon of chemical interaction, and is probably of wide spread significance in the functioning of natural communities. In plant-plant interactions, allelopathy is generally used to denote the process by which plants release phytotoxic compounds (allelochemicals) to the environment, resulting in harmful or beneficial effects on neighboring plants. Utilization of the allelopathic potential of plants for weed control is given great emphasis, because it would reduce the risk of environmental toxicity. Crop species with allelopathic potential have been given greater attention during the last two decades. Cover crops are mostly used in no tillage production system because they provide surface residue which reduces soil erosion, conserves water retention and soil nutrients. Identification of cover crops which provide weed suppression may increase acceptance and utilization of these soil conserving tillage techniques.

We have been engaged in the search for allelopathic plants in order to determine allelochemicals and their mechanism. In the course of this study, we developed some new methods to discriminate and identify allelopathy from other competitive factors such as nutrients, light and water (Fujii *et al.,* 1991a,b,c,d). We have reported allelopathy in velvetbean (Fujii *et al*., 1991a, 1992), hairy vetch (Fujii, 2001), medicinal plants (Fujii *et al.,* 2003), and buckwheat(Iqbal *et al*., 2002, 2003).

In the course of this research, we have developed a new bioassay system that could demonstrate and assess the possibility of allelopathy, named "plant box method," and "sandwich method". The former method involves mixed planting using agar medium, and exudation of allelochemicals from leaves and/or roots. Both methods can show the allelopathic action of root exudates and leaf leachates.

On the other hand, we came to know that it is difficult to use allelopathic plants directory to agriculture by itself, because of the importance of other competition factors such as light and nutrients. We then started to use the allelopathic factor accompanied by competition factors listed above. In this sense, cover crop is a probable outlet of allelopathy. If other competition factors are the same, allelopathy will play an important role in weed suppression in combination with other factors. Some farmers suggested from their experiences that some ground cover plants suppress weeds drastically. There is a possibility



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that some cover crops could be used for practical weed suppression. Subsequently, we began to screen the allelopathic activity of cover plants by plant box method and sandwich method. From these primary selections, and experiences on the fields, we conducted a field experiment to select the most promising cover crop for weed control in orchard garden and abandoned fields.

## MATERIALS AND METHODS

(a) **Chemical prospecting of allelopathic plants.** Such prospecting employed the methods of Fujii *et al.* (1990a, 1990b): Seventy plant species were tested on their allelopathy following Richards' function method, which proved to be suited to germination tests of lettuce and some weed plants. The parameters for germination tests were: onset of germination (Ts), germination rate (R), and final germination percentage (A). A “simplex method” was applied for the computer simulation of germination curves with the Richards' function.

(b) **Velvetbean cultivar:** A cultivar of velvetbean, *Mucuna pruriens* var. utilis cv. Hassjo and cv. Florida veletbean were used for the field test and the extraction of allelochemicals.

(c) **Incorporation of velvetbean leaves into soil:** Two treatments of velvetbean were added to the volcanic ash soil in Tsukuba: one was dry leaves oven-dried at 60℃ overnight, the other was fresh leaves. One g of oven-dried leaves was added to 100 g of soil. The same weight of cellulose powder was added to other pots as a control. Fertilizers added to each pot were as follows: N, P, K of 50, 100, 50 mg/100g-soil d.w. respectively. Available nitrogen containing in the velvetbean residues (1.2%) was added in control pots.

(d) **Weed appearance in the fields with velvetbean stands:** Planting of velvetbean and some other plants were repeated for a period of 2 to 3 years (1988-1990). Plants were grown in lysimeters: each size is 10 $m^2$ and six replications, where the surface soils of 10 cm depth were replaced with uncultivated soils in starting year. Each plot received a standard level of chemical fertilizers: N, P, K of 80, 80, 80 g/10 $m^2$ except for fallow.

(e) **Mixed culture of velvetbean by allelopathy discrimination methods**: Allelopathy of velvetbean in the field was confirmed using Stair-step and Substitutive experiments. The substitutive experiment was modified from the methods of Fujii *et al.*, (1991d).

(f) **Isolation and identification of allelopathic substances:** Some fractions were extracted from fully expanded leaves and roots of velvetbean with 80% ethanol. The acid fraction of the extract inhibited the growth of lettuce (*Lactuca sativa*) seedlings. This fraction was subjected to silica gel column chromatography and HPLC with an ODS column, and the major inhibitor was identical to L-3, 4-dihydroxyphenylalanine (L-DOPA). The identification was confirmed by co-chromatography with an authentic sample using two HPLC column systems (silica gel and ODS) equipped with an electro-conductivity detector.

## RESULTS AND DISCUSSION

### 1) Velvetbean (*Mucuna pruriens*)

(a) **Chemical prospecting of allelopathic plants.** More than 70 plants were investigated with lettuce seed germination tests. It was observed that the activity of velvetbean was distinctively higher *vis-avis* other plant species. Some other plants such as *Artemisia princeps, Houttunia cordata, Phytolacca americana*, and *Colocasia esculenta* also showed the inhibitory response (Fujii *et al.*, 1990b).



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(b) **Incorporation of velvetbean leaves into soil.** An experiment was conducted to examine the effects of velvetbean on the growth of other plants in a mixed culture. The treatment also included an incorporation of velvetbean leaves into soils. Fresh leaves incorporation to soils (1.0% W/W in dry weight equivalent) reduced succeeding emergence of kidney bean (*Phaseolus vulgaris*) up to 60%, plant biomass up to 30% of the control. This deleterious effect diminished two weeks after the incorporation. Dried leaves incorporation showed no inhibition. Later, we found that L-DOPA, an allelochemical from velvetbean was adsorbed onto soils and have no harmful effect to other crops (Fubayashi *et al*., 2007, Hiradate *et al*., 2005, 2010).

(c) **Weed appearance in the fields of velvetbean stands.** Weed populations in spring with continuous cropping fields grown in lysimeters found that velvetbean plot showed a lower population of weeds dominated by sticky chickweed (*Cerastium glomeratum*), than the other plots of eggplant, tomato plant, upland rice and fallow did (Fujii *et al*., 1991b, 1991c).

(d) **Allelopathic compound in velvetbean.** The analysis on effective compound of velvetbean in restraining the growth of companion plants confirmed its association with L-DOPA (Fujii *et al*., 1991a) (Fig 1). It is well known that velvetbean seeds contain high concentrations of L-DOPA (6% - 9%), which plays a role of chemical barrier to insect attacks. In mammalian brain, L-DOPA is the precursor of dopamine, a neurotransmitter, and also acts as important intermediates of alkaloids. In animal skin, hair, feathers, fur and insect cuticle, L-DOPA is oxidized through dopaquinone to produce melanin. As L-DOPA is an intermediate and rapidly metabolized, usually normal tissues have little concentrations of L-DOPA. Fresh velvetbean leaves contain as much as 1% of L-DOPA. It actually exudes from root, and its concentration reaches 1ppm in water-culture solution, and 50 ppm in the vicinity of roots. This concentration is high enough to reduce the growth of neighbouring plants and the growth inhibition in a mixed culture is shown in Agar-medium culture. L-DOPA also leaches out from leaves with rain drops or fog dew. Since velvetbean produces 20 - 30 tons of fresh leaves and stems per hectare, approximately 200 - 300kg of L-DOPA may be added to soils a year.

(e) **Mode of action of L-DOPA:** Some effects of L-DOPA on germination and growth of the selected crops and weeds were examined. L-DOPA at 50 ppm suppresses the radicle growth of lettuce and chickweed 50% lower than the control. However, it was less effective against hypocotyl growth and practically had no effect on seed germination. L-DOPA strongly inhibited plant growth of *Cerastium glomeratum*, *Spergula arvensis* (both Caryophyllaceae), *Linum usitatissimum* and *Lactuca sativa*, and moderately inhibited the growth of Compositae, while with very limited effects on Gramineae and Leguminosae (Nishihara *et al*., 2004). Such selective inhibitiveness is comparable with other candidates of allelochemicals. The L-DOPA contained in fresh velvetbean leaves fully attributes to the plant growth inhibition through its crude extract. The results that L-DOPA displayed strong suppression in the growth of chickweed parallels those weed inhibition of growth in the velvetbean field. All these data suggest that L-DOPA function as an allelopathic substance (Fujii, 1999, 2003, Tomita-Yokotani *et al.*, 2003). Recently we reported a novel pathway by L-DOPA (Golisz *et al.*, 2011).



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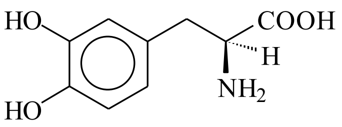
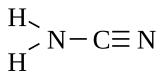
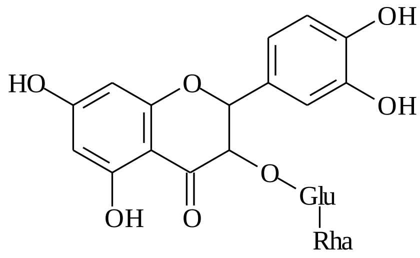


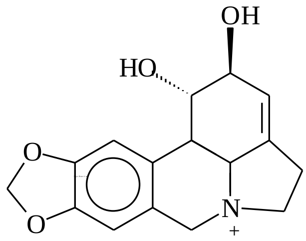
**L-DOPA (1)**
**(3,4-dihydroxy-L-phenylalanine)**



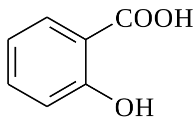
**cyanamide (2)**



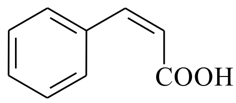
**rutin (quercetin 3-rutinoside) (3)**



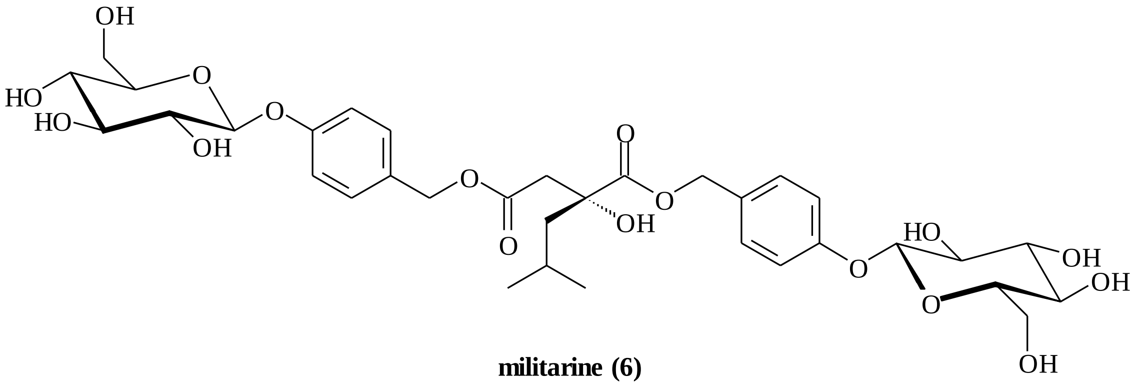
**lycorine (4)**



**salicylic acid (5)**



***cis*-cinnamic acid (7)**



**militarine (6)**

**Fig.1.** Allelochemicals isolated from crops (velvetbean, hairy vetch, buckwheat, red spider lily, dwarf mondo grass, hyacinth orchid and Thunberg spiraea)

Since velvetbean has special abilities such as weed smothering (Fujii *et al*., 1991b), companionship to corn (Fujii *et al.*, 1991d), tolerance to pests, suppression of nematode population, and soil improvement in its physical structure, it could be more widely used to reduce applications of synthetic chemicals to a lower level. Velvetbean seed yields are very high in the tropics, and the seed contains a high level of protein with a useful protein score and rich in anti-oxidative chemicals (Ibe *et al*., 2012). If detrimental factors such as L-DOPA and trypsin inhibitors could be eliminated through proper cooking, it would also contribute to alleviation of the food problems in some tropical countries.

**2) Hairy vetch (*Vicia villosa*).** We have found weed suppression activity of hairy vetch (Fujii, 2001, 2003, Horimoto et al., 2002, Araki *et al*, 2007), and found cyanamide as allelochemical (Kamo *et al.,* 2003). Up to now, the precursor of cyanamide in vetch is unknown, it is clear that this plant synthesize cyanamide itself (Kamo *et al*., 2006, 2008, 2009, 2010, 2012). Hairy vetch is now widely distributed in Japan as cover crop for weed control.

**3) Buckwheat and other plants.** Weed suppression ability of buckwheat is well known. We have evaluated the contribution of allelopathy in buckwheat (Iqbal *et al.*, 2002, 2003, 2007) and found that rutin, rich in this plant, is responsible for the activity (Golisz *et al*.,



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2007, 2008). Allelochemical(s) and their candidates were also reported in dwarf mondo grass (Iqbal *et al*., 2004a, 2004b), in red spider lily (Iqbal *et al*., 2006), in Thunberg spiraea (Hiradate *et al*., 2004, 2005, Abe *et al*., 2012), in hyacinth orchid (Sakuno et al., 2010), yoguso-minebari (Araya *et al.*, 2012), but need more research on chemicals and mode of action for practical use.

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# *Technical Papers*

## *Invasive Weeds, Ecology and Management*

### INVASION OF *ACACIA NILOTICA* INTO SAVANNAS INSIDE BALURAN NATIONAL PARK, EAST JAVA, INDONESIA

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**ABSTRACT** *Acacia nilotica* was imported to Indonesia in 1850's, with an idea of extracting Arabic gum from the plant. It was studied in Bogor Botanical Garden, despite its prolific growth it produced only very small low quality Arabic gum and the studies were abandoned after 40 year works, and seeds were sent to Bali and Palu, Central Sulawesi. *Acacia nilotica* was introduced to Bekol savanna in 1969 with good intention, i.e. to fence teak plantation nearby from fire annually erupted in that savanna. While it was successful in protecting teak forest from fire, *A. Nilotica* successfully also invaded to Bekol savanna. The spread was rapid due to a symbiotic relationship with herbivores, and practically all savannas in the park have been invaded at various degree. Vegetation map developed using landsat ETM-7 and Geo-Eye images from Google earthestimated spatial distribution of *A. nilotica* in Baluran National Park was 6,222.22 ha, with 7 different combinations of vegetation covers, this is the first quantitative estimate of spatial distribution of *A. nilotica* invasion in Baluran National Park. High population density of *A. nilotica* was shown in previously open savanna, while savanna with sparse distributed trees allowed the invasion at lower density, and still lower of *A. nilotica* population was observed on savanna with a denser shrub.

***Keywords****: Acacia nilotica*, Baluran National Park, Vegetation map, landsat ETM-7, Geo-eye, Google Earth.

## INTRODUCTION

Teijsman (1950) reported that *Acacia nilotica* (syn. *A. arabica*) was imported to East Indies (Indonesia) from Calcutta Botanical Garden, India and confirmed by Hasskarl (1986), with an intention of extracting Arabic gum out from this plant. The plant was planted in Bogor Botanical Garden, and found to grow rapidly and prolifically, rising a very high expectation to harvest a great amount of Arabic gum, a highly valued commercial product at that time, therefore, a handsome profit. To their surprise, *A. nilotica* produced only very small amount of low quality gum, it was then realized that it was wrong species, the correct species was *Acacia tanganika* which was developed by Germany, and after 40 years the experiment was abandoned (Sastraparaja, 1978). Some of the seeds were sent to Bali and Palu, Central Sulawesi (Hellinga, 1949), the plant is still growing around Tadulako University.

*Acacia nilotica* was introduced to Savanna Bekol inside Baluran National Park in 1969, with a good reason, to prevent savannas fire from encroaching teak plantation nearby. It was planted as a fence, 2 km length, along the periphery of teak plantation. It grew and spread rapidly. It started flowering in early wet season and pod matured and dropped in the dry season. During dry season grasses and almost other herbage palatable to herbivores in this savanna were dry out. Therefore, juicy and nutritious dropped pods were consumed by herbivore in-preference to dried grasses and other vegetation. However, *A. nilotica* seeds were not harmed during their passage along the herbivores digestive tracts, and excreted out with their faces. In this way, *A. nilotica* seeds were practically distributed everywhere wherever those animals wandered around, facilitating rapid spread and invasion of *A. nilotica* in the park.



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Aerial photograph taken in 1980 showed only a little expansion of *A. nilotica*, but in 1985 a wide invasion was detected and researches to control it including chemical as well as mechanical were initiated. The various control of *A. nilotica* conducted by the management of Baluran National Park (BNP) has created various different types of invasions. Schuurmans (1993) estimated *A. nilotica* invaded 420 ha in Bekol, 600 ha in Balanan, 200 ha virtually the whole Kramat savanna. In Balanan savanna except the northern and western sites *A. nilotica* formed a closed canopy, like those in Bekol and Kramat savanna. Samsoedin *et al* (1996) estimated the invaded area reached 5.000 ha. This rapid spread of *A. nilotica* invasion impacted negatively and created different types of savanna ecosystem alteration. While it is imperative to control this invasive *A. nilotica,* it is important to study the distribution of different types of invasion. This paper reported our research works in delineating different vegetation types in Baluran National Park.

## MATERIALS AND METHODS

Spatial data were obtained free from GeoEye-1 and Landsat ETM-7. Baluran National Park location was available in a great detail from Geo-Eye (Google Earth). GeoEye-1 has a resolution of 0.41-meter on panchromatic and 1.65-meter on multispectral imagery (www.geoeye.com/CorpSite/products/earth-imagery/geoeye-satellite .aspx.). The image of GeoEye-1 can be enlarged to obtain a detailed object (for example a type of *A. nilotica* invasion), with the scale of 1: 20.000. To cover the whole area of Baluran National Park, many such small GeoEye-1 images were constructed and stored in JPEG files to be merged and integrated into one mosaic of GeoEye-1 image. Finally the mosaic image was rectified or geo-referenced using coordinate data from Google Earth and also by overlaying on Landsat image as a control of geometric correction technique. The data were projected into WGS 1984-UTM, Zone_49S. In this way a detailed vegetative map was developed. The interpretation of satellite images followed that of TREES-Technical Notes Arcview (Feldkotter, 1999). The identification of vegetation covers from satellite image utilized the method of Lillesand, and Kiefer (2004), while to estimate crown density the authors adopted Photo interpretation guide for Forest Resource Inventories (Smelser and Patteson, 1975).

Spatial vegetation covers were subsequently identified and their distributions were delineated manually. Types of *A. nilotica* cover were ground checked with appropriate vegetation analysis. To quantify each area of classified vegetation cover after being delineated, an extension program of SIG ArcView was utilised to obtain area ($m^2$) and converted to hectare.

Ground checks on selected *A. nilotica* dominated vegetation covers were carried out with point centered quarter method. A line transect along 100 m was layed down randomly and at an interval of 20 m, points of observation were established, there were 6 points. At each of these points, an imaginary line perpendicular to the transect line was drawn. This line and the transect line divided space around the point into four quarters. In each points the following data were recorded: (a). Number of quarter (I, II, III, IV), (b). Distance to the nearest tree (in meter). (c). Species of the tree, (d). Diameter of tree at 130 cm height from the ground.

If the density of tree of *A. nilotica* dominated vegetation is $\lambda$ tree/$m^2$ , then $1/\lambda = m^2$/tree, .i.e. unit area occupied by a tree. The distance to nearest tree (r) represents the distance between two trees, $r = \sqrt{(1/\lambda)}$. The average of this distance $\bar{r}$ was calculated from the data of all nearest distance.

$$\bar{r} = \frac{\sum_{i=1}^{n} \sum_{j=1}^{4} R_{ij}}{4n}.$$

And the accompanying average density was calculated using the following formula.

$$\bar{\lambda} = \frac{1}{\bar{r}^2} = \frac{16n^2}{\left(\sum_{i=1}^{n} \sum_{j=1}^{4} R_{ij}\right)^2}.$$



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With this formula total tree density dan be calculated, and density of each species can be worked out with this following equation, where k represent individual species

$$\hat{\lambda}_k = \frac{\text{Quarters with species } k}{4n} \times \hat{\lambda}.$$

The cover or dominance of an individual tree is measured by its basal area or cross-sectional area. If, r, c, and A denote the diameter, radius, circumference, and basal area of a tree, respectively, since the area of a circle is $A = \pi r^2$, it is also $A = \pi(d/2)^2 = \pi d^2/4$. Since the circumference is $c = 2\pi r$, then the area is also $A = c^2/4\pi$. Either $A = \pi d^2/4$ or $A = c^2/4\ \pi$ can be used to determine basal area. The basal area of the entire tree sampled were calculated and summed to get the total amount. This total amount becomes a reference for calculating means of individual tree's basal area. The basal area of each species is thus mean of basal area individual tree's multiply by absolute population of the trees.

## RESULTS AND DISCUSSION

Baluran National Park derived from GeoEye-1 is presented in Fig. 1. It was interesting to note that Baluran National Park specified by a single peak of Mount Baluran was geographically delineated from the background soil, with distinct geo genesis, and with the existing dry climate indeed the area has developed into savanna ecosystem, the only savanna ecosystem in Java Island. The classification of vegetation cover especially on *A. nilotica* dominated vegetation, following modified National Standardization Agency of Indonesia (NSAI), enriched with experience in the field these were shown in Figs. 2-4.

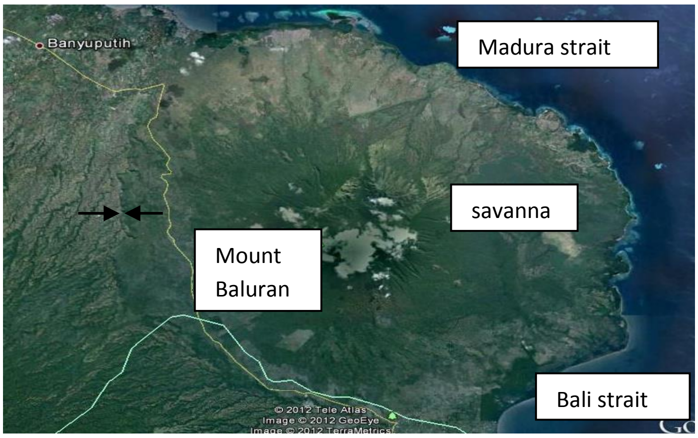


**Fig. 1**. Mount Baluran, with distinct geography, seperated (→←) from the background soil systems . In the northern and eastern site of Mt. Baluran the environmental system was condusive to the development of savanna ecosystem, which was the home of banteng (*Bos javanicus*) beside deer and peacock.

**Fig. 2**. Very dense *Acacia nilotica* population. It looks like a thick carpet. Notice the different between dense and sparse *A. nilotica* population.

**Fig. 3**. *Acacia nilotica* population mixed with light coloured vegetation, made of shrubs mainly *Thespesia lampas*, or *Vernonia cymosa*.



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**Fig. 4**. An area that has been cleared from *A. nilotica* through mechanical control using bulldozer. *Acacia nilotica* invasion has threatened savanna ecosystem and the government has spent a considerable fund to control invasive *A. nilotica* and in this particular site the eradication was

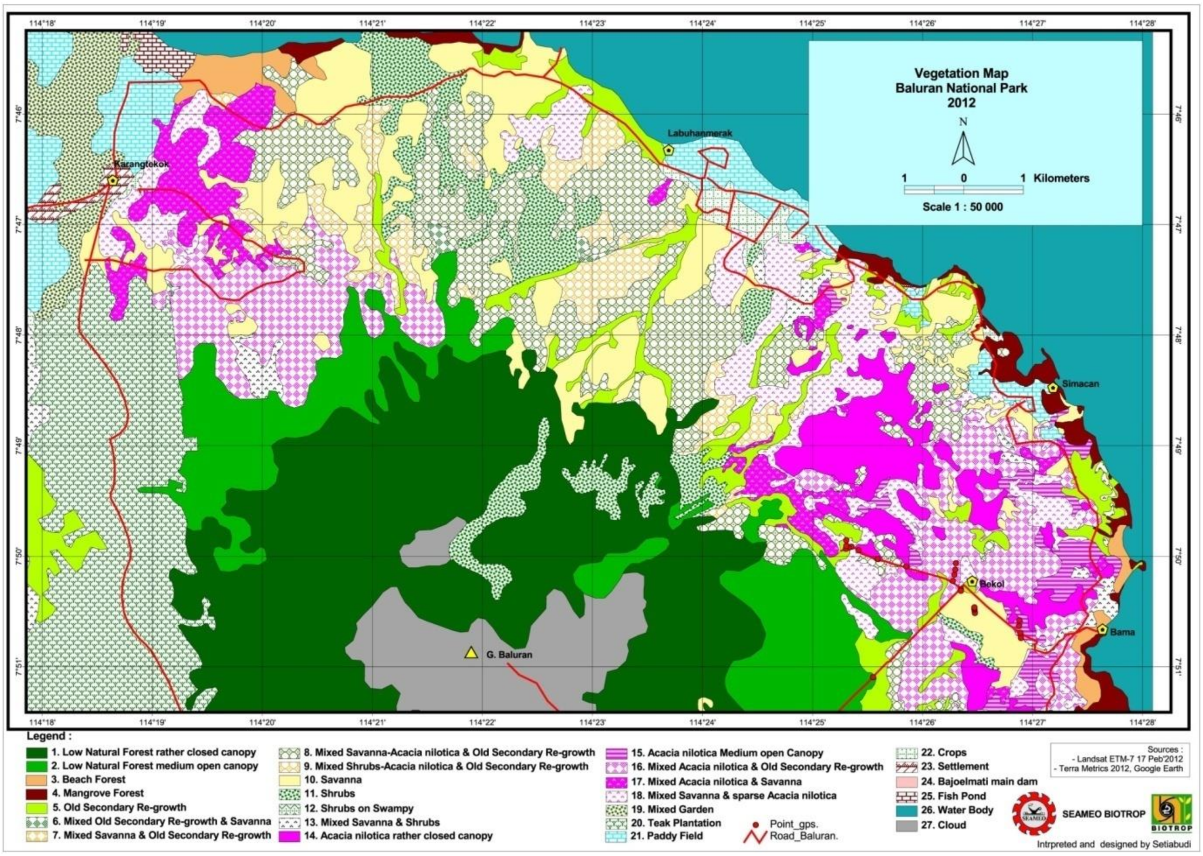


**Fig. 5**. Developed Vegetation Map of Baluran National Park. Scale: 1:50.000.

Where this invasive species prevails, what is the density, for the manager to design its control or eradication. Therefore, to classify *A. nilotica* dominated vegetation cover determined by the population of *A. nilotica*. In the above vegetation map there is a vegetation cover classified as dense *A. nilotica* (Fig.2), not "belukar" as it may be, when it is a local species. This is appropriate as it is well related to the interest of knowing and understanding

Pink colour (full or pattern) is representing areas occupied by *A. nilotica* population, dark green is natural forest with dense canopy, light green is medium canopy of natural forest, while yellow is representing savanna, and pattern green is area occupied by shrubs. There were 17 types of natural vegetations, 4 types of cropping systems, 3 types of other systems and another is cloud.

There were 7 types of vegetation cover associated to *A. nilotica* (1) *A. nilotica* with closed canopy (611.12 ha); (2) *A. nilotica* with medium open canopy (208.35 ha); (3) Mixed *A. nilotica* & old secondary re-growth (1,396.88 ha) ; (4) Mixed *A. nilotica* and savanna (532.16 ha); (5) Mixed savanna – sparse *A. nlotica* (921.48 ha); (6) Mixed savanna- *A. nilotica*, and old secondary re-growth (2,018.41 ha); and (7) Mixed Shrubs- *A. Nilotica* & old Secondary re-growth (533.82 ha).



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The total areas with various vegetation that were invaded by *A. nilotica* was 6,222.22 ha, although we did ground check only part of the areas, the figures were realtively accurate. This is the first quantitative estimation of spasial distribution of *A. nilotica* invasion in Baluran National Park differentiating into various combination of vegetation cover, different from those specified under NSAI. The figures provide information to managers to prioritise areas where *A. nilotica* will be eradicated.

The classification of *A. nilotica* canopy coverage based on high resolution image, was good enough to estimate the distribution of *A. nilotica*, however, the actual *A. nilotica* density as well as other trees and the accompanying vegetation growing under those canopies of different *A. nilotica* population also differed considerably.

Table 2 provides a description of *A. nilotica* invasion in Baluran National Park. The Community i, ii, were under savanna ecosystems, with well developed pasture. This open savanna ecosystems were apparently very suitable for the invasion of *A. nilotica*. The management intervention in efforts to control it, created further development of invasion, where mechanical control by cutting stems of *A. nilotica* did not kill it, rather inducing the stem to sprout and produce coppice leading to multistem plant with overlapping canopies and shown in satellite imagery as a thich carpet. This mainly in Bekol savanna, *A. nilotica* trees were small with very high density reaching more than 2000 tree/ha. The competition among them was very severe, in the field authors noticed some smaller individues were pushed to death. When left alone the population density of this *A. nilotica* community in time would decline leaving smaller number but big trees of *A. nilotica*.

Savanna with sparsely dispered trees mainly *A. leucophloea* and *Zizipus rotundifolia* showed different pattern of *A. nilotica* invasion. *A. leucophoea* when fully grown was a big tall magnificant tree, usually underneath the tree, being shady, was utilized by herbivores (mainly deer) to rest. These herbivores carried seeds of broadleaved weed attached to the fur such *Bidens biternata, Vervonia cymosa, Achiranthes aspera, Eleutheranthera ruderalis,* deposited on the soil around the tree. During wet season the seeds will germinate and grew very fast, flowered, fruited and by the following dry season seeds were dispered to savanna area nearby. Grasses in savanna, especially *Schlerachne punctata, Brachiaria reptans,* grew very fast become very thick, accumulating a considerable biomass and would easily cover down weed seedling and in open savanna those weed seedling rarely able to surface or grew poorly. During dry season that biomass become inflammable and the fire will burn down those poor-growth weeds; in this way savanna was sustainably maintained. However, however, with the invasion of *A. nilotica* which interfered with sun light, reduced the growth of grasses but little affected to those broadleaved weeds, and this was the beginning of savanna ecosystem alteration. Grasses were reduced progressively while broadleaved weeds were becoming highly numerous, as these weeds were able to obtain sufficient light even under the canopy of *A. nilotica*, indeed this *A. nilotiva* invasion threatened the existence of savanna ecosystems.

Savannas with "belukar" or secondary growth in general, the type of this savanna were Kramat savanna. The area was relatively in a high elevation; accessibility was less than those located in Bekol. The area was not a favourite for intervention by manager, leaving *A. nilotica* to grow well without interference, becoming big trees, with grasses *Themeda triandra*, and *Schlerahne punctata*. The pre existing secondary growth of shrubs effectively reduced the establishment of high density *A. nilotica,* but providing a condition to grow into a big tree.



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**Table 1**. Classification of Vegetation Cover identified using high resolution satellite

| No. | Vegetation Cover | Count | Code | Hectare |
|---|---|---|---|---|
| | **Natural Vegetation** | | | |
| 1 | Low Natural Forest with rather closed canopy | 2 | 1 | 6413.52 |
| 2 | Low Natural Forest with open canopy | 15 | 2 | 3414.47 |
| 3 | Beach Forest | 5 | 3 | 179.37 |
| 4 | Mangrove Forest | 16 | 4 | 404.45 |
| 5 | Old Secondary Re-growth | 55 | 5 | 1990.77 |
| 6 | Mixed Old Secondary Re-growth & savannah | 3 | 6 | 27.58 |
| 7 | Mixed savanna-*A. nilotica* & Old Secondary Re-growth | 54 | 8 | 2018.41 |
| 8 | Mixed Shrubs-*A. nilotica* & Old Secondary Re-growth | 20 | 9 | 533.82 |
| 9 | Savanna | 70 | 10 | 1798.65 |
| 10 | Shrubs | 9 | 11 | 764.50 |
| 11 | Shrubs in Swampy area | 7 | 12 | 50.57 |
| 12 | Mixed savanna &shrub | 15 | 13 | 192.47 |
| 13 | *Acacia nilotica* rather closed canopy | 24 | 14 | 611.12 |
| 14 | *Acacia nilotica* medium open canopy | 12 | 15 | 208.35 |
| 15 | Mixed *A. nilotica* & Old Secondary Re -growth | 11 | 16 | 1396.88 |
| 16 | Mixed *A. nilotiica* & Savanna | 15 | 17 | 532.16 |
| 17 | Mixed savanna and sparse *A. nilotica* | 26 | 18 | 921.48 |
| | **Plantation** | | | |
| 18 | Mixed garden | 6 | 19 | 171.32 |
| 19 | Teak Forest | 2 | 20 | 3428.96 |
| 20 | Paddy Field | 9 | 21 | 340.42 |
| 21 | Upland crops | 5 | 22 | 122.50 |
| | **Others** | | | |
| 22 | Settlement | 9 | 23 | 12.10 |
| 23 | Bajulmati main Dam Tunnel | 1 | 24 | 8.68 |
| 24 | Fish pond | 3 | 25 | 0.12 |
| 25 | Cloud | 2 | 26 | 885.73 |
| | TOTAL | | | 26.428.40 |

**Table 2**. Different *Acacia nilotica* population in various ecosystems.

| | *A. nilotica* closed canopy (i) | | *A. nilotica* medium opened canopy (ii) | | Mixed shrubs & *A. nilotica* & old 2nd re-growth (iii) | | Mixed *A. nilotica* & Old 2nd Re-growth (iv) | |
|---|---|---|---|---|---|---|---|---|
| Species | Absolute density (trees/Ha) | Total Basal Area ($M^2$/Ha) | Absolute density (trees/Ha) | Total Basal Area ($M^2$/Ha) | Absolute density (trees/Ha) | Total Basal Area ($M^2$/Ha) | Absolute density (trees/Ha) | Total Basal Area ($M^2$/Ha) |
| *Acacia nilotica* | 2163.09 | 11.15 | 621.65 | 4.68 | 98.84 | 2.22 | 181.53 | 4.23 |
| *Azadirachta indica* | | | 13.23 | 1.03 | | | 6.48 | 0.03 |
| *Litsea* sp. | | | | | | | 12.97 | 0.28 |
| *Schleichera oleosa* | | | | | 2.75 | 0.028 | | |
| *Randia* sp. | | | | | 2.75 | 0.03 | 19.45 | 0.45 |
| *Ziziphus rotundifolia* | | | | | 13.73 | 0.18 | 6.48 | 0.28 |
| *Grewia* sp. | | | | | 5.491 | 0.004 | 45.38 | 0.87 |
| *Acacia leucophloea* | | | | | 8.24 | 0.09 | 19.45 | 0.22 |
| *Premna oblongata* | | | | | | | 12.97 | 2.97 |



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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Rauwolfia* sp. | | | | | | | 6.48 | 0.45 |
| T O T A L | | | | | | | | |
| *A. nilotica* | 2163.09 | 11.15 | 621.65 | 4.68 | 181.53 | 4.23 | 98.84 | 2.22 |
| Non- *A. nilotica* | | | 13.23 | 1.03 | 129.66 | 5.55 | 32.95 | 0.32 |


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# INVASIVE SPREAD OF WATER HYACINTH IN VEERANUM IRRIGATION SYSTEM AND THE IMPACT OF HERBICIDAL CONTROL ON AQUATIC ENVIRONMENT

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**ABSTRACT** The lake Veeranum has a command area of 18,000 ha connected through 27 distributary channels. Recent survey on the infestation of aquatic weeds in these channels indicated that many of these channels were infested heavily with water hyacinth. A research programme supported by Ministry of Water Resources, Government of India was taken up to study the impact of herbicides on the control of water hyacinth, to optimize their doses and time of application and to study their impact on aquatic environment, through a series of green house and watershed studies. Among the different herbicides tried *viz.*, glyphosate, 2, 4-D Na salt and paraquat, glyphosate proved more efficient with higher magnitude of plant height reduction, weed mortality (cent percent) and reduction in bio-mass. The doses of 2.5 kg ha$^{-1}$, 1.5 kg ha$^{-1}$ and 1.5 kg ha$^{-1}$ were found to be optimum for glyphosate, 2, 4-D Na salt and paraquat, respectively. Different seasons did not vary in influencing the impact of herbicides on the weed. Glyphosate also caused least fish mortality of 23.30 per cent after 32 days. Different organs like gills, brain, liver and kidney of the fishes were studied for histology and the observations showed tissue distractions in glyphosate treated fish. In the watersheds also, glyphosate proved more efficient and safe on water quality in terms of pH, EC, DO, COD and mineral content, of treated water. The glyphosate treated water was also found to be safe for irrigating crops with 87, 58 and 62 per cent germination of paddy, cotton and ladysfinger.

***Keywords:*** Fish mortality, Herbicides, Irrigability, Water hyacinth, Water quality.

## INTRODUCTION

The aquatic weed water hyacinth *Eichhornia crassipes* [Mart.] Solms-laubach is considered to be the world's worst aquatic weed. The lake Veeranum in Tamilnadu, a constituent southern state of India, has a command area of 18,000 ha connected through 27 distributary channels, and recent days have been heavily infested with water hyacinth (Kathiresan, 2012). It is believed to occupy over 0.2m ha of water surface in India (Murugesan *et al.*, 2005). Excessive infestations of the weed deleteriously affect water traffic, fishing potential, infrastructure for pumping, hydro electricity generation, water use and biodiversity, other damages include water loss due to evapotranspiration which is 1.02 to 9.8 times higher than evaporation from an open surface (Singh and Gill, 1996). *E. crassipes* also affects the water quality by reducing water temperature, pH, bicarbonate, content, DO and increasing BOD, free carbonate and nutrient level that ultimately makes water unfit for livestock and human use (Deivasigamani and Kathiresan, 2013). Based on the above facts, several mechanical, chemical and biological methods have been used to control this alien weed. Frequent mechanical removal of this weed is highly expensive, laborious and time consuming process. Biological control require a minimum of several years, usually 3 to 5 years, for insect population to increase to density that could bring down the weed stand to a substantial decline (Harley *et al.,*1996; Kathiresan, 2000). Chemical control using weed killers such as glyphosate, paraquat and 2, 4-D Na salt seems to be effective and fast acting.

## MATERIALS AND METHODS

The studies were conducted at the Department of Agronomy, Faculty of Agriculture, Annamalai University during 2010-11, to screen different herbicides for managing water hyacinth and to



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trace their impact on non-target flora and fauna. These studies comprised a series of green house, pond experiments and laboratory studies. Screening of different herbicides was taken up in cement pots of dimension 2' x 2.5' x 2' with water filled up to three fourth of the pot's height, holding six water hyacinth plants. Graded doses *viz*. 1.25, 1.5 and 1.75 kg $ha^{-1}$ of 2, 4-D Na salt, 2, 2.5 and 3 kg $ha^{-1}$ of glyphosate and 1.25, 1.5 and 1.75 kg $ha^{-1}$ of paraquat were compared. These herbicides were sprayed using a spray fluid of 500 *l* $ha^{-1}$ of water, using knapsack sprayer fitted with flood jet deflector nozzle and 12 *lb* $inch^{-2}$ of pressure. After standardizing the dose, different time of application *viz*. month of May, August and November were also compared. Based on the results of these green house experiments, a pond experiment was conducted. A farm pond located in the university premises of dimension 70 m x 10 m with a water depth of 1.5 m was divided into four compartments using polyethene sheets stretched and nailed at the ends and middle to bamboo poles. Each compartment was accommodating water hyacinth subjected to treatments *viz*. untreated control, 2, 4-D Na salt 1.5 kg $ha^{-1}$, glyphosate 2.5 kg $ha^{-1}$ and paraquat 1.5 kg $ha^{-1}$. All the green house and pond experiments were laid out in RBD with four replications and observed for reduction in plant height, biomass, reduction in chlorophyll content and mortality percentage. To be precise, only biomass reduction and mortality percentage are presented in this paper. The studies on non-target flora and fauna comprised laboratory studies for comparing the effect of treated water obtained from the pond studies on seed germination percentage of paddy, cotton and ladysfinger. Further, the fishes *viz*. Common carp, Mrigal and Rohu were compared for their response to treatment with 2, 4-D Na salt 1.5 kg $ha^{-1}$, glyphosate 2.5 kg $ha^{-1}$, paraquat 1.5 kg $ha^{-1}$ and untreated control in the presence of water hyacinth, in cement pots as tried for screening herbicides. The tissues of gills, brain, liver and kidneys from the fishes in these treatments were fixed in neutral buffered formalin 10% dehydrated in ascending grades of ethanol, embedded in soft paraffin, sectioned at 5 nm thickness and stained with Hematoxylin and Eosin (H&E) for comparison. The water quality in terms of pH, EC, dissolved oxygen and chemical oxygen demand, were observed at 15 days interval. Mortality percentage of the weed, was calculated using following formula,

$$\text{Mortality of } E.crassipes\ (\%) = \frac{\text{No.of plant died tank}^{-1}}{\text{Total No.of plant stocked tank}^{-1}} \text{X100}$$

The fish mortality was calculated on 32 DAS using the formula.

$$\text{Mortality of fishes } (\%) = \frac{\text{No.of fishes died tank}^{-1}}{\text{Total No.of fishes stocked tank}^{-1}} \text{X}100$$

The experimental data were statistically analyzed using the methods described by Panes and Sukhatme (1978). After subjecting the data to analysis of variance, least significant difference was worked out a 0.05 per cent probability level. The data on percentage values were transformed by angular transformation before analysis.

## RESULTS AND DISCUSSION

### Pot culture experiments - Herbicide effect on weeds

Among the different doses tried, the highest doses *viz*. 1.75 kg $ha^{-1}$ of 2, 4-D Na salt, 3.0 kg $ha^{-1}$ of glyphosate and 1.75 kg $ha^{-1}$ of paraquat recorded the least biomass on 50 DAS and highest mortality of the weed on 35 DAS (Table 1).

**Table. 1.** Effect of different doses of herbicides sprayed on biomass (g) and morality percentage of *E. crassipes*

| Treatments | Biomass (g) 50 DAS | Mortality (%) 35 DAS |
|---|---|---|
| **2, 4-D Na salt** | | |
| Control (Unsprayed check) | 283.51 | 0.01 (0.00)* |
| 2, 4-D Na salt @ 1.25 kg $ha^{-1}$ | 16.31 | 60.0 (75.10) |
| 2, 4-D Na salt @ 1.50 kg $ha^{-1}$ | 14.30 | 76.1 (94.25) |



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| | | |
|---|---|---|
| 2, 4-D Na salt @ 1.75 kg $ha^{-1}$ | 13.41 | 77.0 (95.00) |
| $SE_D$ | 0.89 | 0.37 |
| CD(p=0.05) | 0.90 | 0.75 |
| **Glyphosate** | | |
| Control (Unsprayed check) | 283.51 | 0.01 (0.00) |
| Glyphosate@ 2.0 kg $ha^{-1}$ | 25.91 | 51.3 (61.0) |
| Glyphosate@ 2.50 kg $ha^{-1}$ | 23.87 | 76.2 (95.0) |
| Glyphosate@ 3.0 kg $ha^{-1}$ | 19.93 | 90.0 (100.0) |
| $SE_D$ | 0.95 | 0.57 |
| CD(p=0.05) | 1.90 | 1.15 |
| **Paraquat** | | |
| Control (Unsprayed check) | 283.51 | 0.01 (0.00) |
| Paraquat @1.25kg $ha^{-1}$ | 20.10 | 54.8 (66.85) |
| Paraquat @1.50kg $ha^{-1}$ | 19.50 | 72.9 (90.25) |
| Paraquat @1.75kg $ha^{-1}$ | 15.70 | 74.1 (92.50) |
| $SE_D$ | 1.90 | 0.60 |
| CD(p=0.05) | 3.80 | 1.20 |

*Figures in parenthesis are original values

Among the different seasons compared for application of these herbicides *viz*., May, August and November, no significant difference was observed. Based on the observations it could be inferred that these herbicides can be sprayed during any part of the year, regardless of the seasonal variation (Table 2).

Among the herbicides tested, glyphosate was observed to be more efficient in suppressing the growth of the weed plant by virtue of reducing biomass and mortality percentage. This is because of the efficient absorption and translocation to all the parts of the plant in addition to the effective interruption of biosynthesis of essential amino acids through EPSP synthase activity. Though 2, 4-D Na salt also happens to be a translocated herbicide, the comparatively less efficiency performance is due to comparatively slower process of interruption of protein synthetic mechanism and the ability of water hyacinth to regenerative compensatory growth from vegetative propagules from runners and smaller plant lets. Paraquat, though very effective in tissue disruption by virtue of free radical and superoxide activity and inhibition of photosystem-I, with the activity being mainly contact and restricted to plant parts of exposure, failed to compare with glyphosate (Kannan and Kathiresan, 2002).

**Pond experiment**

Among the herbicides tested, glyphosate showed highest reduction in biomass with a biomass of 5.0g $plant^{-1}$ at 50 DAS which was followed by paraquat and 2, 4-D Na salt. Untreated control showed the highest biomass of 162.40g. The glyphosate application caused highest mortality percentage of 87.80 at 21 DAS. However all the herbicides exerted 100 per cent mortality on 35 DAS (Fig. 1).

**Table 2**. Effect of time of application of selected herbicides on biomass (g) and mortality percentage (%) of *E. crassipes.*

| Treatment | Biomass (g) | | | Mortality (%) | | |
|---|---|---|---|---|---|---|
| | Control (Unsprayed check) | 2, 4-D Na salt @ 1.50 kg $ha^{-1}$ | Glyphosate @ 2.50 kg $ha^{-1}$ | Control (Unsprayed check) | 2, 4-D Na salt @ 1.50 kg $ha^{-1}$ | Glyphosate @ 2.50 kg $ha^{-1}$ |
| Months | 50DAS | 50DAS | 50DAS | 35 DAS | 35 DAS | 35 DAS |
| MAY | 281.56 | 12.75 | 8.21 | 0.01 (0.00) | 75.60(93.80) | 90.00(100.00) |
| AUGUST | 282.41 | 10.21 | 6.27 | 0.01(0.00) | 71.56(90.0) | 90.00(100.00) |



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| | | | | | | |
|---|---|---|---|---|---|---|
| NOVEMBER | 282.40 | 9.70 | 5.00 | 0.01(0.00) | 75.20(93.50) | 90.00(100.000 |
| S.ED | NS | NS | NS | NS | NS | NS |
| CD(p=0.05) | NS | NS | NS | NS | NS | NS |

*NS-Non-Significant

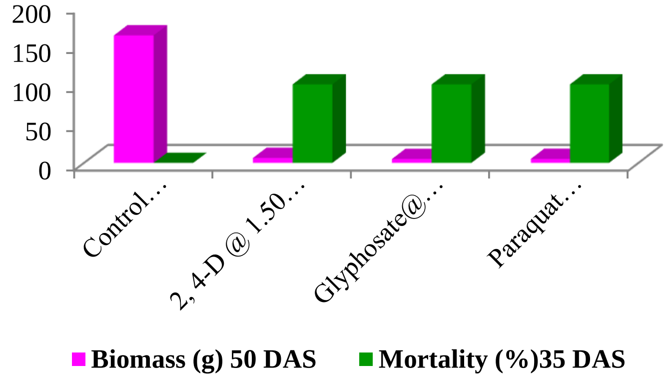


**Fig. 1**. Effect of different Herbicide sprayed on biomass (g) and mortality percentage of *E. crassipes.*

**Effect of treated water on crops and fishes**

Considering the impact of herbicide treated water on the germination of crops also, glyphosate appeared to be safe, recording germination percentages comparable with untreated control, while that of paraquat and 2, 4-D Na salt treated water were slightly inhibitory, recording significantly lesser germination percentages. Similarly, all the fishes *viz*., Common carp, Mrigal and Rohu suffered mortality when subjected to treatment with weed and herbicidal spray. But glyphosate proved comparatively safe, with significantly lesser fish mortality. The treatment with weeds without any herbicidal treatment was also lethal to fishes, recording fish mortality of 27.30 per cent, which is significantly higher than treating the weed with glyphosate (that has shown significantly lesser fish mortality of 26.57 per cent). Similar results were obtained by Kannan and Kathiresan (2002) and Varshney *et al.* (2008). Fishes in water free from water hyacinth as well as any herbicide treatment alone showed no mortality percentage (Table 3).

**Table 3**. Effect of herbicide treated water on germination percentage of crops and fish mortality percentage on 32 DAS.

| Treatments | Germination percentage | | | Mortality percentage of fish | | |
|---|---|---|---|---|---|---|
| | Paddy (7 days) | Cotton (7 days) | Ladysfinger (7 days) | Common carp | Mrigal | Rohu |
| | Irrigation after 24 hours | Irrigation after 24 hours | Irrigation after 24 hours | | | |
| Control (Unsprayed check) | 95.00 | 65.00 | 70.00 | - | - | - |
| 2, 4-D Na salt @ 1.50 kg ha$^{-1}$ | 90.00 | 59.00 | 62.00 | (46.00) 42.70 | (33.33) 35.26 | (25.00)* 30.00 |
| Glyphosate @ 2.50 kg ha$^{-1}$ | 93.00 | 63.00 | 67.00 | (23.30) 28.86 | (16.60) 24.05 | (20.00) 26.57 |



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| | | | | | | |
|---|---|---|---|---|---|---|
| Paraquat @1.50kg ha$^{-1}$ | 90.00 | 57.00 | 63.00 | (42.00)<br>40.39 | (50.66)<br>46.73 | (25.00)<br>30.00 |
| Fish alone | - | - | - | (0.00)<br>0.01 | (0.00)<br>0.01 | (0.00)<br>0.01 |
| Control (Unsprayed check with *E. crassipes* + fish) | - | - | - | (14.50)<br>22.38 | (50.00)<br>45.0 | (21.70)<br>27.30 |
| S.E$_D$ | 1.00 | 1.00 | 1.00 | 3.16 | 2.62 | 0.36 |
| CD (p=0.05) | 2.00 | 2.00 | 2.00 | 6.32 | 5.25 | 0.73 |

*Figures in parenthesis are original values before angular transformation

**Herbicides effect on water quality**

Chemical Oxygen Demand (COD), the amount of oxygen required to oxidise all oxidisable substrate present in the water is used as a typical measure of contamination. In all herbicide treated system, COD was significantly higher over control and this shows the presence of oxidisable organic matter by virtue of dying and decaying plant (Table 4). The 2, 4-D Na salt application caused highest COD of 38 and 59 ppm at 15 and 30 DAS followed by paraquat and glyphosate. The dissolved oxygen, an estimate of oxygen as dissolved in water implies its supportive ability to organism living in aquatic environment like fishes. The DO of untreated water was 7.82 and 7.20 ppm on 15 and 30 DAS, respectively. The DO content was declining significantly in all treatments of which 2, 4-D Na salt recorded the lowest of 6.89 and 5.02 ppm on 15 and 30 days after spray, respectively. This decline in dissolved oxygen was in agreement with a previous study by Kannan and Kathiresan (2002). The pH and electrical conductivity were also affected by the treatments. Glyphosate was observed to be safe, regarding the impact considering all these water quality characters.

**Table 4**. Effect of different herbicide spray on physicochemical parameters of water

| Treatments | pH | | COD (ppm) | | Conductivity (milli mhos) | | DO (mg/lit) | |
|---|---|---|---|---|---|---|---|---|
| | 15 DAS | 30 DAS | 15 DAS | 30 DAS | 15 DAS | 30 DAS | 15 DAS | 30 DAS |
| Control unsprayed check | 7.01 | 6.94 | 32 | 43 | 11.5 | 11.1 | 7.82 | 7.20 |
| 2,4-D Na Salt@ 1.5 kg ha$^{-1}$ | 7.06 | 6.90 | 38 | 59 | 11.7 | 11.0 | 6.89 | 5.60 |
| Glyphosate@ 2.5 kg ha$^{-1}$ | 7.12 | 6.89 | 36 | 49 | 11.9 | 10.9 | 7.02 | 6.32 |
| Paraquat @.1.5 kg ha$^{-1}$ | 7.0 | 6.93 | 36 | 53 | 11.0 | 10.6 | 6.95 | 6.20 |
| S.E$_D$ | NS | NS | 1.00 | 3.0 | NS | NS | 0.15 | 0.17 |



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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| CD (P=0.05) | NS | NS | 2.00 | 6.0 | NS | NS | 0.19 | 0.22 |

NS – Non Significant, COD: Chemical Oxygen Demand, DO: Dissolved oxygen

**Effect on histopathology study of fish organs**

All the herbicides imparted tissue destruction in all the different types of organs compared. Regarding the gills, congestion of blood vessels in the primary lamellae and hyperplasia of branchial arch were some of the injuries suffered. Liver suffered vacuolization, focal necrosis and common lesion with different herbicides. Increased granular layer and swelling of pyramidal cells in the brain, degeneration in tubular epithelium and expanded renal tubules of the kidney are also observed with herbicide treatments (Figure 2, 3, 4 & 5). Similar results were obtained by Kathiresan and Ramah (2000); Bharat Bhusan Patnaik *et al*. (2011) and Reza Sayrafi *et al.* (2011).

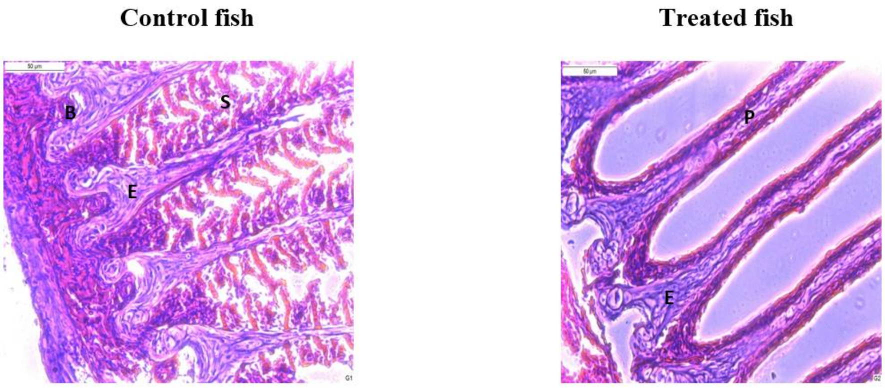


**Fig. 2.** GILLS B-Basement membrane, E-Epithelium layer S-Secondary lamellae, P-Primary lamellae.

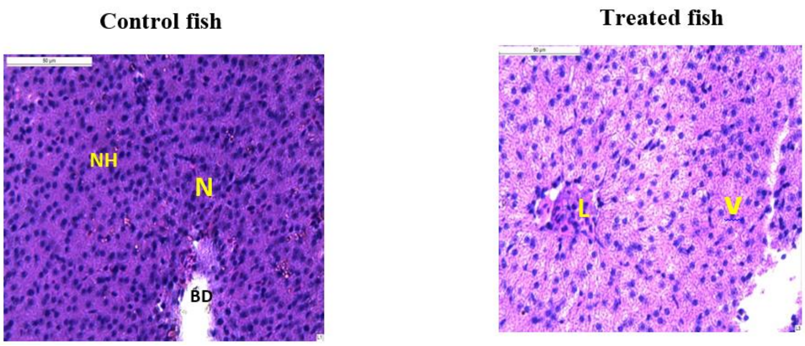


**Fig. 3.** LIVER NH-Normal hepatocytes, BD- bile duct, N-nucleus, L-Leukocytes, VH-vacuolization hepatocytes.

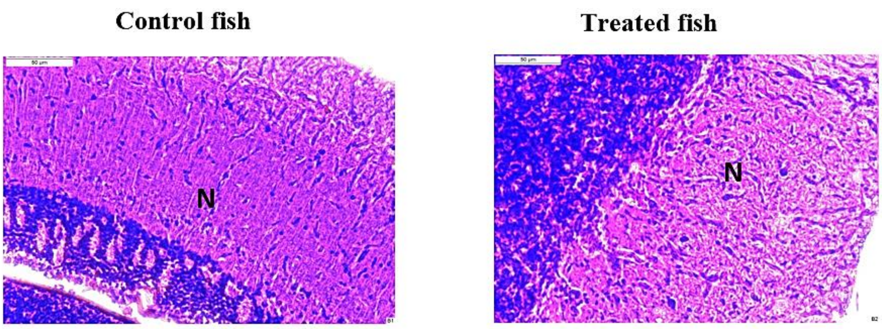




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**Fig. 4.** BRAIN N-Neuroglial cells.

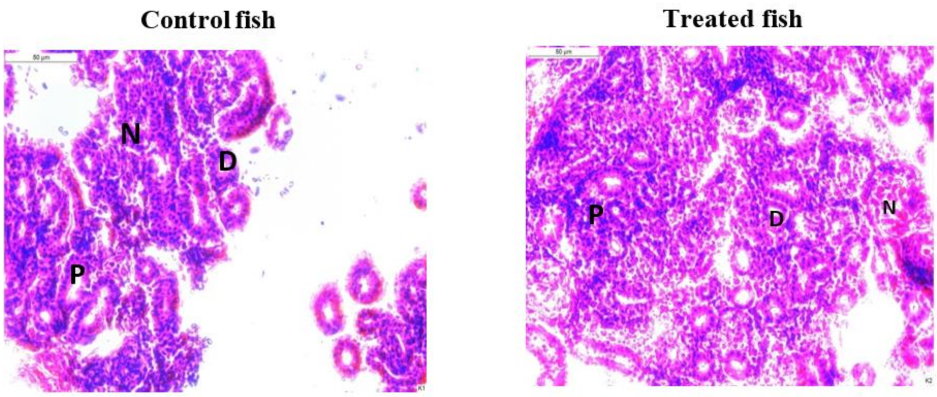


**Fig. 5.** KIDNEY D-Distal tubule, P-Proximal tubule, N-Nephron.

## CONCLUSIONS

Herbicides like 2, 4-D Na salt, glyphosate and paraquat offer rapid and efficient control of water hyacinth and their weed control efficiency is unaltered by the differing seasons. The treated water is safe for crops. The water quality remain altered in terms of Dissolved Oxygen and Chemical Oxygen Demand, where as no significant difference was observed in other characters. Fishes were shown to suffer mortality and tissue damage by the herbicides.

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# OBSERVATION ON THE DEVELOPMENT OF IMPORTANT WEEDS AND INVASIVE ALIEN PLANT SPECIES IN INDONESIA

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**ABSTRACT** Weeds inventory in Indonesia has been conducted and published since the colonial time, dealing with weed species in specific conditions and continuing up to now. Weed problems varied with different types of crops & habitats and differed from time to time. Weeds in agricultural production systems have long been recognized, recently environmental weeds or known as Invasive Alien Plant Species (IAPS) have received increasing attention in Indonesia because their recognized impact on native biodiversity. Since the issue on Invasive Alien Plant Species (IAPS) raised during the "Earth Summit Conference" in Brazil in 1992, IAPS have drawn more attention in Indonesia, and Indonesia has ratified the Convention on Biological Diversity in 1994. After the year of 2000, articles and list of IAPS scattered in several publications, mostly reported from National Park and Botanical Garden. Some species recorded have drawn more attention, because of it potential invasiveness and threat to the community. Some important species are described in this paper.

***Keywords:*** *Weeds, invasive alien plant species, Indonesia*

## INTRODUCTION

In common usage, weeds have been defined as plants that are growing in a place where it is unwanted (Booth 2003). This terminology is very familiar for their impacts on agricultural system. They create competition with crop plants, increase production costs, reduced quality of crops and animals, or other serious problems. The invasive alien plant species, some of them have been well recognized as weeds in agricultural production system, while weed in natural ecosystem are recognized as environmental weed or as invasive alien plant species (IAPS).

Recently, the problem of IAPS requires urgent responses due to its impacts and threatening natural habitat after habitat destruction of natural ecosystem. The presence of IAPS creates a global problem. Ecologically, IAPS not only inflict a considerable agricultural loss, but also could transform the structure and species composition of ecosystem by repressing or excluding native species, altering the ecosystem, and reducing the biodiversity. Therefore some people known IAPS as environmental weeds.

IAPS raised during the "Earth Summit Conference" in Rio de Janeiro, Brazil in 1992. The conference produced Convention on Biological Diversity (CBD) on the effort to prevent the introduction, eradicate alien species that threaten ecosystem, habitat and native species. Indonesia as one of mega biodiversity country that was ratified the CBD through UU No.5 Tahun 1994 (Wijanarko 2001). It is show that Indonesia has a seriousness to restrict and regulate the IAPS as an effort to avoid or minimize the threat of biodiversity.

Indonesia with an area of 7 400 000 km2, with ocean area 74.4%, terrestrial area 25.5% and total number of islands 17 508 has a diverse problems in between their island. With these large areas surveillance throughout Indonesia should be conducted intensively and has to be up dated from time to time.

## INVENTORY OF WEEDS AND INVASIVE ALIEN PLANT SPECIES

The problems of weed are different on each habitat, cultivated area, regions and vary from time to time. Some weeds were not known yet long time ago, but it is now reported as invasive weeds. Therefore a comprehensive weeds and IAPS inventory is needed to assist the management of weeds and IAPS in Indonesia.



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Weeds inventory has been conducted and published since the colonial time, dealing with weed species in specific conditions and continuing up to now. In 1890-2011, several books on brief description on weeds species in agro-ecosystem was written by Kooders (1898), Blekkink (1902), Wigman (1908), Backer and van Slooten (1924), Jochem (1932), Kern (1952), Meijer (1957). Then, there are several comprehensive books that released on 1970-1980 i.e. 50 important weeds in the plantations (Soedarsan & Rivai 1975), Weeds on Horticultural Land (Everaats 1981), Weeds on Aceh and North Sumatra Plantations (Nasution 1986), and the famous book Weeds of Rice in Indonesia (Soerjani *et al.* 1987). SEAMEO BIOTROP has also been published the 24 serial number of Weed Info Sheet in 1990-1994, and Tjitrosoedirdjo *et al.* (2011) on the weeds of cacao agroforestry at Central Sulawesi Indonesia.

Ten important aquatic weeds in Indonesia were reported by Soerjani (1996): *Ceratophyllum demersum J.G. Klein ex Cham.*, *Hydrilla verticillata* (L.f.) Royle, *Eichhornia crassipes* (Mart.) Solms, *Mimosa pigra* L., *Najas indica* (Willd) Cham., *Nelumbo nucifera* Gaertn. , *Panicum repens* L., *Potamogeton malaianus* Miq. *Salvinia molesta* D.S. Michell, and *Scirpus grossus* L. He also has been reported the weeds of plantations in Indonesia, as shown in Table 1.

**Table 1.** List of plantation's weeds in Indonesia based on Soerjani *et al*. (1986)

| No. | Species Name | Comodities |
|---|---|---|
| 1 | *Imperata cylindrica* (L.) Raeuschel | rubber, tea, oil palm, coffee, kina, coconut, fiber plants |
| 2 | *Mikania micrantha* Kunth. | rubber, oil palm, coconut |
| 3 | *Paspalum conjugatum* Berg. | rubber, oil palm, coconut, cocoa, kina |
| 4 | *Cyperus rotundus* L. | coffee, sugarcane, fiber plant, tobacco |
| 5 | *Mimosa invisa* Mart. | coconut, tobacco |
| 6 | *Borreria alata* (Aubl.) DC. | tea, cocoa, coffee |
| 7 | *Euphorbia prunifolia* Jacq. | tea, coffee, tobacco |
| 8 | *Chromolaena odorata* (L.) R.M. King & H. Robinson | cocoa, kina, coconut |
| 9 | *Ageratum conyzoides* L. | cocoa, coffee, sugarcane, tobacco |
| 10 | *Cynodon dactylon* (L.) Pers. | sugarcane, fiber plant |

This list is indicated only for plantation crops. Soerjani *et al*. in 1987 in their book "Weeds of Rice in Indonesia" reported 266 species of weeds in Indonesian rice field. Among this number there are 35 species reported as noxious, important and serious weeds, 16 species of grasses, 7 species of sedges and 12 species of broad leaves. Almost 45.5% of the important weeds represented by grasses. Sumadijaya (2011) added five alien grasses species in Indonesia as a new records which are *Digitaria adcendens* (H.B.K.) Henr. (Taiwan); *Diplachne polystachya* Backer from Japan, Europe and India; *Isachne pulchella* Roth. Ex R & S. from Asia; *Leptochloa fusca* (L.) Kunth from South China and India; *Setaria pallide-fusca* (Schum.) Stapf & Hubb. From India.

SEAMEO BIOTROP began to expand the research field, not only about weeds as well but also IAPS in Indonesia. Inventory on the introduce species base on the existing literature was published by SEAMEO BIOTROP and Ministry of Environment in 2003 (Anonymous, 2003), reporting 36 introduced species. There are 17 % of it is indicated of being invasive. Following this publication a data base on invasive alien plant species was developed including 144 sp and available at the BIOTROP website. The data base is in process of updating.

Tjitrosoedirdjo (2005) was added list of important plants that are recognized as weeds and IAPS (Table 2). The list was described based on a threat of their distribution in Indonesia.



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After the year of 2000, articles IAPS scattered in several publication, mostly reported from National Parks and Botanical Garden (Hartemink, 2010; Haryanto, 1997; Irianto & Tjitrosoedirdjo, Irianto et al, 2011, Master *et al.*, 2013; Mutaqien et al, 2011; Siel & Padmanaba, 2011; Suryanto et al 2010; Sutomo, 2010; and Tituhurua & Sunaryo, 2012).

**Table 2**. List of weeds and IAPS in Indonesia prepared by Tjitrosoedirdjo (2005)

| No. | Species Name | Distribution |
|---|---|---|
| 1 | *Acacia nilotica* (L.) Willd. Ex Del. | West and East Java, Papua |
| 2 | *Austroeupatorium inulifolium* (Kunth) R.M. King & H. Rob | West Java, Bengkulu, West Sumatra |
| 3 | *Chromolaena odorata* (L.) R.M. King & H. Rob. | Throughout Indonesia |
| 4 | *Clibadium surinamense* L. | Sumatra, Central Sulawesi |
| 5 | *Eichhornia crassipes* (Mart.) Solms | Sumatra, Kalimantan, Nusa Tenggara, Papua |
| 6 | *Eupatorium sordidum* Less | West Java |
| 7 | *Hydrilla verticilata* (L.f. Royle) | Java, Sumatra, Kalimantan, Sulawesi |
| 8 | *Mikania micrantha* Kunth | West and East Java, South Sumatra, Papua |
| 9 | *Mimosa diplotricha* C. Wright ex Sauvalle | Troughout Indonesia |
| 10 | *Mimosa pigra* L. | Java, Sumatra, Kalimantan, Papua |
| 11 | *Passiflora edulis* Sims | West and Central Java |
| 12 | *Penisetum polystachion* L. Schult. | West Java |
| 13 | *Piper aduncum* L. | East Kalimantan, Central Sulawesi, Jayapura, Papua |
| 14 | *Salvinia molesta* D.S. Mitchell | Java, Sumatra, Kalimantan, Papua |
| 15 | *Stachytarpeta jamaicensis* (L.) Vahl. | Java, Sulawesi, Timor, Papua |

As people move around the world, they bring together plants with them. Expanding global trade in agriculture, forestry and other industries that depend on raw materials has allowed the transport of species to various parts of the world including Indonesia. Alien species are imported to Indonesia for cultivation such as food crops, horticulture, plantation, forest plantation, ornamental aquarium plants, collection of the botanical garden, experimental plants or other curiosities. Aside from plants purposely imported one there are also introduced plant propagules contaminating imported agricultural products (Tjitrosoedirdjo, 2005).

Not all the alien species are harmful, most of them are crop or ornamental plants. Some species are naturalized and adapted well to the local environment, some become invasive. Some of the species just recognized as weeds and IAPS after they infesting certain areas. The weeds and IAPS would be a big problem while they expand its distribution in Indonesia. There are some national parks as well as some botanical gardens in Indonesia suffering from the occupation of invasive species. Some species recorded have drawn more attention, because of it potential invasiveness and threat to the community (Table 3). Most of them are IAPS. It shows that IAPS has threatened the forests of Indonesia.

**Table 3.** List of important weeds and IAPS in Indonesia

| No. | Species Name | Family | Invaded Area |
|---|---|---|---|
| 1 | *Acacia decurrens* Willd. | Mimosaceae | Mt. Merapi National Park, Yogyakarta (Suryanto *et al.* 2010) |
| 2 | *Acacia nilotica* | Mimosaceae | Baluran National Park, East Java |



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| | | | |
|---|---|---|---|
| 3 | *Achyranthes aspera* Duss | Amaranthaceae | Baluran National Park, East Java |
| 4 | *Arenga obtusifolia* Mart. | Arecaceae | Ujung Kulon National Park in West Java, and several region of Java & Sumatra (Haryanto 1997) |
| 5 | *Bartlettina sordida* (Less.) R.M.King & H.Rob. | Asteraceae | Mt. Gede Pangrango National Park, West Java |
| 6 | *Bellucia pentamera* Naudin | Melastomataceae | Mt. Palung National Park, West Kalimantan |
| 7 | *Bidens biternata* (Lour.) Merr. & Sherf | Asteraceae | Baluran National Park, East Java |
| 8 | *Brugmansia suaveolens* (Willd.) Sweet | Solanaceae | Mt. Gede Pangrango National Park (Mutaqien *et al.* 2011) |
| 9 | *Cecropia pachystachya* Trécul | Urticaceae | Invasive within the Bogor region (Webber *et al.* 2011) |
| 10 | *Cecropia peltata* Billb. ex Beurl. | Urticaceae | Invasive within the Bogor region (Webber *et al.* 2011) |
| 11 | *Cestrum aurantiacum* Lindl. | Solanaceae | Mt. Gede Pangrango National Park & Wornojiwo forests of Cibodas Botanic Garden, West Java(Mutaqien *et al. 2011*) |
| 12 | *Chimonobambusa quadrangularis* Makino | Poaceae | Mt. Gede Pangrango National Park & Wornojiwo forests of Cibodas Botanic Garden, West Java(Mutaqien *et al. 2011*) |
| 13 | *Cobaea scandens* Cav. | Cobaeaceae | Mt. Gede Pangrango National Park |
| 14 | *Eleutheranthera ruderalis* Hitchc. | Asteraceae | Baluran National Park, East Java |
| 15 | *Flemingia lineata* (L.) W.T.Aiton | Fabaceae | Baluran National Park, East Java (Irianto *et al.* 2011) |
| 16 | *Maesopsis eminii* Engl. | Rhamnaceae | Mt. Gede Pangrango National Park, West Java (Tihurua & Sunaryo 2012) |
| 17 | *Merremia peltata* (L.) Merr. | Convolvulaceae | Bukit Barisan Selatan National Park, Lampung (Irianto & Tjitrosoedirdjo 2010) |
| 18 | *Montanoa grandiflora* DC. | Asteraceae | Mt. Gede Pangrango National Park & Wornojiwo forests of Cibodas Botanic Garden, West Java; It is also found in the north of Bandung (Mutaqien *et al. 2011*) |
| 19 | *Passiflora edulis* L. | Passifloraceae | Mt. Gede Pangrango National Park, West Java |
| 20 | *Piper aduncum* L. | Piperaceae | Lowland forests of Papua New Guinea (Hartemink 2010), Wornojiwo forests of Cibodas Botanic Garden & roadsides of West Java (Mutaqien *et al.* 2011), the herbarium specimens were collected from Java, Sumatra, Kalimantan, Sulawesi, & West Papua |
| 21 | *Strobilanthes hamiltoniana* (Steud.) | Acanthaceae | Mt. Gede Pangrango National Park & Wornojiwo forests of Cibodas Botanic Garden, West Java(Mutaqien *et al. 2011*) |
| 22 | *Thespesia lampas* (Cav.) Dalzell | Malvaceae | Baluran National Park, East Java (Irianto *et al.* 2011) |



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Bukit Barisan Selatan National Park in Sumatra has an area of 365 000 Ha and 7 000 Ha of the area infested by *Merremia peltata* (L.) Merr. *M. peltata* is an indigenous species, however when the forest disturbed it is become invasive and grows very fast (Irianto & Tjitrosoedirdjo, 2010; Master *et al.,* 2013).This liana was covering the soils, forming a dense network, smothers the tree and causing the death due to it is inhibit their photosynthesis (Master *et al.* 2013). This plant is a climber it could cover the tree up to more than 25 meter high. It is also reported could reach 9 m to climb within 3 months. The dense mats of *M. peltata* were impeding the activity of tigers, rhinoceros, and elephants. There is a tendency fauna moved to plantation and residential areas. As a result, potentially lead to conflict between human and fauna as well as animals hunt.

In Baluran National Park in West Java, *Acacia nilotica* has invaded 7 500 ha area of savanna from the total area of 25 000 ha savanna. This native plant of South Africa was altering the savanna ecosystem and threats the biodiversity of national park (Djufri 2004). Its presence was reduces the habitat and grazing ground for Banteng (*Bos javanicus*) and the other mammals. *A. nilotica* has also been reported as colonizer at Mount Gede Pangrango Nasional Park in West Java, Wasur National Park in Papua and Komodo National Park in Komodo Island.

In Baluran National Park, broad leave weeds under the canopy of *A. nilotica* were mainly dominated by *Eleutherantera ruderalis*, *Bidens biternata*, and *Achiranthes aspera*. The other problem appeared after Baluran National Park eradicating the *A. nilotica*, the vegetation replaced by broad leaves *Thespesia lampas* and *Flemingia lineata* (Irianto *et al.* 2011). In fact, the existence of these broadleaved weeds was aggravating the grazing area, changing savanna vegetation into shrub that suppresses the growth and production of the grass.

In 2010, *Acacia decurrens* spread at Mount Merapi Nasional Park (Gunawan *et al.* 2013). If allowed to dominate, they will inhibit the growth of another species. Previously, *A. deccurrens* were introduced to Mount Merapi when the area has yet declare as national park. *A. decccurens* were planted with pine. Currently this plant began invaded after Mount Merapi eruption. The succession process contributed to the growth of this species. Invasion of *A. deccurens* was reached 100 ha of 6400 ha total area of national park.

In Ujung Kulon National Park, there is problem with the sugar palm *Arenga obstusifolia* (langkap). Indeed, this plant is local species of national park but the growth is very fast and dominated the national park. They cover an estimated 18 000 hectares of the Ujung Kulon peninsula (GISD 2011), home to the 'Critically Endangered (CR)' Javan Rhinoceros (*Rhinoceros sondiacus*). The spread and dominance of *A. obtusifolia* has impacts on undergrowth that comprises the food of the Javan Rhino; including seedling regeneration because of shading and poor light conditions caused by the canopy of the palm (Haryanto 1997). The invasion of langkap is estimated as part of a succession process after the eruption of Mt. Krakatau in 1883.

Invasive species also occur in many other forests area. *Bartlettina sordida* previously known as *Eupatorium sordidum* is highly invasive in the Mount Gede Pangrango National Park together with *Passiflora edulis*, *Brugmansia suaveolens*, *Cestrum aurantiacum, Chimonobambusa quadrangularis, Clidemia hirta, Cobaea scandens, Eupatorium riparium, Maesopsis eminii,* and *Strobilanthes hamiltoniana*. Some of them firstly planted at Cibodas Botanic Garden, escaped and spread to the forest.

*Bartlettina sordida,* native plant to Mexico was introduced to Indonesia through Cibodas Botanic Garden in October 1899. This plant is planted as ornamental plant and is commonly known as Babakoan. Now, this plant was easily found along the climbing path and open area toward Cibeureum waterfall, Mt. Gede Pangrango National Park. They are threatening the local flora of Mount Gede Pangrango National Park and remove the endemic plants (Sunaryo *et al.* 2012). *B. sordida* invaded the region by forming dense clumps that shade out other plants. Their distribution is in West Java as far as known.



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*Chimonobambusa quadrangularis* was introduced from Japan to Cibodas Botanic Garden in 1994. The bamboo originated from China and was reported as invasive species in Hawaii. This plant is used as bulkhead between Cibodas Botanic Garden and Mt. Gede Pangrango National Park. This bamboo is potentially invade the national park due to it is grow very fast. According to Gunawan *et al.* (2013), bamboo is allelopathic and has the dense canopy that can suppress the growth of another plant. They potentially invasive and can limit the growth of the other vegetation.

*Maesopsis eminii* (kayu afrika) is found predominantly in the area of Bodogol, Mt. Gede Pangrango National Park. It is native to Tropical Africa and brought for the first time to Indonesia in Java, 1920. This species able to adapt at an altitude 500 m-1500 m, have tolerance to several types of soil, spread by seeds which is distributed by birds and mammals, the seeds have dormancy period for more than 200 days, but it can be faster in humid soil conditions. The saplings of this plant are also tolerant to shade, so that it can grow well in forests that have not been disturbed (Sunaryo *et al.* 2012).

*Cobaea scandens* is originally from Central and South America, reported as common weed in New Zealand (Froude 2002) and now has been spread in Mt. Gede Pangrango National Park, West Java, Indonesia. It is fast growing vine that can grow up to 12 m in a year. The vines can grow over trees and shrubs forming a dense canopy, smothering native plants.

Some species of a genus *Cecropia* have been introduced to Bogor Botanic Garden in 1997 (Weber *et al.* 2011). However only two species i.e. *C. peltata* and *C. pachystacya* that were spread in Bogor region, and *C. peltata* was reported become highly invasive within Bogor. It has reached Jakarta to the north and Mt. Gede Pangrango National Park to the south (Sheil & Padmanaba 2011). This tree is native to the Neotropics (American Tropics and subtropics). In Bogor, this species easily found in open and disturbed areas, such as down slope and along river valleys.

Some alien species also occurred in Wornojiwo forest of Cibodas Botanic Garden (KRC) such as *Montanoa grandiflora, M. hibiscifolia, Sanchezia nobilis, Calathea lietzei, Brugmansia candida, Cestrum elegans* (Mutaqien *et al.* 2011). Most of the species is not recorded as invasive in the other area, but it is possible that eventually this species is going to be invasive. For example, recently *Montanoa grandiflora* can be seen in the forest of Mt. Gede Pangrango National Park. It is also can be found in the north of Bandung. Recognizing the invasive species is needed to assist preventing, controlling and eradicating them.

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# INVASIVE WEEDS AND VEGETATION DYNAMICS IN ASSAM

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**ABSTRACT** Assam, representing the valley areas of north-east India, holds nearly 132 species of primitive angiosperms and 23 gymnosperms. The dicotyledonous flora of Assam is composed nearly of 21% exotic species. The number of exotic monocots is also equally high as the understory ground coverage of tropical and subtropical rainforests, riverrine grasslands and swampy ecosystems are rich niches of monocot flora. Being the transitional belt of the Eastern Himalayas and NE India biogeographic zones, Assam is experiencing emigration of Indian biodiversity and immigration of Eurasian, Australian and Pantropical biodiversity since long past. The exotic species find suitable niches in undulating lands and climatic regimes. The invasiveness of alien species is gradually modified, neutralized and digested by ecological functions of dynamic vegetation of Assam. Twenty such invasive weeds with high aggressivity are the most dominant invaders since last couple of decades which have havocked not only the farming community, but also the indigenous vegetation through their serious interference in different cropland and forest ecosystems. Of these, *Ageratum houstonianum, Chromolaena odorata and Eichhornia crassipes* are the best examples of 'driver' species of declining indigenous elements and alteration of native ecosystems. The species, whose activities can be discussed under the Fluctuating Resource Hypothesis, viz. *Imperata cylindrica* and *Lantana camara,* can be grouped as 'passenger' species. However, the species which are best fitted with 'back-seat diver' model, where interactions between disturbance and invasion paid an important role in invasion-dynamics are: *Parthenium hysterophorus and Mimosa diplotricha* that possess strong allelopathic potential and tremendously high competitive ability. Several ecosystems and nearly a hundred of indigenous plants are under direct pressure of these invaders in Assam; and indirectly a number of endemic species are under threat because of multidimensional influences of these invasive weeds.

***Keywords:*** Assam, ecosystem change, invasive weeds, vegetation dynamics,

## INTRODUCTION

In searching the origin of Phanerogams, Takhtajan (1969) found the place from Assam to Fiji, where flowering plants are believed to be originated or cradled. The present political Assam, representing the valley areas of north-east India, holds nearly 131 species of primitive angiosperms (Barua et al. 1988) and 23 gymnosperms (Chowdhury 2005), out of 4020 flowering plants. The dicotyledonous flora of Assam is composed nearly of 21% exotic species (Goswami 2007). The number of exotic monocots is also equally high as the understory ground coverage of tropical and subtropical rainforests, riverrine grasslands and swampy ecosystems are rich niches of monocot dominated vegetation. Assam is in the transitional belt within the biogeographical zones of Sino-Himalayan and north-east India (Rodgers 1985). This subtropical region is experiencing emigration of Indian biodiversity and immigration of Eurasian, Australian and Pantropical biodiversity due to availability of suitable niches (Rao 1994; Barua and Bhagabati 2007). However, in recent decades, human triggered plant migration becomes considerably faster; many of these appear as 'invasive' invaders. In terrestrial ecosystems, plants interact most dramatically with environmental and landscape changes, often reverberating to higher trophic levels (Vila et al. 2007). The potential influence of invaders on change in species composition is the result of a variety of functions involving succession mechanisms and competition factors at local scales depending upon resource availability. The invasiveness of alien species is gradually modified, neutralized and digested by ecological



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functions of dynamic vegetation, which is rather prominent in the context of Assam. Keeping these issues in mind, a study was conducted to explore the most invasive weeds in Assam and their role in vegetation dynamics.

## MATERIALS AND METHODS

Assam state is basically the valley area of north-eastern India. Two mighty rivers, Brahmaputra and Barak pass through the state with innumerable wild streams and tributaries. A region of low to medium hills has separated the Brahmaputra and Barak valleys. The soil is composed of old alluvium (lateritic) in hills and new alluvium in valley areas. The geographical location of this place in the subtropical region of northern hemisphere and the geology of the land comprising of archean to recent formations and the high humid climate make this place ideal shelter for migratory biodiversity.

The invasive weed flora has regularly been monitored since 1983 in different districts of Assam under the All India Coordinated Research Project (AICRP) on Weed Control at Assam Agricultural University, Jorhat. One square meter quadrates were used in randomized manner to quantify the floristic resources. Since 1990, the density, frequency, area coverage and their relative values and finally the importance value index were computed to determine the dominance spectrum of recorded weed species. Based on this spectrum, only the most dominant species were detected, and their variations and authenticity in identification were confirmed after a series of critical taxonomical studies including consultation of herbarium of Botanical Survey of India, Forest Research Institute and that of KEW. Twenty most dominant invasive weeds in last five years were selected from the series of information under this project and their status in Assam was evaluated from the information collected since 1983. These weeds were classified into; driver', 'passenger' and 'Back-seat-Driver' groups based on their role in influencing the associated vegetation. The 'driver' species of declining indigenous elements and alteration of native ecosystems introduced traits or biological processes leading to fundamental alterations of ecosystem properties and changes in ecosystem function of invaded areas with the consequence that the entire native community may be altered or eradicated (Vitousek et al. 1987; Bauer 2012). In contrary, the 'passenger' species (Didham et al. 2005; MacDougall and Turkington, 2005; Didham *et al.*, 2005) become abundant with decline of native plant communities, but that co-relation is largely a result of the association of the 'passenger' species with ecosystem change; removal of 'passenger' is likely to have significant benefits for the native ecosystem. Bauer's (2012) 'back-seat drivers' require or benefit from disruptions of ecosystem processes or properties that lead to declines of native species, but also contribute to changes in ecosystem properties and further decline of native species.

## RESULTS AND DISCUSION

Speciation because of meeting of variety of species as well as overall impact of rising of Himalayas (Janaki 1960) is a common feature of this region which is instrumental in development of indigenous species coupled with increasing endemism. Takhtajan's search for the place of origin of angiospermic plants in Assam is also supported by the geology, geographic position and its indigenous biodiversity. However, the capacity of the land in sheltering migratory flora including the alien invasive weeds has created threats for existence of many indigenous species by offering high degree of competition in the process of plant succession. The increasing aggressivity of the invasive species is accelerated by the advancement in transportation facilities, lake of natural enemies, free migration and anthropogenic factors. The present study has shown the changes in dominant invasive weeds in Assam during the last few decades and helped in enlisting 20 most invasive species that dominated the present cropland and non-cropland situations (Table 1).

Amongst these invasive weeds, *Ageratum houstonianum* was probably introduced in India in the early part of $19^{th}$ century. During this period of 200 years, it has occupied the niches of many herbaceous upland species including that of *A. conyzoides*. With its flushes of



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simultaneously emerged seedlings, it easily smothers the associated plants as 30,000-40,000 seedlings/m$^2$ and 8-20 mature plants/m$^2$ are usually recorded in agricultural fields. This facultative weed has invaded almost all upland and medium lowland crops of Assam and survived under 7-10 days shallow submergence. In shifting cultivation areas ('*jhum'*), *A. houstonianum* is one of the few early colonizing aggressive species. It propagates both by seeds and prostrate portion of the stem, and shows perennating habit. The 'driver' invasive species like *Ageratum houstonianum* and *Borreria articularies* with number of seedlings germinated at a time in succession flashes and covered the ground by smothering other species in the beginning and later making colonies with close canopies, and straggling branches of *Borreria* controlled the growth of the associated species. Changes in species composition and heavy reduction of species richness, particularly in the herbaceous vegetation of crop fields, roadside and forest edges are normal incidences of infestation by such invasive species.

*Hyptis suveolense* is one of the most dominant species of sugarcane fields, '*jhum*' fields and along the edges of forests, tea gardens and road sides. On the other hand, *Cassia tora* and *Chomolaena odorata* have extensively spread in the upland open places, along the roadsides, floors of the plantation forests, grazing fields, bordering playground and woodlands. *Chromolena odorata* has shown more aggressivity and competitive ability by propagating pappus bearded flying seeds and drooping perennating stems. *Mikania micrantha* is the 'driver' species of climbing habit and invaded both cropland and non-cropland areas. Grassy species, *Dichanthium assimile* has extended its distribution from the hills of Mizoram to the tea gardens of the Barak valley region of Assam, and created havoc by acquiring glyphosate resistance capacity. Its increasing invasiveness can easily be correlated with the effects of climate change. In aquatic ecosystems in the flood-prone region, the most dominant 'driver' invaders are: *Echhornia crassipes* and *Leersia hexanda*. *Rosa multiflora* is dominant weed in Kaziranga National Park. Overgrowth of the clumps of this species is responsible for quick depletion of the grazing areas. Moreover, its straggling thorny branches and canopy of the clamps are making passing through impossible even for the rhinoceros and elephants. This species is a native of Japan and migrated to north-east India particularly to the Kaziranga National Park during 1960s. *Rosa multiflora* is responsible for eliminating a number of grassy and grass-associated aquatic and semi-aquatic species, and simultaneously altering the natural grassland ecosystem of the Park.

Introduction of *Ludwigia linifolia* dates back to 40-50 years. It has dominated the rice fields of both upland and medium lowland situations from seedling stage to harvest and sometimes even after harvest of the crop. A study revealed that this highly competitive weed maintained almost 1:1 ratio in height with associated rice varieties, developing wider bushy canopies and competing for nutrients with its long spreading shallow root systems (Anonymous 1996). In non-cropland areas, this species is confined to shallow water bodies and survives as half-submerged, marginal or anchored emerged weed in the same habitat of *Cyperus digitatus, Acanthospermum hispidum* and *Leersia hexanda*. Along with *L. linifolia,* aquatic species like *Cyperus iria, Ludwigia adscendens* and terrestrial species *Lantana camara, Imperata cylindrica* and *Mimosa pudica* are found to be the 'passenger' species in Assam. These cannot be considered as the limiting factors for native species because their eradication helps in restoration of ecosystem. *Ludwigia decurance* is a new addition to this group, migrated to Assam 10-15 years back and spread very fast in transplanted rice lands.

*Parthenium hysterophorus* was first recorded as invasive weeds in and around Guwahati city during the 1980s. Despite all efforts for managing this, it has spread to almost all cities and towns, and along the major roads and railway tracks of the state. It has now become one of the top three invasive weeds in the hill zone of Assam. Fast growing nature, quick colony forming habit, high competitive ability and allelopathic potential have made *Parthenium* a typical example of 'Back-Seat-Driver', which is directly responsible for elimination of about 94 pre-existing species (Table 2). *Mimosa diplotrica* appeared as a troublesome invader in the Kaziranga National Park in 2000s. It has spread not only to the forest ecosystems of both



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northern and southern banks of Brahmaputra but also to the tea-gardens, sugarcane fields and many other cropland and non-cropland areas. Its thorn less variety (var. *innermis*) has also shown mimosine toxicity in the ruminants, while the thorny variety (var. *diptotrica*) has developed high smothering affects and thick colonies damaging the associated vegetation. Almost similar monopoly has also been shown by *Ipomoea carnea* var. *fistula* in the aquatic ecosystem, particularly along the transplanted rice growing areas. By destroying almost all associated species, *Ipomoea* forms a thick mat of prostrate stems and blocks the natural water flows and thus triggers artificial flood followed by siltation. *Ipomoea carnea* has probably been introduced to Assam during 1970s. This species became the most troublesome anchored aquatic and marginal weeds next to *Eichhornia crassipes* during the last 30-40 years. Of these three prominent 'Back-Seat-Driver' weeds, *Ludwigia peruviana,* with its semi-aquatic bushy nature, has been coming up as another threat in aquatic and marshy lands of Assam. This species was first recorded in the Diphu town during 2003 (Barua 2010). The survey conducted in 2013 revealed its extensive spread towards the lower elevations of Karbi-Anglong Hills along the springs, marsh-lands, and medium lowland crop fields and more extensively in the perennial swampy areas (peat lands). Presence of this weed has drastically reduced species richness in the marshy areas. In peat lands, it has offered tremendous competition to *Alpinia allughus, Arundo donax, Acorus calamus* etc. *Ageratum houstonianum, Colocasia asculanta, Cyperus digitatus, Mikania micrantha* and *Sphaerostephanos unitus* are found to be closely associated species of *Ludwigia peruviana*.

## CONCLUSIONS

The presence of invasive species in Assam is of great significance as this region is a repository of unique biodiversity, comprising of primitive land plants, gymnosperms and various other interesting floristic (and fanatic) elements, including considerably high endemic species. Twenty most dominant weeds revealed their severity in pre-existed plant resources. *Parthenium hysterophorus* alone is responsible for replacing 94 pre-existing species. Similarly, *Rosa multiflora* and *Mimosa diplotricha* have created havoc in Kaziranga National Park as 'driver' and 'back-seat driver' species, respectively. Severe infestation of *Ludwigia peruviana* has been recorded as newly introduced invasive species that is expanding towards the lower elevations of Karbi Anglong hills in aquatic and marshland ecosystems. Migration of *Dichanthium assimile* from Mizoram hills to Barak valley is also a serious threat to local flora.

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**Table 1.** List of dominant invasive species in Assam and their status.

| Name of species | Family | Status in different times | | | | Classification | | | Place of origin | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1980s | 1990s | 2000s | 2010s | Driver | Passenger | Back-Seat Driver | | |
| *Ageratum houstonianum* Mill. | Asteraceae | ■ | ■ | ■ | ■ | √ | | | South America | Adhikari and Babu (2008) |
| *Borreria articularis* (L. f.) F. N. Will. | Rubiaceae | ■ | ■ | ■ | ■ | √ | | | Tropical America | www.isws.in/downloads /Invasive PlantsOfIndia-2. |
| *Cassia tora* L. | Fabaceae | ■ | ■ | ■ | ■ | √ | | | Cosmopolitan | Pradhan et al.(2005) |
| *Chromolaena odorata* (L.) King & Robins. | Asteraceae | ■ | ■ | ■ | ■ | √ | | | Tropical America | Reddy (2008) |
| *Cyperus iria* L. | Cyperaceae | ■ | ■ | ■ | ■ | | √ | | Afro-Asiatic | Koul and Naqshi (1988) |
| *Dichanthium assimile* (Steud.) Deshp. | Poaceae | | | ■ | ■ | √ | | | Asia | |
| *Echhornia crassipes* (C. Martius) Solms | Pontederiaceae | ■ | ■ | ■ | ■ | √ | | | South America | Adhikari and Babu (2008) |
| *Hyptis suaveolens* (L.) Poit | Lamiaceae | ■ | ■ | ■ | ■ | √ | | | Tropical America | Reddy (2008) |
| *Imperata cylindrica* (L.) Raeusch | Poaceae | ■ | ■ | ■ | ■ | | √ | | Tropical America | Reddy (2008) |
| *Ipomoea carnea* Jacq. subsp. *fistula* (Mart.ex Choisy) Austin | Convolvulaceae | ■ | ■ | ■ | ■ | | | √ | South America | Pradhan *et al.*(2005) |
| *Lantana camara* L. | Verbenaceae | ■ | ■ | ■ | ■ | | √ | | Tropical America | Reddy (2008) |
| *Ludwigia adscendens* (L.) Hara | Onagraceae | ■ | ■ | ■ | ■ | | √ | | South America | Adhikari and Babu (2008) |
| *Ludwigia decurrens* Walter | Onagraceae | | | ■ | ■ | | √ | | North and South America | http://www.ars-grin.gov |
| *Ludwigia linifolia* Poir | Onagraceae | ■ | ■ | ■ | ■ | | √ | | Pan Tropic | http://www.iucnredlist.org/details /177204/0 |
| *Ludwigia peruviana* (L.) Hara | Onagraceae | | | | ■ | | | √ | Central and South America | www.ars-grin.gov |
| *Mikania micrantha* Kunth | Asteraceae | ■ | ■ | ■ | ■ | √ | | | Tropical America | Reddy (2008) |
| *Mimosa diplotricha* C. Wright | Mimosaceae | | | ■ | ■ | | | √ | Brazil | Anonymous (2013) |
| *Mimosa pudica* L. | Mimosaceae | ■ | ■ | ■ | ■ | | √ | | Brazil | Reddy (2008) |
| *Parthenium hysterophorus* L. | Asteraceae | ■ | ■ | ■ | ■ | | | √ | West Indies and North and South America | Jain and Malhotra (1977) |
| *Rosa multiflora* Thunb. | Rosaceae | | ■ | ■ | ■ | √ | | | Asia Temperate | http://www.ars-grin.gov/ |



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**Table 2.** Affected species due to infestation of *Parthenium hysterophorus* in Assam.

| Affected species | Family |
|---|---|
| *Acalypha indica* L. | Euphorbiaceae |
| *Achyranthes aspera* L. | Amaranthaceae |
| *Achyranthes porphyristachya* Wall | Amaranthaceae |
| *Ageratum houstonianum* Mill. | Asteraceae |
| *Alocasia cucullata* (Lour.) Schott | Araceae |
| *Alocasia fornicata* (Roxb.) Schott | Araceae |
| *Alocasia indica* (Lour.) Koch | Araceae |
| *Alocasia odora* (Roxb) Koch | Araceae |
| *Alternanthera sessilis* (L.) R.Br. ex DC. | Amaranthaceae |
| *Alternanthera tenella* Colla | Amaranthaceae |
| *Alternanthera paronychioides* A. St.-Hil. | Amaranthaceae |
| *Amaranthus polygonoides* L. | Amaranthaceae |
| *Amaranthus spinosus* L. | Amaranthaceae |
| *Amaranthus viridis* L. | Amaranthaceae |
| *Boerhaavia diffusa* L. | Nyctaginaceae |
| *Borreria articularis* (L.f.) F.N. Williams | Rubiaceae |
| *Borreria ocymoides* (Burm. f.) DC. | Rubiaceae |
| *Cannabis sativa* L. | Cannabaceae |
| *Cardiospermum halicacabum* L. | Nyctaginaceae |
| *Cassia hirsuta* L., | Caesalpiniaceae |
| *Cassia occidentalis* L. | Caesalpiniaceae |
| *Cassia tora* L. | Caesalpiniaceae |
| *Centella asiatica* (L.) Urban | Apiaceae |
| *Chromolaena odorata* (L.) King & H.E. Robins. | Asteraceae |
| *Chrysopogon aciculatus* (Retz) Trin | Poaceae |
| *Clerodendrum colebrookianum* Walp. | Verbenaceae |
| *Clerodendrum serratum* (L.) Moon. | Verbenaceae |
| *Clerodendrum viscosum* Vent. | Verbenaceae |
| *Colocasia antiquorum* Schott | Araceae |
| *Colocasia esculenta* (L.) Schott | Araceae |
| *Colocasia mannii* Hook.f. | Araceae |
| *Crassocephalum crepidioides* (Benth.) S. Moore | Asterareae |
| *Crotalaria pallida* Aiton | Fabaceae |
| *Crotalaria striata* DC | Fabaceae |
| *Croton bonplandianum* Baill. | Euphorbiaceae |
| *Cuphea balsamana* Cham et Schld. | Lythraceae |
| *Cynodon dactylon* (L.) Pers | Poaceae |
| *Cyperus bravifolius* (Rottb.) Hassk | Cyperaceae |
| *Cyperus ratundus* L. | Cyperaceae |
| *Dichanthium annulatum* (Forsk.) Staff | Poaceae |
| *Dicrocephala integrifolia* (L.f.) Kuntze | Asterareae |
| *Digitaria setigera* Roth | Poaceae |
| *Digitaria violascens* Link | Poaceae |
| *Diplazium esculentum* (Retz.) Sw. | Athyriaceae |
| *Eleucine indica* (L.) Gaertn. | Poaceae |
| *Euphorbia hirta* L. | Euphorbiaceae |
| *Gynura bicolor* (Roxb. ex Willd.) DC. | Asterareae |


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| | |
|---|---|
| *Hedyotis auricularia* L. | Rubiaceae |
| *Hedyotis corymbosa* (L.) Link. | Rubiaceae |
| *Hedyotis diffusa* Willd. | Rubiaceae |
| *Heliotropium indicum* L. | Heliotropiaceae |
| *Hydrocotyle sibthorpioides* Lamarck | Araliaceae |
| *Hypericum japonicum* Thunberg | Hypericaceae |
| *Jatropha gossipifolia* L. | Euphorbiaceae |
| *Leonurus sibiricus* L. | Lamiaceae |
| *Leucas aspera* (Willd.) L. | Lamiaceae |
| *Leucas indica* L. | Lamiaceae |
| *Lygodium flexuosum* (L.) Sw. | Lygodiaceae |
| *Lygodium japonicum* (Thunb.) Sw. | Lygodiaceae |
| *Melastoma malabathricum* L. | Melastomataceae |
| *Merremia pentaphylla* (Linn.) Hall. f. | Convolvulaceae |
| *Merremia umbellata* (L.) Hallier f. | Convolvulaceae |
| *Merremia vitifolia* (Burm. f.) Hallier f. | Convolvulaceae |
| *Mikania micrantha* Kunth | Asteraceae |
| *Ocimum americanum* L. | Lamiaceae |
| *Oplismenus burmannii* (Retz.) P. Beauv. | Poaceae |
| *Oplismenus compositus* (L.) Beauv | Poaceae |
| *Osbackia nepalensis* J. D. Hooker | Melastomataceae |
| *Oxalis corniculata* L. | Oxalidaceae |
| *Oxalis debilis* Kunth var. *corymbosa* (DC.) Lourteig | Oxalidaceae |
| *Paspalum consugatum* Bergius | Poaceae |
| *Phyllanthus debilis* Klein ex Willd. | Euphorbiaceae |
| *Phyllanthus urinaria* L. | Euphorbiaceae |
| *Plantago erosa* Wall. | Plantaginaceae |
| *Polygonum strigosum* R. Brown | Polygonaceae |
| *Pouzolzia indica* (L.) Gaudich. | Urticaceae |
| *Ricinus communis* L. | Euphorbiaceae |
| *Rumex maritimus*L. | Polygonaceae |
| *Sacciolepis myosuroides* (R. Brown) A. Camus | Poaceae |
| *Setaria glauca* (L.) Beauv | Poaceae |
| *Setaria pumila* (Poir.) Roem & Schult | Poaceae |
| *Sonchus asper* (L.) Hill | Asterareae |
| *Sonchus oleraceus* L. | Asterareae |
| *Sonchus wightianus* DC. | Asterareae |
| *Sphaerostephanos unitus* (L.) Holttum | Thelypteridaceae |
| *Spilanthes calva* DC. | Asteraceae |
| *Spilanthes paniculata* Wall. ex DC. | Asteraceae |
| *Stellaria media* (L.) Vill. | Caryophyllaceae |
| *Stephania japonica* (Thunb.) Miers | Menispermaceae |
| *Synedrella nodiflora* (L.)Gaertn | Asteraceae |
| *Taraxacum officinale* F.H. Wigg | Asterareae |
| *Typhonium trilobatum* (L.) Schott | Araceae |
| *Vernonia cinerea* (L.) Less. | Asteraceae |
| *Youngia japonica* (L.) DC. | Asteraceae |


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# THE ANALYSIS OF WEED COMMUNITY AND DOMINANT WEED SPECIES CHANGING THROUGH GROWING SEASON/ CROP GROWTH STAGES IN LOWLAND RICE FIELD

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**ABSTRACT** The objectives of this research were to analyse the changing of weed community and dominant weed species through growing season/rice crop growth stages. It was taken 156 lowland rice field sample at various elevations by stratified random sampling method. Weed surveys to all of sample locations were conducted four times to get data of weed at vegetative stage in rainy season, generative stage in rainy season, vegetative stage in dry season, and generative stage in dry season. Results showed that composition of weed community in the rice field structurally consisted of 34 broadleaves, 19 grasses, and 17 sedges, while floristically consisted of 70 weed species, 44 genus, and 23 families. Weed community in this rice field were dominated by broadleaves, followed by grasses and sedges. Dominant weed species at vegetative stage in rainy season were *Ludwigia octovalvis*, *Alternanthera philoxeroides*, *Echinochloa crus-galli*, *Monochoria vaginalis*, and *Limnocharis flava*; at generative stage in rainy season were *Echinochloa crus-galli*, *Ludwigia octovalvis*, *Alternanthera philoxeroides*, and *Fimbristhyllis littoralis*; at vegetative stage in dry season were *Echinochloa crus-galli*, *Ludwigia octovalvis*, *Alternanthera philoxeroides*, *Fimbristhyllis littoralis*, and *Limnocharis flava*; and at generative stage in dry season were *Echinochloa crus-galli*, *Ludwigia octovalvis*, *Fimbristhyllis littoralis*, *Alternanthera philoxeroides*, and *Cyperus iria*. There were three dominant weed species at vegetative stage in rainy season that had broad distribution, i.e. *L. octovalvis*, *E.crus-galli*, and *A. philoxeroides*. At generative stage in rainy season until generative stage in dry season showed that *E.crus-galli* and *L. octovalvis* also had broad distribution, while in generative stage in dry season showed that *F. littoralis* had relatively broad distribution. Range of location distribution of *L. octovalvis*, *E. crus-galli*, and *A. philoxeroides* was in lowland until highland, whereas *F. littoralis* was only in lowland.

***Keywords*****:** weed community, weed dominance, changing, rice field.

## INTRODUCTION

Weeds are actual problem always found at paddy field area which in turn create main biological constraint for paddy field rice cultivation (De Datta, 1995; Moody, 1995; Paller *et al*., 2003). Weeds create competition with paddy field rice in utilization of nutrients, water, light and space. Improper handling practice of weeds may decrease the production of paddy field rice in the range of 18 to 35 % (Burhan, 1994).

Weeds composition at paddy field rice area is dynamic in nature due to specific factors. These factors may include weed species characteristics or external effects such as macro environment and micro environment as well as cultivation practices (Aldrich, 1984). The macro environment is regional environment having extensive scale which consisted of several aspects of soil and climate, whereas micro environment is small scale 171ylindrical which is affected by micro topography, rocks, organic matter, nutrients and so forth (Radosevich *et a*l., 1997). On the other hand, cultivation practice has ecological and evolutionary effects on weed community and intensification of cultivation practice frequently produce the emergence of noxious weed species through interspecific and intragenotypic selections (Janiya *et al*., 1999). Therefore, weed community may change due to the effect of cultivation practices such as land preparation, soil tillage, crop cultivars, planting distance, irrigation method, fertilizer



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application and weed control including the use of herbicides (Moody, 1995; Sundaru dan Pane, 1984; Vongsaroj, 1995).

The knowledge of weed community as a part of agroecosystem is very important in order to compose its management strategy so that weed management can be conducted properly as a part of integrated management for agricultural production system (Soerjani, 1991; Tjitrosoedirdjo *et al*., 1984). In addition to economic consideration, the composing of weed management strategy is also based on ecological consideration and weed community characteristics. The I, competitive and adaptive growth characteristics of weed toward the change of environmental condition are important consideration because adaptation potential of weeds are different according to their species (Radosevich *et al*., 1997). Therefore, the study related to weed community is important to be conducted before any practice toward weed growth at paddy field rice is implemented. This study was conducted with objectives to analyse weed species composition and dominant weed species based on their important values as well as the change occurrence during growing season periods or growth stage of paddy field rice.

## MATERIALS AND METHODS

This study was conducted at paddy field area of Ciliwung-Cisadane Watershed which was distributed from upstream to downstream areas. The study period was consisted of two planting seasons, i.e during wet season and dry season. Samples for paddy field area and farmers as the owner was determined by using method of stratified purposive random sampling with elevation as main factor or main theme (Van Gils *et al*., 1990). Paddy field area from upstream to downstream sides was divided into several elevation classes, i.e. 0 – 25, >25 – 50, >50 – 100, >100 – 200, >200 – 300, >300 – 400, >400 – 500, >500 – 600, >600 – 700, >700 – 800, >800 – 900, >900 – 1000, >1000 – 1100, >1100 – 1200, and >1200 m from mean sea level (msl). Paddy field sample locations was decided for each elevation class by considering the proportion, distribution and accessibility aspects. Therefore, this study used 156 units of paddy field sample locations. There was 5 observation plots for each location unit having size of 1 m x 1 m. Observation at each planting season will be conducted two times, i.e. at 3-5 weeks after planting (21-35 days after planting) and at 9-11 weeks after planting (63-77 days after planting). Observation for weed community was consisted of (a) the existing weed species, (b) individual numbers for each weed species, (c) coverage of each weed species, and (d) height of each weed species.

Analysis was conducted through study of several quantitative variables related to weed interaction with crops (Paller *et al*., 2003; Tjitrosoedirdjo *et al*., 1984) that consisted of absolute density (Km), relative density (Kn), absolute frequency (Fm), relative frequency (Fn), absolute dominance (Dm), relative dominance (Dn) and important value (NP). Subsequently, the importance value was used as variable to determine dominance of weed species. Weed species having the highest important value was declared as dominant weed species at each paddy field area.

## RESULTS AND DISCUSSION

Observation results of weed community at paddy field rice within several locations having different elevation and amongst growing seasons/crop growth stages showed that in overall there was 70 weed species. These weed species based on their structures/habitats can be classified as 34 broadleaves, 19 grasses, and 17 sedges. Based on floristical aspect, these weed species were consisted of 44 genus and 23 families. Weed species composition amongst growing seasons/growth stages of paddy field rice in general had shown an alteration or change. Relatively different weed species composition, general weed species and dominant weed species were found at each growing season/growth stage of paddy field rice (Table 1, 2, 3 and 4).

Weed species composition at vegetative stage in wet season was shown in Table 1. From 62 weed species found at this area, weed species abundance per location was in the range of 20



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to 26 species with average value of 23.18 species. There were 5 dominant weed species of all weed species that consisted of *Ludwigia octovalvis*, *Alternanthera philoxeroides*, *Echinochloa crusgalli*, *Monochoria vaginalis* and *Limnocharis flava.* These weed species showed consistent dominance from lowland to highland, even the dominance of *Echinochloa crusgalli* was tend to increase in accordance to elevation increase of location. Other relatively dominant weed species were consisted of *Fimbristhyllis littoralis*, *Sphenoclea zeylanica* and *Salvinia molesta*, whereas the less dominant weed species were consisted of *Cyperus iria*, *Echinochloa colonum*, *Fimbristhyllis grifithii*, *Paspalum distichum* and *Marsilea crenata*.

**Table 1**. Weed species composition of young rice crop in wet season at several elevations of location/paddy field landscape elevations.

| No | Weed Species | Important Value (%) at each elevation of location | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | VII | VIII | IX | X | XI |
| 1 | *Alternanthera* | 17.1 | 38.7 | 28.1 | 49.1 | 42.7 | 38.8 | 27.9 | 31.9 | 24.8 | 19.1 | 17,5 |
| 2 | *Amaranthus lividus* | - | - | - | - | - | - | - | 6.4 | - | - | - |
| 3 | *Amaranthus* | - | - | - | - | - | - | - | - | - | - | 7. 4 |
| 4 | *Azolla pinnata* | 7.4 | - | - | - | - | - | - | - | 7.5 | - | - |
| 5 | *Bacopa* | - | - | - | - | - | - | 6.3 | - | - | - | - |
| 6 | *Bergia capensis* | 6.9 | - | - | - | 3.4 | - | - | - | - | - | - |
| 7 | *Commelina* | - | 5.3 | - | 8.6 | 9.0 | 5.4 | - | 2.7 | 4.9 | 14.9 | 6.4 |
| 8 | *Commellina* | 5.3 | 8.5 | 3.2 | - | 5.4 | 9.4 | - | 10.4 | 2.5 | 4.0 | 8.4 |
| 9 | *Cynodon dactylon* | - | 3.5 | 2.5 | - | 3.0 | - | - | - | - | 2.4 | - |
| 10 | *Cyperus* | - | - | - | - | - | - | 7.5 | - | - | - | - |
| 11 | *Cyperus* | - | - | - | - | - | - | - | 4.3 | - | - | - |
| 12 | *Cyperus difformis* | - | - | 5.9 | 8.9 | | | 4.8 | | | | 5.8 |
| 13 | *Ciperus digitatus* | 14.2 | 20.0 | - | - | - | - | - | - | - | - | - |
| 14 | *Cyperus elatus* | 3.4 | - | - | - | - | - | - | - | - | - | - |
| 15 | *Ciperus halpan* | - | - | 10.3 | 6.7 | 7.1 | - | 2.5 | - | 7.9 | 9.6 | - |
| 16 | *Cyperus iria* | 8.6 | 8.1 | - | 15.3 | 7.3 | 11.8 | 19.5 | 17.3 | - | 7.1 | 8.9 |
| 17 | *Cyperus kyllingia* | - | - | - | - | - | - | 7.4 | - | - | - | - |
| 18 | *Cyperus pillosus* | - | - | 4.5 | - | 6.1 | - | - | - | - | - | - |
| 19 | *Cyperus* | - | - | 5.1 | 3.5 | - | 6.0 | - | - | - | - | 4.1 |
| 20 | *Digitaria ciliaris* | - | 7.9 | 10.7 | 8.4 | 8.8 | 13.4 | 4.8 | 4.7 | 2.3 | 1.7 | - |
| 21 | *Digitaria* | - | - | - | 2.5 | - | - | 3.5 | - | - | - | 6.5 |
| 22 | *Digitaria* | 7.6 | 9.0 | 9.3 | - | 3.7 | - | - | - | - | - | - |
| 23 | *Digitaria nuda* | - | - | - | - | - | - | - | 3.3 | - | - | - |
| 24 | *Digitaria setigera* | - | - | - | - | - | 4.4 | - | - | - | - | - |
| 25 | *Digitaria ternata* | - | - | - | 7.5 | - | - | - | - | - | - | - |
| 26 | *Echinochloa* | 5.3 | - | - | 12.3 | 14.0 | 22.4 | 9.1 | 7.4 | 8.2 | 8.0 | 6.9 |
| 27 | *Echinochloa crus-* | 8.3 | 16.6 | 15.4 | 17.3 | 12.1 | 28.4 | 41.2 | 48.5 | 52.7 | 39.6 | 44.2 |
| 28 | *Eclipta prostata* | - | - | - | - | - | - | - | 6.4 | 8.1 | 9.5 | - |
| 29 | *Eleusine indica* | - | 7.7 | - | 11.0 | 10.1 | 9.5 | 4.1 | - | - | - | - |
| 30 | *Enhydra fluctuants* | - | - | - | - | - | - | - | - | - | - | 6.3 |
| 31 | *Eragrostis* | - | - | - | - | - | 7.0 | 7.9 | 7.5 | 6.8 | 6.3 | - |
| 32 | *Euphorbia hirta* | - | - | - | - | 8.6 | - | - | 4.3 | - | - | - |
| 33 | *Fimbristhylis* | - | - | - | - | - | - | 3.0 | 9.1 | - | - | 9.9 |
| 34 | *Fimbristhylis* | 4.9 | - | 14.8 | 14.1 | 8.4 | 6.1 | - | 10.6 | 10.7 | 19.4 | 16.2 |
| 35 | *Fimbristhylis* | 19.0 | 16.5 | 14.4 | 17.5 | 7.7 | 11.2 | 10.7 | 10.4 | 15.4 | 10.3 | - |



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| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 36 | *Ipomoea aquatica* | 9.5 | 8.8 | - | - | - | - | - | - | - | - | - |
| 37 | *Ischaemum* | - | - | 5.3 | - | - | - | - | - | - | - | - |
| 38 | *Leersia hexandra* | 24.9 | 19.6 | - | - | - | 6.1 | - | - | - | - | - |
| 39 | *Leptochloa* | - | - | - | - | 8.7 | 1.5 | - | - | - | - | - |
| 40 | *Limnocharis flava* | 9.8 | 11.0 | 13.5 | - | 11.9 | 12.3 | 18.2 | 14.7 | 12.5 | 13.6 | 13.7 |
| 41 | *Lindernia ciliata* | - | - | - | - | - | - | 6.3 | - | - | - | - |
| 42 | *Lindernia* | - | - | - | - | - | 3.3 | - | 4.1 | - | - | - |
| 43 | *Lindernia* | - | - | - | - | - | - | - | 4.5 | - | - | - |
| 44 | *Ludwigia* | 19.5 | 6.4 | 10.7 | - | 9.7 | - | 13.5 | 4.8 | 4.9 | - | 10.3 |
| 45 | *Ludwigia* | - | - | 5.2 | 6.5 | - | - | 6.2 | - | 5.8 | 3.2 | - |
| 46 | *Ludwigia* | 30.9 | 39.8 | 46.1 | 41.7 | 45.0 | 47.8 | 37.2 | 37.1 | 31.9 | 40.3 | 28.5 |
| 47 | *Marsilea crenata* | 10.0 | 13.5 | - | - | - | - | 5.3 | 15.8 | 14.4 | 24.2 | 8.9 |
| 48 | *Monochoria* | 10.0 | 13.5 | 14.0 | 10.6 | 18.8 | 11.9 | 12.9 | 11.6 | 22.6 | 32.3 | 16.8 |
| 49 | *Myriophyllum* | - | - | - | - | - | - | - | - | 5.2 | - | 23.9 |
| 50 | *Paspalum* | - | - | 6.1 | - | - | - | - | - | - | - | - |
| 61 | *Paspalum* | 20.2 | 8.3 | 19.8 | 19.1 | 10.7 | 9.7 | 10.3 | - | 4.0 | 4.2 | 4.8 |
| 52 | *Panicum repens* | 10.6 | - | 13.1 | 9.3 | - | - | - | 2.2 | - | - | - |
| 53 | *Phyllanthus debilis* | - | - | 4.6 | - | - | 4.2 | - | - | - | - | - |
| 54 | *Pistia stratiotes* | 8.6 | - | - | - | - | - | - | - | - | - | - |
| 55 | *Politrias amaura* | - | - | 3.0 | 9..3 | 7.1 | 8.8 | 11.9 | 9.2 | 5.3 | - | - |
| 56 | *Portulaca oleracea* | - | - | - | - | 10.6 | - | - | - | - | - | - |
| 57 | *Richardia* | - | - | - | - | - | 2.1 | - | - | - | - | - |
| 58 | *Rotala indica* | - | - | - | - | - | - | 3.8 | - | - | - | - |
| 59 | *Sacciolepsis* | 25.4 | - | - | - | - | - | - | - | - | - | - |
| 60 | *Sagittaria* | - | - | - | - | - | - | - | - | - | - | 17.2 |
| 61 | *Salvinia molesta* | - | 2.4 | - | - | - | - | 14.2 | 10.8 | 29.6 | 30.1 | 27.6 |
| 62 | *Sphenoclea* | 10.6 | 17.5 | 34.6 | 21.1 | 20.1 | 16.7 | - | - | - | - | - |

*Remarks : I = 0 – 25, II = 25 – 50, III = 50 – 100, IV = 100 – 200, V = 200-300, VI = 300 – 400, VII = 400 – 500, VIII = 500–600, IX = 600 – 700, X = 700 – 800 and XI = 800 – 900 m from msl.

**Table 2.** Weed species composition of mature rice crop in wet season at several elevations of location/paddy field landscape elevations.

| No | Weed Species | Important Value (%) at each elevation of location empat/elevasi | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | VII | VIII | IX | X | XI |
| 1 | *Alternanthera philoxeroides* | 18.5 | 25.0 | 25.2 | 27.1 | 39.4 | 27.4 | 27.6 | 25.0 | 22.3 | 17.3 | 14.5 |
| 2 | *Amaranthus spinosus* | - | - | - | - | - | - | - | - | - | - | 6.5 |
| 3 | *Bergia capensis* | 8.4 | - | - | 8.7 | - | - | - | - | - | - | - |
| 4 | *Commellina benghalensis* | - | - | - | - | 5.0 | 6.2 | - | - | - | 8.3 | 8.4 |
| 5 | *Commellina diffusa* | 9.8 | 8.3 | - | - | 5.3 | 5.7 | - | 4.3 | - | - | 3.2 |
| 6 | *Cynodon dactylon* | - | 7.7 | - | - | 7.8 | - | - | - | - | 6.4 | - |
| 7 | *Cyperus brevifolius* | - | - | - | - | - | - | - | 6.5 | - | - | - |
| 8 | *Cyperus difformis* | - | - | 8.7 | 7.4 | - | - | 9.1 | - | - | - | 10.1 |
| 9 | *Ciperus digitatus* | 21.2 | 20.7 | | - | - | - | - | - | - | - | - |
| 10 | *Cyperus elatus* | 6.6 | - | - | - | - | - | - | - | - | - | - |
| 11 | *Ciperus halpan* | - | - | 8.8 | 10.3 | 5.1 | - | - | 5.6 | - | 6.9 | - |
| 12 | *Cyperus iria* | 11.0 | 16.5 | - | 24.3 | 11.2 | 18.0 | 5.8 | 5.7 | 4.7 | - | 4.5 |



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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | *Cyperus kyllingia* | - | - | - | - | - | - | 7.3 | - | - | - | - |
| 14 | *Cyperus pillosus* | - | - | 4.2 | - | 10.0 | - | - | - | - | - | - |
| 15 | *Cyperus platystilys* | - | - | 5.6 | - | - | - | - | - | - | - | - |
| 16 | *Cyperus polystachyos* | - | - | 8.7 | 8.1 | - | 6.0 | - | - | - | - | - |
| 17 | *Digitaria ciliaris* | - | 3.5 | 6.8 | - | 5.2 | 10.8 | 10.2 | 10.4 | 8.4 | - | - |
| 18 | *Digitaria fuscescens* | - | - | - | - | - | - | - | - | - | - | 8.4 |
| 19 | *Digitaria longiflora* | 7.0 | 7.7 | 7.4 | - | 2.8 | - | - | - | - | - | - |
| 20 | *Digitaria setigera* | - | - | - | - | - | 6.2 | - | - | - | - | - |
| 21 | *Digitaria ternata* | - | - | - | 4.5 | - | - | - | - | - | - | - |
| 22 | *Echinochloa colonum* | 9.7 | - | - | 22.2 | 16.0 | 11.8 | - | 12.8 | 24.0 | 22.9 | 20.4 |
| 23 | *Echinochloa crus-galli* | 28.3 | 36.8 | 38.4 | 32.6 | 23.5 | 47.8 | 62.6 | 64.1 | 71.6 | 71.3 | 67.9 |
| 24 | *Eclipta prostata* | - | - | - | - | - | - | - | 8.7 | - | 7.3 | - |
| 25 | *Eleusine indica* | - | 10.0 | - | 8.9 | 3.6 | 5.3 | 8.6 | - | - | - | - |
| 26 | *Enhydra fluctuants* | - | - | - | - | - | - | - | - | - | - | 7.0 |
| 27 | *Eragrostis unioloides* | - | - | - | - | - | 2.2 | 9.5 | - | - | 7.9 | 6.5 |
| 28 | *Euphorbia hirta* | - | - | - | - | 5.9 | - | - | - | - | - | - |
| 29 | *Fimbristhyllis aphylla* | - | - | - | - | - | - | - | - | - | 3.2 | 8.4 |
| 30 | *Fimbristhylis grifithii* | - | 9.4 | 11.2 | 16.1 | 10.0 | 4.7 | 15.2 | 13.2 | 13.3 | 17.3 | 15.7 |
| 31 | *Fimbristhylis littoralis* | 12.6 | 34.9 | 31.8 | 28.0 | 27.4 | 20.3 | 25.3 | 21.7 | 29.5 | 19.8 | - |
| 32 | *Ipomoea aquatica* | 11.4 | 9.9 | - | - | - | - | - | - | - | - | - |
| 33 | *Leersia hexandra* | 26.8 | 12.4 | 20.3 | - | - | 4.4 | 2.1 | - | - | - | - |
| 34 | *Leptochloa chinensis* | - | - | - | - | 11.0 | 5.0 | - | - | - | - | - |
| 35 | *Limnocharis flava* | 10.4 | 12.4 | 12.6 | - | 12.5 | 15.9 | 15.6 | 14.4 | 8.4 | 8.2 | 6.1 |
| 36 | *Lindernia 175ylindr* | - | - | - | - | - | - | - | 5.6 | - | - | - |
| 37 | *Lindernia crustacea* | - | - | - | - | - | - | - | 7.0 | - | - | - |
| 38 | *Lindernia procumbens* | - | - | - | - | - | 3.2 | - | - | - | - | - |
| 39 | *Ludwigia adscendens* | 15.1 | - | 10.6 | - | - | - | 2.7 | 6.8 | 7.2 | - | 6.7 |
| 40 | *Ludwigia hyssofipolia* | - | 5.2 | 5.8 | - | 5.6 | - | 2.9 | 5.5 | 6.9 | - | - |
| 41 | *Ludwigia octovalvis* | 30.3 | 38.3 | 44.9 | 45.3 | 45.6 | 45.5 | 39.8 | 22.3 | 22.1 | 21.7 | 20.4 |
| 42 | *Marsilea crenata* | - | - | - | - | - | - | 10.3 | 10.1 | 11.0 | 15.9 | 9.8 |
| 43 | *Monochoria vaginalis* | 8.9 | 8.0 | 7.7 | 11.2 | 10.8 | 9.6 | 9.2 | 9.1 | 21.3 | 21.7 | 10.9 |
| 44 | *Myriophyllum aquaticum* | - | - | - | - | - | - | - | - | 17.0 | - | 19.0 |
| 45 | *Panicum repens* | 11.7 | - | - | 9.4 | - | - | - | - | - | - | - |
| 46 | *Paspalum commersonii* | - | - | 4.4 | - | - | - | - | - | - | - | - |
| 47 | *Paspalum distichum* | 6.4 | 7.7 | 8.9 | - | 7.4 | 10.8 | 10.6 | 7.0 | 3.2 | 10.2 | 22.1 |
| 48 | *Phyllanthus debilis* | - | - | 5.7 | - | - | - | - | - | - | - | - |
| 49 | *Pistia stratiotes* | 16.1 | - | - | 8.2 | - | - | - | - | - | - | - |
| 50 | *Politrias amaura* | - | - | - | - | 7.2 | 8.9 | 8.5 | 7.7 | 7.5 | - | - |
| 51 | *Portulaca oleracea* | - | - | - | - | 7.7 | - | - | - | - | - | - |
| 52 | *Rotala indica* | - | - | - | - | - | - | - | 6.0 | - | - | - |



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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 53 | *Sacciolepsis interrupta* | 24.6 | - | - | - | - | - | - | - | - | - | - |
| 54 | *Sagittaria guayanensis* | - | - | - | - | - | - | - | - | - | - | 11.9 |
| 55 | *Salvinia molesta* | 4.6 | 4.9 | - | - | - | - | 17.1 | - | 22.2 | 23.7 | 11.6 |
| 56 | *Sphenoclea zeylanica* | - | 19.2 | 22.4 | 28.0 | 24.0 | 24.4 | - | 20.5 | - | - | - |

*Remarks : I = 0 – 25, II = 25 – 50, III = 50 – 100, IV = 100 – 200, V = 200-300, VI = 300 – 400, VII = 400 – 500, VIII = 500–600, IX = 600 – 700, X = 700 – 800 and XI = 800 – 900 m from msl.

**Table 3.** Weed species composition of young rice crop in dry season at several elevations of location/paddy field landscape elevations.

| No | Weed Species | Important Value (%) at each elevation of location | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | VII | VIII | IX | X | XI |
| 1 | *Alternanthera philoxeroides* | 17.7 | 18.7 | 25.0 | 31.9 | 28.1 | 28.4 | 34.3 | 19.4 | 32.1 | 24.5 | 21.3 |
| 2 | *Azolla pinnata* | - | - | - | - | - | - | - | 6.4 | 4.1 | 3.5 | 3.7 |
| 3 | *Bacopa rotundifolia* | | - | - | - | - | - | 4.7 | - | - | - | - |
| 4 | *Bergia capensis* | 12.2 | - | 9.6 | 9.2 | 9.0 | - | - | - | - | - | - |
| 5 | *Commellina benghalensis* | - | - | 8.3 | 7.6 | - | 7.2 | 9.3 | 10.8 | 10.6 | 15.3 | 14.5 |
| 6 | *Commellina diffusa* | - | 7.6 | 5.7 | 6.9 | - | - | - | 3.5 | 5.2 | 6.1 | - |
| 7 | *Cynodon dactylon* | - | 12.2 | 12.2 | 10.8 | 12.2 | 13.6 | 9.7 | 4.8 | - | 2.5 | - |
| 8 | *Cyperus brevifolius* | - | - | - | 5.7 | - | - | - | - | - | - | - |
| 9 | *Cyperus difformis* | - | 9.5 | - | - | - | - | 5.7 | - | - | - | - |
| 10 | *Ciperus digitatus* | 8.4 | - | - | - | - | - | - | - | - | - | - |
| 11 | *Ciperus halpan* | - | - | - | - | 4.5 | - | - | - | 7.7 | - | - |
| 12 | *Cyperus iria* | - | 27.3 | - | 27.7 | 22.9 | 17.7 | 17.2 | 8.7 | - | - | 24.4 |
| 13 | *Cyperus kyllingia* | 8.7 | - | - | 4.8 | - | - | - | - | - | - | - |
| 14 | *Cyperus pillosus* | - | - | 3.2 | - | 4.7 | 4.7 | - | - | - | - | - |
| 15 | *Cyperus polystachyos* | - | - | 6.2 | 4.6 | 7.1 | - | - | - | - | - | - |
| 16 | *Digitaria ciliaris* | - | 11.4 | 8.5 | - | 7.6 | 5.5 | 5.5 | - | - | - | - |
| 17 | *Digitaria fuscescens* | - | - | - | - | - | - | - | 4.1 | - | - | - |
| 18 | *Digitaria longiflora* | 8.9 | - | - | - | 3.5 | - | - | - | - | - | - |
| 19 | *Digitaria nuda* | - | - | - | - | - | - | - | 2.0 | - | - | - |
| 20 | *Echinochloa colonum* | 9.1 | - | - | 10.1 | - | - | - | - | - | - | 8.7 |
| 21 | *Echinochloa crus-galli* | 8.4 | 24.8 | 34.1 | 33.3 | 40.5 | 60.4 | 67.5 | 65.9 | 56.9 | 76.9 | 58.6 |
| 22 | *Eclipta prostata* | - | - | - | - | 5.0 | - | - | 4.4 | 2.0 | 6.7 | - |
| 23 | *Eleusine indica* | - | 10.3 | - | 3.1 | 3.6 | - | - | - | 9.9 | - | - |
| 24 | *Eragrostis unioloides* | - | - | - | - | 3.2 | - | - | - | 2.4 | 8.5 | - |
| 25 | *Euphorbia hirta* | - | - | - | 5.8 | 2.9 | - | - | 5.8 | - | - | - |
| 26 | *Fimbristhyllis aphylla* | 4.6 | - | - | - | - | - | - | - | - | - | - |
| 27 | *Fimbristhylis grifithii* | - | 7.0 | 8.6 | - | - | - | 11.3 | 11.7 | 12.5 | 13.6 | 16.8 |
| 28 | *Fimbristhylis littoralis* | 35.0 | 32.7 | 34.3 | 27.5 | 21.9 | 20.5 | 16.1 | 15.3 | 14.6 | 12.3 | 11.5 |
| 29 | *Ipomoea aquatica* | 11.0 | - | - | - | - | - | - | - | - | - | - |
| 30 | *Leersia hexandra* | 27.7 | 25.7 | - | 8.3 | - | - | - | - | - | - | - |
| 31 | *Leptochloa chinensis* | - | 20.7 | 15.7 | - | - | - | 7.4 | - | - | - | - |
| 32 | *Limnocharis flava* | 15.6 | 14.9 | 13.6 | 11.0 | 16.2 | 16.2 | 18.2 | 12.8 | 7.7 | 6.8 | 9.2 |
| 33 | *Lindernia ciliata* | - | - | - | - | 4.4 | - | - | - | - | - | - |
| 34 | *Lindernia crustacea* | - | - | - | - | - | - | - | 4.1 | - | - | - |
| 35 | *Lindernia procumbens* | - | - | - | - | - | - | - | 4.9 | - | 6.5 | - |
| 36 | *Ludwigia adscendens* | 22.7 | - | 17.7 | - | - | - | 7.3 | 7.8 | 9.2 | - | 9.3 |
| 37 | *Ludwigia hyssofipolia* | 5.7 | - | 4.8 | - | 3.3 | 8.6 | 6.9 | - | 3.7 | - | - |
| 38 | *Ludwigia octovalvis* | 23.2 | 30.6 | 37.1 | 41.1 | 46.0 | 44.3 | 23.1 | 51.6 | 52.2 | 34.4 | 25.6 |
| 39 | *Marsilea crenata* | 6.6 | - | - | 8.2 | 12.7 | 11.0 | 13.0 | 12.4 | 11.9 | 16.1 | 21.4 |
| 40 | *Monochoria vaginalis* | 5.5 | - | 3.5 | 3.5 | 8.5 | 9.3 | 8.3 | 12.4 | 17.1 | 15.7 | 16.4 |
| 41 | *Myriophyllum aquaticum* | - | - | - | - | - | - | - | - | - | - | 23.5 |



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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 42 | *Panicum repens* | 12.7 | 11.5 | - | 6.1 | - | - | - | - | - | - | - |
| 43 | *Paspalum commersonii* | - | - | 9.2 | - | - | - | - | - | - | - | - |
| 44 | *Paspalum distichum* | 21.1 | 17.4 | 18.5 | 14.5 | 12.0 | 15.9 | 9.9 | 9.9 | 9.3 | 9.8 | - |
| 45 | *Phyllanthus debilis* | - | - | 3.6 | - | - | - | - | - | - | - | - |
| 46 | *Pistia stratiotes* | 3.7 | - | - | - | - | 5.5 | - | - | - | - | - |
| 47 | *Politrias amaura* | - | - | 4.5 | 4.6 | - | 6.9 | 7.0 | - | 6.9 | 8.3 | - |
| 48 | *Sacciolepsis interrupta* | 26.6 | - | - | - | - | - | - | - | - | - | - |
| 49 | *Sagittaria guayanensis* | - | - | - | - | - | - | - | - | - | 9.7 | 13.8 |
| 50 | *Salvinia molesta* | 5.0 | 4.0 | - | - | - | - | 17.6 | 15.6 | 18.0 | 17.9 | 21.4 |
| 51 | *Scirpus juncoides* | - | - | - | - | - | - | - | - | - | 4.9 | - |
| 52 | *Sphenoclea zeylanica* | - | 11.7 | 16.1 | 13.9 | 20.2 | 24.3 | - | 5.7 | - | - | - |

*Remarks : I = 0 – 25, II = 25 – 50, III = 50 – 100, IV = 100 – 200, V = 200-300, VI = 300 – 400, VII = 400 – 500, VIII = 500–600, IX = 600 – 700, X = 700 – 800 and XI = 800 – 900 m from msl.

**Table 4.** Weed species composition of mature rice crop in dry season at several elevations of location/paddy field landscape elevations.

| No | Weed Species | Important Value (%) at each elevation of location | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | VII | VIII | IX | X | XI |
| 1 | *Ageratum conyzoides* | - | - | - | - | 8.1 | 5.4 | - | - | - | - | - |
| 2 | *Alternanthera philoxeroides* | 24.2 | 18.8 | 12.6 | 23.7 | 23.0 | 20.4 | 17.7 | 13.2 | 10.3 | 9.3 | 9.7 |
| 3 | *Bergia capensis* | 5.2 | - | - | 8.2 | - | - | - | - | - | - | - |
| 4 | *Borreria laevis* | - | - | 5.4 | - | - | - | - | - | - | - | - |
| 5 | *Commellina benghalensis* | - | - | 7.3 | 10.3 | 6.7 | - | 6.4 | 8.6 | 7.8 | 16.1 | 15.7 |
| 6 | *Commellina diffusa* | - | - | 12.8 | 6.1 | - | 8.5 | - | 15.4 | 5.5 | 6.2 | - |
| 7 | *Cynodon dactylon* | 9.6 | 16.1 | 15.8 | 8.6 | 9.7 | 10.4 | 5.0 | 4.7 | 2.3 | - | - |
| 8 | *Cyperus babakan* | 12.8 | - | - | - | - | - | - | - | - | - | - |
| 9 | *Cyperus brevifolius* | - | - | - | 6.6 | - | - | - | - | - | - | - |
| 10 | *Cyperus difformis* | - | 11.3 | 4.5 | - | - | - | - | 4.8 | - | 12.6 | - |
| 11 | *Ciperus digitatus* | 8.9 | - | - | - | - | - | - | - | - | - | - |
| 12 | *Ciperus halpan* | - | - | - | - | 6.2 | - | - | 11.7 | 13.7 | 13.9 | - |
| 13 | *Cyperus iria* | 10.5 | 20.6 | 11.6 | 30.7 | 18.8 | 18.7 | 25.4 | 8.8 | 8.5 | 20.4 | 17.2 |
| 14 | *Cyperus kyllingia* | - | - | - | - | - | - | - | - | - | - | 4.1 |
| 15 | *Cyperus pillosus* | - | - | 5.4 | - | 6.8 | 10.4 | - | - | - | - | 5.6 |
| 16 | *Cyperus polystachyos* | - | - | - | 4.2 | - | - | - | - | - | - | - |
| 17 | *Dactyloctenium aegyptium* | - | 6.9 | - | - | - | - | - | - | - | - | - |
| 18 | *Digitaria ciliaris* | - | 6.3 | - | - | 24.0 | 13.1 | - | - | - | - | - |
| 19 | *Digitaria longiflora* | 6.1 | - | - | - | - | - | - | - | - | - | - |
| 20 | *Echinochloa colonum* | - | 17.5 | 18.3 | 23.2 | - | - | - | - | 4.3 | - | 14.6 |
| 21 | *Echinochloa crus-galli* | - | 19.7 | 26.2 | - | 53.4 | 31.9 | 77.5 | 60.5 | 68.0 | 55.3 | 75.9 |
| 22 | *Eclipta prostata* | - | - | - | - | 6.9 | - | - | - | - | - | - |
| 23 | *Eleusine indica* | - | 16.2 | - | 10.0 | 7.0 | 13.6 | 6.3 | - | 3.4 | - | - |



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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | *Eragrostis unioloides* | - | - | - | - | - | - | - | - | 5.1 | - | 2.4 |
| 25 | *Euphorbia hirta* | 7.9 | - | - | 14.2 | 3.8 | 5.8 | - | 8.8 | - | - | - |
| 26 | *Fimbristhylis grifithii* | - | - | - | - | - | 8.3 | - | 4.2 | 5.5 | 6.5 | 6.2 |
| 27 | *Fimbristhylis littoralis* | 29.5 | 34.7 | 37.4 | 24.2 | 25.0 | 15.1 | 34.8 | 22.8 | 36.0 | 10.7 | 4.1 |
| 28 | *Ipomoea aquatica* | 12.5 | - | - | - | - | - | - | - | - | - | - |
| 29 | *Leersia hexandra* | 19.1 | 8.5 | - | 11.2 | - | - | - | - | - | - | - |
| 30 | *Leucas lavandulaefolia* | - | - | 3.8 | - | - | - | - | - | - | - | - |
| 31 | *Leptochloa chinensis* | 11.6 | 18.2 | 16.9 | - | - | - | 2.7 | - | - | - | - |
| 32 | *Lindernia ciliata* | - | - | - | - | 3.9 | 6.7 | - | - | - | - | - |
| 33 | *Lindernia crustacea* | - | - | - | - | - | - | - | 8.8 | - | - | - |
| 34 | *Lindernia procumbens* | - | - | - | - | - | - | - | - | - | 4.4 | - |
| 35 | *Limnocharis flava* | 17.8 | - | 5.5 | 5.7 | - | 17.7 | 11.8 | 22.3 | 11.0 | 11.0 | 2.6 |
| 36 | *Ludwigia adscendens* | 23.3 | - | 13.8 | - | - | - | 7.1 | 6.7 | 10.0 | 10.3 | 15.8 |
| 37 | *Ludwigia hyssofipolia* | - | - | 8.6 | - | - | 12.9 | - | - | 6.5 | 7.3 | 5.4 |
| 38 | *Ludwigia octovalvis* | 25.1 | 35.1 | 30.0 | 51.0 | 43.8 | 45.3 | 27.9 | 39.5 | 51.5 | 49.5 | 14.2 |
| 39 | *Marsilea crenata* | - | - | - | 5.4 | - | 6.4 | 15.7 | 19.0 | 15.3 | 14.3 | 23.7 |
| 40 | *Monochoria vaginalis* | 5.8 | - | 5.0 | 14.1 | 3.5 | 4.2 | - | 13.6 | 10.5 | 13.7 | 14.6 |
| 41 | *Myriophyllum aquaticum* | - | - | - | - | - | - | - | - | - | - | 23.5 |
| 42 | *Panicum repens* | 11.6 | 17.0 | - | 10.3 | - | - | - | - | - | - | - |
| 43 | *Paspalum commersonii* | - | - | 14.3 | 7.6 | - | - | - | - | - | - | - |
| 44 | *Paspalum distichum* | 14.4 | 32.3 | 14.3 | 7.9 | 11.4 | - | 19.9 | - | 8.6 | 10.3 | 10.7 |
| 45 | *Phyllathus debilis* | - | - | 3.8 | - | - | - | - | - | - | - | - |
| 46 | *Pistia stratiotes* | 11.6 | - | - | - | - | 5.3 | - | - | - | - | - |
| 47 | *Politrias amaura* | - | - | 15.1 | 6.2 | 4.8 | 11.4 | 13.2 | 6.0 | 5.1 | 5..3 | - |
| 48 | *Portulaca oleracea* | - | - | - | - | - | - | - | 2.9 | - | - | - |
| 49 | *Rotala indica* | - | - | - | - | - | - | 7.6 | - | - | - | - |
| 50 | *Sacciolepsis interrupta* | 21.3 | - | - | - | - | - | - | - | - | - | - |
| 51 | *Sagittaria guayanensis* | - | - | - | - | - | - | - | - | - | 3.7 | 18.4 |
| 52 | *Salvinia molesta* | - | - | - | - | - | - | 21.0 | 5.4 | 11.4 | 13.4 | 15.6 |
| 53 | *Scirpus juncoides* | - | - | - | - | - | - | - | - | - | 6.2 | - |
| 54 | *Sphenoclea zeylanica* | 11.2 | 10.8 | 11.7 | 10.7 | 33.2 | 28.6 | - | 4.0 | - | - | - |
| 55 | *Synedrella nodiflora* | - | - | - | - | - | - | - | 8.3 | - | - | - |

* Remarks : I = 0 – 25, II = 25 – 50, III = 50 – 100, IV = 100 – 200, V = 200-300, VI = 300 – 400, VII = 400 – 500, VIII = 500–600, IX = 600 – 700, X = 700 – 800 and XI = 800 – 900 m from msl.

Moreover, some weed species that only found at lowland area having relatively high important value (NP) were consisted of *Cyperus digitatus*, *Leersia hexandra* and *Sacciolepsis interrupta*. On the other hand, other weed species having relatively high important value (NP) that only found at high land area were consisted of *Myriophyllum aquaticum* and *Sagittaria guayanensis*.

Weed species composition at generative stage in wet season was shown in Table 2. From 56 weed species found at this area, weed species abundance per location was in the range of 17 to 24 species with average value of 20.36 species. There were 4 dominant weed species of all



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weed species that consisted of *Echinochloa crusgalli*, *Ludwigia octovalvis*, *Alternanthera philoxeroides* and *Fimbristhyllis littoralis*. These weed species showed consistent dominance from lowland to highland. The dominance of *Echinochloa crusgalli* was tend to increase in accordance to elevation increase of location, whereas the dominance of *Ludwigia octovalvis* and *Fimbristhyllis littoralis* was tend to decrease. Other relatively dominant weed species were consisted of *Echinochloa colonum*, *Sphenoclea zeylanica*, *Monochoria vaginalis*, *Fimbristhyllis grifithii*, dan *Limnocharis flava*. Moreover, some weed species having relatively high important value (NP) were consisted of *Cyperus digitatus*, *Leersia hexandra* and *Sacciolepsis interrupta*, whereas weed species having relatively low important value (NP) that consisted of *Ipomoea 179ylindr*, *Panicum repens* and *Pistia stratiotes* were only found at low land area. On the other hand, weed species of *Myrriophyllum aquaticum* having relatively high important value (NP) and *Sagittaris guayanensis* having relatively low important value (NP) were only found at high land area.

Weed species composition at vegetative stage in dry season was shown in Table 3. From 53 weed species found at this area, weed species abundance per location was in the range of 16 to 23 species with average value of 20.36 species. There were 5 dominant weed species of all weed species that showed consistent dominance from lowland to highland areas which consisted of *Echinochloa crusgalli*, *Ludwigia octovalvis*, *Alternanthera philoxeroides*, *Fimbristhyllis littoralis* and *Limnocharis flava*. Dominance level of *Echinochloa crusgalli* and *Ludwigia octovalvis* from lowland to highland tends to increase, but it tends to decrease again at certain elevation. The decrease of dominance level with the increase of location elevation was also occurred on *Fimbristhyllis littoralis* and *Limnocharis flava*. Other relatively dominant weed species were consisted of *Cyperus iria*, *Paspalum distichum* and *Marsilea crenata*. Moreover, some weed species having relatively high important values (*Leersia hexandra* and *Sacciolepsis interrupta*) were only found at low land area. On the other hand, two weed species that consisted of *Myrriophyllum aquaticum* with relatively high important value and *Sagittaria guayanensis* with relatively low important value were only found at highland.

Weed species composition at generative stage in dry season was shown in Table 4. From 55 weed species found at this area, weed species abundance per location was in the range of 16 to 23 species with average value of 20.18 species. There were 5 dominant weed species of all weed species that showed consistent dominance from lowland to highland areas which consisted of *Echinochloa crusgalli*, *Ludwigia octovalvis*, *Fimbristhyllis littoralis*, *Alternanthera philoxeroides* and *Cyperus iria*. Dominance level of *Echinochloa crusgalli* from lowland to highland was tend to increase, whereas dominance level of *Ludwigia octovalvis* was also tend to increase, but it tends to decrease again. The decrease of dominance level from lowland to highland was also occurred on *Fimbristhyllis littoralis* and *Alternanthera philoxeroides.* Some relatively dominant weed species were consisted of *Paspalum distichum* and *Sphenoclea zeylanica*. Distribution of *Paspalum distichum* from lowland to highland was relatively uneven and its dominance level was also tend to decrease. *Sphenoclea zeylanica* was tend to be dominant only at lowland to intermediate land areas. Moreover, some weed species having relatively high important values (*Leersia hexandra* and *Sacciolepsis interrupta*) were only found at low land area. On the other hand, two weed species consisting of *Myrriophyllum aquaticum* with relatively high important value and *Sagittaria guayanensis* with relatively low important value were only found at highland area.

The above results showed composition change of weed species amongst growing seasons/crop growth stages of paddy field rice. The change was not only occurred on the existing weed numbers and weed species, but substantially was also occurred on their important values (NP). The change of weed species composition based on their important values was shown in Fig. 1.



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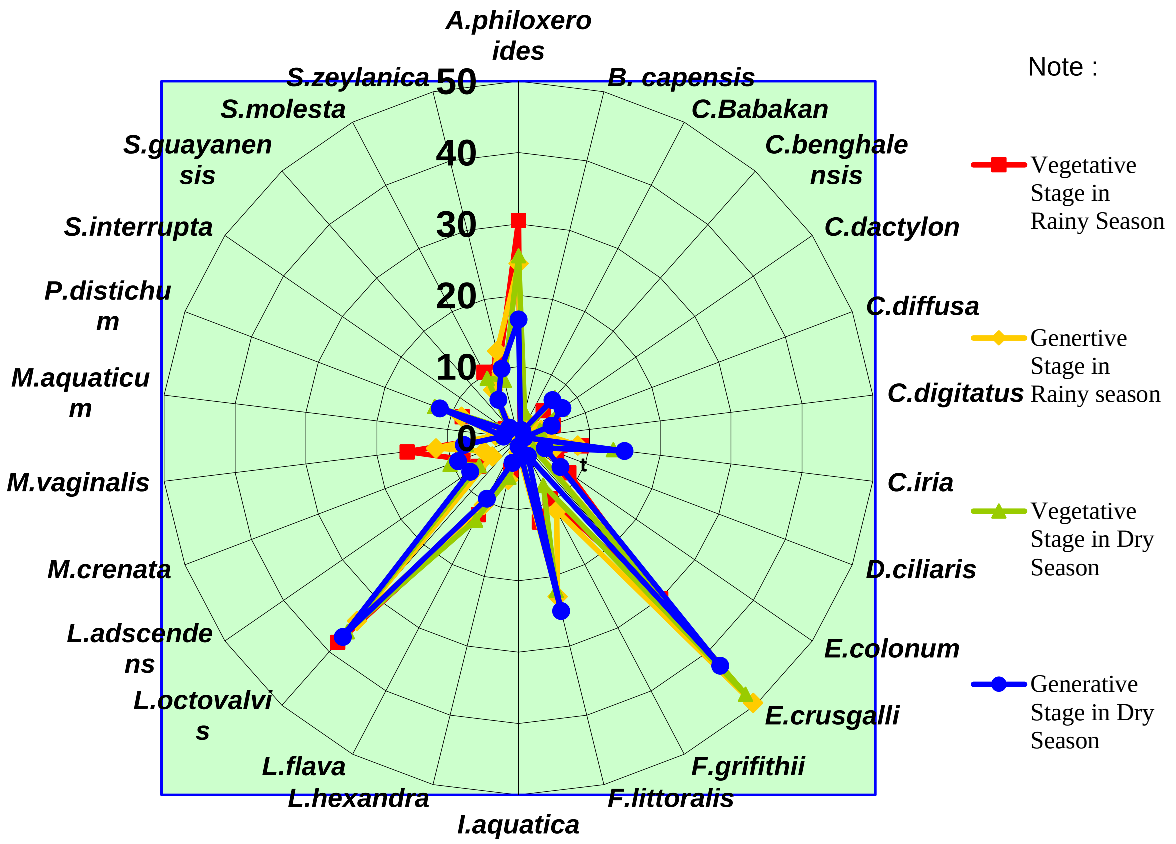


**Fig. 1.** The change of weed species composition based on their important values amongst growing seasons/crop growth stages of paddy field rice at Ciliwung-Cisadane Watershed area.

Figure 1 showed that important values for each weed species at each growing season/crop growth stage as well as amongst seasons/crop growt stages were different. There were four weed species that had high important values which consisted of *A. philoxeroides*, *E. crusgalli*, *F. littoralis* and *L. octovalvis*. From vegetative stage at wet season to generative stage at dry season, the important value of *A. philoxeroides* was tend to decrease, whereas important values of *E. crusgalli* and *F. littoralis* were tend to increase and important value of *L. octovalvis* was relatively stable. Important value for each weed species had significant role in determining the characteristics of weed species composition, especially related to status of general weed species and/or dominant weed species.

From 5 dominant weed species found at vegetative stage during wet season, there were 4 weed species of broadleaves (*Ludwigia octovalvis*, *Alternanthera philoxeroides*, *Monochoria vaginalis* and *Limnocharis flava*) and 1 weed species of grasses (*Echinochloa crusgalli*). This was due to the fact that weed species of broadleaves and grasses had high adaptation and high tolerance toward relatively flooding condition of paddy field area (Janiya dan Moody, 1989). The change of flooding condition on paddy field area might be the main factor for the shifting occurrence of dominant weed species at subsequent growing season/rice crop growth stage. The dominant weed species found at generative stage during wet season were *Echinochloa crusgalli*, *Ludwigia octovalvis, Alternanthera philoxeroides* and *Fimbristhyllis littoralis*, respectively. The decrease of irrigation water flooding and broken seeds dormancy had stimulated the increase of seedlings and population of *Echinochloa crusgalli* which made it to be the most dominant weed species. Weed species of *Echinochloa crusgalli* was hold out as the most dominant weed during two subsequent observations at dry season. The decrease of irrigation water flooding was also important factor which made *Fimbristhyllis littoralis* as one of dominant weed during this observation because the decrease of irrigation water flooding might stimulated its seedling and growth (Ampong-Nyarko dan De Datta, 1991; Holm *et al.*,



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1977; Soerjani *et al*., 1987). *Fimbristhyllis littoralis* showed consistent dominance and in fact tend to increase during two subsequent observations at dry season. The decrease of irrigation water flooding and environmental change occurred during generative stage at wet season could be tolerated and adapted by *Ludwigia octovalvis* and *Alternanthera philoxeroides* (Everaarts, 1981; Soerjani *et al*., 1987) so that these weeds became the dominant weeds although their dominances were not as high as *Echinochloa crusgalli*. Dominance of these weeds was hold out until two subsequent observations at dry season. On the other hand, dominance level of *Monochoria vaginalis* and *Limnocharis flava* was decreased so that they were no longer as dominant weeds during generative stage at wet season. This was due to the fact that decrease of fooding water was hindered their growth and their relatively low habitat could not compete with rice crops (Lamid dan Paller, 1984). The dominant weed species on young rice crop at dry season were *Echinochloa crusgalli*, *Ludwigia octovalvis*, *Alternanthera philoxeroides*, *Fimbristhyllis littoralis* and *Limnocharis flava*, respectively. The first four of dominant weed species were dominant during previous observation on generative stage at wet season, whereas the fifth dominant weed species (*Limnocharis flava*) had ever been dominant weed species on vegetative stage at wet season. The re-emergence of *Limnocharis flava* as one of dominant weed species was especially due to relatively high quantity of flooding water on vegetative stage at dry season which in turn stimulated its growth and development (Soerjani *et al*., 1987). The dominant weed species found during generative stage at dry seasons were consisted of *Echinochloa crusgall, Ludwigia octovalvis, Fimbristhyllis littoralis, Alternanthera philoxeroides* and *Cyperus iria*, respectively. The first four of dominant weed species were dominant during previous observation on vegetative stage at dry season, whereas the fifth dominant weed species (*Cyperus iria*) was newly emerged during this observation. Meanwhile, *Limnocharis flava* was not emerge as dominant weed species due to its poor growth as a results of decrease flooding of irrigation water and poor competition with rice plant that had higher growth level (Pons *et al*., 1987). On the other hand, decrease flooding of irrigation water had also affected dominance level increase of *Fimbristhyllis littoralis,* whereas coverage effect of higher rice crop height had results in dominance level decrease of *Alternanthera philoxeroides* which had low habitat (Soerjani *et al*., 1987; Everaarts, 1981). The emergence of *Cyperus iria* as one of dominant weed species was not only due to decrease flooding of irrigation water, but also because of illumination (light) increase during dry season. These two factors were capable to increase seedling and growth rates of this weed species which in turn made it as one of dominant weed species (Galinato *et al*., 1999).

From dominant weed species mentioned above, there were 4 weed species that consistently always appeared as dominant weed species on paddy field rice area which consisted of *Echinochloa crusgalli*, *Ludwigia octovalvis*, *Fimbristhyllis littoralis* and *Alternanthera philoxeroides*. These weed species had high adaptation capability and high tolerance toward the changes of environmental condition which were occurred amongst growing seasons/growth stage of paddy field rice. These weed species had high reproduction rate, fast initial growth, efficient utilization of resources and capable to complete their life cycle within relatively short time. Therefore, these weed species were difficult to be controlled because their population became very high in favourable growth condition. As a result, these weed species had high potential to create severe competition with paddy field rice which in turn can resist the growth and decrease production of rice crop (Ampong-Nyarko dan De Datta, 1991; Galinato *et al*., 1999; Holm *et al*., 1977; Soerjani *et al*., 1987).

In addition, there were some weeds species that consistently found only at lowland paddy field rice or highland paddy field rice areas. Some weeds species that consistently found only at lowland paddy field rice were *Leersia hexandra*, *Sacciolepsis interrupta*, and *Ipomoea 181ylindr*. These weeds species had good adapation toward condition of lowland environment and less tolerance toward condition of highland 181ylindrical so that they were typical weeds of lowland paddy field. Other weeds species that consistently found only at highland area were *Myrriophyllum aquaticum* and *Sagittaria guayanensis*. These two weeds species had good



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adapation toward condition of highland environment and less tolerance toward condition of lowland environment so that they were typical weeds of highland paddy field. All of five weeds species require and occupy flooding paddy field so that irrigation water condition was not a factor that differentiate their adaptation characters. The difference in adaptation character is assumed more or less due to air temperature factor because lowland area had relatively hot air temperature, whereas highland area had relatively cold air temperature. In this case, some weeds species that consistently found at lowland area had good adaptation and tolerance toward relatively high temperature, whereas some weeds species that consistently found at highland area only had good adaptation and tolerance toward relatively cold air temperature. Adaptation capability and tolerance ranges toward environmental condition are the results of selection and evolution processes which taken place for long time period and important to maintain life sustainability at environmental condition of habitat (Aldrich, 1984; Chozin *et al*., 1991; Radosevich *et al*., 1997; Sastroutomo, 1990).

## CONCLUSIONS

We derived several conclusions from this study, *viz.* (a). Weeds species composition of paddy field rice had changed due to the change of growing seasons/crop growth stages as well as location altitude or elevation, but there were 7 general weeds species that always found at every growing season/growth stage of paddy field rice and had relatively even distribution from lowland area to highland area which consisted of *Alternanthera philoxeroides*, *Fimbristhyllis littoralis*, *Ludwigia octovalvis*, *Echinochloa crusgalli*, *Limnocharis flava*, *Monochoria vaginalis* and *Paspalum distichum;* (b). The composition change of dominant weeds species was occurred amongst growing seasons/crop growth stages. In this case, dominant weeds species on vegetative stage at wet seasons were consisted of *L. octovalvis, A. philoxeroides*, *E. crusgalli*, *M. vaginalis* and *L. flava*; dominant weeds species on generative stage at wet season were consisted of *E. crusgalli*, *L. octovalvis, A. philoxeroides* and *F. littoralis*; dominant weeds species on vegetative stage at dry season were consisted of *E. crusgalli*, *L. octovalvis, A. philoxeroides*, *F. littoralis* and *L. flava*; and dominant weeds species on generative stage at dry season were consisted of *E. crusgalli, L. octovalvis*, *F. littoralis*, *A. philoxeroides* and *C. iria,* and (c). There were some weeds species that consistently found growth only at lowland paddy field rice and others weed species that consistently found growth only at highland paddy field rice. Based on the growth location consistency, there were some typical weeds for lowland paddy field rice which consisted of *Leersia hexandra*, *Sacciolepsis interrupta* and *Ipomoea 182ylindr* as well as some typical weeds for highland paddy field rice which consisted of *Myrriophyllum aquaticum* and *Sagittaria guayanensis*.

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# WEED MAPPING IN TWO CORN (*ZEA MAYS*) PRODUCTION CENTERS IN WEST JAVA PROVINCE OF INDONESIA

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**ABSTRACT** The principal objectives of this study were to map and determine the spread of weeds in the districts of Majalengka and Kuningan, the two central areas of corn production in West Java, Indonesia. This information generated can be used to control weeds in the crop. This study was conducted from March to June 2011. The experimental method used was descriptive with survey methods. Six observation areas were selected in each district, making a total o 12 of observation areas with different altitude and micro-climates, but with the ages of corn that were almost similar between 5 to 8 weeks. The spread of vegetation was determined through analysis of all weed species, summed of dominant ratio (SDR), coefficient of weed community population and dry weight of weeds. Questionnaires were given to the farmers to find out the history of the studied area of corn, *viz.* crop varieties, cropping patterns, types of fertilizer, weed control techniques, age of corn, row spacing and types of tillage. The dominant broadleaved weed species that found both in Majalengka and Kuningan research areas were *Ageratum conyzoides* L. and *Alternanthera philoxeroides* (Mort.) Griseb, whereas grass species were *Echinochloa colona* (L.) Link and *Eleusine indica* (L.) Gaernt. The dominant weed found in Majalengka district research areas were eight broad leaves species and three grass species, whereas the dominant weed found in Kuningan district research areas were seven broad leaves species, four grass species and one sedge species.

***Keywords*****:** Corn, community coefficient, dominant weed species, weed mapping.

## INTRODUCTION

Corn is one of the agricultural food commodities that have good prospects in Indonesia, because domestic demand is likely to increase from year to year as a result of increasing of consumption, growth of population and income (Rizal and Purnomowati, 2007). The efforts to improve corn productivity can be achieved by planting high yielding varieties, control of pest, plant diseases and weeds. Weeds can reduce crop yields through competition of nutrients and water in the soil, sunlight for photosynthesis, and space. The presence of weeds in cultivated crops decrease both quantity and quality of crop, so weeds in corn should be controlled to prevent yield losses (Rukmana and Saputra, 2003). Yield loss due to weeds can exceed the yield losses due to pests and diseases. Several studies have shown a negative correlation between weed dry weight and yield of corn, with a decrease in the yield can be up to 95% (Violic, 2000). According to Clay and Aquilar (1998) corn that planted with monoculture system and use low input does not give a high yield as a result of competition with weeds. The amount of reduction in corn due to weed competition was ranged from 16 to 62% (Sasmita, *et al*., 2003). Therefore, weed control is necessary to reduce weed infestation and crop losses.

In Indonesia there are 140 types of broad leaf weeds, 36 species of grass weeds, and 51 species of sedges weeds (Laumonier *et al*., 1986). Weeds that commonly found in corn from class of Gramineae are *Eleusine indica*, *Echinochloa colonum, Paspalum vaginatum, Cynodon dactylon, Panicum spp., Imperata 184ylindrical* and *Polytrias amaura*, whereas from class of Cyperaceae are: *Cyperus rotundus, Cyperus Iria* and *Cyperus compressus,* and from class of broad leaf weeds are: *Mimosa invisa, Ageratum conyzoides* and *Althernanthera sessilis* (Warisno, 1998).

Mapping of weeds in corn in various ecosystems and environmental conditions in two production centers of corn in West Java province is required in order to design the control



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method of weed in corn effectively. Corn production centers in West Java Province in accordance with West Java corn-belt program, is concentrated in the districts of Kuningan, Majalengka, Sumedang, Ciamis, Tasikmalaya, Garut, Bandung and Sukabumi.

At this present time in Indonesia there is no specific information regarding the type of weed species that exist on the corn field at various stadium, location, soil type, altitude, agro-climate and previous cropping patterns. Weed mapping in corn in a production center of Majalengka and Kuningan Districts are necessary for planning weed control efforts systematically. The purpose of this study was to determine the spread of corn weeds in Majalengka and Kuningan districts and this information can be used as a strategy to control weed in corn earlier

## MATERIALS AND METHODS

The experiments were conducted in the corn field owned by local farmers in districts of Majalengka and Kuningan.. The experiment areas were located at the different altitude, soil type, climate condition and different cropping pattern. The experiment was carried out from March 2011 to June 2011. The materials and tools used are 1) maps of Majalengka and Kuningan, 2) questioner, 3), shovels, 4) hoe, 5) plastic strap, 6) plastic bags, 7) electrical scales and 8) drying oven.

The experimental design used was descriptive with survey methods. In each district, six areas of corn field were determined that spread in a variety of different environmental conditions, but with the age of corn that are almost similar between the age of 5 to 8 weeks. The vegetation analysis was carried out on each corn crop area by using the method of minimum plot technique. The spread of vegetation was determined through analysis of all weed species, summed of dominant ratio (SDR), coefficient of weed community population and dry weight of weeds. Questionnaire was given to the farmer to find out the history of the studied area of corn, such as crop varieties used, cropping patterns used, kind of fertilizer used, weed control technique, age of corn, row spacing and type of tillage.

The observations used are percentage of summed dominance ratio (SDR), coefficient of weed population community, and total dry weight of the dominant weed species (broad leaves, grasses and sedges).

## RESULTS AND DISCUSSION

**Characteristics of study areas.** Table 1 summarizes the general traits of the study area including the annual rainfalls alongside the soil tillage, crop cultivars, agronomic and pest and diseases control measures in the districts of Majalengka and Kuningan.

**Table 1**. Characteristic of study areas in Districts of Majalengka and Kuningan

| Category | District of Majalengka | District of Kuningan |
|---|---|---|
| Altitude | Sub district Patuanan 61 m<br>Sub district Cisambeng 73 m<br>Sub district Karamat 80 m<br>Sub district Sindangkasih 139 m<br>Sub district Kulur 130 m<br>Sub district Kutamanggu 126 m | • Sub district Pakembangan 477 m<br>• Sub district. Bandorasa wetan 510 m<br>• Sub district Manis kidul 536 m<br>• Sub district Cirendang 695 m<br>• Sub district Cisantana 982 m<br>• Sub district Puncak 985 m |
| Crop cultivar used | Hybrid Pioneer 12 & 21 | Hybrid Pioneer 12 & 2 |
| Cropping pattern | Mostly monoculture and a few polyculture | Mostly monoculture and a few polyculture |
| Soil tillage | Full tillage | In upland areas zerro tillage and in lowland areas minimum tillage |



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| | | |
|---|---|---|
| Spacing | 100 x 60 cm (harvest time >100 days)<br>20 x 70 cm (harvest time 80-100 days) | 100 x 60 cm (harvest time >100 days)<br>20 x 70 cm (harvest time 80-100 days) |
| Pest, diseases and weed control | Mostly mechanic control and a few chemical control | Mostly mechanic control and a few chemical control |
| Previous crop used | Cereal, rice and corn | Chili, rice, corn and onions |
| Climatic condition at year 2010 | Annual rainfal is 2697 mm | Annual rainfal is 3596-3744 mm |
| Previous corn yield per ha | 4-7.5 ton | 7 ton |
| Irrigation supply | Available | Not available |
| Fertilizer used | Urea, phosphate | Urea, phosphate and organic fertilizer |

**Vegetation analysis**

The dominant weed species found in Majalengka district research areas were eight species of broad leaf weeds: *Aeschynomene indica* L, *Ageratum conyzoides* L, *Alternanthera philoxeroides* Griseb, *Cleome rutidosperma*, D.C, *Commelina diffusa* Burn F, *Polygala paniculata* L, *Synedrella nodiflora* L. and *Tridax procumbens* and three species of grasses: *Echinochloa colona* (L.) Link, *Eleusine indica* (L.) Gaernt, and *Eragrotis tenela* (L) (Beaur ex R&S) (Table 2). It also showed that the dominant of weed species found in the research area of Majalengka District vary, but generally dominated by broad leaf weeds. The broadleaved weed of *Alternanthera philoxeroides* Griseb was the most dominant weed and found in four research areas, namely Patuanan, Sindangkasih, Kulur and Kutamanggu. Weed of *Eleusine indica* (L) belongs to the most dominant grass weed species found in four research areas of Patuanan, Karamat, Kulur and Kutamanggu. There was no dominant sedge weed that found in Majalengka district.

**Table 2.** Weeds dominant in six corn fields in district of Majalengka

| **No** | **Species** | **Summed Dominance ratio (SDR ) %** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **A** | **B** | **C** | **D** | **E** | **F** |
| | **Broad leaf :** | | | | | | |
| 1 | *Aeschynomene indica* L. | 21.3 | 10.0 | | | | |
| 2 | *Ageratum conyzoides* L | | | 11.7 | | | |
| 3 | *Alternanthera`philoxeroides* (Mort) Griseb | 17.8 | | 17.4 | 20.4 | 12.4 | |
| 4 | *Cleome rutidosperma* D.C | | 10.6 | | | 17.5 | 21.0 |
| 5 | *Commelina diffusa* Burn F | 11.3 | 10.4 | | | 14.4 | |
| 6 | *Polygala paniculata* L. | 11.6 | | | 10.2 | | 10.0 |
| 7 | *Synedrella nodiflora* (L.) Gaernt | | | 13.9 | 10.7 | | |
| 8 | *Tridax procumbens* L. | | | | | | 11.4 |
| | **Grass :** | | | | | | |
| 9 | *Echinochloa colona* (L.) Link. | | 31.3 | | | | |
| 10 | *Eleusine indica* (L.) Gaernt | 12.3 | 10.4 | | 19.0 | 10.5 | |
| 11 | *Eragrotis tenela* (L.) Beaur ex R&S | 10.9 | | 15.3 | 12.3 | | 18.3 |
| | Others weeds species | 14.8 | 27.3 | 41.7 | 27.4 | 45.2 | 39.3 |

**Note:** District of Majalengka **:** A-F = villages of Patuanan, Karamat, Sindangkasih, Kulur, Kutamanggu and Cisambeng.

The dominant weed species that found in the research area of Kuningan district were six broad leaf weed species: *Ageratum conyzoides* L, *Alternanthera philoxeroides* (Mort)



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Griseb, *Amaranthus spinosus* L, *Artemisia vulgaris*, L., *Ephorbia hirta*, L and *Ipomoea Sp*, while the dominant grass weeds species found were *Eleusine indica* (L.) Gaernt, *Rorippa indica* (L) Hiern, *Cynodon dactylon* (L.) Pers and *Echinochloa colona* (L.) Link. Sedge weed species (*Cyperus kyllingia, L)*, was found in one research areas of Cirendang (Table 3). The weed of *Ageratum conyzoides* L and *Alternanthera philoxeroides* (Mort) Griseb were the most dominant broad leaf weed species that found in three research areas. The dominant grass weeds species of *Eleusine indica* (L) Gaernt was found in four research areas of Maniskidul, Cirendang, Pakembangan and Bandorasa wetan (Table 3).

Table 2 and 3 showed that the dominant weed that found in research areas of Majalengka and Kuningan Districts were different, but generally dominated by twelve species of broad leaf weeds, five species of grass weed species and one species of sedge weed species. The dominant broad leaf weed species that found in Majalengka and Kuningan research areas were *Ageratum conyzoides*, L. and *Alternanthera philoxeroides* (Mort) Griseb, while the dominant grass species found was *Eleusine indica* (L.) Gaernt and *Echinochloa colona* (L.) Link According to Mercado (1979) the difference of weed species was due to different crop management such as water control, type of fertilizer used and different cropping system.

**Table 3.** Weeds dominant in six corn fields in district of Kuningan

| No | Species | Summed Dominance Ratio ( SDR ) % | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F |
| | **Broad leaf:** | | | | | | |
| 1 | *Ageratum conizoides*, L | | | 19.4 | | 14.4 | 11.6 |
| 2 | *Alternanthera philoxeroides* (Mort) Griseb | 17.0 | 11.3 | | | 13.8 | |
| 3 | *Amaranthus spinosus*, L | 14.2 | | | | | |
| 4 | *Artemisia vulgaris* L. | | 28.4 | 28.2 | | | |
| 5 | *Euphorbia hirta* L. | | 15.0 | | | | |
| 6 | *Ipomoea* Sp | | | | 10.9 | | |
| | **Grass:** | | | | | | |
| 7 | *Eleusine indica* (L) Gaernt | 21.3 | | | 22.9 | 19.1 | 17.1 |
| 8 | *Rorippa indica* (L.) Hiern | 11.8 | | | | | |
| 9 | *Cynodon dactylon (*L) Pers | | | | 11.5 | | |
| 10 | *Echinochloa colona* (L.) Link. | | | | 13.1 | | |
| | **Sedge:** | | | | | | |
| 11 | *Cyperus kyllingia* L | | | | 12.2 | | |
| Other weeds species | | 35.7 | 45.3 | 52.4 | 29.4 | 52.7 | 71.3 |

District of Kuningan: A-F = villages of Maniskidul, Cisantana, Puncak, Cirendang, Pakembangan and Bandorasa wetan.

**Coefficient of weed population community (C %)**

The value of coefficient of weed population community (C) is a value to compare two type of vegetation from two different areas. The value of coefficient of weed population community (C) in all areas of the District Majalengka and Kuningan are below 75%, which means that the observation area of A - B and so on do not have the same weed population. The weed populations in six research areas of Majalengka were different from the weed population in six research areas of Kuningan. According to Tjitrosoedirdjo (1984) the value of C is smaller than 75% means that no similarity of the population. This is probably due to different altitude, climate, soil fertility, tillage methods, cropping history of corn, row spacing and different varieties of corn used. According to Nasution (1986) altitude affects the dominance of weed species. Weed species that dominate rubber plantations in North Sumatra and Aceh on 0-30 m altitude was sedge weed, whereas at an altitude 30-100 m was dominated by grass weed species. Furthermore, Nasution (1986) states that the type of soil also determines the dominance of



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weeds. The broad leaf weed species found more dominant on podzolic soil. Sedge weeds found more dominant in alluvial soil as compared with podzolic soil. According to Knott (2002) previous cropping pattern and soil type influence the number and species of weeds occurring. Weed species density are influenced by soil type, soil fertility and soil tillage (Aldrich and Kremer, 1997).

**Table 4.** Comparison value of weed population community coefficient from both research areas in Majalengka and Kuningan districts.

| No | District of Majalengka | | District of Kuningan | |
|---|---|---|---|---|
| | **Research areas** | **C (%)** | **Research areas** | **C (%)** |
| 1 | A : B | 50.62 % | A : B | 26.50 % |
| 2 | A : C | 46.24 % | A : C | 23.29 % |
| 3 | A : D | 49.95 % | A : D | 28.51 % |
| 4 | A : E | 43 % | A : E | 57.69 % |
| 5 | A : F | 31.77 % | A : F | 45.70 % |
| 6 | B : C | 26.20 % | B : C | 58.50 % |
| 7 | B : D | 33.68 % | B : D | 23.70 % |
| 8 | B : E | 34.23 % | B : E | 29.56 % |
| 9 | B : F | 27.48 % | B : F | 27.94 % |
| 10 | C : D | 60.58 % | C : D | 34.44 % |
| 11 | C : E | 55.17 % | C : E | 34.28 % |
| 12 | C : F | 43.58 % | C : F | 31.02% |
| 13 | D :E | 46.25 % | D : E | 34.75 % |
| 14 | D : F | 28.16% | D : F | 38.14 % |
| 15 | E :F | 51.39 % | E : F | 59.85 % |

**Note: District of Majalengka :** A-F = villages of Patuanan, Karamat, Sindangkasih, Kulur, Kutamanggu and Cisambeng.

**District of Kuningan:** A-F = villages of Maniskidul, Cisantana, Puncak, Cirendang, Pakembangan and Bandorasa wetan.

**Total dominant weed dry weight of grass, sedges and broad leaves weed species.**

Table 5 showed that the highest total dry weight of weed in district of Majalengka was found in the research area of B (Karamat) and A (Patuanan) with a value of 140.02 g and 139.79 g respectively. This was probably due to the research areas of Karamat and Patuanan have the highest total summed dominant ratio (SDR 72.66% for Karamat and 85.15% for Patuanan). Kutamanggu has the lowest total weight of dominant weed species (34.2 g), where the total SDR value of the research area of Kutamanggu also has the smallest value of total SDR 54.92%.

Table 6 showed that the research area of A (Maniskidul) in Kuningan district has the highest total dry weight of the most dominant weeds (149 g) and also highest total summed dominant ratio (64.33%), while the lowest total dry weight was found in research area of F (Bandorasa wetan) with the value of 64.9 g. Bandorasa wetan has also the lowest total summed dominant ratio (28.77%) as compared with the other research areas in Kuningan district. Table 5 and 6 showed that most of research areas in district of Kuningan has higher total dry weight weed as compared with the research areas in district of Majalengka. This probably due to Kuningan has higher altitude (477-982 m) as compared with Majalengka (61-130 m).

Weed distribution patterns were affected by altitude. Higher altitude areas tend to have higher weed population as compared with the lower altitude areas (Tjitrosoedirjo *et al.*, 1984). Most soil tillage in Kuningan research areas was zero tillage, whereas in Majalengka research areas full tillage. Zerro tillage system will increase weed population and distribution. According to Aldrich and Kremer (1997) soil tillage will influence weed species density.



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**Table 5.** Total dominant weed dry weight of grass, sedges and broad leaves weed species in six research areas in district of Majalengka.

| No | Species | Dry weight of weed in research areas (gram) | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F |
| | **Broad leaf weeds** | | | | | | |
| 1 | *Ageratum conyzoides* L | | | 14.4 | | | |
| 2 | *Aeschynomene indica* L. | 17.1 | 9.1 | | | | |
| 3 | *Alternanthera philoxeroides* Griseb | 34.6 | | 23.6 | 23.3 | 5 | |
| 4 | *Cleome rutidosperma* D.C | | 8.3 | | | 15.9 | 26.2 |
| 5 | *Comelina diffusa* Burn F | 22.2 | 8.4 | | | | |
| 6 | *Polygala paniculata*, L | 4.4 | | | | | |
| 7 | *Synedrella nodiflora* L. Gaernt | | | 13.9 | 11.4 | | |
| 8 | *Tridax procumbens* L. | | | | | | 7.9 |
| | **Grasses** | | | | | | |
| 1 | *Echinochloa colona* L. Link.) | | 99.5 | | | | |
| 2 | *Eleusine indica* L. Gaernt | 32.9 | 14.5 | | 55.4 | 13.3 | |
| 3 | *Eragrotis tenella* (L.) Beaur ex R & S | 28.5 | | 17.6 | 3.4 | | 7.9 |
| | **Total** | 139.8 | 140.0 | 69.5 | 93.42 | 34.2 | 42.0 |

**Note: District of Majalengka :** A-F = villages of Patuanan, Karamat, Sindangkasih Kulur, Kutamanggu and Cisambeng

**Table 6.** Total dominant weed dry weight of grass, sedges and broad leaves weed in six research areas in district of Kuningan.

| No | Species | Dry weight of weed in research areas (gram) | | | | | |
|---|---|---|---|---|---|---|---|
| | | **A** | **B** | **C** | **D** | **E** | **F** |
| | **Broad leaves weeds** | | | | | | |
| 1 | *Ageratum conizoides* L | | | 39 | 9.9 | 22.7 | 22.7 |
| 2 | *Amaranthus* L | 21 | | | | 6.26 | |
| 3 | *Artemisia vulgaris* L | | 44.4 | 54 | | | |
| 4 | *Alternanthera philoxeroides* (Mort) Griseb. | 32.7 | 19.6 | | | 12.1 | 4 |
| 5 | *Euphorbia hirta*, L | | 38.2 | | | | |
| 6 | *Ipomoea Sp* | | | | 5.5 | | |
| | **Grasses** | | | | | | |
| 1 | *Cynodon dactilon* (L.) Pers. | | | | 6 | | |
| 2 | *Eleusine indica* (L.) Gaernt. | 81.4 | | | 53.7 | 38.2 | 38.2 |
| 3 | *Rorippa indica* (L.) Hiern | 13.9 | | | | | |
| 4 | *Echinochloa colona* (L.) Link. | | | | 25.2 | | |
| | **Sedges** | | | | | | |
| 1 | *Cyperus kyllingia* L. | | | | 22.2 | | |
| Total | | 149 | 102.2 | 93 | 122.5 | 79.26 | 64.9 |

**Note: District of Kuningan:** A-F = villages of Maniskidul, Cisantana, Puncak, Cirendang, Pakembangan and Bandorasa wetan.

## CONCLUSIONS

The dominant broad leaf weed species that found both in Majalengka and Kuningan research areas *Ageratum conyzoides* L. and *Alternanthera philoxeroides* (Mort.) Griseb, whereas grass species were *Echinochloa colona* (L.) Link and *Eleusine indica* (L.) Gaernt.



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Weed composition in corn in districts of Majalengka and Kuningan were different. The dominant weed that found in Majalengka district were eight broad leaves species: *Aeschynomene indica* L. *Ageratum conyzoides* L, *Alternanthera philoxeroides* Griseb, *Cleome rutidosperma* D.C, *Comelina diffusa,* L, *Polygala paniculata,* L, *Synedrella nodiflora* L. Gaernt, and *Tridax procumben*, L, three species of grasses *: Echinochloa colona* (L.) Link, *Eleusine indica* (L.) Gaernt, *Eragrotis tenela* (L.) Beaur ex R&S. The dominant weed species that found in Kuningan district were six species of broadleaves weeds: *Ageratum conyzoides L, Alternanthera philoxeroides* (Mort) Griseb, *Amaranthus spinosus,* L., *Artemisia vulgaris*, L, *Euphorbia hirta*, L and *Ipomoea Sp.,* four species of grasses weed: *Eleusine indica* (L.) Gaernt, *Rorippa indica* (L.) Hiern, *Cynodon dactylon* (L.) Pers and *Echinochloa colona* (L.) Link*, one species of sedge: Cyperus kyllingia,* L.

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# DISTRIBUTION PATTERN OF PREDOMINANT WEEDS IN WET SEASON AND THEIR MANAGEMENT IN WEST BENGAL, INDIA

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**ABSTRACT** A survey on predominant weeds in nine districts of Purulia, Bankura, Birbhum, Burdwan, South Dinajpur, North Dinajpur, Cooch Behar, Jalpaiguri and Darjeeling in West Bengal, India revealed that *Ludwigia parviflora* (with a frequency of 42 to 83.85%), *Marselia quardifolia* (33.95 to 78.85%), *Cynodon dactylon* (13.85 to 45%), *Digitaria sanguinalis* (13.72 to 23.16%), *Echinochloa colonum* (5.39 to 22%), *Eclipta alba* (3.5 to 56%), *Cyperus iria* (10 to 83%), *Cyperus compressus* (13 to 42.6%) and *Alternanthera philoxeroides* (5 to 19%) were the most pre-dominant invasive species in wet season paddy field of almost all nine districts of West Bengal. In non-cropped areas, *Croton bonplandianum* (40 to 80%), *Cynodon dactylon* (20 to 36.05%), *Cassia tora* ( 40.77 to 60%), *Parthenium hysterophorus* (7.5 to 63%), *Blumea lacera* (12.15 to 69%), *Euphorbia hirta* ( 21.5 to 35%), *Ageratum conyzoides* (19 to 57%), *Xanthium strumarium* ( 6 to 30%) and *Aerva lanata* (12 to 18%) appeared frequently in all the districts of South Bengal whereas *Pteris spp.* ( 26 to 68%), *Lantana camara* (12 to 52.6%), *Tephrosia purpurea* (F 27.3 to 51.9%), *Cassia occidentalis* (16 to 46%), *Cannabis sativa* (3.5 to 15%) and *Parthenium hysterophorus* (15.3 to 38%) became aggressive with higher frequency and Importance Value Index (IVI) in the districts of northern part of West Bengal. Integrated approach using herbicides and mechanical weeding are the effective means of weed management in wet season paddy field in West Bengal. Biological control involving Mexican beetle - *Zygogramma bicolorata* and local competitive plants has been found promising against *Parthenium.* There is opportunity to utilize weeds as compost, vermicompost, green manures, mulching materials, fuel, fodder, medicine, biogas production, craft making, making basket, bags and other materials from the weeds of non-cropped areas in West Bengal.

***Keywords***: Predominant weeds, distribution, West Bengal, weed management, utilization

## INTRODUCTION

Weeds are an integral part of each and every agro-phytocoenosis. Thus, their interference with plant is natural. Because of high competitive ability and allelopathic interference, weeds cause an irreversible damage to plants. Some weeds are poisonous to human being and livestock. Out of total 826 weed species reported in the country, 80 are considered as very serious and 198 as serious weeds (NRCWS, 2007). The losses caused by them are more than either disease or insect and they create problem in intensive cultivation. The economic impact of weeds on the Indian economy as estimated at the Directorate of Weed Science Research (DWSR), Indian Council of Agricultural Research, reflects that nearly one third of oilseeds, half of the food grains, and an equal amount of pulses produced currently are lost due to weeds and proper weed management technologies if adopted can result in an additional production of 103 million tonnes of food grains, 15 million tonnes of pulses, 10 million tonnes of oilseeds and 52 million tonnes of commercial crops per annum, which in few cases are even equivalent to existing annual production. This amounts to an additional income of rupees 1,05,036 crores per annum (Varshney and Prasad Babu, 2008). Simply by adopting improved weed management practices in rice, mustard, wheat and potato, a gain of rupees 2.17 crores was estimated in parts of Birbhum, Bankura and Burdwan district of West Bengal during the period from 2001-2005 (Duary *et al.*, 2007).



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With nearly 72 per cent of the population living in the rural areas, agriculture is the predominant occupation in West Bengal, an agriculturally important province in eastern part of India. Irrespective of the variability in soil type, topography and rainfall in different parts of the state, the basic feature of cropping is the predominance of rice in the state. In wet season more than 80 per cent of the land is covered with paddy, with some other crops like jute, maize and pulses. Diversity in soil, climate and physiographic condition of West Bengal not only facilitate establishing weeds in crop field, but also in non-cropped areas. Climate change and variability have also played important role in establishment and redistribution of species in different agro-climatic zones. Recently import of food grains also facilitated many invasive species to enter, establish and spread in non-cropped and garbage areas of the State. Many weeds of non-cropped areas are potential source to enter into crop field just like Parthenium, which has now been found in many crop fields causing additional problem to the farmers (Ankure and Duary, 2010). These invasive species are potential threat to biodiversity, recreational and aesthetic values of land, and human and animal health.

Spectrum of weed flora is dynamic across habitats. To know the dynamics and relative importance of a species in a particular phytosociety, studies on flora and vegetation characteristics assume great relevance. Since the extent of damage depends upon the nature of weed, intensity of infestation, the association with the crop and other biotic and edaphic characteristics, proper knowledge on composition of weed flora of a specific region along with their identification, characterization and distribution are necessary to formulate an economic and effective weed management strategy. With this perspective an attempt was made to survey weed flora associated with wet season paddy and non-cropped areas of nine districts of West Bengal and their management.

## MATERIALS AND METHODS

Weed survey was conducted during wet season of 2010 in nine selected districts of West Bengal lies between 22° 38′ N to 27° 13′ N latitude and 85° 75′ E to 89° 54′ E longitude. The districts were Purulia, Bankura, Birbhum, Burdwan, South Dinajpur, North Dinajpur, Cooch Behar, Jalpaiguri and Darjeeling. For recording observations on the composition of weed flora, a stop was made after every 10-12 km on the selected route. The site for recording observation was selected about 200 meter away from the main road in case of cropped area so that it could represent an undisturbed situation with natural weed flora. For non-cropped area, the observation was recorded from road side mainly. Difficult-to-indentify weeds associated with the wet season paddy as well as non-cropped areas were identified after blooming. However, familiar or known weeds were identified even prior to flowering. The size of the quadrat was 1 m × 1 m. Twenty spots were considered in each village for a particular habitat. Species wise weed count was made using the list count quadrat method suggested by Mishra (1968). After recording the data, daily data sheet was arranged according to cropped and non cropped areas for a district. Hence, district-wise data sheets were arranged. Ecological analysis of weed flora was done on the basis of density (D), frequency (F), relative frequency (RF), relative density (RD) and relative dominance (RDo). The importance value index (IVI) was calculated to express the ecological success of the species with a single value only as per the method used by Mishra (1968).

## RESULTS AND DISCUSSIONS

During the survey in transplanted wet season paddy in nine districts of West Bengal, weeds under 24 botanical family were recorded, in which larger number of species were under Poaceae, ranging from 7 in North Dinajpur to 12 in Birbhum, followed by Cyperaceae, three in Darjeeling to 6 in most other districts, Amaranthaceae upto 5 species in Birbhum, Commelinaceae, one in Bankura to 4 in three other districts. Again weeds under 30 botanical family were observed in non-cropped areas, in which larger number was under Astereceae, ranging from 7 in four districts and 10 in Purulia, upto 9 species under Poaceae, followed by



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Fabaceae - 4 in North Dinajpur to 8 in Birbhum, Amaranthaceae - 4 in five districts to 7 in Birbhum, Euphorbiaceae - 2 in most of the districts to 6 in Birbhum, Malvaceae and Solanaceae upto 3 each, Verbinaceae and Commelinaceae - 2 each.

**Distribution of weeds in wet season paddy**

In Purulia district, out of total 36 species recorded the most frequently distributed weeds in wet season paddy were *Ludwigia parviflora* (with 78% frequency and IVI value of 55) followed by *Eclipta alba* (55% and 44.2), *C. iria* (49.2% and 25.19), *Marsilea quadrifloia* (63.16% and 21.06), *C. compressus* (42.6% and 18.8), *C. difformis* (40.2% and 15.8) and *F. miliacea* (35.26% and 14.53). A total of 24 species were recorded in Bankura district and out of which the most predominant weeds were *Ludwigia parviflora* (with 83.85% frequency and IVI value of 61.54), followed by *C. iria* (60.77% and 33.15), *Marsilea quadrifloia* (73.85% and 28.18), *C. difformis* (59.23% and 26.84), *Commelina nudiflora* (49.23% and 24.96), *Eclipta alba* (28.46% and 16.48), *Hydrolea zeylanica* (43.08% and 15.56) and *E. colona* (26.9% and 15.29). *Ludwigia parviflora* (with 62.79% frequency and IVI value of 41.42), *A. sessilis* (47.21% and 22.62), *Commelina benghalensis* (50.93% and 22.44), *Cynodon dactylon* (45% and 20.61), *F. miliacea* (45.12% and 19.80), *E. colona* (34.65% and 13.82), *Marsilea quadrifloia* (33.95% and 13.72), *C. nudiflora* (33.14% and 13.6), *C. iria* ( 31.16% and 14.41) and *Cyperus difformis* (31.05% and 12.79) were the most frequently distributed weeds in Burdwan district. In Birbhum, 45 weeds were recorded in paddy. *Ludwigia parviflora* (with 66.26% frequency and IVI value of 52.65), *Marsilea quadrifloia* (61.78% and 22.91), *C. iria* (54.3% and 26.3), *F. miliacea* (50.19% and 18.75), *Commelina benghalensis* (40.84% and 18.58), *Cyperus difformis* (33.46% and 12.59), *Cynodon dactylon* (32.24% and 16.87), E. *alba* (28.97% and 23.24), *C. compressus* (42.6% and 18.8) and *C. difformis* (40.2% and 15.8) were major weeds in paddy field. Thirty four weed species were observed in paddy field of S. Dinajpur where *Ludwigia parviflora* (with 42.5% frequency and IVI value of 40.10) was the most frequently distributed weed. Other major weeds were *Cyperus digitatus* (50% and 33.82), *A. sessilis* (38.13% and 21.07), *C. benghalensis* (36.88% and 19.08), *F. miliacea* (33.75% and 17.9), *E. colona* (25.63% and 17.56), *C. dactylon* (36.88% and 17.17), *M. quadrifolia* (31.25% and 13.92), *Monochoria vaginalis* (28.13% and 12.8), *Dactyloctenium aegyptium* (21.25% and 11.10) and *C. iria* (18.13% and 9.46). The paddy field of North Dinajpur district was infested with a total of thirty three weed species, out of which *Ludwigia parviflora* (with 42.0% frequency and IVI value of 31.47) was the most predominant weed. *Cyperus digitatus* (44% and 29.65), *A. sessilis* (52.67% and 29.33), *F. miliacea* (42% and 21.68), *C. benghalensis* (44.67% and 21.04), *C. dactylon* (42.67% and 18.35), *M. quadrifolia* (35.33% and 14.34), *Monochoria vaginalis* (29.33% and 12.86), *C. nudiflora* (24.67% and 11.06) and *Dactyloctenium aegyptium* (24.0% and 10.63) were the other dominant weeds in the paddy field of this district. *Ludwigia parviflora* (with 60.42% frequency and IVI value of 46.52) was found most frequently in paddy fields of Cooch Behar district also where thirty weed species were found. *C. iria* (54.38% and 36.97), *C. dactylon* (55.83% and 30.43), *E. colona* (43.96% and 24.6), *A. philoxeroides* (52.71% and 27.24), *C. benghalensis* (51.885 and 25.96), *E. indica* (36.67% and 20.59), *F. miliacea* (38.96% and 17.85), *Dactyloctenium aegyptium* (28.96% and 16.63) and *C. nudiflora* (22.29% and 10.29) were the other weeds found more frequently in the paddy field. A total of forty weed species were observed in Jalpaiguri district. *Ludwigia parviflora* was the most pre-dominant weed (with 54.29% frequency and IVI value of 35.67) species found in paddy field, followed by *C. dactylon* (40.82% and 26.05), *A. sessilis* (34.69% and 22.66), *C. benghalensis* (36.53% and 22.58), *C. iria* (28.98% and 20.55), *F. miliacea* (31.63% and 19.37), *E. colona* (29.59% and 19.45), *Dactyloctenium aegyptium* (20.61% and 13.64), *C. digitatus* (20.61% and 18.61) and *C. nudifolra* (18.57% and 11.31). *Ludwigia parviflora* (with 60% frequency and IVI value of 53.45), *C. iria* (52.14% and 38.38), *Cynodon dactylon* (44.29% and 25.38), *Dactyloctenium aegyptium* (37.14% and 22.37), *E. india* (38.57% and 21.28), *C. benghalensis* (35.71% and 18.02), *E. colona* (29.29% and 15.39), *A. philoxeroides* (27.14% and 14.46), *F. miliacea* (30%



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and 13.40) and *C. nudiflora* (22.14% and 10.99) were the most frequently distributed weeds in Darjeeling district.

From the survey in different districts, an idea of pattern of distribution of different weeds could be visualized to understand the impact of individual weed on the wet season paddy. The most frequently observed weeds infesting transplanted wet season paddy under the study area were *L. parviflora*, *C. iria*, *F. miliacea*, *Cynodon dactylon*, *M. quadrifolia*, *Commelina benghalensis*, *C. nudiflora*. Similar weed flora was also reported by several workers (Duary, 1994; Mukherjee and Singh, 2005 and Gogoi *et al.*, 2005). Continuous water standing due to heavy rainfall in the wet season, probably determined the composition of weed flora in transplanted rice in which broadleaved species were more in number and more dominant than the total grasses or sedges in all the districts (Fig. 1). Besides, any crop grown in wet season is heavily infested with all categories of weeds because of warm and humid weather, characterized by high temperature and rainfall. Many workers have reported all categories of weeds to be present in different rice ecosystems (Mukhopadhyay and Duary, 1995; Duary *et al.*, 2005a; Mondal *et al.*, 2005, Moorthy and Saha, 2005 and Bhattacharjee and Ray, 2007). *Ludwigia parviflora* was the most dominant species in paddy throughout all the districts under study. During last two decades, it has been observed that this weed appeared as the most predominant weed in different rice ecosystems. The probable reason for its wide ecological amplitude might be the adaptation by special structure like periderm and pneumatophores as reported by Duary and Mukhopadhyay (1999).

*Commelina benghalensis*, *C. nudiflora*, *Marsilea quadrifolia* and *Cynodon dactylon* are the weeds which propagate mainly through vegetative means. Recently the increased use of tillage implements (power tiller and tractor-drawn) while tilling the soil, has aggravated the problem of such weeds by cutting into pieces which further grow and multiply as separate individuals. This corroborates the findings of Duary and Mukhopadhyay (2004) who reported that mechanisation like use of tractor and power tiller greatly elevated the problems of species like *Marsilea* which propagates vegetatively in rice-rice system of West Bengal.

**Distribution of weeds in non-cropped area**

In non-cropped areas also the number of broadleaved weeds was much higher than grass and sedge (Fig. 2). In Purulia district a total of sixty two weeds were observed. *Croton bonplandianum* (with highest frequency of 47.31 % and IVI value of 33.99), *Cassia tora* (27.96% and 24.09), *Cynodon dactylon* (28.82% and 21.18), *Amaranthus viridis* (30.86% and 16.18), *Ipomoea carnea* (36.88% and 14.99), *Ageratum conyzoides* (31.4% and 14.08), *Cyperus rotundus* (20.86% and 12.87), *Euphorbia hirta* (21.51% and 11.22), *Calotropis procera* (30% and 10.65), *Xanthium strumarium* (28.28% and 10.87), *Chromolaena odorata* (25.05% and 10.42) and *Solanum sisymbrifolium* (16.56% and 6.01) were the most dominant weeds in the non-cropped areas. *Parthenium hysterophorus* was observed with a frequency of 7.42% in the district. In Banukra district a total of thirty seven weed species were found, out of which *C. bonplandianum* (56.92% and 28.09),*C. tora* (with frequency of 40.77 % and IVI value of 28.29), *Parthenium hysterophorus* (35.38% and 21.48), *Phylanthus niruri* (31.54% and 20.51), *E. hirta* (33.85% and 16.30), *Tephrosia purpurea* (30.77% and 15.17), *A. viridis* (32.3% and 14.07), *A. spinosus* (30.77% and 13.19), *Achyranthus aspera* (53.08% and 12.97), *Blumea lacera* (31.54% and 10.92), and *S. sisymbrifolium* (32.3% and 10.27) were the most dominant weed. *Xanthium strumarium* was observed with a frequency of 20%. Total fifty five weed species were found in Burdwan district, out of which *C. tora* (with frequency of 48.95% and IVI value of 25.83), *C. bonplandianum* (35.93% and 15.05), *C. dactylon* (36.05% and 16.76), *C. occidentalis* (37.21% and 18.69), *P. hysterophorus* (35.47% and 15.96), *Ageratum conyzoides* (37.21% and 15.86), *T. purpurea* (24.53% and 12.03), *Xanthium strumarium* (27.91% and 10.33), *Sida rhombifolia* (26.05% and 12.22), *Lantana camara* (20.35% and 8.61), *C. rotundus* (28.95% and 12.78), *A. aspera* (32.91% and 11.25) and *S. sisymbrifolium* (22.3% and 8.81) were the most dominant weed. *C. bonplandianum* (53.46% and 30.33) recorded the



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highest value of frequency and IVI among the total sixty two weeds found in non-cropped areas of Birbhum district. *C. dactylon* (36.36% and 22.03), *X. strumarium* (40.47% and 17.11), *Aerva lanata* (33.46% and 14.53), *I. carnea* (35.60% and 14.51), *P. niruri* (23.83% and 14.32), *C. tora* (27.1% and 14.17), *Colocasia esculenta* (31.21% and 12.96), *A. viridis* (23.08% and 11.13), *A. Conyzoides* (24.86% and 10.65), *A. Aspera* (35.14% and 10.31) and *S. sisymbrifolium* (25.79% and 11.03) were the other weeds found more frequently. The frequency of *Parthenium* was 8.69% in roadside of the district. In S. Dinajpur district total thirty nine weeds were found. *S. rhombifolia* recorded the highest value of frequency and IVI (46.25% and 32.40). Other dominant weeds were *C. occidentalis* (33.75% and 24.91), *C. tora* (36.25% and 24.53), *T. purpurea* (31.25% and 22.99), *Pteris sp.* (26.25% and 22.21), *Vernonia cineria* (33.75% and 21.07), *A. conyzoides* (31.88% and 16.66), *C. dactylon* (18.75% and 10.96), *X. strumarium* (16.25% and 10.75), *Tridax procumbense* (18.75% and 10.08), *L. camara* (13.13% and 9.06), *P. hysterophorus* (15.0% and 8.84) and *S. sisymbrifolium* (10.63% and 7.35). *C. tora* (with frequency of 43.33% and IVI value 29.63), *C. occidentalis* (38.67% and 29.08), *Pteris sp.* (26.00% and 22.05), *Vernonia* (34.67% and 19.23), *A. conyzoides* (30.67% and 18.92), *T. Purpurea* (27.33% and 17.18), *C. dactylon* (30.67% and 15.78), *L. camara* (26% and 17.98), *S. rhombifolia* (23.33% and 14.58), *T. procumbens* (28% and 12.97), *A. aspera* (26% and 11.07), *P. hysterophorus* (10.67% and 6.76) and *S. sisymbrifolium* (7.33% and 4.48) were found more frequently and recorded the higher IVI value out of thirty six weed species found in N. Dinajpur district. *Xanthium strumarium* was found with a frequency of 10%. *C. occidentalis* (53.75% frequency), *Sida rhombifolia* (52.29%) *Pteris* sp. (47.71%) and *Lantana camara* (46.67%) were most frequently observed in the non-cropped areas of Cooch Behar district. *Pteris sp.* (with frequency of 47.71% and IVI of 33.90), *T. purpurea* (46.46%), *C. benghalensis* (32.5% and 17.49), *C. dactylon* (32.71% and 13.97), *C. tora* (31.46% and 12.05), *C. bonplandienum* (30.83% and 11.33) were distributed more frequently out of 39 total weed species recorded. *X. strumarium, P. hysterophorus* and *S. sysimbrifoilum* were recorded with a relatively lower frequency of 8.13, 6.04 and 1.67%, respectively. In Jalpaiguri district a total of forty weeds were found, out of which *Pteris sp.* recorded the highest value of frequency and IVI (64.28% and 44.81). Among the others, *L. camara* (52.65% and 31.65), *T. purpurea* (51.84% and 25.32), *C. occidentalis* (45.71% and 21.72), *S. rhombifolia* (43.88% and 17.44) *C. tora* (38.98% and 17.20), *C. bonplandianum* (38.57% and 16.46), *T. procumbens* (30.0% and 11.09), *C. benghalensis* (23.88% and 11.05), C*leorodendron* (16.73% and 10.54), *X. strumarium* (20.41% and 9.30) and *P. hysterophorus* (15.31% and 6.11) recorded higher value of frequency as well as IVI. A total of Thirty five weed species were found in the non-cropped areas of Darjeeling district. Out of these, *L. camara* recorded the higher value of frequency (42 %) and highest value of IVI (48.23). *Pteris sp*. was found more frequently with second highest IVI value. Other dominant weeds were *C. rotundus, Cassia occidentalis, C. benghalensis, Sida rhombifolia, Tephrosia purpurea, Amaranthus spinosus, Croton bonplandianum, Cassia tora, A. aspera, C. dactylon* etc. *Parthenium hysterophorus* was observed with a frequency of 8.57% in the district.

*Parthenium hysterophorus, Xanthium strumarium, Solanum sysimbrifolium* were distributed almost all the districts under study. *Parthenium hysterophorus* has spread like a forest fire in all the states of India in non-cropped areas owing to its unique characteristics like higher seed production potentiality, absence of dormancy of seeds, photo- and thermo-insensitiveness, allelopathic effect, completion of life cycle thrice a year in West Bengal etc. as also reported by Duary *et al*. (2005b).

**Five pre-dominant weed species in nine districts of West Bengal**

*Ludwigia parviflora* was the most predominant weed flora in transplanted wet season paddy in all the districts of West Bengal (Table 2). Among the sedges *C. iria* was one of the five most predominant weeds in seven districts. *Commelina benghalensis, A. sessilis and Marsilea quadrifolia* were one of the five major weeds in six, four and three districts, respectively.



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*Cynodon dactylon* and *F. miliacea* also appeared as one of the five most predominant weeds in four and three districts under study respectively.

**Table 2.** Five pre-dominant weed species in transplanted wet season paddy

| Districts | Pre-dominant weed species in transplanted wet season paddy | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Purulia | *L. parviflora* (55.21)* | *E. alba* (44.19) | *C. iria* (25.19) | *M. quadrifolia* (21.01) | *C. compressus* (18.80) |
| Bankura | *L. parviflora* (61.54) | *C. iria* (33.15) | *M. quadrifolia* (28.18) | *C. difformis* (26.84) | *C. benghalensis* (24.96) |
| Burdwan | *L. parviflora* (41.42) | *A. sessilis* (22.62) | *C. benghalensis* (22.44) | *C. dactylon* (20.61) | *C. digitatus* (20.46) |
| Birbhum | *L. parviflora* (52.65) | *C. iria* (26.30) | *E. alba* (23.24) | *M. quadrifolia* (22.91) | *F. miliacea* (18.75) |
| S. Dinajpur | *L. parviflora* (40.10) | *C. digitatus* (33.82) | *A. sessilis* (21.07) | *C. benghalensis* (19.08) | *F. miliacea* (17.90) |
| N. Dinajpur | *L. parviflora* (31.47) | *C. digitatus* (29.65) | *A. sessilis* (29.33) | *F. miliacea* (21.68) | *C. benghalensis* (21.04) |
| Cooch Behar | *L. parviflora* (46.52) | *C. iria* (36.97) | *C. dactylon* (30.43) | *A. philoxeroides* (27.24) | *C. benghalensis* (25.96) |
| Jalpaiguri | *L. parviflora* (35.67) | *C. dactylon* (26.05) | *A. sessilis* (22.66) | *C. benghalensis* (22.58) | *C. iria* (20.55) |
| Darjeeling | *L. parviflora* (53.45) | *C. iria* (38.38) | *C. dactylon* (25.38) | *D. aegyptium* (22.37) | *E. indica* (21.28) |

* Figures in parentheses are IVI values.

**Table 3.** Five pre-dominant weed species in non-cropped areas of different districts

| Districts | Pre-dominant weed species in non-cropped area | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Purulia | *C. bonplandianum* (33.99)* | *C. tora* (24.09) | *C. dactylon* (21.18) | *A. viridis* (16.18) | *I. carnea* (14.99) |
| Bankura | *C. tora* (28.29) | *C. bonplandianum* (28.09) | *P. hysterophorus* (21.48) | *P. niruri* (20.51) | *E. hirta* (16.30) |
| Burdwan | *C. tora* (25.83) | *C. occidentalis* (18.69) | *C. dactylon* (16.76) | *P. hysterophorus* (15.96) | *A. conyzoides* (15.86) |
| Birbhum | *C. bonplandianum* (30.30) | *C. dactylon* (22.03) | *X. strumarium* (17.11) | *A. lanata* (14.53) | *I. carnea* (14.51) |
| S. Dinajpur | *S. rhombifolia* (32.40) | *C. occidentalis* (24.91) | *C. tora* (24.53) | *T. purpurea* (22.99) | *Pteris sp.* (22.21) |
| N. Dinajpur | *C. tora* (29.63) | *C. occidentalis* (29.08) | *Pteris sp.* (22.05) | *V. cinerea* (19.23) | *A. conyzoides* (18.92) |
| Cooch Behar | *L. camara* (34.72) | *Pteris sp.* (33.90) | *S. rhombifolia* (23.72) | *T. purpurea* (22.66) | *C. occidentalis* (21.64) |
| Jalpaiguri | *Pteris sp.* (44.80) | *L. camara* (31.65) | *T. purpurea* (25.32) | *C.occidentalis* (21.72) | *C.bonplandianum* (17.2) |
| Darjeeling | *L. Camara* (25.42) | *Pteris sp.* (24.58) | *C. rotundus* (20.69) | *C. occidentalis* (19.48) | *C. benghalensis* (19.13) |

* Figures in parentheses are IVI values



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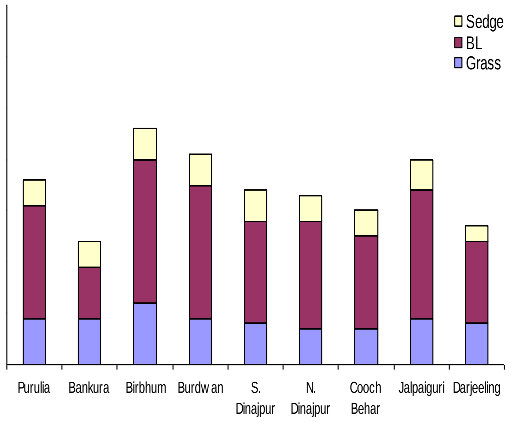


**Fig.1**. Number of weeds under different categories in paddy

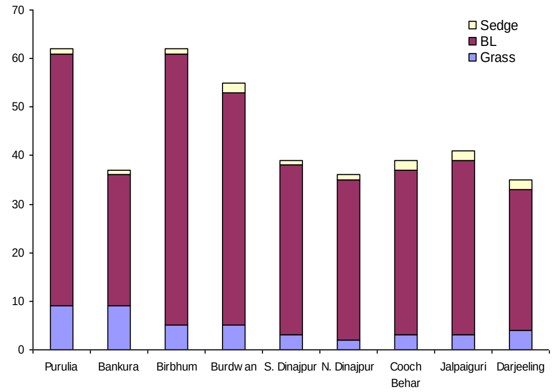


**Fig.2**. Number of weeds under different categories in non-cropped area

Again in non-cropped areas, *Cassia occidentalis* and *C. tora* were one of the most five predominant weeds in six and five districts of West Bengal under study, respectively. In most of the districts, *Croton bonplandianum* and *Tephrosia purpurea* were also recorded as one of the five dominant weeds in wet season (Table 3). *Cynodon dactylon* was one of the major weeds in three districts. However, in North Bengal *Pteris sp.* and *Lantana camara* appeared as one of the most pre-dominant weeds in non-cropped areas. *P. hysterophorus* was observed in all the districts with frequency from 6.04 to 35.47% and it was one of the five major pre-dominant weeds in 2 districts of West Bengal under study.

**Management**

Indian agriculture is labour intensive. Until recent past, manual weeding was the predominant weed management practice in West Bengal. But non-availability of labourers in peak period of demand due to implementation of National Rural Employment Guarantee Scheme of Govt. of India has compelled the farmers to search for alternative weed management strategies. Farmers are not able to tackle the new weed problems with their existing weed management practices. Severe crop losses due to delayed weeding or no weeding are common. As a result demand for low cost, effective, time saving, improved weed management strategies in different agro-ecological situations has increased from various corners of West Bengal. Farmers started to think of alternatives and herbicides are becoming obvious choice. Recently, the use of herbicide is increasing. Herbicides like butachlor, pyrazosulfuron -ethyl are used in dry bed and wet bed nursery. In transplanted wet season paddy depending upon the nature and intensity of weed flora glyphosate as pre-planting, followed by pyrazosulfuron-ethyl, pretilachlor, butachlor as pre-emergence or Almix, sodium bispyribac and ethoxysulfuron to a limited extent as early post emergence are recommended and practised. Almix has performed quite satisfactorily in managing weeds like *Marsilea*, *Jussia*, *Commelina* and other broadleaved weeds along with sedges. Sodium bispyribac has been found effective in rice nursery as well as main field where *E. crusgalli*, *E. grabrescence* are problem.

In non-cropped areas *Parthenium* was distributed in almost all the districts. In smaller area of infestation herbicides are used in a very limited scale. Metribuzin at 2.0 kg $ha^{-1}$, glyphosate, 2, 4-D at 2.0 and 1.0 kg caused significantly higher mortality of *Parthenium hysterophorus* (Duary *et al.*, 2005b). Biological control of *Parthenium* involving competitive local self-perpetuating plants like *Tagetis erecta, Cassia tora, Cassia occidentalis, Tephrosia purpurea, Ocimum americana, Sida rhombifolia* has been found very effective in suppressing *Parthenium*. Mexican beetles (*Zygogramma bicolorata*) had been established in Birbhum district of West Bengal after 4-5 years continuous release. In established site, 80-95% damage to *Parthenium* by the beetle was noticed during September – October. This was the first time



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large scale establishment of the biocontrol agent (*Zygogramma bicolorata*) and considerable damage to *Parthenium* in West Bengal. Integrated approach using glyphosate at 1.0 lit/ha and biological methods involving competitive plants *Cassia tora and Tephrosia purpurea* and insect *Zygogramma bicolorata* suppressed *Parthenium* to the extent of more than 90% (Anonymous, 2011).

Tribal people of West Bengal (Tribal like *Santal*, *Kheria* and *Moora* of Khatra region of Bankura district) have been observed to use an infusion of the whole plant of cocklebur (*Xanthium*) to recover from cough. The leaf extract has significant anti-cough effect in experimentally induced cough reflex in mice like the standard drug - codeine phosphate (Mandal *et al.*, 2005). *Xanthium strumarium* has been recorded in West Bengal to have their use as vegetable in addition to medicine. Young twigs are used as vegetable. Whole plant is used in chronic malaria, ulcers, syphilitic and piles as also reported by Biswas and Mondal (2012).

People in different districts utilize the water hyacinth as fodder, fuel, soil enhancers, mulch on soil, water cooler, compost and making craft as also reported by Ghosh and Purkait (2007). Green leaves are collected and used as fodder. The plants are collected and used as mulching materials for growing potato, taro, elephant foot yam etc. Fuel briquettes, preparation of compost, vermicompost, craft making, making furniture, cards, basket etc. are the other uses of water hyacinth. In eastern India the water hyacinth's stems are used as a braiding material and a source of fibers. Strings of dried fibers are woven or interlinked together to form a braid or cord used for making bags, footwear, wreaths, hats, vases and more decorative materials. Dried stems are used for baskets and furniture.

*Croton bonplandianum* was reported to have many medicinal uses. The parts which have medicinal value are seed and seed oil. This plant is rich in N, P, K, especially in potash. The farmers generally incorporate the plants into the soil with the onset of monsoon. The plants after collection and drying are also used as fuel for domestic purposes. Local people in the remote area of West Bengal, use its root against snake bite and the leaf extract against high fever. Leaves of this plant are used for controlling high blood pressure and for the treatment of skin diseases, cut and wounds. Various extracts of this plant are also known to possess antimicrobial activity and antitumour activity (Mandal *et al.*, 2009).

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# AUTECOLOGY OF INVASIVE SPECIES *CYPERUS ROTUNDUS* L. IN FOREST PHENOTYPIC AND GENOTYPIC VARIATIONS AMONG WEEDY RICE (*ORYZA SATIVA* F. *SPONTANEA*) POPULATIONS IN MATARA AND KURUNAGALA DISTRICTS OF SRI LANKA

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**ABSTRACT** Weedy rice (*Oryza sativa* f. *spontanea*) has posed a novel threat to the sustainability of rice cultivation in Sri Lanka and thus it is apparent to control weedy rice (WR) to enhance the quality and quantity of economical yield of rice. Present study focuses to determine the level of phenotypic and genotypic diversity of WR populations occur in rice fields of Matara and Kurunagala Districts, Sri Lanka. Seeds of presumed WR bio-types were collected from five different locations in each district. Five replicates of each bio-type were planted in plastic pots with representative paddy soils from each location. Replicates were arranged in Complete Randomized Design (CRD). Agro-morphological characterization was made using thirty six characters according to Standard Characterization Catalogue. Collected data was subjected to reduction of dimension using Classification and Regression Tree analysis (CART), on which four out of 8 variables (lemma and palea color, seed coat color, panicle shattering and awning after full heading) were chosen based on the time, labor and cost benefit. Results of the Cluster analysis (CA) agreed with the results obtained from CART. The reduced data were subjected to CA for clustering WR bio-types with cultivated rice varieties as a reference. Cluster formation was evaluated by calculating Cophenetic Coefficient and Inconsistency Index. The cluster analysis classified the WR bio-types collected from both districts into three groups at 50% phenone level (Cophenetic Co-efficient = 0.89). Calculated Inconsistency Indices showed a considerable regularity in grouping of WR bio-types in the clusters produced. On the analysis of data with respect to districts, three possible groups of bio-types and six possible groups of bio-types represented the Matara and Kurunagala Districts respectively. Results of molecular studies using SSR markers ($RM_{21}$ and $RM_{11}$) also revealed a higher genetic diversity among WR populations in two different districts in the country.

***Keywords:*** Weedy rice, Agro-morphological variation, *Oryza sativa* f. *spontanea*, Sri Lanka.

## INTRODUCTION

Weedy rice (*Oryza sativa* f. *spontanea*) of the family Poaceae is a weed, which is morphologically highly variable and shows similarities to wild and cultivated rice. Weedy rice (WR) is commonly distributed in rice-growing areas especially in South-East Asia, North America and Southern Europe (Mortimer *et* al, 2000) and the problem is most prominent in areas where direct wet and dry seeded rice growing systems are available (Ferrero and Finassi, 1995). Varying degrees (40%-80%) of WR infestations are reported from many tropical countries (De Souza, 1989, Diallo, 1999, Garcia and Rivero, 1999, Fletes, 1999). The WR incidents was first reported in Sri Lanka in Ampara District (Marambe and Amarasinghe, 2000; Abeysekera *et al*., 2010) which is at present spreading into many areas irrespective of the agro-ecological zones in the country. Although WR was first reported in mid 1990s, the knowledge on its genetic diversity along the mechanisms of distribution is limited, which preclude the WR



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control and management in the country. Therefore, it is necessary to study WR populations in different agro-ecological zones using multivariate statistical procedures to compare agro-morphological and molecular variability. This enables the understanding of the level and spatial distribution of the diversity of WR populations in the country. Morphological and topographical characteristics of the plants have been the criteria in identification and classification of WR bio-types (Qinjin *et al*., 2006). The objective of the present study is to determine the level of phenotypic and genotypic variation of weedy rice populations that occur widely in two different rice cultivating agro-ecological zones of Sri Lanka.

## MATERIALS AND METHODS

Seeds of presumed different WR bio-types were collected from five different locations each at Matara District (Wet zone; agro-ecological region $WL_2$) of the southern province and Kurunagala Districts of the north western province (intermediate zone; agro-ecological zone $IL_1$) of Sri Lanka (Table 1), subjected to dormancy breaking treatments, and sown in plastic trays in a plant house at the Open University of Sri Lanka.

A total of five replicates of each bio-type were planted in plastic pots with representative paddy soils from each location. Replicates were arranged in Complete Randomized Design (CRD). Agro-morphological characterization (using thirty six characters) of WR bio-types and cultivated rice varieties were made using the Standard Characterization Catalogue (PGRC, 1999). The WR bio-types and respective cultivated rice varieties, which were separated well in Cluster Analysis (CA) and Principal Component analysis (PCA) were chosen for molecular characterization to ascertain the diversity of WR bio-types as well as to confirm the results obtained from the statistical procedures employed in the study.

The total genomic DNA was extracted from 7-day old seedlings of the respective WR and cultivated rice varieties using Pure Link® plant total DNA purification kit (Invitrogen, Catalog No. K 1830-05). The DNA samples were stored at -20$^o$C until use and the DNA quantification was carried out using Bio-spec Nano Drop Spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). Two SSR primer pairs ($RM_{11}$ and $RM_{21}$) were selected from Rice Genes data base and labeled (http://gramene.org).

The primer sequence of the primer pairs are given in Table 2. The polymerase chain reaction (PCR) was performed with denaturation period of 1 min, at 95 $^o$C followed by 35 cycles of 30 sec at 95 $^o$C, 30 sec at 56.9 $^o$C and 1 min at 72 $^o$C, and subsequently 7 min at 72 $^o$C for the final extension. Reactions were carried out in a volume of 25 µl containing 1 x buffer, 1mM dNTPs, 2µM SSR primers, 2mM $Mgcl_2$, 50 ng of genomic DNA and 0.5 U of Taq Polymerase. The PCR products were separated on 6 % polyacrylamide denaturing gels and silver stained in accordance with Song *et al*. (2003).

### Analysis of data

The data were subjected to data pre-processing to transform into homogenous variables. The dimension reduction of transformed variables were performed on the data using Classification and Regression Tree (CART) analysis and for the confirmation of CART results, a cluster analysis of variables was carried out on the same data set. The CA and PCA were carried out on the reduced data to observe the grouping patterns of WR bio-types and cultivated rice varieties in Kurunegala and Matara Districts. Cophenetic Correlation and Inconsistency Indices were calculated for the clusters produced in CA as a diagnostic test. In addition, the PCA was carried out on the district wise split data set.

**Table 1.** Sampling sites, GPS location, Presumed weedy rice bio-types and accompanied cultivated rice varieties use in the study

| District / Sampling sites site | Presumed weedy rice biotype | Cultivated variety | GPS value |
|---|---|---|---|
| **Kurunegala** | | | |



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| | | | |
|---|---|---|---|
| Kurunagala, Bulunahala Yaya | KKBW1, KKBW2 | Bg 358 (C1) | $7^{o}$ 38' 48.3" N $80^{o}$ 30' 28.8" E |
| Kumbukwawa, Dahampala Yaya | KKDW1, KKDW2, KKDW3 | Bg 379-2 (C2) | $7^{o}$ 35' 19.2" N $80^{o}$ 28' 38.4" E |
| Kuliyapitiya, Hambalawa Yaya | KKLHW1, KKLHW2 | Bg 358 (C3) | $7^{o}$ 26' 16.5"N $80^{o}$ 13' 18.2" E |
| Ibbagamuwa, Bulunwawa Yaya | KIBW1, KIBW2 | Bg 359 (C4) | $7^{o}$ 35' 19.9" N $80^{o}$ 28' 38.4" E |
| Kurunagala, Kuliyapitiya Ahala Yaya | KKLAW1 | Bg 379-2 (C5) | $7^{o}$ 27' 20.1" N $80^{o}$ 5' 15.8" E |
| **Matara** | | | |
| Welligama, Mudugamuwa | MWMW1, MWMW2, MWMW3, MWMW4 | Bg 379-2 (MWMC) | $5^{o}$ 97' 33.3" N $80^{o}$ 42' 25.0" E |
| Mapalana, Kamburupitiya | MMKW1, MMKW2, MMKW3, MMKW4 | Bg 307 (MMKC) | $6^{o}$ 06' 66.5" N $80^{o}$ 57' 75.5" E |
| Palolpitiya, Akurugoda | MPAW1,MPAW2,MPAW3,MPAW4 | Bg 352 (MPAC) | $6^{0}$ 02' 74.4" N $80^{o}$ 57' 50.9" E |
| Hakmana, Komangoda | MHKW1,MHKW2,MHKW3 MHKW4 | At 362 (MHKC) | $6^{0}$ 84' 62.2" N $80^{o}$ 65' 2" E |
| Morawaka | MMW1,MMW2,MMW3 | Bg 379-2 (MMC) | $6^{0}$ 26' 52.8" N $80^{o}$ 49' 1" E |

**Table 2**. The SSR primer pairs used for DNA amplification

| **Primer name** | **Sequence (5'-3')** |
|---|---|
| $M_{13}RM_{11}F$ | TGTAAAACGACGGCCAGTTCTCCTCTTCCCCCGATC |
| $PigtRM_{11}R$ | GTTTCTTATAGCGGGCGAGGCTTAG |
| $M_{13}RM_{21}F$ | TGTAAAACGACGGCCAGTACAGTATTCCGTAGGCACGG |
| $PigtRM_{21}R$ | GTTTCTTGCTCCATGAGGGTGGTAGAG |

## RESULTS AND DISCUSSION

Three variables were extracted for WR bio-types of both districts after data were subjected to dimension reduction using CART. The cluster analysis (CA) resulted in eight clusters. The results indicated that four variables namely, lemma and palea color, seed coat color, panicle shattering and awning after full heading could be used for characterization of WR bio-types. The total data set classified the WR bio-types into three groups at 50 % phenone level (Cophenetic Coefficient = 0.89) (Fig. 1).



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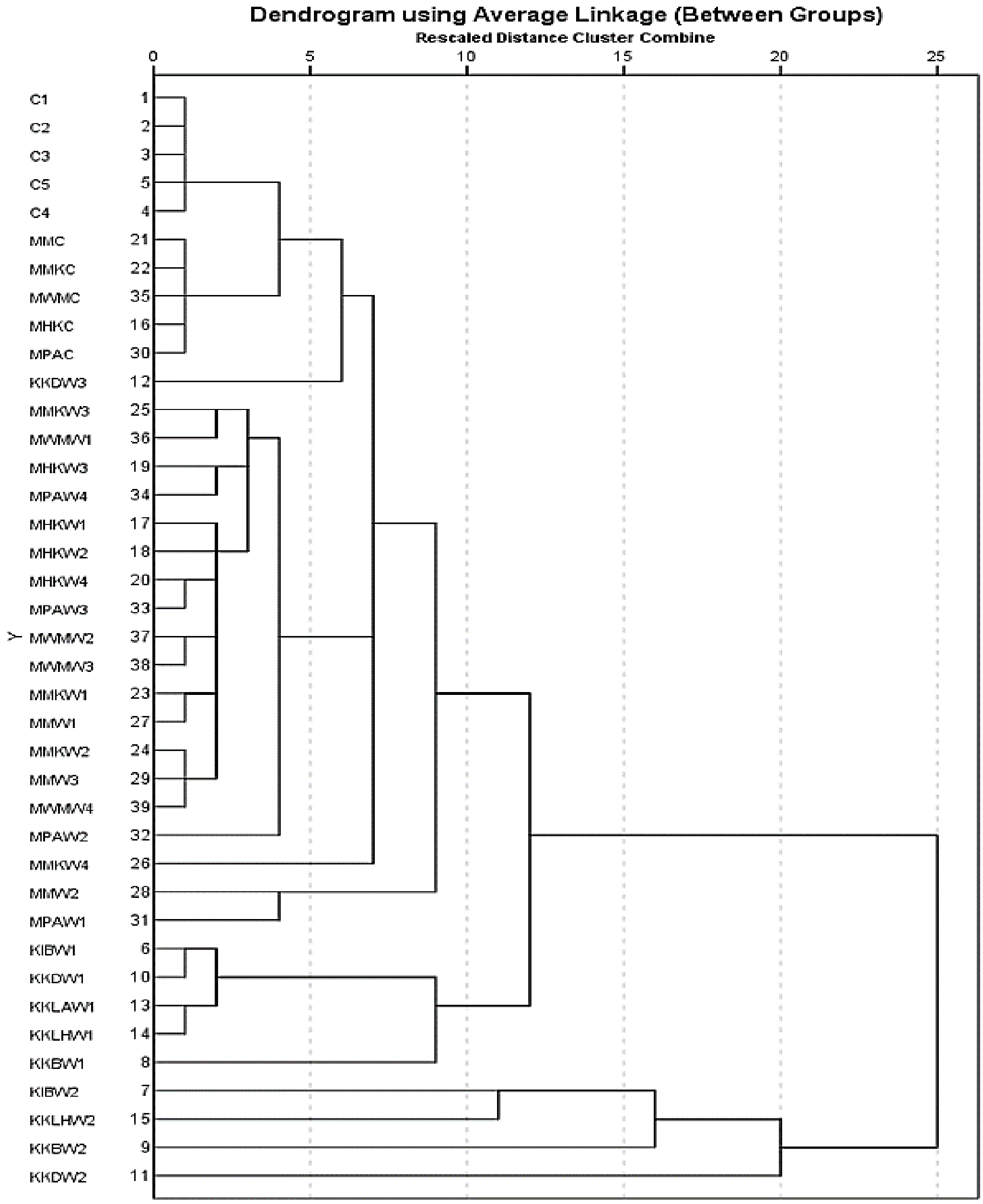


**Fig. 1**. Dendrogram plotted from the phenotypic (agro-morphological) characters of weedy rice accessions and cultivated varieties collected from Kurunagala and Matara Districts of Sri Lanka.

The calculated Inconsistency Index showed a considerable consistence of the clusters produced indicating that the resulted groups were more or less homogenous. Further, the biplot of principle component analysis (PCA) 1 with PCA 2 of the data collected on WR-biotypes from the Kurunagala District showed that there were six possible groups, reflecting that they were well-separated into distinct groups with respect to cultivated rice varieties (Fig. 2A). Similarly, biplot of Matara showed that the WR-biotypes cluster into three possible groups, which were more or less well-separated from the cultivated rice varieties (Fig. 2B). Further, the patterns reflected in the CA and PCA are confirmed by the molecular analysis (Fig. 3).

The result obtained from the CA and PCA on the variation of morphological characters of WR-biotypes indicated that morphological characters are vary widely among the different biotypes *i.e.*, there were certain degree of overlapping of the groups in PCA biplot and misclassification of weedy rice biotypes in the dendrogram resulting from the CA. Therefore, care must be exercised when using phenotypic characters in characterization of weedy rice.



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Similar conclusions have been reached on the study of phenotypic diversity of land races of rice in West Bengal (Chakravorty *et al*., 2013). However, in the present study, there was equal importance in genotypic and phenotypic characters in characterization of weedy rice biotypes in Sri Lanka.

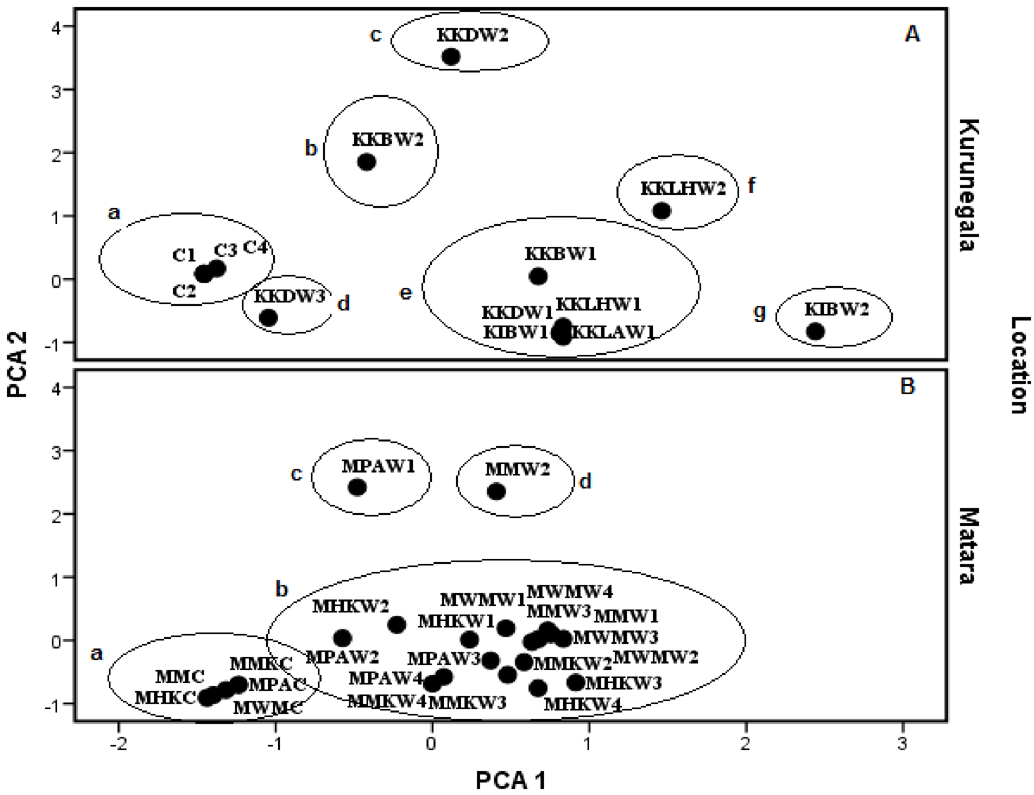


**Fig. 2.** Biplot derived from the PCA 1 and PCA 2 for selected weedy rice accessions and cultivated rice varieties from Matara and Kurunegala Districts (PCA 1 = 43.539%, PCA 2 = 24.436%; Cumulative = 68.975%).

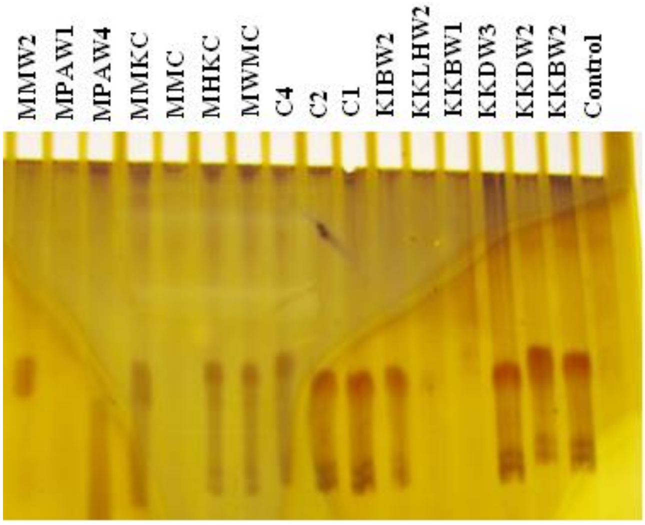


**Fig. 3.** Acrylamide gel obtained for selected weedy rice accessions and cultivated rice varieties in the study with the primer $RM_{21}$. Please refer Table 1 for abbreviations. ($RM_{11}$ gel is not included as the banding patterns were not prominent due to insufficient anneal).



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## CONCLUSIONS

Phenotypic and genotypic variations found in the weedy rice populations collected from two rice growing Districts of the Sri Lanka seems to be area specific. A higher phenotypic variation of weedy rice occurs in the location at intermediate zone ($IL_1$) when compared to that of the wet zone of the country. Molecular studies also supported this observation. However, sequencing data is necessary to confirm such observations. Use of fewer agro-morphological characters through reduction of dimensions applying multivariate statistical procedures in identifying weedy rice bio-types is important for a cost effective characterization.

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# AGRO-MORPHOLOGICAL VARIATIONS OF WEEDY RICE POPULATIONS (*ORYZA SATIVA SPONTANEA*) IN SRI LANKA

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**ABSTRACT** Weedy rice (*Oryza sativa* f. *spontanea*) (Poaceae), identified for the first time in Sri Lanka in 1990s in the Eastern Province of Sri Lanka, has now become a serious threat to the rice production in many areas of the country. Weedy rice is characterized by high seed-shattering, variable seed dormancy and competitiveness with cultivated rice. A remarkable feature observed among weedy rice populations in Sri Lanka was the variability of the plant architecture. Therefore, an island-wide collection of weedy rice populations amounting to >4849 morpho-types was made during 2009/2012 to characterize their agro-morphological features as the first to explore possibilities of utilizing important characteristics in the varietal improvement program. Higher degrees of variations were observed among grain characters such as; presence / absence of awn, length of the awn, colour of the awn, hull and pericarp, grain size and shape, etc. Among the plant characters variations were observed in number of panicle per hill, spikelets per panicle, percentage grain filling, grain weight etc. Morphological characterization indicated that weedy rice types have intermediate characters to those of cultivated and wild rices (*Oryza nivara*, *Oryza rufipogon*). Weedy rice collected from the southern and eastern coastal areas found to have similarities with wild rice species. For example, presence of awn >80 %, red pericarp >80%, long slender grain type >60%, light seeds >90%, higher seed shattering >90%, long seed dormancy >75%. Furthermore, the weedy rice collection had higher number of tillers/hill, higher number of panicles/plant and less number of spikelets/panicle. Weedy rice present inland areas of the country showed similarities to traditional rice cultivars grown in those areas like; low degree of seed shattering 40-70%, different grain shape; (long 20%/ intermediate 30%/ bold 50%), red /white pericarp and no awns (70%), less tillers/plant (80%), less panicles/plant. The characteristics of weedy rice morpho-types were recorded for further improvement of rice breeding program at Rice Research and Development Institute in Sri Lanka.

***Keywords***: Accessions, morphological characters, variations, weedy rice

## INTRODUCTION

Emergence of off-types of rice which are morphologically and physiologically different from the inbred varieties, their parental material, land races and traditional varieties in farmer fields has been a significant phenomenon ever since broadcast sowing became popular a few decades back. However, this trend became a major issue when the seeds of these off-types show high degree of shattering at immature stage – for obvious reason -- for their survival-- that we now referred as weedy rice. Weedy rice, reported for the first time in the Eastern Provinces of Sri Lanka in 1990s, has now spread into many rice growing areas irrespective of the agro-ecological zones in the country. Efforts were made over the last decade to understand their epidemiology, morphological and physiological properties to help design control methods and to utilize important characters in varietal improvement. Some of the distinct features of weedy rice are; diverse grain color; shape, presence of awn; leaf color and angle and panicle characters. In addition, weedy rice collected from different locations showed some features specific to the location. Of the more than 5,000 weedy rice types collected so far, agro-morphological features of 1,300 distinct weedy rice types were evaluated. Weedy rice has well developed roots system as compared to cultivated types indicating in support of their high competitive nature. The seed



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of weedy rice has a high dormancy period and a lengthy viability (> 80 weeks) under submerged conditions in rice soils as compared to cultivars. This feature facilitates weedy rice to develop a seed bank in paddy soil. Weedy rice has various biological properties, for instance more than 30% weedy rice types tested found to possess resistance to brown plant hopper.

In Sri Lanka, more than 90% of farmers practice direct seeding. Weedy rice is a serious threat due to its competitiveness with cultivated rice. Rapid growth of weedy rice avoids penetration of solar radiation to the ground and it causes more fungal and bacterial infections. Higher seed shattering will increase soil seed bank and end up with higher infestation of weedy rice in next season. Seed dormancy is another important feature in weedy rice as it can stay in the soil up to seven years. This weed affects quality and quantity of rice yield and create greater problem on market value of the rice. However, it is difficult to describe the extent and severity of weedy rice threat precisely, because of the higher variability in morphological and phenotypical characters of weedy rice. Therefore this study is conducted to report the distribution and morphology of weedy rice in Sri Lanka.

## MATERIALS AND METHODS

Panicle samples were collected from weedy-rice infested fields in different regions of Sri Lanka from 2009 to 2012. In order to make the sampling more representative, 10- 15 panicles showing different morpho-types were collected from each selected location. Collected samples were categorized according to the grain characters; presence of awn, length of awn, colour of awn, hull and pericarp. The weedy rice collection was grouped on the basis of the above grain characteristics and from each group 5 samples were randomly selected for planting in cement pots separately. Morphological characters such as; plant height, number of tillers, number of panicles per plant, panicle type and grain characters were recorded. Useful characters of weedy rice accessions were identified and selected for further improvement of the rice varietal improvement program at Rice Research and Development Institute of Sri Lanka.

## RESULTS AND DISCUSSION

A total of 4,849 weedy-rice morpho-types were collected from 14 districts in Sri Lanka (table 1 and fig 1). These morpho-types were categorized approximately into 251 groups according to their grain characteristics. We observed a large percentage of weedy rice had black or straw coloured hull. However, there were equal number of weedy rice possessing red and white pericarp and long (>20mm) and short grains (<20mm) (table2). A majority of weedy rice did not have awn and a majority of those with awn found to be red. The number of weedy rice possessing awn measuring < 1cm and 1cm < found to be equal (table 3). These observations suggested that the weedy rice morpho-types collected in Sri Lanka had all possible combinations of the grain features (in terms of presence and absence of own, color of the own, hull and pericarp)

**Table 1.** Number of weedy rice phenol-types collected from different districts of Sri Lanka during 2009- 2012 and number of morpho-type identified

| District | No. of weedy rice morpho-types collected | No. of groups identified |
|---|---:|---:|
| Ampara | 522 | 28 |
| Matara | 485 | 24 |
| Puttalam | 245 | 22 |
| Batticoloa | 210 | 16 |
| Polonnaruwa | 372 | 18 |
| Kandy | 222 | 4 |
| Galle | 278 | 12 |
| Kegalle | 125 | 16 |



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| | | |
|---|---|---|
| Anuradapura | 325 | 21 |
| Hambantota | 280 | 14 |
| Rathnapura | 360 | 18 |
| Kurunegala | 585 | 24 |
| Paranthan | 452 | 16 |
| Kalutara | 388 | 18 |
| Total | 4,849 | 251 |

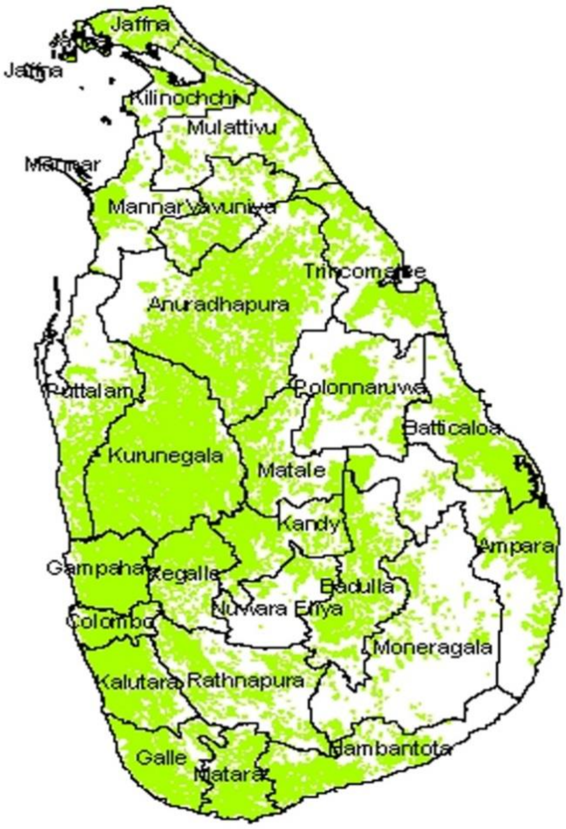


**Fig 1.** District-wise distribution of rice lands in Sri Lanka

**Table 2.** Weedy rice morpho-types classified according to grain characters

| *Districts* | *Hull colour* | | | *Pericarp colour* | | *Grain shape* | |
|---|---|---|---|---|---|---|---|
| | *Black* | *Brown* | *Straw* | *Red* | *White* | *Short <20mm* | *Long >20mm* |
| Ampara | 108 | 212 | 202 | 408 | 114 | 312 | 210 |
| Matara | 98 | 165 | 222 | 298 | 187 | 265 | 220 |
| Puttalam | 170 | 70 | 5 | 180 | 65 | 172 | 73 |
| Batticaloa | 150 | 24 | 36 | 196 | 14 | 185 | 25 |
| Polonnaruwa | 132 | 46 | 194 | 210 | 162 | 169 | 203 |
| Kandy | 98 | 24 | 100 | 108 | 114 | 115 | 107 |
| Galle | 135 | 41 | 102 | 96 | 182 | 116 | 162 |
| Kegalle | 78 | 46 | 01 | 104 | 21 | 78 | 47 |
| Anuradapura | 165 | 44 | 116 | 210 | 115 | 142 | 183 |
| Hambantota | 210 | 28 | 42 | 186 | 94 | 164 | 116 |
| Rathnapura | 162 | 34 | 164 | 132 | 228 | 146 | 214 |



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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Kurunegala | 180 | 74 | 331 | 301 | 284 | 352 | 233 |
| Paranthan | 128 | 58 | 266 | 231 | 221 | 236 | 216 |
| Kalutara | 165 | 76 | 147 | 210 | 178 | 165 | 223 |
| Total | 1,979 | 942 | 1,928 | 2,870 | 1,979 | 2,617 | 2,232 |
| (%) | 40.8% | 19.4% | 39.8% | 59.18% | 40.82% | 54.0% | 46.0% |

**Table 3.** Grain characters of weedy rice accessions classified into awn characters

| Districts | Awn | | Awn colour/ Awn length (cm) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | present | absent | Red | | White | | Straw | |
| | | | 0-1 | 1-3 | 0-1 | 1-3 | 0-1 | 1-3 |
| Ampara | 218 | 304 | 32 | 74 | 45 | 35 | 18 | 14 |
| Matara | 176 | 309 | 66 | 32 | 26 | 28 | 12 | 12 |
| Puttlam | 108 | 137 | 45 | 41 | 0 | 8 | 8 | 6 |
| Batticlo | 130 | 80 | 32 | 42 | 9 | 9 | 21 | 17 |
| Polonnaruwa | 92 | 280 | 8 | 6 | 20 | 12 | 18 | 28 |
| Kandy | 14 | 208 | 2 | 1 | 2 | 1 | 6 | 2 |
| Galle | 130 | 148 | 26 | 26 | 26 | 24 | 14 | 14 |
| Kegalle | 30 | 95 | 10 | 8 | 3 | 6 | 2 | 1 |
| Anuradapura | 107 | 218 | 33 | 34 | 8 | 4 | 16 | 12 |
| Hambantota | 95 | 185 | 12 | 22 | 4 | 11 | 8 | 38 |
| Rathnapura | 82 | 278 | 2 | 18 | 9 | 22 | 21 | 10 |
| Kurunegala | 283 | 302 | 12 | 6 | 69 | 36 | 95 | 65 |
| Paranthan | 174 | 278 | 85 | 40 | 13 | 18 | 8 | 10 |
| Kalutara | 124 | 264 | 53 | 32 | 8 | 7 | 13 | 11 |
| Total | 1,763 | 3,086 | 418 | 382 | 242 | 221 | 260 | 240 |
| % | 36.4 | 63.6 | 23.7 | 21.6 | 13.7 | 12.5 | 14.7 | 13.6 |

The selected 251 morpho-types of weedy rice showed very high degree of diversity in terms of tillering ability, number of panicles/ plant (when grown under uniform condition) and number of spikelets / panicle. There were equal number of morpho-types having <100 and >100 spikelets / panicle. A majority of the weedy rice morpho-types had grain weight less <20g/ 1000 grains and the percentage filled grains were <70% (Tables 4A and 4B).

**Table 4A.** Distribution of agronomic characters of the 251 selected groups of weedy rice morpho-types (%)

| Districts | Number of tillers/hill | | | Number of panicles/hill | | | Number of spikelets/plant | |
|---|---|---|---|---|---|---|---|---|
| | <3 | 3-6 | >6 | <5 | 5-10 | >10 | <100 | >100 |
| Ampara | 39 | 29 | 32 | 14 | 43 | 43 | 36 | 64 |
| Matara | 17 | 33 | 50 | 38 | 17 | 46 | 58 | 42 |
| Puttalam | 18 | 45 | 36 | 9 | 32 | 59 | 55 | 45 |
| Batticaloa | 19 | 44 | 38 | 44 | 6 | 50 | 44 | 56 |
| Polonnaruwa | 67 | 22 | 11 | 67 | 17 | 17 | 39 | 61 |
| Kandy | 50 | 25 | 25 | 75 | 25 | 0 | 25 | 75 |
| Galle | 42 | 33 | 25 | 50 | 25 | 25 | 33 | 67 |
| Kegalle | 38 | 38 | 25 | 38 | 19 | 44 | 56 | 44 |
| Anuradapura | 38 | 19 | 43 | 48 | 33 | 19 | 48 | 52 |
| Hambantota | 21 | 21 | 57 | 21 | 21 | 57 | 43 | 57 |
| Rathnapura | 11 | 56 | 33 | 28 | 39 | 33 | 67 | 33 |
| Kurunegala | 63 | 25 | 13 | 54 | 21 | 25 | 54 | 46 |



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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Paranthan | 19 | 38 | 44 | 56 | 13 | 31 | 56 | 44 |
| Kalutara | 61 | 6 | 33 | 44 | 11 | 44 | 61 | 39 |
| | 35% | 31% | 34% | 38% | 24% | 38% | 49% | 51% |

**Table 4B.** Distribution of agronomic characters (100 grain wt and % filled grains) of the 251 selected groups of weedy rice morpho-types (as %)

| Districts | 1000 grain weight (g) | | % fill grain | |
|---|---|---|---|---|
| | <20 | >20 | <70 | >70 |
| Ampara | 68 | 32 | 71 | 29 |
| Matara | 67 | 33 | 79 | 21 |
| Puttalam | 64 | 36 | 73 | 27 |
| Batticaloa | 50 | 50 | 56 | 44 |
| Polonnaruwa | 33 | 67 | 44 | 56 |
| Kandy | 50 | 50 | 50 | 50 |
| Galle | 67 | 33 | 67 | 33 |
| Kegalle | 38 | 63 | 56 | 44 |
| Anuradapura | 57 | 43 | 43 | 57 |
| Hambantota | 57 | 43 | 57 | 43 |
| Rathnapura | 61 | 39 | 39 | 61 |
| Kurunegala | 38 | 63 | 46 | 54 |
| Paranthan | 56 | 44 | 38 | 63 |
| Kalutara | 44 | 56 | 44 | 56 |
| | 54.1% | 45.8% | 55.7% | 44.2% |

Furthermore, we observed that the number of tillers/hill (at vegetative stage) in Polonnaruwa and Kurunegala populations (inland districts) were lower than those in the coastal districts. It was evident by the fact that more populations taken from inland districts such as Kandy, Kurunegala, Polonnaruwa and Parantan had less number of panicles/hill than those populations taken from coastal areas. The weedy rice populations taken from coastal areas like Ampara, Batticaloa, Hambantota, Matara, and Galle had higher number of morpho-types with 1000grain weight <20g and <70% filled grain/panicle. These evidences suggest that weedy rice in Sri Lanka have emerged from different origins and the likely sources could be wild rice species abundant in some areas and traditional rice cultivars grown in by farmers in other areas.

Weedy and cultivated rice have evolved from wild *Oryza* species (Vaughan et al, 2005). Of the 21 wild rice species in the genus *Oryza,* 9 are tetraploid (BBCC, CCDD) and the rest are diploid. Several hypotheses have been put forward by scientists to explain the origin of weedy rice. Some Scientists consider weedy rice as natural hybridization between different cultivars as well as between wild and cultivated rice or cultivated rice between cultivated rice are the main reasons of the development of many morphological varieties of weedy rice in rice fields. Some scientists consider them to be due to natural mutations or both. Weedy rice could occur due to several factors like, gene flow between wild and cultivated japonica types, old rice varieties becoming feral as well as crosses between cultivated rice and wild rice such as *Oryza nivara* and *Oryza rufipogon* (Marambe and Amarasinghe, 2000). Diversification in to different groups of *oryza* probably occurred in China about 800 years ago. Cultivated rice and most of weedy rices have the same genome; AA and the same number of chromosome, 2n=24. Species with same genome group are of most concern as they can easily hybridize and produce weedy off type of *Oryza* (Ahangangoda, 2008; Baltazar et al. 2000; Chauhan *et al.* 2010 a, b; Dharshana *et al*. 2006). Subasighe et al 2007 morphological analysis and molecular techniques indicated that Weedy rice samples collected from Puttalam and Galle show intermediate characteristics to those of cultivated rice (Bg 300) and annul wild rice (*Oryza nivara*).



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Many types of morphological variants of weedy rice are found in different rice growing areas in Sri Lanka. The evaluation done on 6 morphological variants of weedy rice on the basis of samples collected in and Puttalam area helped identified six different variants of weedy rice types during 2000-2002. Many types of morphological variants of weedy rice were identified in different rice growing areas in the county. Darshana et al, 2007 reported that weedy rice in southern Sri Lanka showed a very high morphological diversity. And eleven distinct types were identified based on the grain morphology.

Abeysekara *et al*. (2010) reported that from the 1500 weedy rice accession collected from different rice growing areas during 2002-2004, 517 morphologically different weedy rice types were identified. The weedy rice accessions collected from different rice growing areas in the country during 2009- 2012helped identify 251 distinct and abundant and morpho-types variants of weedy rice types have been observed. These variants showed differences in grain characters; colour, presence or absence of awn, panicle type, panicle length, grain size, grain shape and plant characters. ; Plant height, leaf colour, leaf angle, no of tillers. In general, weedy rice plants are taller, produce more tillers and panicles/hill, have greater panicle length and more grain /panicle, longer flag leaf and have an earlier flowering habit than cultivated rice. Although they show close similarities to modern cultivars, they differ from the latter in terms of plant height, pericarp pigmentation, possession of an awn and longer panicles.

The morphological analysis indicated that weedy rice show intermediate characters to those of cultivated and wild rice (*O. nivara, O. rufipogan*). It varies from location to location and depends on the types and age of the growing rice cultivar. Most of the weedy rice identified near to the coastal areas in the country viz Ampara, Mannar Puttalam, Matara are similar to wild rice in that they have high degree of seed shattering>90%, long seed dormancy, >95% red peri carp, long awn, light seeds, and .tillers /plant, panicles /plant are always higher than the cultivated rice although they look like cultivated rice during the vegetative stage. Samples collected from inside the country viz. Kurunegala, Polonnaruwa, Anuradhapura, they have less degree of seed shattering 40-70%, different grain shape long, intermediate and bold, red and white pericarp ,black, golden or brown hull, with and without awn, always taller than the cultivated rice and very early flowering less tillers/plant and less panicles /plant very similar to traditional rice cultivars it also very difficult to distinguish from cultivated rice in vegetative period (unpublished data Abeysekara)

As natural hybridization between different cultivars as well as between perennial and annual wild rice and cultivated rice are the main reasons of the development of weedy rice. Studies conducted in Sri Lanka, were observed that 1300 different variants and from that 90% found to be taller plant ranging from 90 to 165cm, the number of panicles/hill ranged from 6-21.the flag leaf measured 24-68cm and angle erect, semi erect and droopy. More than 68% of the weedy rice had black hull and mostly 64% were red pericarped. About 54% variants had grain with long awn. The apiculus colour ranged from purple to brown. The awn length ranged from 0.1 to 7.0cm, awn colour from straw to dark brown. The grain shape ranged from long long, medium to bold and the fertility ranged from30 to 80%.weedy rice variants had high grain shattering (Abeysekara *et al*. 2010). Growth pattern of cultivated rice and weedy rice were different but it is difficult to distinguished from cultivated rice in early vegetative stage but it is easy to distinguished while after tillering stage, and early flowering stage due to many morphological variances in comparison with the cultivated rice; longer and slender tillers, droopy and pubescent leaves, rooting in stem nodes, tall plants, early flowering, and easy shattering before the maturity of cultivated rice.

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# EMERGENCE AND GROWTH OF WEEDY (*ORYZA SATIVA F. SPONTANEA)* AND CULTIVATED RICE (*ORYZA SATIVA*) IN RESPONSE TO FLOODING AND SEED BURIAL DEPTHS

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**ABSTRACT** Weedy rice (*Oryza sativa f. spontanea*) causes serious losses in direct-seeded rice in Asia, and is difficult to control in a rice crop with selective herbicide as it is the same species as the cultivated rice. A pot experiment was conducted to test the effect of flooding and burial depths on the emergence and growth of weedy rice from Philippines (BWR), Vietnam (VWR) and cultivated rice genotypes, IR64 (intolerant of submergence during germination) and Khao Hlan On (KHO; tolerant). Seeds of rice genotypes and weedy rice were sown to three different soil depths (1, 5 and 15 cm) and three water regimes (aerobic, saturated and flooded to 2 cm depth). There was greater emergence of all rice genotypes and weedy rice when sown under shallow burial depth in saturated or aerobic soil, compared with 2 cm flooding. The combination of 2 cm flooding and 1 cm burial depth resulted in only 2 % emergence in BWR and VWR at 21 days after seeding (DAS) while KHO and IR64 had 79 % and 12 % emergence, respectively. The combination of 2 cm flooding and seed burial to 5 cm resulted in a 99 % reduction in emergence of all weedy rice and cultivated varieties. Burial to 15 cm combined with 2 cm flooded and aerobic condition resulted in no emergence in all genotypes. Dry matter production by 60 DAS of rice genotypes and weedy rice was greatly reduced (99 %) by the combination of 5 cm burial and 2 cm flooding. Furthermore, seed burial to 15 cm reduced dry matter of rice genotypes and weedy rice by 70 – 99 % regardless of moisture regimes. The emergence and growth of weedy rice was greatly influenced by the flooding and depth of seeding. A combination of deep sowing and flooding could provide valuable cultural means to manage weedy rice infestation in rice fields.

***Keywords***: Burial depth, direct seeding, flooding, *Oryza sativa*, weedy Rice

## INTRODUCTION

Weedy rice (*Oryza sativa f. spontanea)* is a serious threat to the sustainability of rice production in many rice producing countries in South and Southeast Asia, including Philippines and Vietnam, causing serious yield losses. In the Philippines, weedy rice infestation in rice fields is from 1 - 48% (Baltazar and Janiya, 2000). In Vietnam, weedy rice occurrence is mostly in direct seeded rice with an estimated average yield loss of 18 % (Chin, 1997). Severity of the yield loss due to weedy rice infestation depends on the relative density of weedy rice to rice and the duration of interference with the rice crop (Vidotto and Ferrero, 2009). Some of the traits associated with weedy rice, like seed shattering, seed dormancy, greater nutrient absorbing ability and higher tillering ability provide better competitive advantage over cultivated rice varieties, resulting in increased crop losses.

Direct wet-seeded rice is widely practiced in South and Southeast Asia and in many other rice producing areas such as Latin America, USA and Australia. With expansion of direct seeding, high weed infestations are becoming a major constraint. Chemical weed control has therefore increasingly been used as an efficient weed management practice. Among the common weeds in direct seeded rice, weedy rice is by far, the most serious problem in several Asian countries. Management of weedy rice is difficult as there is no effective selective herbicide to be used for its control in rice fields and weedy rice is hard to distinguish from



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cultivated rice varieties due to their close similarities in growth and morphology during the vegetative stage, as they both belong to same species. Further, Bakar *et. al.* (1998) suggested that weedy rices will continue to evolve morphologically by inter-crossing with modern cultivars, making them difficult to control chemically. Therefore, for sustainable management of weedy rice, emphasis should probably rely more on integrated agronomic and cultural means of weed control rather than solely on the use of herbicides.

Factors such as irrigation interval, flood, duration, depth of flooding, stage of development of the weeds (Kent and Johnson, 2001) and soil depth of the weeds seeds (De Datta, 1981) are among the important factors that could affect the efficacy of water management and flooding to control weed species. Flooding was frequently suggested as a potential means to control weedy rice. The germination and emergence of both rice and weed seeds is directly related to the depth at which seeds were buried (Harper and Obeid, 1967). Weedy rice problems associated with the direct seeded rice could therefore, be alleviated by means of proper seeding depth and water management at the early stages of crop establishment. Potential of using flooding and seeding depth as important components of integrated weedy rice management where flood-tolerant rice genotypes are used is timely and need evaluation. The objective of this study was to determine the effects of flooding and burial depth on the emergence and growth of *Oryza sativa* f. *spontanea* and cultivated rice genotypes with variable tolerance of flooding during germination and early growth.

## MATERIALS AND METHODS

A greenhouse experiment was conducted at the International Rice Research Institute (IRRI), Los Baños, Philippines, with two different weedy rice, Philippines Bicol Region (BWR), Vietnam (VWR) and two cultivated rice genotypes, IR64 (intolerant of submergence during germination) and Khao Hlan On (KHO; tolerant). The experiment was performed using a split-split plot arrangement within a randomized complete block design with three replicates. Treatments consisted of seeds buried at 1, 5 and 15 cm depths, and three different water regimes, aerobic, saturated and 2 cm flooding. Irrigation treatments constituted the whole plots, burial depths as split plots, and weedy rice/rice genotypes as split-split plots. Plastic buckets with the length of 58 cm, width of 38 cm and height of 29 cm were filled with 45 kg of soil. The soil (clay 37 %; sand 15 %; silt 48 %; pH 6.11 and organic carbon 0.95 %) used for the experiment was sterilized before experiment. Twenty seeds of each genotype were placed on nylon net bags (9 cm height and 7.5 cm diameter). Nylon bags were buried at 1, 5 and 15 cm depth to achieve treatment burial depth. Each experimental unit consisted of eight sampling units. All buckets were watered daily to maintain aerobic condition (50% of water holding capacity, 1000 g of dried soil + 250 ml of water). To maintain saturated condition watering was done daily (1000g of dried soil plus 500ml of water), whereas for flooding treatment, 2-cm water head was maintained above the soil in each bucket. Seed samples were exhumed at 7, 14, 21, 28, 35, 42, 49 and 60 days after burial to determine total dry biomasses and emergence counts were recorded at 21 DAS. Data were subjected to analysis of variance (ANOVA) using STAR for Windows version 1.2 (IRRI, 2013).

## RESULTS AND DISCUSSION

### Seedling emergence

There were significant interaction effects ($p<0.05$) of burial depth and water regime on emergence of all weedy rice and cultivated rice genotypes. In all rice genotypes and weedy rice, while seed germination occurred at all burial depths, a higher percentage of the seedling emergence was observed at 1 cm and 5 cm burial in both saturated and aerobic conditions compared with 2 cm flooding (Fig.1). The combination of 2 cm flooding and 1 cm burial depth resulted in only 2 % emergence in BWR and VWR after 21 days of sowing, while KHO and IR64 had 79 % and 12 % emergence, respectively. No seedling emergence was observed when seeds were buried at 15 cm, either under aerobic or flooded condition, but emergence of BWR



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was 8 % under the combination of 15 cm and saturated condition. There was a significant decline in emergence at 15 cm burial depths for BWR, VWR, KHO and IR64 (Fig. 2).

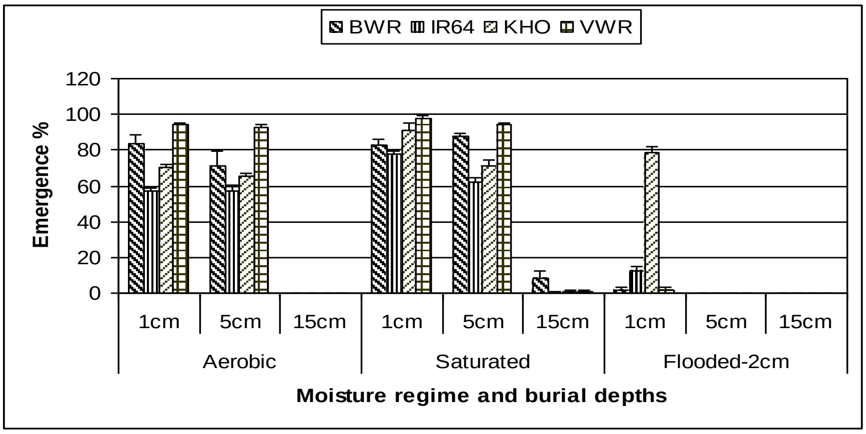


**Fig. 1**. Percent emergence of seedling at 21 days after sowing of two rice genotypes and two weedy rices grown at different burial depths and different moisture regimes. The vertical bars represent standard errors.

Similar trend of emergence of all weedy rice and cultivated rice genotypes were observed with 1 cm and 5 cm burial under both saturated and aerobic moist condition but not under 2 cm flooded condition. All burial depths greatly reduced the emergence of all rice genotypes and weedy rices when the soil was flooded with 2 cm of water, with greater reductions in BWR and VWR under this water regime, and less reduction in KHO (Fig 3). Even 1 cm sowing depth and 2 cm of flooding, resulted in significant reduction in emergence of weedy rice. No emergence was observed with combination of 5 cm and 15 cm burial with 2 cm of flood. Results showed that deeper burial reduces seedling emergence of all weedy rice and cultivated rice genotypes regardless of the moisture regime.

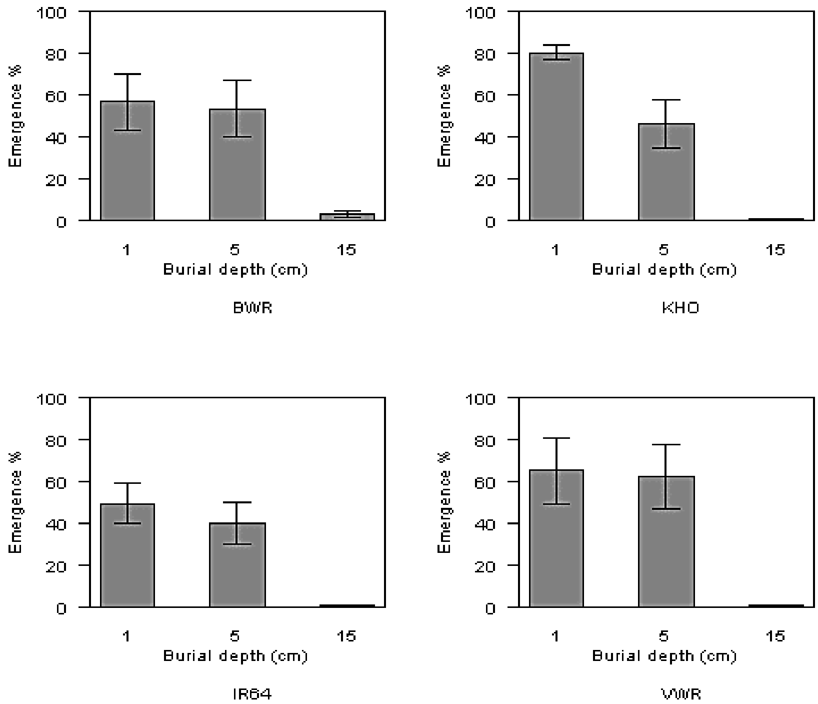


**Fig. 2.** Percent emergence of seedlings at 21 days after sowing of two rice genotypes and two weedy rice sown at different soil depths and across water regimes. The vertical bars represent standard errors.



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Burial depth is an important factor for seed germination and seedling emergence (Harper *et al*., 1986). Deeper seed burial and flooding were reported to decrease the emergence and biomass production of weedy rice (Chauhan, 2012). In this study, seed germination of both rice genotypes and weedy rice occurs at all burial depths (1, 5 and 15 cm). However, as burial depth increased, seedling emergence decreased. Performance of the different genotypes varied with the combination of burial depth and moisture regime. The data also showed that a proportion of seeds had germinated but the seedlings failed to emerge above the soil surface. Buried seeds either died prior to germination due to infection with pathogens and/or predation (Davis and Renner, 2007) or young seedlings senescence before reaching the soil surface (Telewski and Zeevaart, 2002). Successful germination and emergence could occur when seedlings eventually emerge from the soil and maintain contact with air. In some cases, seeds may not germinate when their dormancy was not sufficiently broken (Vleeshouwers *et al*., 1995). Experimental results showed that, seedling emergence were reduced with deeper sowing. According to Zorner *et al.* (1980) this was mainly due to depth-mediated suicidal germination caused by reduction of reserves.

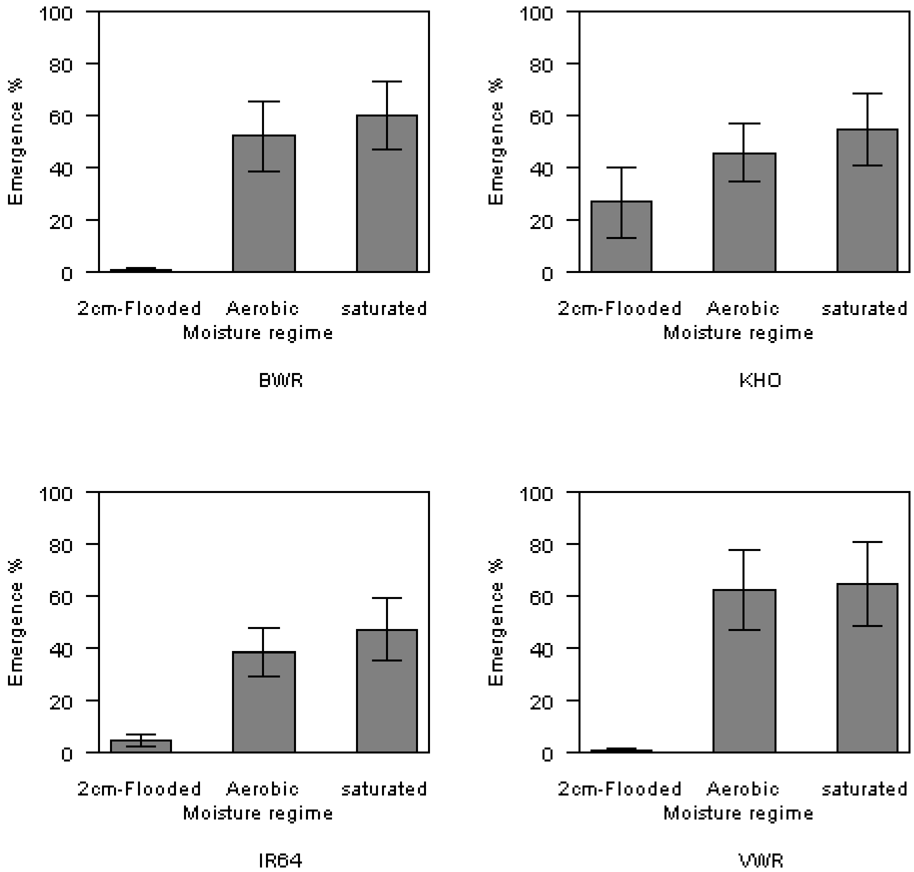


**Fig. 3.** Percent emergence of seedling at 21 days after sowing of two rice genotypes and two weedy rices under different moisture regime and across sowing depths. The vertical bars represent standard errors.

**Plant biomass**

The interactions of seed burial with water regime on total biomass of all weedy rice and cultivated rice genotypes were significant. Dry matter production of rice genotypes and weedy rice was greatly reduced (99 %) under the combination of 5 cm burial and 2 cm flooding condition. Furthermore, sowing seeds at 15 cm depth reduced dry matter of both rice genotypes and weedy rice by 70 to 99 % regardless of the moisture regimes. Total seedling biomass of rice genotypes and weedy rice was significantly influenced by burial depth (Fig. 4). Biomass reduction under 5cm burial depth was lower compared to the 15 cm burial depth. BWR showed



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higher biomass production when sown at 5 cm, compared with VWR and the two rice genotypes. However a general trend of decreasing biomass accumulation was observed with increasing burial depth. Biomass production was significantly affected by moisture regime regardless of the burial depth (Fig. 5). Higher biomass production was observed under saturated and aerobic condition compared to the 2 cm flooded condition. All genotypes had the lowest biomass in 2-cm flooded conditions regardless of burial depth. Saturated and aerobic soil conditions favored the survival and growth of weedy rice but numbers were significantly reduced by flooding to a depth of 2 cm.

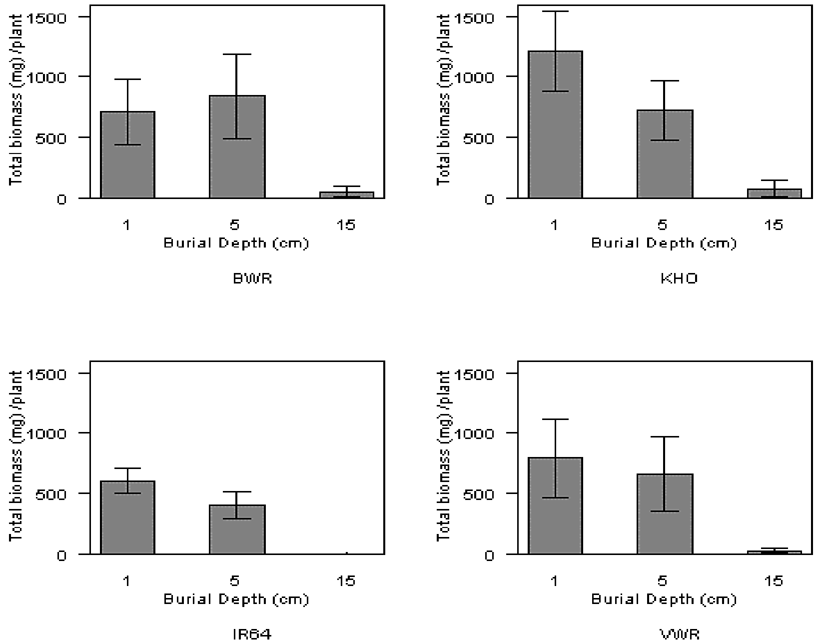


**Fig. 4.** Biomass production at 60 days after sowing of two rice genotypes and two weedy rice sown at different depths and across moisture regime. The vertical bars represent standard errors.

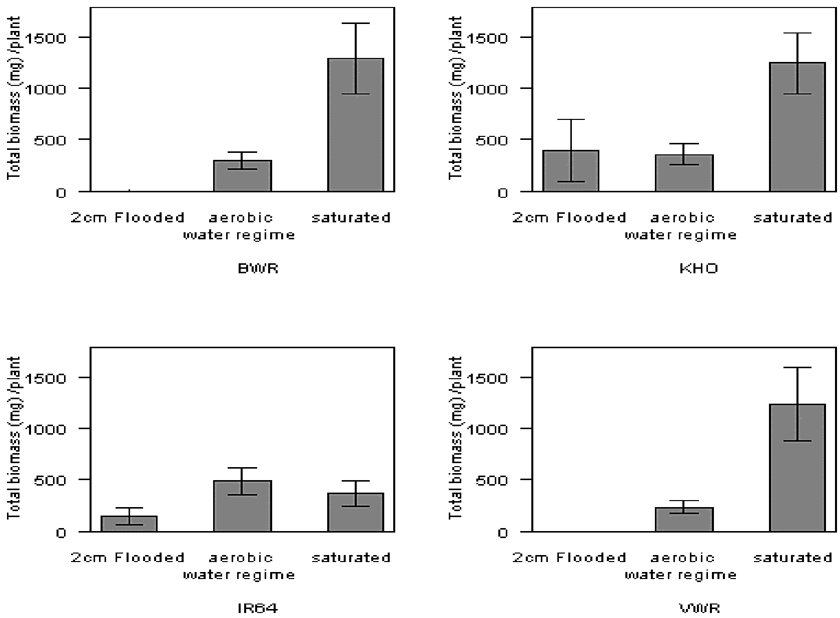


**Fig. 5**. Biomass production at 60 days after sowing of two rice genotypes and two weedy rice grown under different moisture regime and across sowing depths. The vertical bars represent standard errors.



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Flooding delayed emergence and growth of the weeds in rice fields (Moody and De Datta 1982; Kent and Johnson 2001). Civico and Moody (1979) found that seedling depth and flooding reduce germination, survival and growth of *E. glabrescence,* and Tuong *et al.* (2000) suggested flooding as an effective method to control weeds in direct seeded rice. Use of rice genotypes which can germinate and emerge in flooded soil reduce the risk of early flooding and enhance the efficiency of weed management (Ismail *et al*. 2009; 2012). Our results suggested that, flooding had greater suppressive effects on emergence and biomass production of the weedy rice. Burial depth also had a pronounce effect on emergence and growth of rice genotypes and weedy rice. A combination of 2 cm of flooding with the 5 and 15 cm burial gave excellent control of weedy rice. Under flooded condition the flood-tolerant rice genotype (KHO) had higher survival compared with the intolerant rice genotype (IR64). Apparently, flooding is an effective cultural practice for managing weedy rice especially in fields when tolerant rice varieties are used. The results of this study showed that it is important to combine optimum sowing depth, flooding and suitable rice genotype to ensure effective suppression of weedy rice in direct seeded rice. More research is needed to validate these measures and to establish an integrated management strategy for effective control of weedy rice in infested rice fields.

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# MAIZE PRODUCTIVITY AND WEED SPECIES SHIFTS DUE TO ORGANIC MATTER TREATMENTS AND NPK FERTILIZER APPLICATIONS IN SUMATERA UPLANDS, INDONESIA

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**ABSTRACT** The aim of this study was to investigate the effects of organic matter treatments and NPK fertilization at several rates on weeds population, and the growth and yields of maize. The studies were conducted at Agro Techno Park Indralaya, from September 2010 through July 2011. The methods used were quadratic method for weeds observation, with split plot design to evaluate organic matter and NPK fertilizer effects. The organic matter treatments consisted of K1 = soils planted with mucuna, K2 = soils planted with cowpea, K3 = soils with mucuna compost, K4 = soils with dried corn stalks compost, K5 = corn stalks incorporated, K6 = soils incorporated with cowdung, and K7 = soils left fallow. The organic matter used was 3 ton ha$^{-1}$. The area that were applied with organic matter 3 months before was planted with maize with NPK fertilizer treatments. The organic matter represented the main plot, while NPK fertilizer treatments were as sub-plots. The NPK fertilizer applications consisted of : $P_0$ = without NPK fertilizer, $P_1$ = 25%, $P_2$ = 50%, $P_3$ = 75%, and $P_4$ = 100% of standard rate (urea 400 kg ha$^{-1}$ , SP-36 100 kg ha$^{-1}$ dan KCl 50 kg ha$^{-1}$). The dominant weeds before treatments were *Panicum maximum, Borreria laevis* and *Mucuna pruriens.* Weed species shifts, fortified by changes in diversity indices, were registered during the studies. Following treatments with organic matter, *Richardia brasiliensis* and *Eleusine indica* were dominant. The diversity index before treatments was 2.47 while these indices declined with organic matter treatments and NPK fertilizer applications registering values ranging values from 1,52 to 2,10. Cowpea (*Vigna unguiculata*) gave the highest maize yield. The use of organic matter was able to reduce the use of NPK fertilizer. The combination of incorporated maize stalks with 50% of NPK fertilizer registered the highest corn yield.

***Keywords***: Weeds population, organic matters, rotation, fallow, corn.

## INTRODUCTION

Dynamics of weed populations in arable fields are influenced by environmental and soil characteristics and also by cropping system and management practices. Crop rotation, the tillage system, application of agrochemicals and other agricultural practices affect the soil seed bank and weed flora. Weed seed banks may reflect the status of weed population in the present and the past, and could be regarded as an indicator of the impact of soil and crop management (Buhler *et al*., 2001). Weed presence may cause more problems in some cropping systems, especially in organic farming where application of synthetic agrochemicals is not allowed.

Extensification program toward upland soil is one of the Indonesian government program to maintain self supporting of food. Upland acid soil in Indonesia is about 122 million ha or about 67.5% of total agricultural area which mostly scattered outside Java (Dierolf *et al*., 2001). This area is dominated by ultisol soil with an area of 45.8 million ha and classified as marginal land (Subagyo *et al*., 2000). Upland acid soil offers opportunities for agricultural development despite having complex soil chemical and physical constraints. Because of its high potential, it had been developed for food crops in the last few years. One of them is maize which is an important commodity. Maize production in Indonesia in 2012 was at 18.95 million tones,



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of this number about 30% for export. While, maize consumption in Indonesia is currently about 6 million tones. (Central Statistic Indonesia, 2012).

The ultisol is marginal soil that has characters: poor of nutrient content, high aluminium saturated, low of organic matter, and low of pH (Notohadiprawiro, 2006). So it is not beneficial for plant growth. Therefore, it needs to be amended to increase productivity of soil by addition of fertilizers and application of organic matter (Rachman *et al*., 2008).

Conventional agriculture use chemical fertilizers and pesticides rate higher and higher, finally it could be accumulated that cause soil pollution. Soil also will be damaged and saturated resulted by using high technology input (Utami and Handayani, 2003). Balasubramanian and Nguimgo (1993), stated that it is better to use organic matter and fallow rotation system with leguminous plant to fulfill corn plant nutrient needs and to decrease chemical fertilizer.

Besides of increasing plant growth, fertilizer also affected weeds growth. Competitive relationships between weeds and corn for nutrient and water will occur if weeds are not controlled (Fadhly and Tobri, 2008).

Rotation is effective way to control weeds because it create condition that affect the growth and reproduction of weeds, so it decrease weeds density (Marenco and Avilla, 1999). Rotation also will affect weeds species that grow because in one season weeds species will be different for each plant species. Findings were noted by Koocheki *et al*., (2009), shifting of weeds species was happened in plant rotation system.

Farmers in Rwanda planted some of leguminous species such as *Mucuna pruriens* and C*rotalaria ochroleuca* in fallow period before planting of corn, soybean and shorgum to maintain soil fertility (Balasubramanian and Nguimgo, 1993). A study in Africa showed that corn yield in the next period of planting 89% higher after Mucuna and 52% after Sunnhemp (C*rotalaria ochroleuca*), compared with corn yield after natural fallow period. Furthermore, farmers in Tanzania also use Sunnhemp in fallow period as alternative or as fertilizer addition in food cultivation. Marenco and Avila, (1999) also observed that *Crotalaria paulina* Schrank and *Mucuna atterima* Piper & Tracy rotation reduced weed cover, total weed dry matter accumulation and weed density by about 70, 80 and 90% respectively, in comparison to continuous rice. These reductions may be important in crop production system since weed competition is considered to be critical during the first weeks of crop growth (Moody, 1993), especially when it is taken into account that no herbicides were used during the experiment. Furthermore, cowpea and crotalaria rotation reduced weed population, cowpea-rice: total of weeds 187.50, crotalaria-rice: 131.25, compared to continuous rice: 684.38.

Weed population dynamics in agriculture area is affected by environment and soil characteristic, management practice, planted pattern system. Weed density is higher in continue planting than in rotation. Changing in weed species will happen if plant management and rotation change (Koocheki *et al*., 2009).

Although much work has been done to determine the effects of organic matter to the growth and yield of maize, more studies need to be conducted to ascertain the effects of organic matter and chemical fertilizer to maize production and weed growth.

The objective of this study was two-fold: a) to determine the effects of organic matter in combination with NPK fertilizers on maize productivity in order to decrease the amount of chemical fertilizer application, and b) to study the impact of organic treatments on weeds.

## MATERIALS AND METHODS

The experiment was conducted at Agro Techno Park Indralaya South Sumatera Indonesia from September 2010 through July 2011. The research was consisted of 3 steps. The first step was weed identification before study, the second step was organic matter application, and the third step was planting of maize with N, P, and K fertilizer. The soil was Ultisol (pH 4.72; O.M. 3.30%; N-total 0.21%; available P 47.36 ppm; and available K 0.21 me per 100 g). Organic matter treatments were applied 3 months before planting of maize.

The methods used were quadratic method for weeds observation, with split plot design to evaluate organic matter and NPK fertilizer effects. The organic matter treatments consisted



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of K1 = soils planted with mucuna, K2 = soils planted with cowpea, K3 = soils with mucuna compost, K4 = soils with dried corn stalks compost, K5 = corn stalks incorporated, K6 = soils incorporated with cowdung, and K7 = soils left fallow. The organic matter was applied at 3 ton $ha^{-1}$. The area that were applied with organic matter 3 months before was planted with maize with NPK fertilizer treatments. The organic matter represented the main plot, while NPK fertilizer treatments were as sub-plots. The NPK fertilizer applications consisted of : $P_0$ = without NPK fertilizer, $P_1$ = 25%, $P_2$ = 50%, $P_3$ = 75%, and $P_4$ = 100% of standard rate (urea 400 kg $ha^{-1}$ , SP-36 100 kg $ha^{-1}$ dan KCl 50 kg $ha^{-1}$). Seeds were planted manually in 5 m x 4 m plot with two seeds per hole. The plant spacing was 75 cm x 25 cm, so each plot consisted of 5 rows of plant with 20 plants per row after the plants were thinned to one per hole at two weeks after planting (WAP). Fertilizers (SP-36, KCl and 1/3 of Urea) were applied at planting time and the rest of urea was applied at 4 WAP. At harvest maturity all the ears in each plot were hand harvested; dried in the drying room for 2 weeks and weighted (13% moisture). Ear weight per plot (kg) was converted into yield (ton) $ha^{-1}$.

The kinds of weed species and their densities were determines by the quadrat sampling method. For observation before study, weeds from a 1 m x 1 m quadrat were hand-pulled from the soil, with the number of quadrat was 10. The observation of weeds at the end of research using 5 sample plots with a size 0.5 m x 0.5 m in each treatment plot. All the weeds were identified and the numbers of each species was counted. The weed variables collected were weed species, weed dry weight, weed diversity index. Weed diversity index was calculated by Shannon index using Past Program (Hammer *et al*., 2001). Weed dry weight and all data of maize were subjected to analysis of variance (ANOVA). Data were analyzed, using excel program and differences between treatments were compared using Honest Significant Difference (HSD) test at the $P$ = 0.05 level (Gomez and Gomez, 1984). Weed dominance was expressed as the summed dominance ratio (SDR) of relative density (RD) and relative dry weight (RDW) calculated as followed:

$$SDR = \frac{RD + RDW}{2}$$

$$\text{Where } RD = \frac{\text{No. of individuals in a species}}{\text{Total no. of individuals in all species}} \times 100$$

$$\text{and } RDW = \frac{\text{Dry weight of individuals in a species}}{\text{total dry weight of individuals in all species}} \times 100$$

## RESULTS AND DISCUSSION

### Maize productivity

Different organic matters application did not give significant effects on chlorophyll number, biomass dry weight, and corn yield per ha (Table 1). Application of NPK fertilizer affected significantly to all observed parameters (Table 2). There was not interaction between organic matters and NPK fertilizer treatments to all observed parameters.

Soil which was planted with cowpea (K2) gave the highest chlorophyll number, biomass dry weight, and corn yield per ha. Cowpea is leguminous plant that could fix atmospheric N, because there is legume-rhizobia symbiotic association in root nodules. Therefore cowpea can increase soil fertility by fixing the atmospheric nitrogen. Biological fixation of atmospheric N is the major known source of N for plants in natural system (Radosevich, 2007). The role of N



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in soil for plant are as basic substance for protein and chlorophyll maker, where chlorophyll is important for the photosynthesis process. The increasing photosynthesis process due to increasing chlorophyl amount could give accumulated photosynthate result in corn ear. Beside that, cowpea also contains higher phosphor substance than mucuna.

Growth and yield of corn were affected significantly by NPK fertilizer, those were indicated by almost all of parameters. P4 treatment (100% of standard rate) had the highest for almost all of parameters (Tabel 2), but it was not different to P2 (50% of standard rate) and P3 (75% of standard rate of NPK fertilizer). While P3 was different significantly with P0 (without fertilizer) and P1 (25% of standard rate). That mean that application of NPK fertilizer on 50% of standard rate in combination with organic matter had been able to produce the same corn yield with 100% of standard rate.

**Table 1.** Effects of organic matters on plant growth and yield of corn.

| Parameters[1] | Organic matters | | | | | | |
|---|---|---|---|---|---|---|---|
| | K1 | K2 | K3 | K4 | K5 | K6) | K7 |
| 1 | 36,50 | 39,75 | 35,99 | 36,14 | 38,35 | 37,64 | 35,16 |
| 2 (gr) | 387,41 | 449,92 | 447,06 | 429,97 | 438,96 | 406,57 | 408,71 |
| 3 (ton) | 3,43 | 4,34 | 3,87 | 3,67 | 4,08 | 4,24 | 4,02 |

[1] Chlorophyll number (1), Biomass dry weight (2), Corn yield per ha (3).

**Table 2.** Effects of N, P, K fertilizer on plant growth and yield of corn.

| Parameter [2] | NPK Fertilizer | | | | | HSD 0,05 |
|---|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | P4 | |
| 1 | 32,26a | 36,19b | 38,19b | 39,18c | 39,55c | 2,82 |
| 2 (g) | 326,12a | 389,85b | 430,53b | 485,55c | 488,39c | 50,06 |
| 3 (ton) | 2,40a | 3,65b | 4,44c | 4,46c | 4,80c | 0,60 |

[1] Numbers in the same row followed by the same letter are not significantly different at the 5% level of probability according to HSD's Test

[2] Chlorophyll number (1), Biomass dry weight (2), Corn yield per ha (3).

**Changing in composition of weeds**

The result of calculation of the dominance total value showed that weed composition changed after organic matters and NPK fertilizer treatments. At the beginning of the experiment area was dominated by *Panicum maximum* (SDR 23.30%), *Borreria laevis* (SDR 13.39%) and *Mucuna pruriens* (SDR 12.68%) (Table 3).

**Table 3.** Some of dominance weeds before and after treatments of organic matter and NPK fertilizer.

| No | Weed species | Family | Before treatments | After treatments |
|---|---|---|---|---|
| 1 | *Panicum maximum* | Graminae | 23,30 | 2,00 |
| 2 | *Borreria laevis* | Rubiaceae | 13,39 | - |
| 3 | *Mucuna pruriens* | Leguminosae | 12,68 | 2,10 |
| 4 | *Eleusine indica* | Graminae | 8,47 | 26.52 |
| 5 | *Richardia brasiliensies* | Rubiaceae | 7,40 | 39.48 |
| 6 | *Ageratum conyzoydes* | Compositae | 5,24 | - |



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| | | | | |
|---|---|---|---|---|
| 7 | *Cleome rutidosperma* | Capparidaceae | 4,36 | 2,98 |
| 8 | *Mimosa diplotricha* | Leguminosae | 4,31 | 2,82 |
| 9 | *Digitaria ciliaris.* | Graminae | 4,1 | - |
| 10 | *Emilia sonchifolia* | Compositae | 3,92 | - |
| 11 | *Cyanotis cristata* | Commelinaceae | - | 6.17 |
| 12 | *Brachiaria paspaloides* | Gramineae | - | 3,99 |
| 13 | *Borreria alata* | Rubiaceae | - | 3,84 |
| 14 | *Euphorbia prunifolia* | Euphorbiaceae | - | 2,52 |

The dominant weeds after treatments were *Richardia brasiliensis* (SDR 39.48%), *Eleusine indica* (SDR 26.52%) and *Cyanotis cristata* (SDR 6.17%). The areal of research was dominated by *Richardia brasiliensis*. This weed is a perennial broadleaf weed. It blooms all year, drought-tolerant, It produces a deep taproot and will resprout from broken pieces of taproot left in the soil when the plant is pulled. It self sows freely and as volunteer seedlings next season. Broadleaf weeds dominated corn planting area at Agro Techno Park, because broadleaf weeds were able to be adapted and had high tolerance on seasonal cultivated soil. This character was got from its adaptation to seasonal soil cultivation that also became good habitat for weeds germination and weeds growth (Madkar *et al*., 1986).

**Weed Diversity**

The highest weeds diversity index after organic matter and NPK fertilizer treatments was on K6P0 (plot that given cow manure without NPK fertilizer), it was 2.10 (Figure 3). Such a thing was caused cow manure might be still contain a lot of viable weed seeds which was not damaged in cow digestion. Therefore, those seeds would germinate in field that caused weed diversity index in K6P0 was the highest. Manure which is mixed with food remnant can increase soil nutrient and weeds will grow extensively on cultivated field (Mayadewi, 2007).

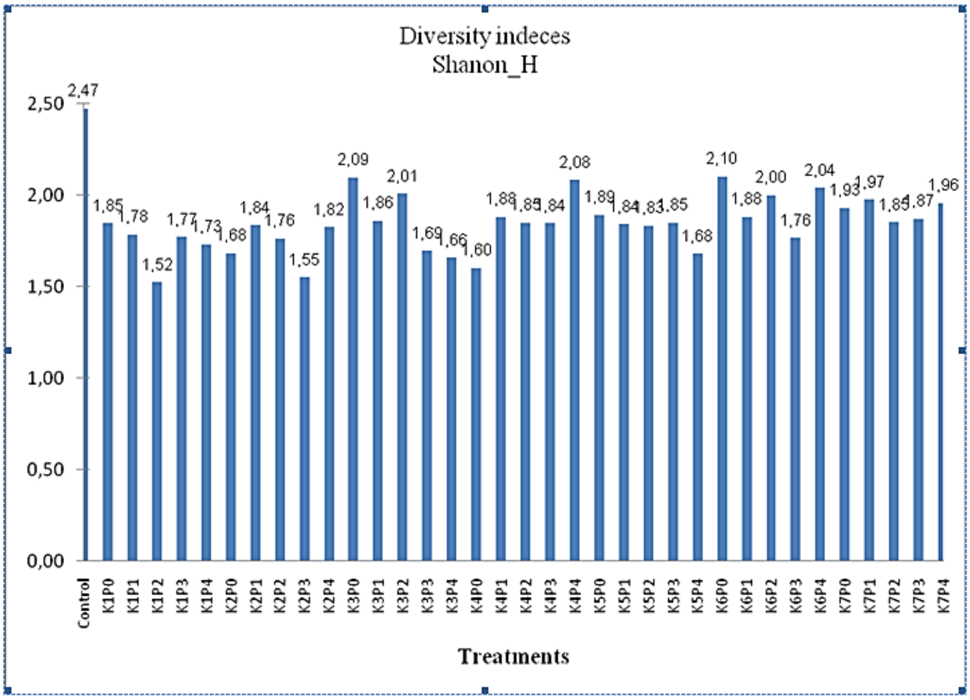


**Fig. 3.** Effects of organic matters and n p k fertilizer on weed diversity



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## CONCLUSSIONS

The following conclusions can be derived from the study, viz. (i) Organic matter treatments did not affect corn yield significantly, but treatment of planted cowpea gave better yield compared to other organic matter treatments, (ii) NPK fertilizer application affected all corn parameters significantly, and 50% of NPK fertilizer had produced best corn yield. Application of 50% NPK fertilizer in combination with organic matter had been able to produce the same corn yield with 100% of NPK fertilizer; (iii) There was weed shifting because of organic matters and NPK fertilizer effects. It was found that the dominant weed species before study was *Panicum maximum* and after treatments the dominant weed species was *Richardia brasiliensis*; and (iv) Weed community diversity decreased after organic matter treatments, it was shown by diversity index before treatments which was 2.47 and after treatments it was 1.52 – 2.10

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# WEED FLORA AND THEIR MANAGEMENT IN AQUATIC ENVIRONMENTS OF ASSAM, INDIA

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**ABSTRACT** The weed flora of different aquatic environments was surveyed and documented since 1983. Under non-crop aquatic situations like ponds, *beels* and other derelict water bodies in the six agro-climatic zones of Assam, predominant aquatic weed flora comprised of different free floating, suspended and submerged, anchored floating, anchored emerged and marginal weeds. The most dominant weed species in deep water rice crop are *Paspalum scrobiculatum* L., S*acciolepis interrupta* (Willd.) Stapf, *Oryza rufipogaon* Griff., *Monochoria hastata* (L.) Solms, *Eichhornia crassipes* (Mast.) Solms, *Cyperus spp*., *Alternanthera philoxeroides* Griseb., *Dichanthium annulatum* (Forssk.) Stapf along with sporadic incidence of *Phragmites karka* (Retz.) Trin. ex Steud.. In transplanted winter rice, dominant weed flora comprised of *Monochoria vaginalis* (Burm.f.) C.Presl ex Kunth, *Ludwigia linifolia*(Vahl.) Bennet, *Sphenoclea zeylanica Gaertn.* from broadleaved weeds, *Cyperus iria* L., *S. juncoides* Roxb., *Fimbristylis littoralis* Gaud., from sedges and *Echinochloa crusgalli* (L.) P. Beauv from grasses were predominant. Experiments conducted at the DWSR-AAU Centre, Jorhat showed successful management of weeds in non-cropped and cropped aquatic environments. Application of glyphosate 41% (2.5, 4.0, 5.0, 7.5 and 10.0 kg/ha) resulted in yellowing and drying of *Eichhornia* and *Spilanthes* with symptoms appearing between 4-6 DAA. At 45 days, there was regrowth of *Eichhornia* and new flowering in *Spilanthes* with glyphosate 2.5 kg/ha. Treatment with paraquat 8 ml/l resulted faster and complete killing of *Eichhornia* (2008-09). A long term trial after 12 years showed butachlor 1.5 kg/ha + 2,4-D 0.75 kg/ha rotated with pretilachlor 0.750kg/ha in a transplanted rice-rice sequence resulted lowest density and dry weight of weed (2009-10). Application of oxadiargyl 70 g/ha under normal method of planting gave best control of weeds in transplanted rice (2002-2003). Lowest weed density and dry weight throughout the growing season of transplanted rice were maintained by application of pretilachlor 1000 g/ha followed by Almix 4 g/ha (2011-12).

***Keywords:*** Cropped aquatic environment, deep water rice, derelict water bodies, non-crop aquatic situation, transplanted rice.

## INTRODUCTION

Aquatic weeds are a potential danger to a water body and they cause various harms. Identification of the weeds is essential for their effective control and maintaining a balance in the ecosystem (Lewis and Miller, 1980). Aquatic environment comprises both non-cropped (freshwater) and cropped (terrestrial) situation between which there is vast difference in the nature of weeds, their intensity and interaction with other components of the ecosystem. Depending upon the specific purpose for which a water body is to be utilized, the weed management strategy needs to be evolved; accordingly the control measures are evaluated, integrated and implemented.

The state of Assam is located in north eastern India and the whole region is characterized by high rainfall, moderate temperature and high relative humidity. The state has 3,73,901 ha of fresh water non-crop water bodies which accounts for 4.74 per cent of total geographical area of the state (Directorate of Economics and Statistics, Govt. of Assam, 2005). Terrestrial ecosystem which is temporarily stagnated with water of varying level and duration is converted to aquatic environment and such aquatic environments are generally put under shallow, medium



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and deep water rice cultivation. A total area of 17 lakh hectares is under such rice out of which 12 lakh hectares are under flood free shallow and medium lowland. A sizeable area under deep water rice is also included under this (Ahmed *et. al.*, 2010).

## MATERIALS AND METHODS

The survey of the aquatic environment was started in 1983 and the work was summarized initially for the period of 1983 to 2000. The changes in the composition of the weed flora under these environments were again monitored for five years during 2008-12. Dominance of the different weed species was recorded by random sampling, counting the individual numbers and ranking them on the basis of their degree of infestation. Accordingly, five dominant weed species in each agro-climatic zone under each group *viz.* free floating, suspended-submerged, anchored floating , anchored emerged and marginal weeds were listed. The entire state of Assam has been delineated into six agro-climatic zones depending on the prevailing rainfall, terrain and soil characteristics. These zones are, i) Upper Brahmaputra Valley Zone (UBVZ), ii) Central Brahmaputra Valley Zone (CBVZ), iii) Lower Brahmaputra Valley Zone(CBVZ), iv) Hill Zone (HZ), v) Barak Valley Zone (BVZ) and vii) North Bank Plain Zone (NBPZ). The agricultural research and development plans of the state are prepared individually for each of these zones. Therefore, weed survey results were compiled zone wise and dominance pattern of different weeds was evaluated for each zone separately.

Individual field experiments were conducted at Directorate of Weed Science Research-Assam Agricultural University Centre, Jorhat to evaluate and identify a suitable weed management practice individually for non-crop as well as cropped aquatic environment. The first experiment on aquatic weed management was carried out in 2001with ten treatments in three replications. The experimental site was a low lying area with water stagnation up to a depth of one metre. The herbicides were applied as per the treatment plan with Knapsack sprayer taking a water volume of 400 liter per hectare. The second experiment was conducted on efficacy of different herbicide against *Eichhornia crassipes* during 2009. The trial was carried under controlled condition in concrete tanks of the dimension of 3 m X 2m X 1m. The tanks were filled up with water up to a height of 0.7 m and ten plants of water hyacinth were (*Eichhornia crassipes)* per $m^2$ were kept for one week. Herbicides as per treatment were applied with a spray volume of 400 litres per ha. The third experiment being continued for last 12 years in a parmanent site at the Instructional-cum-research Farm (ICR Farm) of Assam Agricultural University (AAU), Jorhat comprised of five treatments arranged in randomized block design with 3 replications. Another field experiment entitled 'Weed management in *kharif* rice' included 12 treatments conducted in the ICR Farm of AAU, Jorhat in a randomized block design with 3 replications during 2002-03. The experiment was conducted in. The fifth experiment was conducted at the ICR Farm of AAU, Jorhat during 2011-12 to evaluate herbicide combinations for control of complex weed flora in transplanted rice with 11 treatments replicated thrice and laid in a randomized block design.

## RESULTS AND DISCUSSION:

### Weed flora and their dominance - Non-crop aquatic environment

The weed flora of non-crop aquatic environment comprised of various weed species under the categories of free floating, suspended-submerged, anchored floating, anchored, emerged and marginal weeds (Table 1). There were seven most dominant free floating weed species across the six agro-climatic zones. Likewise, the number of dominant species under suspended-submerged, anchored floating anchored emerged and marginal weeds irrespective of zones was 7, 5, 10 and 10, respectively. It was revealed that *Eichhornia crassipes* and *Trapa natans* in free floating, *Ottelia alismoides*, *Vellisneria natans* and *Hydrilla verticellata* in suspended-submerged, *Nymphoides indicum*, *Nymphaea nouchali*, *Sagittaria gyayanensis* ssp. *lappula*, *Marsilia minuta* and *Euryale ferox* in anchored floating, *Monochoria hastata* and *Eleocharis dulcis*/ *Eleocharis spp.*in anchored emerged and *Leersia hexandra* in marginal group were



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distributed equally in all the six zones. This indicated their wider adaptability and competitiveness. Murphy, 1988 reviewed the weed problems in freshwater ecosystem and recorded *Eichhornia crassipes* as the worst weed in such ecosystem.

**Table 1.** Category-wise dominant weed flora under non-cropped aquatic habitats

| Sl. No. | Weed | Zone |
|---|---|---|
| **Free floating** | | |
| 1. | *Eichhornia crassipes* (Mast.) Solms | UBVZ, CBVZ, LBVZ, HZ, BVZ, NBPZ |
| 2. | *Salvinia natans* (L.) All | UBVZ, CBVZ, HZ, BVZ, NBPZ |
| 3. | *Pistia stratiotes* L. | UBVZ, CBVZ, LBVZ, HZ, |
| 4. | *Hygroryza aristata* Nees | UBVZ, CBVZ, BVZ, BVZ, NBPZ |
| 5. | *Trapa natans* L.var. *bispinosa* | UBVZ, CBVZ, LBVZ, HZ, BVZ, NBPZ |
| 6. | *Lemna minor* L. | LBVZ, HZ, NBPZ |
| 7. | *Salvinia natans* (L.) All – *S. cuculata* Roxb. Ex Bory complex | LBVZ |
| **Suspended & submerged** | | |
| 8. | *Ceratophyllum demersum* L. | UBVZ, CBVZ, HZ, BVZ, NBPZ |
| 9. | *Ottelia alismoides* (L.) | UBVZ, CBVZ, LBVZ, HZ, BVZ, NBPZ |
| 10. | *Utricularia bifida* L. | UBVZ, CBVZ, HZ, BVZ, NBPZ |
| 11. | *Vellisneria natans* (Lour.) Hara | UBVZ, CBVZ, LBVZ, HZ, BVZ, NBPZ |
| 12. | *Hydrilla verticellata* (L.f.) Royle | UBVZ, CBVZ, LBVZ, HZ, BVZ, NBPZ |
| 13. | *Potamogaton nodosus* Poir. | LBVZ, |
| 14. | *Ceratophyllum –Chara-Utricularia-complex* | LBVZ, |
| **Anchored floating** | | |
| 15. | *Nymphoides indicum* (L.) Kuntz. | UBVZ, CBVZ, LBVZ, HZ, BVZ, NBPZ |
| 16. | *Nymphaea nouchali* Burm. f., *Nymphea spp.* | UBVZ, CBVZ, LBVZ, HZ, BVZ, NBPZ |
| 17. | *Sagittaria gyayanensis* ssp. l*appula* (Don) Bogin | UBVZ, CBVZ, LBVZ, HZ, BVZ, NBPZ |
| 18. | *Marsilia minuta* L. | UBVZ, CBVZ, LBVZ, HZ, BVZ, NBPZ |
| 19. | *Euryale ferox* Salsb. | UBVZ, CBVZ, LBVZ, HZ, BVZ, NBPZ |
| **Anchored emerged** | | |
| 20. | *Monochoria hastata* (L.) Solms | UBVZ, CBVZ, LBVZ, HZ, BVZ, NBPZ |
| 21. | *Monochoria vaginalis*( Burm. f.) Presl. | UBVZ, HZ, BVZ, NBPZ |
| 22. | *Limnophylla heterophylla* Benth. | UBVZ, LBVZ, HZ, BVZ |
| 23. | *Oryza rufipogon* Griff. | UBVZ, HZ, |
| 24. | *Eleocharis dulcis* (Burm. f.) Hens., *Eleocharis spp.* | UBVZ, CBVZ, LBVZ, HZ, BVZ, NBPZ |
| 25. | *Scirpus juncoides* Roxb. | CBVZ, LBVZ, BVZ, NBPZ |
| 26. | *Sagittaria sagittifolia* L. | CBVZ |
| 27. | *Ludwigia linifolia* (Vadl) Bennet | CBVZ |
| 28. | *Enhydra fluctuens* Lour. | LBVZ |
| 29. | *Eleocharis acutangula*(Roxb.)Sch. | NBPZ |
| **Marginal** | | |
| 30. | *Leersia hexandra* Swartz. | UBVZ, CBVZ, LBVZ, HZ, BVZ, NBPZ |
| 31. | *Commelina diffusa* Burm. f. | UBVZ, CBVZ, BVZ, |
| 32. | *Ludwigia adsedens* (L.) Hara | UBVZ, CBVZ, LBVZ, BVZ, NBPZ |
| 33. | *Sacciolepsis interrupta*(Willd.) Stapf. | UBVZ, CBVZ, HZ, BVZ, NBPZ |



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| | | |
|---|---|---|
| 34. | *Hymanachne acutigluma* (Steud.) Gillil | UBVZ |
| 35. | *Vetiveria ziznioides* (L.) Nash | CBVZ |
| 36. | *Ipomoea carnea* Jacq. | LBVZ, HZ, BVZ, NBPZ |
| 37. | *Ipomoea aquatica* Forssk. | LBVZ, HZ |
| 38. | *Cyperus distans-C.pilosus* complex | LBVZ, HZ |
| 39. | *Phragmites karka*(Retz.) Steud. | NBPZ |

**Table 2.** Newly spreading weeds in non-crop aquatic environment

| Zone | Weeds |
|---|---|
| NBPZ | Free floating: *Lemna perpusilla* Torry, *Pistia strateotes* L.<br>Suspended & submerged: *Utricularia-Ceratophyllum* complex<br>Anchored emerged: *Alternanthera philoxeroides* (Mart) Greiseb., *Colocasia antiquorum* Schott |
| UBVZ | Suspended & submerged: *Utricularia gibba* L. subsp. *exoleta* (R. Br) P.Taylor<br>Anchored emerged: *Oryza rufipogon* Griff. ,*Sagitteria trifolia* L., *Ludwigia linifolia* Rolla Rao<br>Marginal: *Calamus tenuis* Roxb.*Panicum repens* L., *Polygonum glabrum* Willd. R*anunculus sceleratus* L., *Rumex nepalensis* Spreng. |
| LBVZ | Anchored emerged: *Alternanthera philoxeroides* (Mart) Greiseb. *Eleocharis dulsis* (Burm. f.) Hens. *Ludwigia linifolia* Poir., *Hymanachne acutingluma* (Steud.) Gill. *Sacciolepsis interrupta* (Willd.) Stapf. , *Scirpus grossus* L.f.<br>Marginal: *Vetiveria ziznioides* (L.) Nash, *Saccharum spontaneum* L, *Polygonum spp*., *Ipomoea carnea* Jacq. |
| BBZ | Anchored floating: *Isachne miliacea* Roth, *Ipomoea aquatica* Forssk ,<br>Anchored emerged: *Sphenochlea zeylanica* Gaertn |

Weed species and their dominance spectrum are fluctuating in Central Brahmaputra Valley zone in a rather faster rate because of frequent flood and runoff water. However, the frequency of occurrence of *Vetiveria zizanioides* is increasing considerably during last five years. In Lower Brahmaputra Valley Zone, the aggressivity of *Ludwigia* species has increased considerably in aquatic ecosystems of non-cropped lands along with a number of species of *Cyperus* during last five years (Table 2). Dominance of *Ottelia alismoides* has also increased. In swampy open lands, an ecotype of *Monochoria hastata* with other broadleaves became more common than other species of same habitat. Dominance of *Ipomoea carnea* is increasing tremendously in last few years which has caused havoc in Karimganj and Hailakandi districts of BVZ. Another weed recorded is *Sphenochlea zeylanica* which has also become dominant in stagnant water bodies. In North Bank Plain Zone, most of the areas are highly flood affected and hence, prevalence of *Eichhornia crassipes* is quite noticeable in non-cropped and cropped areas. In the water bodies adjacent to tributaries of the major river Brahmaputra, the presence of *Hygroryza aristata* is exceptionally high. Edges of water bodies are rich in *Phragmites karka* and *Leersia hexandra*.

**Weed flora and their dominance - Cropped aquatic environment**

There was variation in the dominance pattern of the weeds in transplanted *kharif* rice across the agro-climatic zones. However, out of the 15 weed species identified to be dominant in transplanted *kharif* rice in different zones. *Cyperus iria* was the most prevalent weed in five zones (Table 3). The weeds which are common between non-crop and cropped aquatic situation were *Sagitarria guayanensis*, *Sacciolepsis interrupta*, *Monochoria vaginalis*, *Ludwigia linifolia*, *Sphenochloa zeylanica* and *Scirpus jucoides*, while *Cyperus iria*, *Echinochloa crusgalli*, *Ludwigia perennis*, *Fimbristylis littoralis*, *Paspalum scrobiculatum*, *Cyperus difformis*, *Phyllanthus fraternus - P.urinata* complex, *Hydrolea zeylanica* and *Cynodon dactylon* were specific only to the transplanted *kharif* rice ecosystem.



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**Table 3.** Weed flora in transplanted *kharif* rice

| Zone | Weeds |
|---|---|
| UBVZ | *Sagitarria guayanensis* (Don) Bogin, *Sacciolepis interrupta* (Willd.) Stapf., *Cyperus iria* L., *Echinochloa crusgalli* (L.) P. Beauv., *Monochoria vaginalis* (Burm. F.) Presl. |
| CBVZ | *Ludwigia linifolia* (Vahl.) Bennet , *Cyperus iria* L., *Sphenochloa zeylanica* Gaertn., *Ludwigia perennis* L., *Fimbristylis littoralis* Gaud. |
| LBVZ | *Paspalum scrobiculatum* L., *Echinochloa crusgalli* (L.) P. Beauv., *Cyperus iria* L., *Ludwigia linifolia* (Vahl.) Bennet, *Monochoria vaginalis* (Burm. F.) Presl. |
| NBPZ | *Echinochloa crusgalli* (L.) P. Beauv., *Monochoria vaginalis* (Burm. F.) Presl., *Paspalum scrobiculatum* L., *Sacciolepis interrupta* (Willd.) Stapf., *Ludwigia linifolia* (Vahl.) Bennet, |
| HZ | *Sphenochloa zeylanica* Gaertn., *Cyperus iria* L., *Fimbristylis littoralis* Gaud., *Scirpus jucoides* Roxb., *Cyperus difformis* L. |
| BVZ | *Sacciolepis interrupta* (Willd.) Stapf., *Phyllanthus fraternus* Webst. - *P. urinata* L. complex, *Paspalum scrobiculatum* L., *Hydrolea zeylanica* (L.) Vahl., *Cynodon dactylon* (L.) Pers |

**Table 4.** Weed flora in deep water rice

| Zone | Weeds |
|---|---|
| UBVZ | *Cynodon dactylon* (L.) Pers, *Ludwigia octovalvis* sub-sp. s*essiliflora* (Mich.) Raven, *Leersia hexandra* Swartz., *Oryza rufipogon* Griff., *Eragrostis uniloides* (Retz.) Nees *ex* Steud. Complex |
| BVZ | *Sphenochlea zeylanica* Gaertn., *Cynodon dactylon* (L.) Pers, *Ludwigia octovalvis* sub-sp. s*essiliflora* (Mich.) Raven, *Eragrostis uniloides* (Retz.) Nees *ex* Steud. Complex, *Fimbristylis littoralis* Gaud. |
| NBPZ | *Paspalum scrobiculatum* L., *Oryza rufipogon* Griff., *Hygroryza aristata* (Retz.) Nees exW. &A., *Hymanachne acutigluma* (Steud.) Gill., *Sacciolepis interrupta* (Willd.) Stapf. |

In deep water rice, the weed flora varies from terrestrial to aquatic depending upon the water level in the field. Deep water rice cultivation starts in a dry field and gradually water level rises to a maximum, thereafter it decreases. The data presented in Table 4 showed that fourteen different weed species were recorded and *Cynodon dactylon*, *Ludwigia octovalvis* sub-sp. s*essiliflora* and *Eragrostis uniloides* complex were found to be most widely distributed.

## Weed management

**Experiment 1.** The dominant weed species were *Eichhornia crassipes*, *Alternanthera philoxeroides*, *Spilanthes paniculata*, *Brachiaria mutica* and *Ludwigia linifolia*. A gradual decline in weed dry weight was observed in all the treatments. Application of glyphosate resulted yellowing and drying of *Eichhornia* and higher dose of this herbicide (5kg/ha and above) caused inhibition of flowering in *Spilanthes.* However, the results presented in Table 5 revealed that glyphosate @ 5.0 kg/ha and the doses above this resulted significant reduction in weed dry weight at 30 and 45 days after spraying as compared to untreated and there was no regrowth at 45 days. Jimenez, 2003 also concluded the efficacy of 2,4-D and glyphosate on *Eichhornia*.

**Table 5.** Dry weight of aquatic weeds at different days after spraying herbicides.

| Treatment | Weed dry weight (g/m$^2$) at days after spraying | | | |
|---|---|---|---|---|
| | 0 days | 15 days | 30 days | 45 days |
| Glyphosate 2.5 kg/ha | 64.3 | 63.6 | 53.3 | 54.7 |
| Glyphosate 4.0 kg/ha | 63.4 | 60.5 | 51.8 | 53.4 |



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| | | | | |
|---|---|---|---|---|
| Glyphosate 5.0 kg/ha | 61.9 | 57.3 | 52.8 | 49.2 |
| Glyphosate 7.5 kg/ha | 56.2 | 48.6 | 49.3 | 49.8 |
| Glyphosate 10.0 kg/ha | 52.7 | 42.1 | 41.0 | 41.0 |
| Untreated | 66.3 | 61.7 | 59.5 | 58.8 |
| CD (p=0.05) | 5.99 | 5.97 | 6.43 | 5.34 |

**Experiment 2.** The data incorporated in Table 6 from this experiment showed that weeds were completely dried by the second week of the spray irrespective of herbicides. Paraquat applied tanks recorded faster drying of the plants. Lowest weed dry weight at 7 days after spray was recorded with paraquat 8 ml/l of water and highest in untreated control. Weeds were completely killed by 15 days after spray in all the herbicide treated tanks but weed dry weight increased in untreated control. Regrowth of weeds up to 120 days of spray was not observed in treated tanks irrespective of herbicides.

**Table 6.** Weed dry weight and regrowth of aquatic weeds as affected by treatments**.**

| Treatments | Visual rating of drying days after spraying | | Weed dry weight ($g/m^2$) days after spraying | | Regrowth up to 120 days of spraying |
|---|---|---|---|---|---|
| | 7 | 14 | 7 | 14 | |
| Paraquat 4 ml/l | 7 | 10 | 5.2 | - | Nil |
| Paraquat 8 ml/l | 8 | 10 | 3.8 | - | Nil |
| Glyphosate 41SL 5 ml/l | 4 | 10 | 6.8 | - | Nil |
| Glyphosate 41SL 10 ml/l | 6 | 10 | 6.4 | - | Nil |
| 2,4-D Na salt 1.25 g/l | 5 | 10 | 7.1 | - | Nil |
| 2,4-D Na salt 2.50 g/l | 5 | 10 | 6.5 | - | Nil |
| Glufosinate ammonium 6.5 g/l | 4 | 10 | 5.8 | - | Nil |
| Glufosinate ammonium 13.0 g/l | 6 | 10 | 5.8 | - | Nil |
| Metsulfuron methyl 0.05 g/l | 6 | 10 | 7.2 | - | Nil |
| Metsulfuron methyl 0.05 g/l | 6 | 10 | 6.8 | - | Nil |
| Untreated control | 0 | 0 | 56.8 | 71.2 | -- |

**Experiment 3.** Continuous monitoring of the weeds in this trial since last 12 years showed that broad-leaved as well as total weed population decreased gradually in all the plots receiving herbicide treatments after 2007 in autumn rice and after 2005 in winter (*kharif*) rice. There was a heavy reduction in grasses. The only grass species recorded in autumn rice during 2009 and 2010 were *Leersia hexandra* and *Sacciolepsis interrupta* and *Echinochloa crusgalli*, *Isachne globosa*, *Oryza rufipogan* and *Rottboelia exaltata* disapperared after 2008. Grass population also reduced considerably in *kharif* rice. Species like *Fimbristylis littoralis*, *Ipomoea aquatica*, *Isachne himalaica*, *Leersia hexandra* and *Oryza rufipogan* disappeared from the field in *kharif season* after 2009. The data showed that butachlor 1.5kg/ha + 2,4-D 0.75kg/ha rotated with pretilachlor 0.75kg/ha (100 % NPK by fertilizer) resulted the relatively lower density and dry weight of weed and higher grain yield of both autumn and winter rice (Table 7).

**Table 7.** Weed density, weed dry weight and grain yield of autumn and winter rice**.**

| Treatment | Weed density ($No./m^2$) 60 DAT | | Weed dry weight ($g/m^2$) 60 DAT | | Grain yield (q/ha) | |
|---|---|---|---|---|---|---|
| | Autumn | Winter | Autumn | Winter | Autumn | Winter |
| Farmers' practice(1 HW) | 54.8 | 54.0 | 27.83 | 20.2 | 21.2 | 31.9 |
| Butachlor 1.5kg/ha + 2,4-D 0.75kg/ha (100 % NPK by fertilizer) | 34.0 | 49.2 | 18.23 | 15.7 | 26.0 | 35.4 |



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| | | | | | | |
|---|---|---|---|---|---|---|
| Butachlor 1.5kg/ha + 2,4-D 0.75kg/ha (75 % NPK by fertilizer+25% by organic) | 31.0 | 37.3 | 17.18 | 14.5 | 27.5 | 27.1 |
| Butachlor 1.5kg/ha + 2,4-D 0.75kg/ha rotated with pretilachlor 0.75kg/ha (100 % NPK by fertilizer) | 30.8 | 31.3 | 16.53 | 14.1 | 28.8 | 38.4 |
| Butachlor 1.5kg/ha + 2,4-D 0.75kg/ha rotated with pretilachlor 0.75kg/ha (75 % NPK by fertilizer+25% by organic) | 31.8 | 41.3 | 17.10 | 14.8 | 28.0 | 37.6 |
| C.D. (*p*=0.05) | 11.6 | 11.6 | 3.47 | 3.0 | 9.3 | 3.5 |

**Experiment 4.** The most dominating weed species in the experimental site were *Panicum repens*, *Isachne himalaica*, *Leersia hexandra*, *Oryza rufipogon*, *Cyperus halpan*, *C. platystylis*, *Eleocharis acutangula*, *Scirpus juncoides* and *Ludwigia linifolia.* Results presented in Table 8 showed that application of oxadiargyl 70 g/ha under normal method of planting resulted in lowest weed density at 30 DAT while lowest dry weight at 30 DAT was recorded with normal planting + oxadiargyl 70g/h and farmers' planting + fenoxaprop-p-ethyl 70g/ha at 20-25 DAT. The highest grain yield obtained with farmers' planting + oxadiargyl 70g/ha.

**Table 8.** Weed density, Weed dry weight and grain yield of rice due to treatments

| Treatment | Weed density (No./m$^2$) | Weed dry weight (g/m$^2$) | Grain yield of rice (q/ha) |
|---|---|---|---|
| Normal planting+ Oxadiargyl 70g/ha at 3 DAT | 58 | 30.7 | 28.16 |
| Farmers' planting+ Oxadiargyl 70g/ha at 3 DAT | 84 | 36.0 | 38.80 |
| Farmers' planting+ almix 4g/ha at 20-25 DAT | 102 | 36.0 | 37.50 |
| Farmers' planting+ Fenoxaprop-p-ethyl 70g/ha at 20-25 DAT | 84 | 30.7 | 30.30 |
| Normal planting+ pretilachlor 0.75 kg/ha at 3 DAT | 108 | 37.3 | 30.66 |
| Farmers' planting+ pretilachlor 0.75 kg/ha at 3DAT | 148 | 48.0 | 28.83 |
| Normal planting fb. Rotary weeder(25 DAT) | 164 | 36.0 | 23.00 |
| Farmers' practice | 144 | 38.7 | 29.16 |
| Unweeded control | 176 | 50.7 | 19.66 |
| C.D. (*p*=0.05) | 14 | 6.2 | 4.65 |

**Experiment 5.** The weed flora of this experiment comprised of *Monochoria vaginalis*, *Sagittaria guayanensis*, *Cyperus iria*, *Fimbrisylis littoralis*, *Eleocharis acutangula*, *E. dulsis* and *Scirpus juncoides*, *Leersia hexandra*, *Sacciolepsis interrupta*, *Echinochloa crusgalli*, *Isachne himalaica* and *Paspalum* spp. Other aquatic weeds like *Marsilea minuta*, *Alternanthera philoxeroides* and *Hydrolea zeylanica* were also observed. Application of pretilachlor 1000g/ha followed by almix 4g/ha or pyrazosulfuron 20g/ha followed by manual weeding 30 DAT resulted lowest weed density and dry weight at 60 DAT (Table 9). Higher grain yield was obtained from pretilachlor 1.0 kg/ha followed by almix 4g/ha as well as pyrazosulfuron 20g/ha followed by weeding once.



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**Table 9.** Weed density, weed dry weight and grain yield of transplanted rice

| Treatments | Weed density (No/$m^2$) | Weed dry weight (g/$m^2$) | Grain yield (q/ha) |
|---|---|---|---|
| Pretilachlor 1000g/ha | 32.7 | 20.1 | 30.00 |
| Pyrazosulfuron 20g/ha | 31.3 | 21.5 | 31.25 |
| Pretilachlor 1000g/ha followed by ethxysulfuron 18.75g/ha | 26.3 | 20.1 | 30.25 |
| Pretilachlor 1000g/ha followed by almix 4g/ha | 16.7 | 12.6 | 34.83 |
| Pyrazosulfuron 20g/ha followed by manual weeding30DAT | 21.4 | 18.0 | 32.83 |
| Hand weeding 25 and 45 DAT | 27.3 | 14.5 | 30.83 |
| Weedy | 63.0 | 50.1 | 25.17 |
| CD (p=0.05) | 16.2 | 13.9 | 6.7 |

## CONCLUSIONS

There is a great variation in aquatic weed flora under different agro-climatic conditions of Assam. However, there was similarity of weed flora to a great extent between permanently aquatic non-cropped and temporarily aquatic cropped ecosystem. Recent survey showed a changing composition and dominance in the weed flora of non-cropped aquatic environment.

Weed management trials revealed application of glyphosate @ 5kg/ha or 5ml, paraquat 4ml, 2,4-D Na salt 1.25g, glufosinate ammonium 6.5g or metsulfuron methyl 0.05g per liter of water gave very effective control of aquatic weeds. In aquatic ecosystem with transplanted rice, application of oxadiargyl 70g/ha or almix (chlorimuron methyl+metsulfuron methyl) 4 g/ha was very effective. Alternately, pretilachlor 1000g/ha followed by almix 4g/ha as well as pyrazosulfuron 20g/ha followed by manual weeding 30 DAT would be effective in controlling the weeds and increasing grain yield of rice. Further, butachlor + 2,4-D rotated with pretilachlor was found to be most suitable herbicide rotation combination for control of weeds and higher grain yield from rice.

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## *Quarantine, Special Weed Problems and Weeds as Bio-Resources*

# STRATEGIC MANAGEMENT OF INVASIVE PLANT SPECIES WITH REFERENCE TO THE ROLE OF AGRICULTURAL QUARANTINE ON THE PREVENTION OF TRANSBOUNDARY MOVEMENTS.

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**ABSTRACT** In recent years, invasive plant species have become a high-profile policy topic for the Indonesian communities at the national levels which have emphasised the need cross-sectoral coordination between competent institutional and stakeholders at all levels. As it has been indicated that a number of invasive plants species has seriously invaded ecosystems such as water hyacinth (*Eichhornia crassipes*) in Rawa Pening reservoir at Central Java, Siam weeds (*Cromolaena odorata*) in Savana at Kupang, and Morning glory plants (*Merremia peltata*) in Ujung Kulon National Park and South Barisan hill National Park. These might be need cross-sectoral effort to reduce their population. Indonesian Agricultural Quarantine agency is one of the inter-institutional agency which has been mandated to prevent trans-boundary movement of invasive plants species. Prevention effort of invasive plants species introduction in to the Indonesian territory through implementation Pre-border actions such as pre-shipment inspection, recognition and pre-clearance of the consignments in a source country. While, at the point of entry, border controls and quarantine measures need to be used to prevent or minimise the risk of introducing invasive plant species that could become invasive.To manage post-border action of invasive plant species, Ministry of Environment in coordination with other agencies currently prepare a National Strategy and Action Plan for management of invasive alien species (IAS). This concept is consisting of status, strategy and plan of action of invasive alien species in Indonesia. Through plan of action such as prevention, mitigation, and eradication of invasive plan species in some infested ecosystems can be reduced their population.

***Keywords:*** Quarantine, trans-boundary, and Invasive plant species.

## INTRODUCTION

The introduction of invasive plant species beyond their natural range is rising sharply, due to increased transport, trade, travel and tourism and unprecedented accessibility of goods resulting from globalisation. These activities provide vectors and pathways for live plants, animals and biological material to cross biographical barriers that would usually block their way. These might be have significant environmental, economic and public health impacts and present a significant risk of the wholesale homogenisation of ecosystems (Genovesi and Shine, 2003).

In recent years, invasive plat species have become a high-profile policy topic for the Indonesian communities at the national levels which have emphasised the need cross-sectoral coordination between competent institutional and stakeholders at all levels. As it has been indicated that a number of invasive plants species are seriously invaded ecosystems such as water hyacinth (*Eichhornia crassipes*) in Rawa Pening reservoir at Central Java, Siam weeds (*Cromolaena odorata*) in Savana at Kupang, and Morning glory plants (*Merremia peltata*) in Ujung Kulon National Park and South Barisan Hill National Park. These might be need cross-sectoral effort to reduce their population.



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Indonesian Agricultural Quarantine agency is one of the inter-institutional agency which has been mandated to prevent trans-boundary movement of invasive plants species. Prevention effort of invasive plants species introduction in to the Indonesian territory through implementation Pre-border actions such as pre-shipment inspection, recognition and pre-clearance of the consignments in a source country. While, at the point of import, border controls and quarantine measures need to be used to prevent or minimise the risk of introducing invasive plant species that could become invasive.

To manage post-border action of invasive plant species, Ministry of Environment in coordination with others agencies currently prepare a National Strategy and Action Plan for management of invasive alien species (IAS). This concept is consisting of status, strategy and plan of action of invasive alien species in Indonesia. Through plan of action such as prevention, mitigation, and eradication of invasive plan species in some infested ecosystems can be reduced their population. Thus, the objective of this paper is to elaborate inter-agency's responsibilities for management of invasive plant species.

## THE ROLE OF AGRICULTURAL QUARANTINE

Prevention between and within Indonesian territory is generally far more cost-effective and environmentally desirable than measures taken following the introduction and establishment of an invasive plant species. They should be given priority as the first line of defence. Prevention effort need to begin at the place of origin or export (before living organism crosses the biogeographically barrier). Prevention at source is particularly important where there are known is incursion and where interception of species may be difficult due to consignment are packed into containers in a source country and transported to dispersed estimation, often remote from traditional inspection sites at entry points.

At the point of import, border controls and quarantine measures need to be used to prevent or minimize the risk of introducing invasive plant species that are or could become invasive. This requires a framework of rules, trained staff, reference lists of species and risk goods, technical procedures and surveillance.

Agriculture quarantine agency may play an important role in trans-boundary movement of invasive plant species through strengthening quarantine regulations as clearly stated in Law of 12 of 1992 concerning Fish, Animal, and Plant Quarantine, Government Regulation Number 14 of 2002 concerning Plant Quarantine, Minister Decree Number 93 of 2011 concerning List of Quarantine pest, carrier and distribution, and currently under proposed of regulating of the prevention of movement of invasive plant species in to Indonesian territory (Table 1).

**Table 1.** List of Invasive Plant Species as Pests and Weeds

| No | Plant Status | Quantity | Remarks |
|---|---|---|---|
| 1 | Invasive plant species as quarantine pests | 52 species | MoA Decree Number 93 0f 2011 |
| 2 | Invasive plant species as Environmental weeds | 86 Species | Draft of MoA Decree on IAS Prevention into Indonesian Territory |

Current Quarantine policies for the prevention on the trans-boundary movement of invasive plant species is through implementation pre-border, at-border, and post-border of quarantine activities (IPPC, 2004). Pre-border of quarantine activities may consist of pre-shipment, quarantine recognition, and quarantine pre-clearance. The inspection of consignment is conducted in the point of exit prior to import. These might reduce the movement of invasive plant species due to an intensive of quarantine inspection.

Pre-border quarantine action means in intensive of quarantine inspection at the point of entry to the consignments which do not conducted by pre-border quarantine actions. These activities may consist of strengthening quarantine action such as inspection, detention, refusing,



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destroying or releasing. Quarantine inspection at the point of entry may require dedicated quarantine installation or premises, to minimize the escape of invasive plant species associated with consignments. While, Post-border quarantine activities means an inspection of consignment after at-border activities was done through intensive monitoring and surveillance, increase early warning systems, and rapid response in case of any explosive of invasive plant species in the fields (Table 2).

**Table 2**. Quarantine management of action for prevention of trans-boundary movement of invasive plant species.

| No | Place of Inspection | Activities |
|---|---|---|
| 1 | Pre-border | -Pre-shipment inspection;<br>-Recognition of invasive plant species free areas; and<br>-Pre-clearance |
| 2 | At-border | -Quarantine action (inspection, detention, refusal, destroy or release);<br>-Quarantine installation. |
| 3 | Post-border | -Monitoring and surveillance of invasive plant species;<br>-Early warning systems;<br>-Emergency/rapid response<br>-Control measures |

Unintentional movement of weeds seeds and or invasive plant species through importation of grain crops such as wheat grain has shown that some weed species such as *Polygonum convolvulus, Brassica nigra, Lolium perenne* and Avena *fatua* were the most frequent weeds intercepted in the wheat grain (Table 3).

*Polygonum convolvulus* has been evaluated based on Weed Risk Assessment (WRA), and has been recommended to be refusal its presence in Indonesia (Tasrif and Sahrir, 1999). Taking these results of into consideration, it was concluded that attention should be given to the possible introduction of invasive plant species through seed consignments. Tasrif and Sahrir (1999) reported that *Cencherus echinatus* has been intercepted in wheat grain importation from Australia. Currently, this new weed has been found and become a problem in abandon areas of Makassar, South Sulawesi Province (Tasrif, 2013, *unpublished data*).

## INTER-AGENCY COOPERATION ON IPS AND ITS MANAGEMENT

Currently, the Government of Indonesia is under finalized the draft of the National Strategy and Plan of Action of Invasive Alien Species (IAS). The drafts of management status of IAS/IAPS are consist of Policy and law and regulation of IAS/AIPS, research, Management institutional, information systems and data base, economic policy, public awareness and networking through international cooperation and agreement (MoE, 2012).

The establishment of some invasive plant species such as water hyacinth (*Eichhornia crassipes*) in Rawa Pening reservoir at Central Java, Siam weeds (*Cromolaena odorata*) in Savana at Kupang, and Morning glory plants (*Merremia peltata*) in Ujung Kulon National Park and South Barisan Hill National Park These might be need cross-sectoral effort to reduce their population. Control and Eradication program could be taken into consideration sooner, as well as public awareness, otherwise, those invasive plants species may become a serious impacts on economic lost and environment.

**Table 3**. Weeds intercepted through the trans-boundary movement of wheat grain in 2012 to 2013 *).

| No | Commodities | Country of Origin | Weeds Intercepted |
|---|---|---|---|
| 1. | Wheat grain | Australia | 1. *Avena fatua*<br>2. *Lolium perenne* |



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| | | | |
|---|---|---|---|
| | | | 3. *Polygonum convolvulus*<br>4. *Brassica* nigra.<br>5. *Ambrosia* sp.<br>6. *Cleome* sp. |
| 2. | Wheat grain | Canada | 1. *Avena fatua*<br>2. *Brassica nigra*.<br>3. *Polygonum convolvulus*<br>4. *Linum utilissinum*<br>5. *Paspalum* sp. |
| 3. | Wheat grain | India | 1. *Avena fatua*<br>2. *Brassica* nigra.<br>3. *Paspalum* sp. |
| 4. | Wheat grain | USA | 1. *Avena fatua*<br>2. *Lolium perenne*<br>3. *Polygonum convolvulus* |

*) Data collected from Makassar Agricultural Quarantine Service, 2013 (unpublished)

## CONCLUSIONS

The most common approach to preventing introduction has been to target individual species for quarantine in pre-border and at-border activities. Species based quarantine systems are conducted to anticipate risk before harm actually occurs, or to address the unintentional introductions that comprise the majority of invasive plant species introductions.

Meanwhile, managing invasive plant species is thus becoming more challenging and will be a lengthy mandate. Risk and impact assessments, early detection and monitoring and implementation of effective control measures in the Post-border area highly dependent on the availability of information that can keep pace with new invasion threats.

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# BUILDING CAPACITIES IN WEED AND INVASIVE ALIEN PLANT SPECIES CONTROL AND MANAGEMENT IN SOUTHEAST ASIA: THE SEAMEO BIOTROP EXPERIENCE

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**ABSTRACT** Since its establishment in 1968, the SEAMEO Regional Center for Tropical Biology (BIOTROP) in Bogor, Indonesia has recognized the important role of weed science in the context of agricultural production and the need to build capacities on it in Southeast Asia. Thus, the Centre developed and implemented 33 intensive long-term and short-term training courses from 1972-2013 in Indonesia and 3 other countries in the region in collaboration with international development organizations such as the Australian Center for International Agriculture Research (ACIAR), International Development Research Center (IDRC), CAB-International, the Food & Agriculture Organization (FAO) of the United Nation, Global Environment Facility (GEF), the United Nations Development Programme (UNDP), Indonesian Agriculture Quarantine Agency (IAQA), Research Institute of Estate Plantation and Weed Science Society of Indonesia (WSSI). In response to the global call to address the issue on invasive alien plant species (IAPS) put forward during the Earth Summit in Brazil in 1992, SEAMEO BIOTROP also started offering training courses on the management and control of IAPS. Since 2004, the Centre had conducted 10 national and four regional training courses on the subject matter for young scientists, lecturers, staff of the plantation crops, forest rangers, plant quarantine officers, etc. Two of these training courses were specially conducted for 60 Indonesian plant quarantine officers designated at different entry points of the country in cooperation with FAO which were held in 2010 and 2011. These capacity building activities have benefitted a total of more than 600 researchers and teachers. SEAMEO BIOTROP's training alumni on these subject matters have become advocates for management of weeds and IAPS in their respective institutions and countries through their research and teaching works. A considerable number of them pursued and obtained their PhD and MS degrees in weed science.

This paper highlights the development, content scope, processes, accomplishments, and lessons learned by SEAMEO BIOTROP in building capacities in weed science and IAPS management in Southeast Asia.

***Keywords:*** Capacity building, weeds and invasive alien plant species, SEAMEO BIOTROP

## INTRODUCTION

As one of the regional centres of the Southeast Asian Ministers of Education Organization (SEAMEO) established in 1968, the Regional Center for Tropical Biology (BIOTROP), located in Bogor, Indonesia, is mandated to conduct research, training, and information exchange activities aimed at solving critical biological problems toward contributing to the economic development of the region. SEAMEO is an intergovernmental organization whose purpose is to promote cooperation in education, science, and culture in Southeast Asia.

From its early years of operation, SEAMEO BIOTROP had already put attention to weed control as one of the focus areas under its Tropical Agricultural Pest Biology Program. This program, which ran from 1973 to 1998, was the Centre's attempt to address the adverse effects of plants and animal pests and come up with biological measures to control them for human and environmental welfare. Under this program, SEAMEO BIOTROP had provided a series of short-term training courses and long-term intensive training and research programs in



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weed control and research techniques for young scientists who are or will be in a position to either disseminate up-to-date information they obtained from BIOTROP to students and farmers or to develop their own interests in weed research and adaptation of modern techniques to local problems. Participants come from universities or colleges, research institutions, plantations estates, agrochemical companies, and national parks. The intensive courses conducted by BIOTROP filled the gaps, since the subject of weeds left neglected by other institutes in the region (Soerjani, 1971).

In response to the global call to address the issue on invasive alien plant species (IAPS) put forward during the Earth Summit in Brazil in 1992, SEAMEO BIOTROP also started offering training courses on the management and control of weeds and IAPS since 2004.

BIOTROP has an important role in weed science and invasive alien plant species in context of agriculture production and natural ecosystems, and the need to build capacity building on it in South East Asia (Bangun, 1996).

## SHORT- TERM TRAINING COURSES

SEAMEO BIOTROP offered short-term training courses on weed science from 1972 to 2004 and on control and management of invasive alien plant species from 2011 up to present both at the regional and national levels.

### Regional Training Courses

From 1972 to 1990, SEAMEO BIOTROP conducted eight regional training courses on weed science with six weeks duration each. The objectives of these courses were to update the participants on the recent concept of weeds, their biology with various approaches for their control management; to provide opportunities for training in the laboratory and in the field on the developing experimental techniques for the integrated management of weeds; to encourage the establishment of a regional network on the management of weeds. These courses were funded through the SEAMEO Special Education and Development Fund (SEDF) which benefitted 210 people from eight SEAMEO member countries, namely: Cambodia, Indonesia, Laos, Malaysia, Philippines, Singapore, Thailand, and Vietnam. Participants were nominated by their respective governments and selected according to SEAMO BIOTROP set of criteria. Of this number, 77 of them, who were Indonesians, either paid for themselves or sponsored by their respective institutions.

Five of these training courses were held in Bogor while the rest were conducted in Los Banos, Philippines in 1976, Kuala Lumpur, Malaysia in 1977 and Bangkok, Thailand in 1990. The training curriculum covered the whole aspects of weed science from weed taxonomy and biology to weed control. The general training course curriculum is given below (Robson, 1977):

1. Introduction to weeds, weed management and pest management
2. Crop and weed relationships and assessment of crop losses
3. Weed Biology
4. Collection, taxonomy and weed surveys
5. Weed control methods:
    a. Preventive measures
    b. Direct physical methods
    c. Cultural & ecological methods
    d. Biological methods
    e. Chemical methods (including herbicide characteristics, formulations. Behavior, use, residues and registration)
    f. Utilization of weeds
    g. Integrated control
6. Weed control in major food crops, horticulture, Plantation crops, grasslands and pastures, forestry and water



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7. Impact of weed control on the environment (residue analysis, bio-assay techniques, effect on soil microbes etc.)

Participants received lectures and conducted laboratory and field exercises to response to their needs for new knowledge, skills, and attitude in weed control and management. Participants were required to conduct group projects which were presented during the last day of the training for assessment and critiquing.

It is interesting to mention here that for the training courses held in Bogor in 1981 and in Bangkok, the focus was on aquatic weeds.

Starting 1990, SEAMEO BIOTROP shortened the duration of the course to four weeks then to ten days in 2004 due to the limitation in available funds and also the difficulty for some participants to be away from their office for a long period of time. Thus, the time allocated for practical exercises was much lesser than usual.

In response to the global issue on invasive alien species, topics related to it were added during the 2004 course offering, namely: Concept of Invasive Alien Plant Species, Process of Plant Invasions, and Risk Analysis. It was only in 2011 that SEAMEO BIOTROP started offering a full training course on IAS with the following topics and activities as follows: 1). Training course on the management of weeds and invasive alien plant species (2011); 2). Training course on the management of weeds and invasive alien plant species (2012); 3) Biological control of invasive alien plant species (2012); 4). Training course on the management of invasive alien plant species in the framework of forest restoration (2013).

The training in 2012 in cooperation with CAB-International focused on Biological Control of Invasive Alien Plant Species funded by the Global Environment Facility (GEF) and the United Nations Development Programme (UNDP). On the other hand, the training in 2013 focused on IAS control and management in the framework of forest restoration in relation to SEAMEO BIOTROP's program thrust on tropical biology for environmental integrity wherein landscape restoration is one of the focal topics.

In total, SEAMEO BIOTROP trained 342 individuals relating to weed science in general 10 training courses and invasive alien plant species in 5 training courses. Table 1 shows the breakdown of participants by country per year

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**Table 1.** Number of participants in SEAMEO BIOTROP's regional short-term training courses in Weed Science and Control and Management of Invasive Alien Plant Species, 1972-2013.

| No. | Year | Training Duration | No. of Participants per country | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Brunei | Camb. | Ind. | Lao | Mal. | Phil. | Sing | Thai | Timor Leste | Viet. | Obser-vers | Total |
| 1 | 1972 | 6 weeks | - | - | 3 | - | 2 | 3 | 2 | 2 | - | 1 | 30 | 44 |
| 2 | 1972 | 6 weeks | - | 2 | 4 | 2 | 2 | 2 | 2 | 4 | - | 1 | 14 | 33 |
| 3 | 1974 | 6 weeks | - | 2 | 3 | 1 | 2 | 4 | 1 | 2 | - | 1 | 25 | 42 |
| 4 | 1976 | 6 weeks | - | - | 5 | - | 3 | 4 | - | 4 | - | - | 5 | 21 |
| 5 | 1977 | 6 weeks | - | - | 7 | - | 11 | 3 | 1 | 2 | - | - | - | 24 |
| 6 | 1981 | 6 weeks | - | - | 15 | - | 4 | 1 | - | 4 | - | - | - | 24 |
| 7 | 1984 | 6 weeks | - | - | 5 | - | 1 | 2 | - | 2 | - | - | - | 10 |
| 8 | 1989 | 6 weeks | - | - | 9 | - | 2 | 2 | 1 | 2 | - | - | - | 16 |
| 9 | 1990 | 4 weeks | - | - | 3 | - | 2 | 3 | - | 3 | - | - | 1 | 12 |
| 10 | 1991 | 4 weeks | - | - | 10 | - | 3 | 1 | - | 1 | - | - | - | 15 |
| 11 | 2004 | 10 days | - | 1 | 6 | - | - | 1 | - | - | - | 1 | 2 | 11 |
| 12 | 2011 | 1 week | - | - | 17 | - | - | 2 | - | 2 | 1 | - | - | 11 |
| 13 | 2012 | 1 week | 2 | 1 | 19 | - | 1 | - | - | - | - | 2 | - | 25 |
| 14 | 2012 | 1 week | - | 4 | 7 | - | 1 | 4 | - | - | - | 3 | - | 19 |
| 15 | 2013 | 1 week | - | 2 | 22 | - | - | - | - | 1 | 1 | - | - | 24 |
| Total | | | 2 | 12 | 135 | 3 | 34 | 32 | 7 | 29 | 2 | 9 | 77 | **342** |



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**National Courses**

SEAMEO BIOTROP also implemented short-term training courses on weed science and invasive alien plant species from 1984 to 2011 for staff of government universities/colleges, research institutions, national parks, plantation estates and plant quarantine agencies in Indonesia.

The weed science training courses were conducted from 1984 to 2007 which were supported by the Government of Indonesia through DIPA fund. The participants from private companies and commercial firms have to find their own sponsor to join the course. The course duration varied from three to 20 days depending on the scope of topics included. The three-day course in 2007 focused on "Herbicide efficacy using compound active ingredients for controlling weeds", where the participants mostly came from agrochemical companies.

The training courses in 2010 and 2011 were on Control and Management of Invasive Alien Plant Species. Two of these training courses were specially conducted for 42 Indonesian plant quarantine officers designated at different entry points of the country which were funded by FAO in 2010 and 2011 (Table 2). The training for plant quarantine officers focused on the identification of invasive alien plant species and risk analysis.

Just recently, SEAMEO BIOTROP received a request from the Centre for Forest Conservation and Rehabilitation Research and Development, Ministry of Forestry to conduct a four-days training course on the management of Invasive Alien Plant Species for the staff of National Parks in Indonesia by mid-November 2013 with funding support from GEF and UNEP.

**Table 2**. Number of participants in BIOTROP's national short-term training courses in weed science and Invasive Alien Plant Species, 1984-2011.

| No. | Year | Duration of the training | No. of Participants |
|---|---|---|---|
| 1. | 1984 | 12 days | 33 |
| 2. | 1997 | 4 weeks | 22 |
| 3. | 1997 | 2 weeks | 33 |
| 4. | 2000 | 1 week | 15 |
| 5. | 2007 | 3 days | 21 |
| 6. | 2010 | 1 week | 34 |
| 7. | 2010* | 1 week | 22 |
| 8. | 2011* | 1 week | 20 |
| 9. | 2011 | 1 week | 17 |
| Total | | | 221 |

* Training course for Plant Quarantine officers of Indonesia

## LONG-TERM INTENSIVE TRAINING AND RESEARCH FELLOWSHIPS

From 1971 to 1983, SEAMEO BIOTROP provided 12 training cum research fellowships under its Tropical Agricultural Pest Program (Table 3). In total, 58 participants from Cambodia, Indonesia, Lao, Malaysia, Philippines, Thailand and Vietnam got the fellowship who responded to the Centre's call for proposals to conduct research in weed science. The research topics selected varied considerably from the more highly academic studies of herbicide behaviour to the utilization of weeds. Selected participants received introductory and "on-site" training while conducting their research project from six to ten months through the supervision of SEAMEO BIOTROP scientists or visiting scientists seconded to the Centre. Each grantee was required to prepare a scientific report of his/her research project and present it in a seminar. In some cases, the research reports were accepted by the participant's university as part of fulfilling the requirements for a Master of Science degree or other higher degree (Robson, 1977). In 1980-1983, the fellowship was extended to 10 months as a result of the evaluation of previous years' projects.



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**Table 3**. Number of participants in SEAMEO BIOTROP long term training course in weed science.

| No. | Year | Duration of the course (months) | No. of participants per country | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Camb. | Ind. | Lao | Mal. | Phil. | Thai | Viet. | Total |
| 1. | 1971/72 | 9 | - | 2 | - | - | 1 | 2 | 1 | 6 |
| 2. | 1972 | 9 | - | 1 | - | - | 1 | 1 | 1 | 4 |
| 3. | 1972/73 | 6 | 1 | 2 | 2 | - | - | - | - | 5 |
| 4. | 1974 | 6 | 1 | 4 | 1 | - | 1 | 1 | 1 | 9 |
| 5. | 1974/1975 | 6 | 1 | 2 | 1 | - | 1 | 2 | - | 7 |
| 6. | 1975-76 | 6 | - | 2 | - | - | 2 | 2 | - | 6 |
| 7. | 1976-77 | 6 | - | 3 | - | - | - | 3 | - | 6 |
| 8. | 1977-78 | 6 | - | 2 | - | 1 | 1 | 1 | - | 5 |
| 9. | 1978-79 | 6 | - | 1 | - | - | - | - | - | 1 |
| 10. | 1979-80 | 6 | - | 1 | - | - | - | - | - | 1 |
| 11. | 1980-81 | 10 | - | 4 | - | 1 | - | 2 | - | 7 |
| 12. | 1982-83 | 10 | - | - | - | 1 | - | - | - | 1 |
| Total | | | 3 | 24 | 4 | 3 | 7 | 14 | 3 | **58** |

After 1983, SEAMEO BIOTROP stopped giving fellowships for long-term intensive training cum research due fund limitation. However, starting in 2010, the Centre launched its thesis research grants program to Indonesian PhD students. In 2012, one PhD student majoring on weed science at Institute Pertanian Bogor got a thesis research grant from SEAMEO BIOTROP.

## IMPACTS OF SEAMEO BIOTROP'S CAPACITY BUILDING ACTIVITIES IN WEED SCIENCE AND IAS CONTROL AND MANAGEMENT

SEAMEO BIOTROP's short term and long-term capacity building activities in weed science gave birth to the Southeast Weed Information Center (SEAWIC) in 1985 which operated as a project until 1994 under its Tropical Agricultural Pest Biology Program. This project was aimed to properly organize the growing knowledge and experiences from weed research projects and towards addressing the information demand on weed management from various parts of Southeast Asia. As a result, the project came up with 18 volumes of annotated bibliographies of approximately 1,500 weed researches conducted from 187 to 1994 from various countries in the region aside from the 24 information sheets on major weeds. The project also developed a weed database and established the SEAMEO BIOTROP herbarium. At present, the database contains 144 species entries while the Herbarium contains 6125 collections.

SEAMEO BIOTROP's capacity building activities have also been instrumental in developing research institutes in the region such as the "National Institute of Biological Control of Weeds" in Thailand with its first director, Dr. Banpot Napompeth, being an alumnus of SEAMEO BIOTROP. Many universities in Indonesia have adopted the weed science training course curricula of BIOTROP weed training course into curricula given to their students of Agriculture. Many prominent weed scientists in the region are the BIOTROP training alumni such as Prof. Baki Hj. Bakar, Dr.Anwar Ismail, and Dr.Azmi Man of Malaysia, Dr.Umporn Suwunamek and Dr.Banpot Napompeth of Thailand, Dr.Aurora Baltazar of the Philippines, Prof. Jodi Munandir, Dr.Soetikno and Dr. Soekisman of Indonesia.

## LESSONS LEARNT

Generally, SEAMEO BIOTROP's capacity building activities in weed science and control and management of IAS have been well received and considered useful by most of the participants who attended them. However, there have been a number of criticisms, too. Foremost of these



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is that the level of the short-term courses is either too high or too elementary depending upon the background of the participants. Despite the selection criteria, it was difficult for SEAMEO BIOTROP to receive the appropriate batch of participants that were being nominated by their respective countries. Thus, lecturers have to adjust their approach during training implementation to level off with heterogeneous backgrounds of all the participants. An alternative is to offer training courses focused on specific aspects of weed science and IAS instead of the general one. Background knowledge, interest and experience of the participants should also be considers.

Other lessons learned to effectively build capacities in weed science and IAS control and management in Southeast Asia are as follows:

1. Even participants differ in their educational background and experiences on weed science and IAS, it is important that they have very good working knowledge on oral and written English language to facilitate the teaching-learning process.
2. To ensure that the training course could comprehensively cover the aspects of weed science and IAS but at the same time not compelling the participants out from their respective office duties for a long time, the appropriate duration of the training is two weeks.
3. Within this 10-day duration, the training must balance time for lectures, practical works, field trips, and individual project assignments.
4. The selection of resource persons and facilitators is also important. They should have adequate knowledge and experiences on both the theory and practical aspects of the subject matters they will handle during the training.
5. Results of individual and group project assignments must always be presented in class as a way of monitoring the change in knowledge and skills of the participants while on training aside from administering pre- and post-tests.
6. Action planning must be institutionalized as part of training requirement that would indicate how the participants would apply the things they would learn from the training course. The action plan could also be an important document to establish collaboration between SEAMEO BIOTROP and the respective institutions of the participants.
7. Networking should be encouraged among the participants during the training implementation to enable the continuous exchange of knowledge and experiences among them and establish collaborative undertakings between their institutions even after the training. This enhances advocacy for control and management of weeds and IAS. Thus, SEAMEO BIOTROP has maintained a database of training alumni and has also established the Southeast Asian Network for the Advancement of Research and Training in Tropical Biology (SEANARTTropBio) where all training alumni are encouraged to be members. The website for this network has also been launched recently by SEAMEO BIOTROP.
8. It is important to have a good training evaluation instrument that would provide venue for training participants to give feedbacks towards the continuous improvement of the training design.
9. Sufficient funding is a must.

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# CHEMICAL CONTROL OF *ACACIA NILOTICA* UNDER MEDIUM DENSITY REGIME POPULATIONS AND BROADLEAVED WEEDS IN BEKOL SAVANNA BALURAN NATIONAL PARK, EAST JAVA INDONESIA

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**ABSTRACT.** Field experiments were carried to control *A.nilotica* and broadleaved weeds in efforts to reestablish grasses at Bekol savanna, Baluran National Park. This savanna has been invaded by *A.nilotica* at medium population, where vegetation under the canopy was dominated by a mixed of broadleved weeds, such as *Eleutheranthera ruderalis, Hyptis suaveolens, Bidens biternata, Achiranthes aspera, Aeschinomene indica etc.* These broadleaved weeds competed out grasses, leaving grass coverage only 33% consisting mainly *Brachiaria reptans* and *Oplismenus compositus*. During dry season of 2012 an experiment was designed to eradicate *A.nilotica* trees. Trees were cut in June at different height from the ground ( 10, 30, and 50 cm ), combined factorially with triclopyr applied by brushing with soft painting brush on cut stump at 0, 15 ml, 30 ml, 60 ml, and 120 ml GARLON 670 EC /l diesel oil. The plot measured 10 x 10 $m^2$ containing 8 - 9 trees of Acacia at 8-10 diameter. Five months later, it was found that 10 cm height of cut *A.nilotica* stump, was the best treatment in reducing the ability of *A.nilotica* to survive ( $P<0.05$), while the rate of brushing from 15 to 120 ml Garlon /l diesel oil on those cut stump killed *A.nilotica* similarly. In this dry season, the environmental condition varied considerably possibly masking the experimental outcome. The second experiment was conducted in February 2013 to control broadleaved weeds using 6 treatments: fluroxypir (at 0.75 l Starane/ha), triclopyr at 0.5 l and 1.0 l Garlon/ha), 2,4-D amine (at 1.0 l and 2.0 l/ha Weedamine) and control. The herbicides were applied foliarly using a knapsack spayer equiped with T-jet nozzle to deliver 400 l solution/ha randomly on plots measuring 10 x 10 $m^2$. Three months later it was shown that all herbicide rates applied in the experiment was effective in controlling broadleaved weeds reducing percent cover from about 58% to 22% or even 8% and promoting the growth and expansion of grasses from 33% to more than 60% or even 80%. Confirmation of triclopyr rate as GARLON 670 EC when applied to brush 10 cm height cut stump of *A.nilotica*, was carried out in wet season. When evaluated one month after application, rates of 1.0 to 3.0% prevented the cut stump from sprouting, while cut stump under control already showed a prolific sprouting. It is concluded that *A.nilotica* may be eradicated by cutting tree stem at 10 cm height from the ground and brushing with triclopyr formulated as GARLON 670 EC diluted in diesel oil at 1% concentration. Folar application of triclopyr at 0.5 l GARLON 670 EC/ha as well as fluroxypir at 0.75 l Starane/ha) was effective to control brodleaved weeds, when done during wet season when those weeds are actively growing before flowering.

***Keywords:*** *Eleutheranthera ruderalis, Hyptis suaveolens, Aeschynomene indica, Achiranthes aspera, Ocymum conum,,* chemical control, savanna, Baluran National Park.

## INTRODUCTION

Mount Baluran, a small currently non-active volcano indeed creates a fantastic geological environment. Located at the northern foothill of huge Ijen Mountain, at the tip of northeastern of East Java, it looks like a pancake at the edge of a plate. The geographical set up produces a peculiar dry area, leading to the establishment of a savanna ecosystem. The area receives only a meagre rain as the water vapour carried by wet southern wind has been deposited as rain at the other side of the mountain.



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A Dutch concession holder of Labuhan Merak and Gunung Mesigit areas, A.H. Loedeboer, who was also a hunter noticed that Baluran area had a good prospect for large herbivore conservation. In 1930, K.W. Dammerman, director of Bogor Botanical Garden, proposed Baluran to be a protected forest. On 25$^{th}$ September 1937 the Governor General of the Dutch East Indies established Baluran as a Wildlife Conservation Area. It was re-established as such by the Minister of Agriculture and Land Affairs, Republic of Indonesia, on 11$^{th}$ May 1962. On 6$^{th}$ March 1980, on the occasion of the World Strategic Conservation Day, it was declared a National Park by the Minister of Agriculture and then strengthened by a decree of the Ministry of Forestry no 279/Kpts VI/ 1997 dated 24$^{th}$ May 1997. The park covers an area of about 25.000 ha, located in between 7$^0$ 29' 10" and 7$^0$ 55' 55" South Latitude and 114$^0$ 29' 20" and 114$^0$ 39'10" East Longitude, characterized by a single cone of Baluran mountain having a summit at 1,247 m.( Siregar & Tjitrosoedirdjo, 19990

Baluran National Park consists mainly of savannas in the district of Situbondo, East Java province. This national park is the only savanna ecosystem in Java and is very important park to conserve highly valued herbivore, i.e. banteng (*Bos javanicus),* beside rusa timor *(Cervus timorensis),* kijang *(Munticus muntjak),* anjing ajak*( Cuon alpinus),* leopard *( Panthera pardus),* and particularly protected peacock *( Pavo muticus),* in addition to local peasent and monkeys, and feral buffalo *(Bubalus bubalis),*. Those savannas are currently invaded by *Acacia nilotica*. The invasion has been rapid and extensive. *A.nilotica* flowers at the start of wet season, and mature pods are dropped starting toward the end of wet season and continued into dry season when all grasses and most of other vegetation were dried out. It was only natural, therefore, for those herbivores to graze upon those juicy nutritious pods instead of dried vegetation of grasses and other weeds. *A. nilotica* seeds of digested pods are not harmed along herbivore digestive tract and excreted in the feces. Those herbivores wander around in the park and pratically distributes *A .nilotica* seed everywhere, facilitating a rapid spread.

Rescent studies using satelite imagery revealed that all savannas in the park we*re* invaded by *A. nilotica* with varying degree of invasion shown by variability of *A.nilotica* coverage from one site to the other (Setiabudi et al. 2013). Ground checking revealed a population up to more than 1000 trees/ha of *A. nilotica*).

Bekol savanna inside Baluran Nationalm Park is one that is invaded by *A.nilotica*. Invasion of *A.nilotica* has been considered as the main factor altering vegetation composition in the savanna into shrubs, that might be dominated by broad leaved weeds such as *Thespesia lampas, Vernonia cymosa, Bidens biternata, Eleutheranthera ruderalis,* or *Achirantes aspera, etc.* (Tjitrosoedirdjo et al. 2010). The invasion of *A.nilotica* increased soil organic matter also increased cathion exchange capacity; with wet climate such one in 2010 the environment supported the growth of broadleaved weeds at the expend of grasses. (Imam et al, 2013). It is imperative, therefore, to control not only *A.nilotica* but also broadleaved weeds to restore savanna. The authors reported the results of works on the control of broadleaved weeds and *A.nilotica* and its impact on grasses.

## MATERIALS AND METHODS

The work were carried out from 2012 to 2013 during dry and wet seasons at Bekol savanna inside Baluran National Park. The first experiment was carried out during the dry season of 2012 directed toward finding out the best triclopyr concentration to eradicate *A.nilotica*. An effort to control broadleaved weeds during dry season was aborted, as all weeds and grasses were dried out. The treatments consisted of cutting *A.nilotica* trees at various above the ground height ( 10, 30 and 50 cm) and brushing triclopyr (formulated as Garlon 670 EC ) diluted in diesel oil with soft paint brush at different rates on the cut stump, immediately after cutting with a chainsaw. (0, 15, 30, 60, and 120 ml of Garlon/l diesel). The rates of Garlon applied were relatively high to anticipate the effect of dry season that may reduce the physiological impact of triclopyr. The treatments were combined factorially, replicated 3 times and applied randomly in the field. The plots measured 10 x 10 m$^2$ with 8-9 *A.nilotica* trees having diameter varied



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from 8-32 cm. The experiment was carried out in a dry hot season of June and evaluated in November 2012. The evaluation was based on the sprouting ability of treated stumps; stumps that did not sprouce was considered as killed. The data collected were statistically analysed and means were compared at 5% level.

The second experiment was directed toward finding the best herbicide concentration to control broadleaved weeds and was carried out in the wet season late January 2013. The experimental area was savanna, however, under the invasion of *A.nilotica*, grasses under the canopy of *A.nilotica* were competed out and dominated by broadleaved weeds mainly *Eleutheranthera ruderalis*, *Bidens biternata*, *Achiranthes aspera*, *Vernonia cymosa*, etc. The vegetation composition before the experimentation was sampled using quadrat of 1 x 1 $m^2$, ten times. Three selective herbicides (kills broadleaved weeds, injury little to grasses) were applied, i.e.: fluroxypir (formulated as Starane at 0.75 lt/ha), triclopyr (formulated af Garlon at 0.5 and 1.0 lt/ha) and 2,4-D (formulated as weedamin, at 1.0 and 2.0 lt/ha), together with control ( no herbicides) there were six treatments, replicated 4 times and randomised in the field on plots measuring 10 x 10 $m^2$. The herbicides were diluted in water applied using a knapsack sprayer, with T-jet nozle to deliver about 400 l solution /ha. The experiment was carried out while *A.nilotica* trees were still standing with almost full canopy giving good shady working condition. The evaluation was carried out in May 2013, based upon the percent of coverage of the existing vegetation after treatments. The reduction of coverage was due to the death of mainly broadleaved weeds, although new seedlings of some plants were also recoreded. The data collected were analysed statistically to evaluate the impact of treatments.

After evaluating the impact of herbicides on broadleaved weeds, *A.nilotica* trees were cut at 10 cm height from the ground and brushed with triclopyr formulated as Garlon at 0, 1, 1.5, 2, 2,5 and 3 % solution in diesel oil. This experiment was intended to confirm or otherwise the result of previous experiment conducted during dry season. The treatments were randomised completely in the field on plots measuring 10 x 10 m2 carrying 9-10 trees having diameters of 8.00 – 9.00 cm. The experiment was evaluated in June 2013 based upon the ability of treted stumps to sproud.

## RESULTS AND DISCUSSION

**Experiment 1**

During dry season, most of vegetation, grasses or browadleaved weeds were dried out, except *A.nilotica*, this invasive alien plant species was still growing prolifically. The treatment factors did not interact significantly, but the rate of garlon brushed on cut stump reduced the ability to survive significantly (Table 1).

As shown in the table, even control without any herbicide treatment reduced sprouting almost 40%. This was due to inconsistency of plants in the field during dry season, as it was noticed that some trees with cut stump under no herbicide treatment was found also dead, it was simply due to the soil environmental variability as the condition was so dry. The valuable results of the experiment were indicated by these data that herbicide concentration from 15 ml/ l to 60 ml/l diesel oil reduced the sprouting ability of cut stump of *A.nilotica* by more than 80%, in other word the concentraion of triclopyr as Garlon at 1.5 ml/100 diesel oil or about 1.5% (by volume) was sufficient to kill *A.nilotica* trees at those tree diameter when cut at 10 cm above the ground and immediately bruished with soft brush painting. A higher concentration up to 120 ml/lt gave a better result almost killed the tree but still leaving 7% to be retreated again. This concentration will be too expensive, probably it will have a more consistant result using 15 ml/lt diesel oil if workers can be trained to make sure that the herbicide solution was applied correctly and consistantly.

The impact of different stump height on the survival *of A.nilotica* stump was shown in Table 2.



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**Table 1**. The percetage of sprouting of cut stump after herbicide brushing recorded 5 months after treatment.

| | Treatments (Concentration of Garlon ml/l diesel oil.) | | | | |
|---|---|---|---|---|---|
| | 0 | 15 | 30 | 60 | 120 |
| % of sprouting of cut stump | 63.1[a] | 19.4[b] | 15.5[b] | 14.0[b] | 6.6[c] |

Note: numbers followed by different letter differed significantly at 5%.

**Table 2**. Percentage of sprouting ability of stumps cut at different height

| | Treatment ( cm height from the ground) | | |
|---|---|---|---|
| | 10 cm | 30 cm | 50 cm |
| Percetage of sprouting | 11[a] | 27[b] | 35[c] |

Note: Numbers followed by the same letter do not differ significantly at 5%.

The height of 10 cm was the best, it almost kills *A.nilotica tree*, leaving only 11% to survive. This finding was similar to those reported in Australia, that the shorter the height of cut stump bruished with herbicides the better. It is realised that brushing 10 cm height stump with herbicides is a back breaking jobs therefore the supervisor must be able take care and ensure that the calculated dosages of herbicides is delivered to the right place at the right time. Although triclopyr has proven a good herbicide to control *A.nilotica after* cutting trees at 10 cm height from the ground, it must be done consistently, attended from time to time to ensure that survived stumps should be re treated
Immediately.

**Experiment 2**

Vegetation composition before experimentation was shown in Table 3 with SDR as an indication of species contribution to the vegetational composition. SDR (Summed Dpminance Ratio) is actually index of Important Values divided by variables utilized to calculate those IV. The benefit of SDR is that its total is 100, therefore, interpretation of its indices are much easier.

The invasion of *A.nilotica* in this savanna promoted the growth of and subsequently the vegetation was dominated by broadleaved weeds, mainly *E.ruderalis* (26.0), *H. Suaveolens* (11.58), *B. biternata* (9.11), *A. indica* (3.65) , *Achyranthes aspera* (2.86) and others leaving only 24.49 for grasses which was occupied by *B. reptans* and *O. Compositus*. The area was also near a road fenced by *Azadirachta indica.* This tree produces cupious fruit which are eaten by monkey. These flock of monkey rest and consumed those fruit of *A.indica* and dropped those seeds in savanna. The author noticed those dropped seeds germinated well as indicated in the table as seedling of *A.indica*. This distribution of *A.indica* seedling constitutes another threat of tree invasion into savanna. The manager said that the threat is negligible as trees of *A.indica* is much easier to control then *A.nilotica*, certainly the threat will be considerable when the population of *A.indica* reaches 1000 trees/ha.



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**Table 3.** The composition of vegetation before herbicide application recorded during wet season (early February 2013).

| No | Species | Family | SDR | No | Species | Family | SDR |
|---|---|---|---|---|---|---|---|
| 1 | *Achyranthes aspera* | Amaranthaceae | 2.86 | 14 | *Ipomoea sp.* | Convolvulaceae | 2.91 |
| 2 | *Aeschynomene indica* | Fabaceae | 3.65 | 15 | *Merremia emarginata* | Convolvulaceae | 0.52 |
| 3 | *Bidens biternata* | Asteraceae | 9.11 | 16 | *Mimosa diplotricha* | Fabaceae | 1.04 |
| 4 | *Brachiaria reptans* | Poaceae | 3.59 | 17 | *Ocymum canum* | Lamiaceae | 0.57 |
| 5 | *Cayratia trifolia* | Vitaceae | 0.64 | 18 | *Oplismenus compositus* | Poaceae | 20.9 |
| 6 | *Centrosema sp* | Fabaceae | 0.56 | 19 | *Phyllanthus debilis* | Euphorbiaceae | 0.45 |
| 7 | *Cleome gynandra* | Capparidaceae | 1.60 | 20 | *Phyllanthus niruri* | Euphorbiaceae | 1.95 |
| 8 | *Commelina sp.* | Commelinaceae | 0.47 | 21 | S'dling *Acacia nilotica* | Fabaceae | 0.53 |
| 9 | *Corchorus olitorius* | Tiliaceae | 2.10 | 22 | S'dling *Azadirachta indica* | Meliaceae | 1.38 |
| 10 | *Digera arvensis* | Amaranthaceae | 0.45 | 23 | *Thespesia lampas* | Malvaceae | 0.95 |
| 11 | *Eleutheranthera ruderalis* | Asteraceae | 26.0 | 24 | *Vernonia cymosa* | Asteraceae | 0.55 |
| 12 | Hyptis suaveolens | Lamiaceae | 11.58 | T O T A L | | | 100 |
| 13 | *Ipomoea alba* | Convolvulaceae | 6.29 | | | | |

The dominanating broadleaved weeds competed out valuables grasses such as *Dicanthium caricosum*, *Schlerachne punctata* leaving *O.compositus* and *B.reptans* as the last two are able to survive under *A.nilotica* canopy although the populations are progressively declining, while those of broadleaved weeds are progressively increasing.

The results of herbiside teratments were shown in Table 4. The variability of vegetation composition as well as the variability of workers in the filed were considerable, although the experiment was able to show the impact of precribed herbicides at different rate, coefficient of variability was 56.19% for the percentages of broadleaved weed coverage, and 16.68% for grass coverage.



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**Table 4**. The percentages of broadleaved weed and grass coverages 3 months after herbicide application.

| No | Treatments | % broadleaved cocerage | % grass coverage |
|---|---|---|---|
| 1 | Fluroxypir, 0.75 l/ha | 10.58 [b] | 83.23[a] |
| 2 | Triclopyr, 0.5l/ha | 21.79[b] | 62.58[b] |
| 3 | Triclopyr, 1.0 l/ha | 7.66[b] | 82.68[a] |
| 4 | 2,4-D, 1.0 l/ha | 12.71[b] | 78.13[ab] |
| 5 | 2,4 D, 2.0 l/ha | 8.69[b] | 80.52[a] |
| 6 | Control | 58.79[a] | 32.48[c] |

Note: Figures in a column followed by the same letter do not differ significantly at 5%.

The results showed that foliar application of selective herbicides, i.e. fluroxypir (0.75 l/ha), triclopyr (0.5-1.0 l/ha) and 2,4-D amine (1.0 – 2.0 l/ha) were effective in controlling some weeds when applied during wet season in this area ($P<0.05$). It should be emphazied that herbicides must be applied during wet season, i.e. when those weeds are actively growing, before flowering,i.e. in the month of January to March, depending upon the onset of the wet season. Some broadleaved weeds somehow was not much affected by the rate of herbicide applied, such as *Lantana camara*. *Bidens biternata,* a local species which may grow up to 2m height in a very dense population required a higher rate of triclopyr. *Ocymum canum, a* short herb grew under other broadleaved weeds, when those higher weeds were eradicated it emerged to form a dense spotty population. This may become important weed as it has trichome covering leaves, petioles, stem, its whole body that may prevent herbicide droplet from coming into contact with leaf surface, and with considerable gland of mentol it is not eaten by herbivores, although a similar plant is consumed by people, eaten raw as vegetable, in so many dishes, known as kemangi. Herbicide 2,4 D has been rising a considerable controvercies, and in USA has been proposed to be banned, it may not be utilzed here.

The impact of broadleaved weed control using those selective herbicides on the growth of grasses (*B.reptans and O. compositus*) during this experiment was very good indeed, increasing the grass coverage from about 33% to more than 60% or even up to 80%. Another experiment (not recorded here) grasses such *S.punctata, Polytrias amaura* also gained a considerable growth when broadleaved weeds were controlled. It is important to be aware that although foliar application of selective herbicides is able control broadleaved weeds, to facilitate grasses to grow and expand, those left over broadleaved weeds must still be eradicated and prevented from coming back, and of course *A.nilotica* must be eradicated.

The selective herbicides tested seems able to serve the purpose of contolling broadleaved weeds at the same time harming grasses a little if any. From the result it may be concluded that *A.nilotica* may be eradicated by cutting at 10 cm height from the ground and brusing the cut stump with triclopyr at 1% in diesel oil solution, applied during wet season. From the point of practicality it is advisable to control broadleaved weeds first under the canopy of *A.nilotica*, using foliar triclopyr application at 1lt GARLON 670 EC /ha and after controlling those weeds the eradication of *A.nilotica* may be initiated.



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# COMPARISON OF COMPETETIVENESS OF TREE PARASITES, *DENDROPHTHOE FALCATA*, *HELICANTHUS ELASTICA* AND *MACROSOLEN CAPITELLATUM* BY OXYGEN ISOTOPE DISCRIMINATION AND NUTRIENT ANALYSIS

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**ABSTRACT.** Hemiparasitic plants are major weeds on tree crops. *Dendrophthoe falcata*, *Helicanthus elastica* and *Macrosolen capitellatum.*are major parasitic species infesting trees of the tropics. The relative efficiency of the parasite to mobilize nutrients and water from the host is a measure of the competitiveness of the species. To evaluate the relative competitiveness of the parasites, leaf samples of the parasites infecting the same host were used for the estimation of $^{18}O$ composition of the leaf biomass and nutrient content of the leaves. The relative water content of the parasite and their hosts were also studied. Among the three genus, *D. falcata* showed higher $\mathbf{^{18}O}$ isotope accumulation in the leaf biomass. This is reported to have a positive relationship with the mean averaged transpiration rate ( Sheshshayee *et al*,2005) . Assessment of the RWC of the hosts and parasite indicated that a positive water potential gradient is maintained by the parasitic species for the flow of water and nutrients from host to parasite. Increased transpiration by the parasite indicates that it is imparting higher stress to the host plant in terms of water and nutrients. This affects the productivity and growth of the host species *D. falcata* was found to have higher K and Ca content. The accumulation of N, which stimulates vegetative growth was also higher in *D. falcata*. From the above observations it is to be presumed that *D. falcata* is a more competitive parasitic species to the host as compared to *H.elastica* and *M. capitellatum.*

***Keywords*:** Hemiparasites, competitiveness, $^{18}O$, relative water content, nutrients

## INTRODUCTION

Hemiparasitic plants are major weeds on tree crops. *Macrosolen capitellatum*, *Helicanthus elastica* and *Dendrophthoe falcata.* are major parasitic species infesting trees of the tropics. They depend on their host for water and nutrients and absorb them from the vascular bundle of the host through haustorial connections. The relative efficiency of the parasite to mobilize nutrients and water from the host is a measure of the competitiveness of the species. Mark and Reid (1971) have reported that maintenance of a water potential gradient between the host *Pinus contorta* and the parasite *Arceubhotium americanum* was due to the higher transpiration rate of the mistletoes. Water potential differences between host and parasite are maintained by a transpiration-driven reduction in parasite water potential coupled with a high fluid phase resistance in the haustorium (Davidson and Pate, 1992). Recently the enrichment of the heavy isotope of oxygen in leaf water (or that of the biomass) is being adopted to assess variations in transpiration rate and stomatal conductance (gs) (Farquhar and Richards, 1984; Bindumadhava *et al*., 1999). Transpiration pull is the main driving force for the uptake of nutrients from the host plant (Lamont and Southhall, 1982). Osmoregulation is one of the mechanisms for preserving the turgor pressure in plant species (Gunasekera and Berkowiz, 1992). Relative water content (RWC) indicates the balance between water absorbed by plant and consumed through transpiration.

The objective of the study was to compare the transpiration rate and nutrient accumulation in three Loranthaceae members commonly found on the fruit crops of the humid tropics, so as to ascertain their relative competitiveness.



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## MATERIALS AND METHODS

Leaf samples of *Helicanthus elastica*, *Dendrophthoe falcata* and *Macrosolen capitellatum* were collected from the host mango, sapota and jack fruit. Since *H.elastica* and *D.falcata* were found to infect a large number of fruit trees, care was taken to collect these two parasites from the same host plant for isotope discrimination studies and nutrient estimation. *M. capitellatum* was collected from Jack fruit tree as they from the major host of the parasite. *D. falcata* samples were also collected from the same tree for comparison.

The leaves of the parasites were harvested separately, oven-dried at $70^0$ C for 72 h., and finely powdered. $\delta^{18}O_{lb}$ in the leaf biomass of the parasites were measured by pyrolysing 1 mg of leaf samples with glassy carbon catalysts at 1400°C using an elemental analyzer (TC/EA, Thermo electron, Bremen, Germany) interfaced with an isotope ratio mass spectrometer (Delta plus, Thermo electron, Bremen, Germany), working on continuous flow devise at the National Facility for Stable isotope studies, Department of Crop Physiology, University of Agricultural Sciences, Bangalore, India. The analytical uncertainty of isotope measurements was less than 0.2. The enrichment $\boldsymbol{\Delta^{18}O_{lb}}$ in leaf biomass was computed as follows:

$$\Delta^{18}O_{lb}, (^0/_{00}) = \delta^{18}O_{lb} - \delta^{18}O_{dw}$$

$\delta^{18}O_{lb}$ refers to leaf biomass and $\delta^{18}O_{dw\ to}$ distilled water

Nutrient content of the leaf samples of the parasite were estimated using di- acid method in an atomic absorption spectrophotometer (Analyst 400 - Perkin Elmer Ltd.).

For estimation of relative water content (RWC) fresh leaf samples of the host and the parasite (25 each) were weighed, submerged in distilled water overnight wiped free of moister and the turgid weight was taken, the same leaf was than dried in the oven at 70ºC for 48 h to obtain the dry weight. RWC of the leaves of parasite and host was estimated according to Dhopte and Manuel (2002) using the formula.

$$RWC = (FW-DW/TW-DW) \times 100$$

Where, FW is fresh weight, DW is dry weight and TW is turgid weight of leaf samples. Mean and standard deviation of the samples was estimated.

## RESULTS AND DISCUSSION

Estimation of $\delta^{18}O$ content in the leaf samples of the parasitic species showed that *D. falcata* had higher accumulation of $\delta^{18}O$ in the leaf biomass (Fig. 1.) as compared to *H. elastica* and *M.capitellatum* .The $\delta^{18}O$ enrichment of the leaf biomass is by the transfer $^{18}O$ from leaf water into the cellulose biomass (Stermberg *et al*., 1986). Though the values were not subjected to statistical analysis the trend observed was important as the parasites were collected from the same host plants. Increased transpiration has been shown to enrich leaf $\delta^{18}O$ content. (Gonfiantini *et al*., 1965; DeNiro and Epstein, 1979). Sheshshayee *et al* (2005) have reported that $\delta^{18}O$ of leaf biomass could be used as an effective surrogate for mean transpiration rate. Hence it is to be inferred that *D. falcata* has higher transpiration rate than the other two species.



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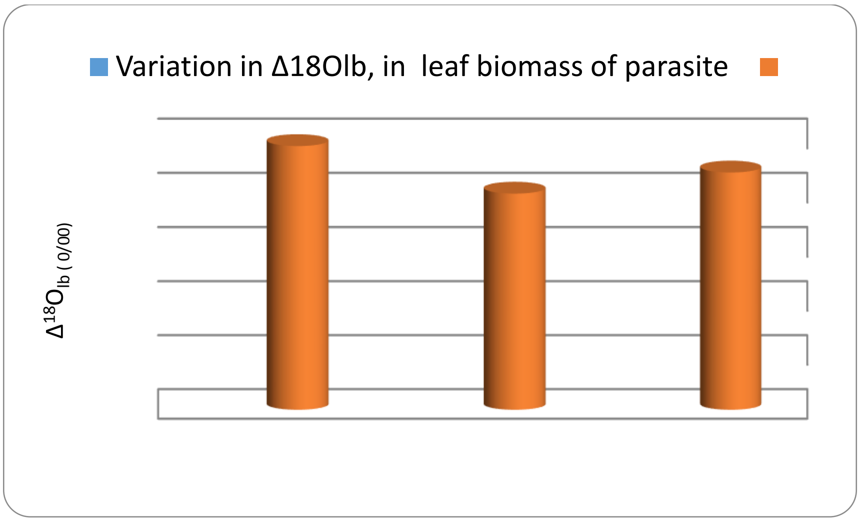


**Fig. 1.** Oxygen isotope ($\Delta^{18}O_{lb\ (0/00)}$) in the leaf biomass of *D. falcata*, *H. elastica* and *M. capitellatum*

Stomata regulate water loss from leaves. Ehleringer and Marshall (1995) have reported that parasite transpires at a higher rate than the host so as to ensure a gradient for the movement of minerals and water from the host to the parasite. Study of the stomatal characters of the three parasitic species showed variation in stomatal size and density. According to Metcalfe and Chalk, 1988, stomatal density and stomatal size determine the leaf diffusive conductance.

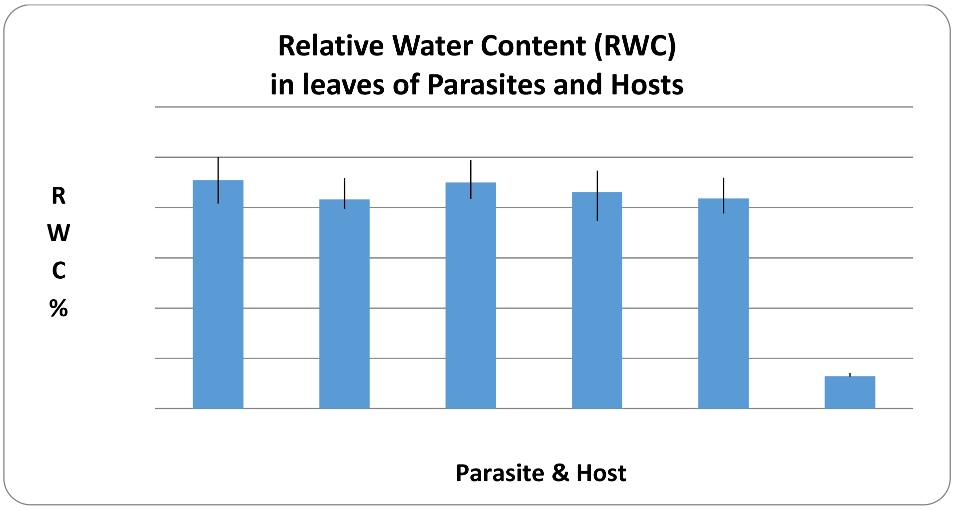


**Fig. 2.** Relative water content (%) of parasite and host. candles on top indicate the standard deviation within samples.

Estimation of RWC at any point of time between the host and parasite (fig. 2) showed that a gradient exist between the host and the parasite. Higher RWC in the parasite indicates higher capacity of the parasite to extract water from the host.

The three parasitic species accumulate more of Ca and K (table 1). Both K and Ca play an essential role in osmoregulation. K is known to play a critical role in the stomatal activity and water relation of plants (Marchner, 1995; Mengel and Kirkby, 2001). *D. falcata* was found to have higher K and Ca contents as compared with the other two parasites. The capacity of plants to maintain high concentrations of K in their tissues and intracellular Ca ions have been reported to impart high tolerance to water stress such as drought and salinity. (Knight *et al.*, 1997; Bartels and Sunkar, 2005). The higher accumulation of N by the parasite accounts for higher vegetative growth of the species.



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**Table 1.** Nutrient content of *H. elastica* and *D. falcata* from the same Mango tree and *M. capitellatum.* from Jack tree .

| | | N | P | K | Ca | Mg | Fe | Mn |
|---|---|---|---|---|---|---|---|---|
| 1 | *H.elastica* | 0.735 | 0.088 | 2.06 | 1.375 | 0.234 | 0.031 | 0.025 |
| | *D. falcata* | 2.415 | 0.005 | 2.72 | 6.29 | 0.188 | 0.036 | 0.020 |
| | *M. capitellatum* | 1.225 | 0.155 | 2.42 | 3.557 | 0.468 | 0.153 | 0.011 |

The efficiency of the parasite to absorb nutrients from the host depends on the transpiration pull and stomatal conductance. Among the three genus *D. falcata* was found to have higher transpiration pull as revealed from $\delta^{18}O$ isotope accumulation in leaf biomass.

## CONCLUSIONS

Increased transpiration by the parasite indicates that it is imparting higher stress to the host plant in terms of water and nutrients. This affects the productivity and growth of the host species. *D. falcata* seems to transpire more than the other two species as evident from the $^{18}O$ signature in the leaf biomass. The capacity to absorb N, Ca and K from the host is also more in the case of *D. falcata*. This in turn will affect the productivity of the host plant. Hence among the three parasitic species studied *D. falcata* seems to be more competitive than *H. elastic* and *M. capitellatum.*

## ACKNOWEDGEMENT

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## *Herbicide Resistance Weeds and Weed Competition*

# SIMULATION MODELLING CAN HELP UNDERSTAND AND PREDICT HOW MANAGEMENT, WEED BIOLOGY AND GENETICS AFFECT THE DEVELOPMENT OF HERBICIDE RESISTANCE

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**ABSTRACT.** The way weeds evolve resistance to herbicides depends on a complex interaction between the underlying genetics, the weed management used by the farmer, and the biology of the weed species. PERTH (Polygenic Evolution of Resistance To Herbicides) is an individual-based model created to simulate these complex interactions and predict the rates and patterns of resistance evolution in a wide range of different conditions. We show how PERTH can help evaluate the efficacy of management options such as herbicide rotation, maintaining robust herbicide rates at high efficacy, avoiding or introducing refuges, and using non-chemical methods of control, and how the efficacy of these options depends on the genetics underlying the resistance. We also show how biological or ecological factors can affect the evolution of resistance, including factors such as seed bank longevity and dormancy, fecundity, seed dispersal, and rates of self-fertilisation versus out-crossing. We also demonstrate how PERTH has recently been adapted to account for spatial heterogeneity, and show how this may affect the evolution of resistance.

***Keywords:*** Herbicide, resistance, weeds, rotation, rate, dose, genetics, genetics, polygenic, monogenic, ecological traits, spatial effects.

## INTRODUCTION

Weeds are a major problem in Australian cropping systems, greatly exacerbated by the increasing prevalence of evolved herbicide resistance (Heap 2012). As weeds evolve resistance, previously effective herbicide options lose efficacy and then fail. Growers must then find new weed control options that are more expensive, less convenient or less environmentally benign; adapt their farming system to include options that are less reliant on the herbicides in question; or in some cases adopt new systems entirely to deal with this problem. Rather than wait until resistance has evolved, it is preferable to look for management options that may help avoid or delay the evolution of resistance.

However, predicting the evolution of resistance under different management options is difficult; because the way weeds evolve resistance to herbicides depends on a complex interaction between the underlying genetics, the weed management used by the farmer, and the biology of the weed species. Moreover, it is a long-term problem that results from rare mutations in huge populations, so experimental results based on short-term studies of relatively small populations are always of limited value. Simulation modelling provides a tool for integrating existing knowledge to predict how these complex interactions will occur across realistic spatial scales and time periods, and thus evaluate the likely efficacy of different management options (eg Maxwell et al. 1990, Diggle et al. 2003, Neve 2008, Thornby and Walker 2009).

PERTH (Polygenic Evolution of Resistance To Herbicides) is an individual-based model created to simulate the complex interactions between the underlying genetics, the weed management used by the farmer, and the biology of the weed species in order to predict the rates and patterns of resistance evolution in a wide range of different conditions (Renton et al. 2011, Renton 2012, Renton et al. 2012, Manalil *et al.* 2012). In this paper, we describe how



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PERTH has been used to help evaluate the efficacy of management options such as herbicide rotation, maintaining robust herbicide rates at high efficacy and using non-chemical methods of control such as strategic tillage, and how the efficacy of these options depends on the genetics underlying the resistance. We also show how PERTH has been used to help predict how biological or ecological factors can affect the evolution of resistance, including factors such as seed bank longevity and dormancy, fecundity, seed dispersal, and rates of self-fertilisation versus out-crossing. Finally we show how a spatially-explicit version of PERTH has been constructed to account for spatial variation in weed densities and resistance allele frequencies across a field, and how these change both spatially and temporally in interaction with the other management, genetic and ecological factors. This paper presents an update to a previous summary (Renton et al. 2012), describing more recent applications and developments with the PERTH model.

## MATERIALS AND METHODS

The PERTH model is an individual-based simulation model that tracks the population of weed plants across single growing seasons and the population of weed plants across multiple years (Renton et al. 2011 for details). The overall model dynamics are illustrated in Fig 1. At the beginning of a model run an initial population of weed seeds is created using specified initial resistance allele frequencies, while in subsequent years the weed seed population carries over from the previous year. In either case, the model represents a collection of weed seeds existing in a dormant weed seed bank at the start of each season. A number of cohorts are simulated through the year. For each cohort, any seed currently in the seed bank has a certain chance of germinating, emerging and becoming established; otherwise it remains in the seed bank. Any established weed seedlings will face certain weed management, such as a pre-emergent herbicide, a post-emergent in-crop selective herbicide, or soil cultivation, depending on which cohort it is part of. Later cohorts will not be affected by the pre-emergent herbicide for example. Soil cultivation also affects the seed bank, possibly killing seeds, but usually just moving them within the soil. Soil residual herbicides may also be simulated, and these will cause mortality to seeds germinating at relevant times and locations in the soil. Any weed that germinates and survives all weed management that it faces, then sets seed.

Genotype and resistance status are represented individually for each weed seed or plant, and all individuals of the same resistance genotype will have the same resistance status. The total number of genes involved in resistance can be set as one (monogenic) or any number greater than one (polygenic). The maximum strength of resistance (the resistance status value for individuals with all possible resistance alleles) must also be specified. The gene effect at each locus can be set as dominant (one allele has the same effect as two), recessive (one allele has no effect) or intermediate (one allele has a partial effect). The gene effects from each locus can be set to combine additively (with neutral or no epistasis), antagonistically (negative epistasis) or synergistically (positive epistasis). In a simulated population, each particular individual has zero, one or two ‘resistance’ alleles present at each gene (locus), and R, the resistance status of that individual (and its genotype), depends on which alleles are present, according to the specified maximum resistance, epistasis and dominance. More complicated relationships between genotype and resistance can be specified if required, to represent multiple resistance statuses regarding multiple herbicides with partial cross-resistance for example (as described below).



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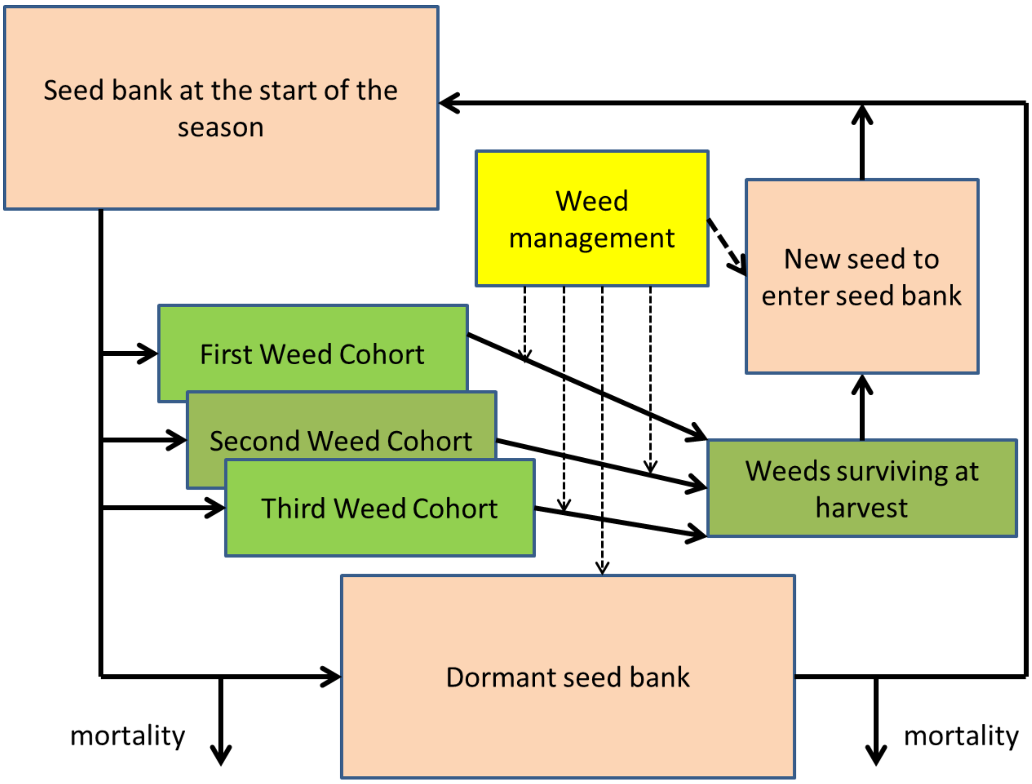


**Fig. 1.** The model simulates how weed seed bank density and genetics changes from year to year, based on ecological processes that occur and management activities that are applied in each year.

Each individual has a certain probability of surviving a management event. For non-chemical management, this probability will usually be set to be the same for all individuals (independent of an individual's genotype and resistance status), and this may also be used for some herbicides, if we are not interested in evolution of resistance to that particular herbicide. However, for the herbicide(s) of interest, the chance of mortality can be set to be dependent on the individual's resistance status, so that more resistant weeds are more likely to survive the herbicide application. In this case, the effective herbicide dose received by an individual weed is assumed to vary around the target application dose, and is then simply divided by its resistance status (R). This procedure results in a family of logistic dose response curves for different genotypes, as illustrated in Figure 2. Note that the R value thus corresponds to the ratio between the $LD_{50}$ (dose needed for 50% kill rate) for the individual's genotype and the $LD_{50}$ for the completely susceptible genotype; this ratio is a commonly used empirical measure of resistance status.

The amount of weed seed produced is calculated using the hyperbolic competition function (Firbank and Watkinson 1985) commonly used in weed population models (eg. Diggle *et al.* 2003). This depends on the relative competitiveness of the weed and crop, the crop density and the density of the surviving weeds. This function also gives the crop yield as a percent of the maximum possible yield. The genotype of each of the new seeds is chosen by randomly selecting a father and a mother from the weed population, and randomly choosing one allele from the mother and one allele from the father at each relevant locus.

The area to be simulated and the initial seed bank density are set at the beginning of a model run, and size of the initial seed bank population is then the product of these two numbers. The initial resistance allele frequency for each gene (locus) related to resistance is also specified at the start of each run and the initial seed bank is then set up according to these initial frequencies. As the simulation continues, the frequencies of allele and genotypes and the total weed population fluctuates according to the management applied and the biological processes simulated. Simulation is generally continued for a specified numbers of years, or until weed



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populations or crop yield penalties reach a specified critical threshold that makes continued cropping unfeasible.

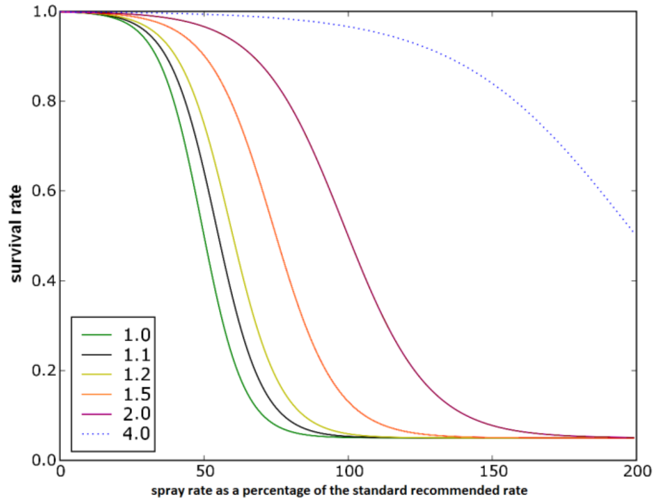


**Fig. 2.** Probability of weeds of different resistance levels (R=1.0 ,1.1 ,1.2 ,1.5 ,2.0, 4.0) surviving a range of different herbicide spray rates.

## RESULTS AND DISCUSSION

The PERTH model has now been applied to investigate a number of herbicide resistance issues, as summarised below.

### Herbicide Rate

The original version of the PERTH model was designed to address the question of whether using herbicides at rates that were lower or higher than a standard registered rate could hasten or delay the evolution of resistance to the herbicide. This study found that the evolution of resistance to an in-crop selective herbicide could be significantly hastened by reducing herbicide rates, but only for certain cases where the underlying resistance genetics was effectively polygenic (Fig. 3, top; Renton et al. 2011).

### Weed biology

The PERTH model was adapted to investigate how the biological or ecological characteristics of a weed species affected the chance of it evolving resistance to a herbicide. The main change to the model was allowing for different mating systems, the degree of outcrossing versus self-fertilisation. Other changes included adding a greater number of plant cohorts within a year, to allow different patterns of dormancy and germination across and between years. In addition we investigated the effect of mortality rates, fecundity, competitiveness and phenotypic variability. We also considered interactions with the usage pattern of the herbicide (pre-sowing versus in-crop) and different underlying genetics. Results show that ecological traits have a strong influence on the evolution of resistance, and this influence depends significantly on genetics and herbicide usage pattern (Fig. 3, bottom).



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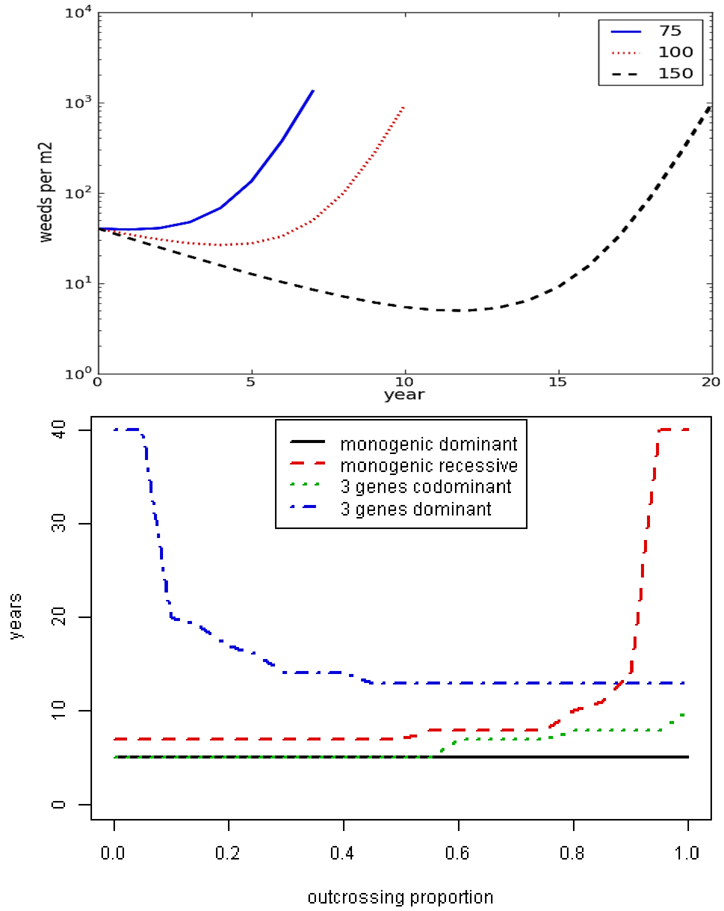


**Fig. 3.** Examples of PERTH output illustrating the different applications discussed in this paper, showing (top) weed populations changing over time under different herbicide rates, and (bottom) number of years to evolve resistance to an in-crop selective herbicide for four genetic scenarios across different levels of outcrossing.

**Tillage effects**

The PERTH model was adapted to investigate whether no-tillage systems were likely to lead to faster evolution of resistance to soil-residual herbicides applied with sowing, compared to more traditional systems based on full-cut or mouldboard tillage. We also investigated whether strategic use of soil inversion with a mouldboard plough could delay resistance by burying resistant seed and thus 'resetting the resistance clock'. The main change to the model was dividing the seed bank into a number of soil layers to represent surface, shallowly-buried and deeply-buried seeds. Other changes included incorporating soil-residual herbicides that only affect germinating seeds into the model. We also considered interactions with different underlying genetics, and biological characteristics such as seed dormancy and mortality rates. The model predicts that no-tillage systems do not increase the rate at which resistance evolves, and that strategic use of soil inversion could delay resistance by burying resistant seed and also by bringing susceptible seed back to the surface (Fig. 4, top). Efficacy of the strategy is affected by underlying genetics, dormancy and mortality rates to some degree.

**Optimal herbicide rotation**

The PERTH model was adapted to investigate optimal herbicide rotation strategies for soil-residual herbicides. This was motivated by the current availability of new herbicide options, and the question of whether these should be immediately included in weed management strategies as an alternative to the currently used soil-residual herbicide to reduce selection pressure, or instead reserved for when resistance to the currently used soil-residual herbicide has emerged. The model had already been adapted to represent soil-residual herbicides, as



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mentioned above, so the main change to the model was extending the link between genotype and resistance status to allow the model to simulate the evolution of resistance to a number of herbicides simultaneously, while accounting for different fitness penalties and different levels of possible cross-resistance to these herbicides. The study predicts that rotating all available herbicides can extend weed management sustainability compared to using herbicides one by one until they are no longer effective, but the difference is very small, and the particular rotation strategy used has very little overall effect (Fig. 4, bottom).

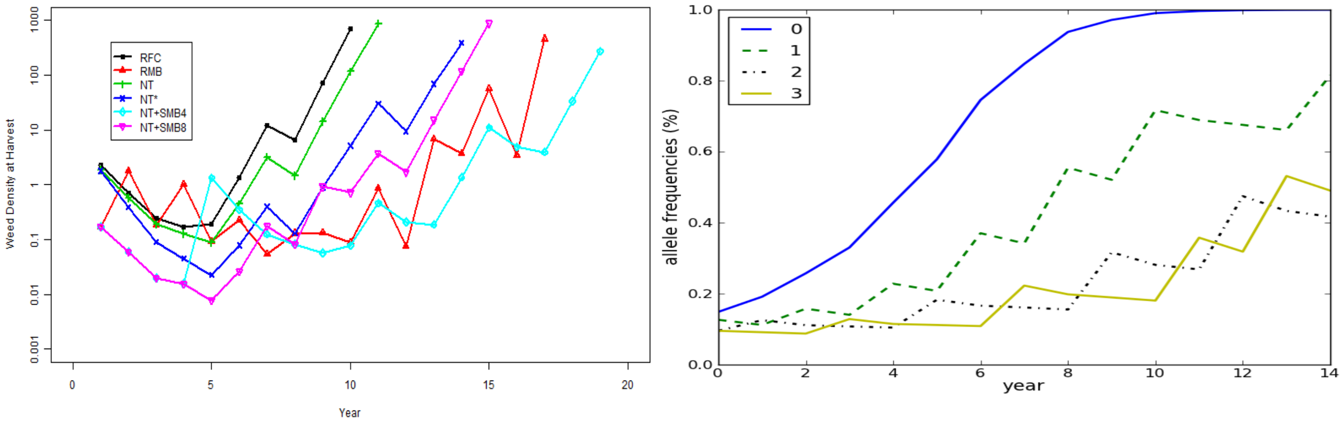


**Fig. 4.** Examples of PERTH output illustrating the different applications discussed in this paper, showing (top) weed populations changing over time under different tillage strategies, and (bottom) resistance allele frequencies changing over time under different herbicide rotation strategies.

## Spatial factors

The PERTH model has recently been adapted to be spatially-explicit, that is, to explicitly represent spatial variability across a field in terms of weed densities and resistance allele frequencies, and how these change with time, management, and assumptions regarding weed ecology and underlying resistance genetics. The spatially explicit version of the model was able to show how different resistance genetics are likely to lead to different spatial patterns of resistance and weed density. When resistance is conferred by a single dominant rare gene, well-defined small patches of resistant weeds appear in the field and quite quickly reach high densities, but spread out across the field only slowly. When the same level of resistance is conferred by a combination of several more common co-dominant genes, resistant patches are much less distinct, emerge and increase in density more slowly, and are generally larger in extent and spread over a larger portion of the field (Fig. 5).



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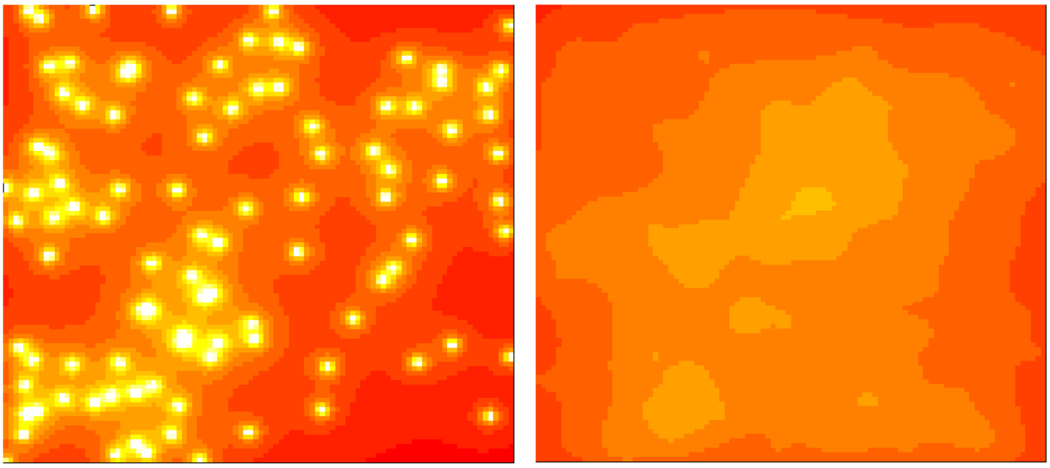

**Fig. 5.** Examples of PERTH output illustrating how the genetics underlying herbicide resistance can affect the spatial patterns of weed densities and resistance levels predicted in a filed. High weed densities and resistance levels are represented in lighter colours. In the example on the left, the underlying genetics was assumed to monogenic and dominant, while in the example on the right it was assumed to be polygenic and co-dominant. Apart from the differences in genetics, the two simulations were based on identical assumptions and simulated evolution of resistance over the same period (12 years).

## CONCLUSIONS

PERTH is an individual-based model that simulates the complex interactions between the genetics underlying herbicide resistance, the weed management used by the farmer, the biology of the weed species, and the spatial distribution of weeds and resistance alleles across a field, in order to predict the rates and patterns of resistance evolution. PERTH can help evaluate the efficacy of management options and predict and provide insights into how genetic, biological ecological and spatial factors can affect the evolution of resistance. Simulation models like PERTH allow us to integrate information, knowledge and understanding from a range of fields in order to gain insights into ecological and evolutionary processes and make predictions over temporal and spatial scales that cannot be addressed with purely empirical approaches. Using an individual-based approach allows us to integrate more aspects of biological reality than simpler population-based approaches (Renton 2012).

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# A POPULATION OF GOOSE GRASS (*ELEUSINE INDICA*) FROM OIL PALM FIELD RESISTANT TO GLYPHOSATE AND PARAQUAT

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**ABSTRACT** Glyphosate herbicide had been used continually three to four times each year for 26 years for general weed control in an oil palm field in Deli Serdang Regency, North Sumatra Province, Indonesia. However, in the last five years glyphosate application had been noticed no longer control goosegrass (*Eleusine indica*) satisfactorily in the field. A pot experiment was carried out to determine whether the goosegrass population has developed resistance to glyphosate. Seedlings originating from seeds of one population collected at Adolina Estate were grown outdoors and showed 7-fold glyphosate-resistance compared with a susceptible population. The glyphosate-resistant population has also confirmed to be resistant to paraquat as the population was found 56-fold more resistant to paraquat than the susceptible population. However, the population was satisfactorily controlled by ammonium glufosinate.

***Keywords*: Herbicide resistance, glyphosate, paraquat, ammonium glufosinate**

## INTRODUCTION

Oil palm is the most important perennial crops cultivated in Indonesia. Indonesia, with the area of nine million hectare of oil palm, has the largest area of oil palm in the world followed by Malaysia. In 2011 crude palm oil (CPO) production was 23 M tonnes. Malaysia, with the area of oil palm 5 million hectares produced 17.6 M tones in 2009. Indonesia is the world's largest CPO producer, with Malaysia second. The two countries account for 85% of world palm oil production.

Weed control plays an important role in oil palm cultivation. The presence of uncontrolled weed could reduce oil pal production both quality and quantity. Weed control may account for 17 to 27 percent of the total upkeep cost in immature or mature oil palm. Chemical method is the most popular to control weed especially those growing within circle weeding in immature and mature oil palm trees. Several herbicides are commonly used in oil palm such as glyphosate, paraquat, glufosinate, and metsulfuron methyl. Herbicides are continually applied three to four times each year.

In a government owned oil palm plantation, PT Perkebunan Nusantara 4, at Adolina Estate was reported that after 26 years of glyphosate and paraquat use the herbicides did not control goosegrass (*Eleusine indica*) satisfactorily any more. The management tried to increase rates of the herbicide but they found results no improvement. The management was not aware why they failed to control *Eleusine* and, due to their experience using glyphosate with good control on the species for a long time, they continually used glyphosate on the area which result in high production cost for the oil palm company.

Goosegrass (*Eleusine indica* (L) Gaertn) is listed as one of the 10 worst weeds of the world (Holm *et al* 1977). Goosegrass is capable of producing 140,000 seeds per plant (Chin and Raja, 1979).

Goosegrass can be easily controlled by herbicide. However, over reliance on herbicide application may result in herbicide-resistant population. Population of goosegrass has been reported resistant to fluazifop-butyl in Malaysia 1989. The fluazifop-butyl resistant goosegrass was cross-resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides (Tiw *et al.*, 1997). The resistance of goosegrass to glyphosate was reported in 1997 (Lee & Ngim, 2000) and to glufosinate ammonium in 2010 (Jalaluddin *et al*, 2010). The development of herbicide resistance in weeds is an evolutionary process in response to selection pressure



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imposed by repeated use of one or more herbicides with the same mode of action for a long time (Powles *et al*. 1998).

## MATERIALS AND METHODS

Mature seeds of goosegrass were collected from around one hundred clumps at Adolina Estate, PT Perkebunan Nusantara IV, Kabupaten Serdang Bedagai, Province of North Sumatra, Indonesia where repeated use of glyphosate gave poor control. The population was regarded as a putative resistant plants. Seeds were transferred to Weed Laboratory test of resistance. Likewise, seeds of susceptible population of goosegrass that had never been sprayed with herbicide (regarded as susceptible population and designated as EFP) were collected from field in Universitas Sumatera Utara Campus in Medan. Seeds of the putative resistant and susceptible biotypes of goosegrass were allowed to germinate in trays that measured 27 cm x 36 cm x 5 cm and that contained soil potting mixture in a shadehouse at the Faculty of Agriculture, Universitas Sumatera Utara. The seedlings of putative resistant plants and the susceptible plants were transplanted 40 plants per tray. Each population was planted in three trays. Plants at three-to four leaf stages were sprayed with 200 g. a.i. $ha^{-1}$ paraquat using knapsack sprayer equipped with system management valve (SMV) making constant flow-rate. All putative resistant plants survived 200 g. a.i. $ha^{-1}$. paraquat application two weeks after planting. In contrast, all the susceptible plants died at the same rate of paraquat. It was concluded that the putative resistant was truly resistant to paraquat and designated as EAD.

For level of resistance test of the EAD population we grew plants of both populations with the same procedure as we conducted previously for testing of resistance as described above. Both populations were germinated in trays. The seedlings of both populations were transplanted into seedling trays that contained the soil potting mixture. Three weeks after transplanting, at four- to five-leaf stage, the seedlings of both the EAD and EFP biotypes were sprayed with glyphosate, paraquat, or glufosinate by using a knapsack sprayer, as described previously. The rates of glyphosate used for the dose–response tests were 0, 120, 240, 340, 480, 600, and 720 g a.i.$ha^{-1}$, for paraquat were 0, 50, 100, 150, 200, 250, and 300 g.$ha^{-1}$. While the rates for glufosinate were 0, 110, 220, 330, 440, 550, 660 g a.i. $ha^{-1}$. Plant survival was measured 3 weeks after spraying (WAS) whereas dry weight was measured 6 WAS. Data were collected and analyzed using standard deviation for plants survival and analysis of variance for plant dry weight.

## RESULTS AND DISCUSSION

Effect of herbicide on goosegrass populations, resistant and susceptible, three weeks after spraying is shown on Figure 1. Resistant plants survived 90.2% to 100% at the rates of 0 to 480 g a.i. glyphosate. $ha^{-1}$. In other words there was no difference of plant survival of the resistant population (EAD) among the rates up to 480 g. a.i. $ha^{-1}$ glyphosate. Moreover, at the rate of 720 g. ai.$ha^{-1}$ glyphosate survived by 70% of the resistant population. In contrast, only 17% susceptible plant population survived at 240 g. a.i. $ha^{-1}$ and all plants were killed at the rate of 600 g. ai. $ha^{-1}$ of glyphosate (Figure 1 A). Based on the LD50 calculation, the resistance level of EAD population was 7-fold of the susceptible population (EFP).

Similarly, dry weight of the resistant plants was no significantly difference between the rate of 0 to 720 g a.i. $ha^{-1}$ glyphosate which is ranging from 17.8 g to 25.8 g per box. In contrast, dry weight of the susceptible plants where glyphosate at 240 g a.i. $ha^{-1}$ could reduce dry weight up to 94.25% (Table 1).

Both populations were sprayed with paraquat herbicide also (Figure 1B). Number of EAD biotype survived paraquat application was ranging from 91.4% at the rate of 50 g a.i. $ha^{-1}$ to 95.8% at the rate 300 g a.i.$ha^{-1}$ whereas the susceptible biotypes (EFP) survived less than 3% only at the rate of 150 g. a.i. $ha^{-1}$. We calculated the LD50 for the EAD biotype and we found that the resistance level of EAD biotype was 56-fold of the susceptible population (EFP).



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Similarly, dry weight of both populations, EAD and EFP, showed significant difference at the corresponding rates of paraquat (Table 2). However, there is no difference of dry weight of the resistant plants (EAD) among the rates of paraquat (0 to 300 g a.i.ha$^{-1}$) which is ranging from 67 to 100 % of control. In contrast, susceptible plants (EFP) was reduced up to 68.5 % even at low rate of paraquat (100 g a.i.ha$^{-1}$).

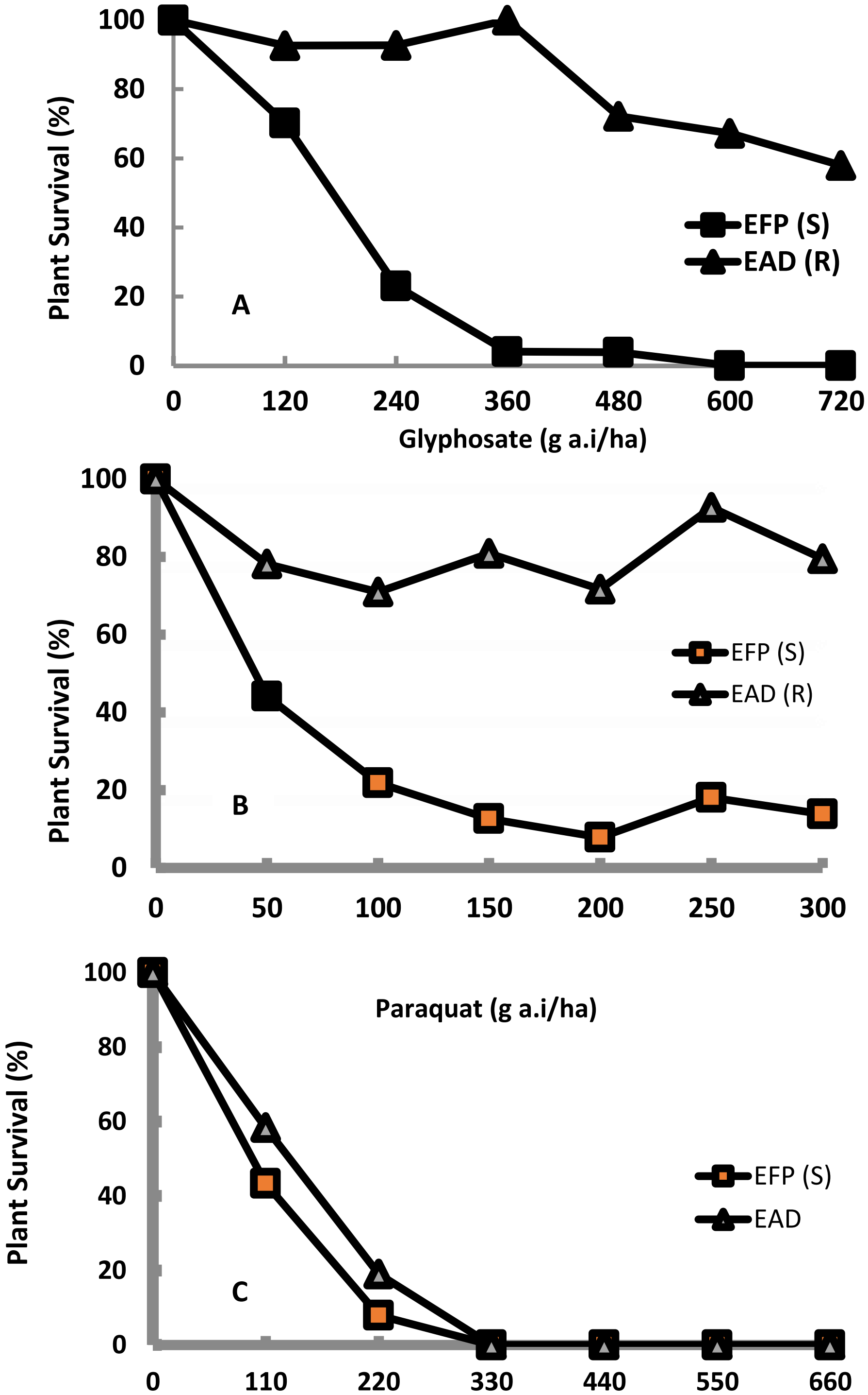


**Fig 1**. Plant survival of resistant plants (EAD) (▲) and susceptible biotype (EFP) (■) after being sprayed with glyphosate (A), paraquat (B), and glufosinate (C) three weeks after spraying.



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**Table 1.** Effect of glyphosate on dry weight of *Eleusine indica* glyphosate-resistant biotype (EAD) and susceptible biotype (EFP) 6 WAS.

| Glyphosate | Dry weight | |
|---|---|---|
| (g a.i.ha$^{-1}$) | EAD | EFP |
| | ………………*g/box*.................... | |
| 0 | 25,8 | 19,1a* |
| 120 | 21,3 | 16,6a |
| 240 | 17,4 | 1,1b |
| 360 | 16,4 | 0b |
| 480 | 18,8 | 0,3b |
| 600 | 15,6 | 0b |
| 720 | 10,9 | 0b |

*Figures followed by the same lowercase letters are not significantly different at 5% level (LSD tests).

**Table 2.** Effect of paraquat on *Eleusine indica* glyphosate-resistant biotype (EAD) and susceptible biotype (EFP) 6 WAS.

| Paraquat | Dry weight | |
|---|---|---|
| (g a.i.ha$^{-1}$) | EAD | EFP |
| | ………………*g/box*.................... | |
| 0 | 25,8 | 19,1a* |
| 50 | 23,9 | 13,5ab |
| 100 | 21,4 | 6bc |
| 150 | 22,1 | 2,1c |
| 200 | 32 | 1,3c |
| 250 | 17,4 | 4,8c |
| 300 | 17,8 | 5,1c |

*Figures followed by the same lowercase letters are not significantly different at 5% level (LSD tests).

**Table 3**. Effect of glufosinate on *Eleusine indica* glyphosate-resistant biotype (EAD) and susceptible biotype (EFP) 6 WAS.

| Glufosinate | Dry weight* | |
|---|---|---|
| (g a.i.ha$^{-1}$) | EAD | EFP |
| | ………………*g/box*.................... | |
| 0 | 25,8c | 19,1a |
| 110 | 10,4b | 4,9b |
| 220 | 1a | 0,3bc |
| 330 | 0a | 0c |
| 440 | 0a | 0c |
| 550 | 0a | 1,9bc |
| 660 | 0a | 0c |

*Figures followed by the same lowercase letters are not significantly different at 5% level (LSD tests).

The EAD population was also sprayed with glufosinate for cross resistance test. The response of EAD and EFP biotypes were shown on Figure 1C and Table 3. Survival of EAD biotype at rates of glufosinate applied was no significantly difference compared to susceptible biotype



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(EFP) at the corresponding rates. All EAD plants were killed at 330 g.ai.ha$^{-1}$ glufosinate which is the same as those occurred with the susceptible plants (EFP). Even at the lower rate of 220 g a.i.ha$^{-1}$, number of EAD plants killed was similar to that of susceptible population which is ranging from 3 to 13% only. Similarly, dry weight of these biotypes were similar at corresponding rates. Therefore, the EAD biotype was effectively controlled by glufosine.

Thus, it is concluded that the EAD biotype is resistant not only to glyphosate but also to paraquat. However, the EAD biotypes has not developed resistant to glufosinate. The three herbicides are common herbicides used in oil palm plantation in Indonesia. This finding is the first resistant case found in oil palm in Indonesia.

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# CROP COMPETITION FOR WEED MANAGEMENT IN CONSERVATION CROPPING SYSTEMS

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**ABSTRACT** Conservation cropping with stubble retention and no-till is very important for improving soil condition and water use, and for increasing soil carbon in the temperate cropping zone of south-eastern Australia. However, tillage and stubble burning are replaced by herbicides for weed control, leading to increased selection pressure for herbicide resistance (especially glyphosate). In addition, retained stubble can reduce crop competitiveness due to poor crop establishment, reduced early crop vigour, and use of wider crop row widths to avoid blockages and facilitate sowing. The opportunities to increase crop competition by choice of crop, cultivar and elevated seeding rates for improved weed management and herbicide performance are reviewed. Crop competition is a simple and cost-effective way for farmers to improve weed control, increase herbicide efficacy and prolong the life of useful chemicals by reducing the rate of development of herbicide resistance. The future performance of herbicides is expected to decline with the spread of resistance and with climate change to hotter and drier conditions. The roles of infrequent strategic burning and tillage, as well as other cultural control tactics for effective weed management in these systems are also discussed.

***Keywords***: Stubble retention, no-till, herbicide resistance, weed suppression.

## INTRODUCTION

No-till and stubble retention replace tillage and stubble burning in conservation cropping systems**,** leading to greater dependence on herbicides for weed control. Conservation cropping can have considerable production and environmental benefits but herbicide resistance is exacerbated, including to important chemicals like glyphosate and paraquat (Heap 2013), both integral to the success of these systems. This is of considerable concern given the lack of new modes of action entering the market place.

Herbicide-based weed management is practiced by most farmers because it is simple and cost-effective. It is not until farmers ‘hit the wall’ with resistance that they generally consider integrating non-chemical control options, even though considerable information on the benefits of integrated weed management has been promoted for the last two decades.

Weeds increase risks to farm businesses and reduce profits, production and confidence. These impediments must be overcome by developing new control tactics and management strategies within sustainable farming systems. Farmers require knowledge and understanding to assess the technological and socio-economic feasibility of new control options. Adoption of new technologies is most likely when they are practical, cost-effective, adaptable, and fit within the preferred farming system.

In this review we examine the features of conservation cropping systems in south-eastern (SE) Australia, impacts on weed management, opportunities to increase crop competitiveness, and other non-chemical options.

## CONSERVATION CROPPING

Stubble retention has been widely adopted in the lower rainfall zones of the southern wheat-belt (<350 mm rainfall per annum) and in the northern wheat-belt of Australia for improved soil condition and greater water availability. However, uptake in SE Australia has been less in



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the medium to high rainfall zones and under irrigated crops, due problems at sowing, including stubble blockages in sowing gear (Scott *et al*. 2010). In addition, the benefits here are less clear than in the north and other regions where soil erosion is more of a problem and stubble decay is quicker.

As a result, up to 50% of farmers often burn stubble in the SE region for improved weed and disease control and ease of sowing, but at the cost of increased greenhouse gas emissions, reduced soil carbon, and degraded soil, air and water quality. To avoid burning, some crops are sown at a wide row spacing (35+ cm compared to the standard 17 cm spacing) to allow sowing through greater quantities and lengths of crop residues, and to minimise blockages and problems of crop establishment. However, wide row spacing can reduce the ability of a crop to compete with weeds (Peltzer *et al*. 2009) and increase dependence on herbicides.

Twenty to thirty years ago it was predicted that changing from conventional to conservation cropping in Australia (e.g. Cornish and Pratley 1987) would lead to changes in weed infestations, including: increasing dominance of difficult-to–control species, especially perennial species; staggered patterns of weed emergence; decreased crop competitiveness; and reduced herbicide efficacy, especially soil applied herbicides (e.g. trifluralin) is often reduced due to poor contact. Possible positive outcomes for weed management are that stubble acts as a physical barrier to weed emergence, exudes suppressing allelochemicals, and enhances soil biological activity for weed seed decay and provides habitat for weed seed predators.

Experience in North America and Europe generally shows that tillage often alters the vertical distribution of weed seeds in the soil (Cousens and Moss 1990; Staricka *et al*. 1990) and seeds are usually concentrated on or near the surface under conservation cropping systems (Feldman *et al*. 1996; Torresen *et al*. 2003).

Changing farming systems often result in weed species shift. Previous research has shown that biennial or perennial species and wind-dispersed species often dominate in no-till systems (Derksen *et al*. 1993). Circumstantial evidence in SE Australia indicates minimum tillage systems have favoured the spread of wind-disseminated species like common sowthistle (*Sonchus oleraceus*) (Widderick *et al*. 2002) and flax-leafed fleabane (*Conyza bonariensis*) (Wu *et al*. 2007).

Brome grasses (*Bromus diandrus* Roth and *Bromus rigidus* Roth) have also been reported to have increased their prevalence as weeds of crops of southern Australia (Gill and Blacklow 1984; Kon and Blacklow 1988). Kleemann and Gill (2006) concluded that the increasing importance of *B. rigidus* in conservation tillage is due to its longer dormancy and light inhibition of germination. Further research has shown that weeds can alter dormancy requirements in response to changing farming systems. Both smooth barley (*Hordeum murinum* ssp. *glaucum*) and *B. diandrus* have been shown to extend their dormancy to escape pre-sowing control measures. Populations of these two grasses from cropping fields possessed much greater dormancy than did those from non-crop habitats (Fleet and Gill 2012; Kleemann and Gill 2013).

However, weed species, climatic and soil interactions cause considerable variation in weed responses to stubble retention and level of cultivation. Recent surveys have documented that herbicide resistance in weeds is an increasing problem in SE Australia because of the heavy reliance on herbicides in conservation cropping (Broster *et al.* 2011a and 2011b; Boutsalis *et al.* 2012). Non-chemical innovations are urgently needed to delay the onset of herbicide resistance. One of the most cost-effective ways to manage weeds in both conventional and conservation cropping is to maintain a diverse crop rotation and to ensure that crops are strongly competitive.

## CROP COMPETITION

Crop competition is well recognised as an important low-cost tactic for weed management (e.g. Lemerle *et al*. 2001; Mohler 2001) using increased crop seed rates and strongly competitive genotypes. In addition, a number of studies show that the efficacy of herbicides can be improved when combined with increased competitive ability in crops (e.g. Lemerle *at al*. 1996; Walker



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*et al*. 2002; Lemerle *et al.* 2013). This is particularly important in the situations where herbicide effectiveness is reduced and unreliable, for example with herbicide resistance and with the predicted climate change to hotter and drier conditions (Howden *et al.* 2010).

Many factors influence crop emergence, vigour and competitive ability, and the following can be manipulated by agronomy to favour the crop: species, cultivar, seeding rate, row spacing, seed size and quality, seeding depth, herbicide treatment, fertiliser timing and placement, correct use of pesticides for disease and pest control, and stubble and tillage.

The crop competitive ability could be further improved by incorporating allelopathic traits (Wu *et al.* 1999). Some elite wheat accessions have been shown to possess strong allelopathic activity against weeds (Wu *et al.* 2000; Bertholdsson 2005 and 2012). The total genetic variation explained by wheat suppressing activity was found to be in the range of 0-21%, while as high as 58% was found for barley (Bertholdsson 2005).

Research has been progressed to breed wheat allelopathic activity for weed suppression (Bertholdsson 2010). Genetic manipulation of wheat allelopathic activity has been made by introducing rye genetic materials (Bertholdsson 2012; Bertholdsson *et al.* 2012). Bertholdsson (2012) documented that wheat lines with rye chromatin, all or part of a rye chromosome, showed high allelopathy. It is therefore possible to introduce elite weed-suppressing genes from alien species such as barley and rye into wheat to significantly improve wheat's natural defense on weeds.

## OTHER CULTURAL CONTROL TACTICS

Reviews of the use of other cultural or non-chemical control for weed management are available (e.g. Radosevich *et al.* 1997; Lemerle and Murphy 2000; Bond and Grundy 2001; Hatcher and Melander 2003; Upadhyaya and Blackshaw 2007). Techniques include: preventing the spread of weeds between fields, rotating crops and pastures, delaying sowing, strategic tillage, thermal weeding (flaming) and burning, grazing by livestock, and using living mulches, and intercropping and cover crops.

Recent advances in thermal weed control have seen the development of infrared and microwave weeders in which infrared weeders heat ceramic or metal surfaces to generate the infrared radiation directed at the target weeds (Ascard 1998; Bond and Grundy 2001), while the microwave radiation causing dielectric heating of plant tissue water that eventually kills the plant (Sartorato *et al.* 2006). A prototype of microwave weeder has also been recently experimented in Australia ((Brodie 2013).

These thermal weed control methods offer a potential alternative to herbicides in areas where weeds have developed herbicide resistance. Although highly effective in killing plants, it remains a real challenge to kill weeds seeds the soil (Brodie 2013).

Making silage or cutting hay can be an effective non-chemical control option for certain weed species (Blackshaw and Rode 1991; Stanton *et al.* 2012). Stanton *et al.* (2012) investigated the impact of ensiling and digestion on the viability of seeds of 11 weed species and three pasture species in Australia and found seed from all grass weeds except annual ryegrass were rendered unviable after being ensiled, whereas some broadleaf weed seeds remained viable. Similar effects were also achieved with digestion. The viability of marshmallow seed (*Malva parviflora* L.) and the three pasture species biserrula (*Biserrula pelecinus*), bladder clover (*Trifolium spumosum*) and French serradella (*Ornithopus sativus*) were tolerant to either ensilage or digestion treatment. Westerman *et al.* (2012) also reported that success of ensilage depends on weed species.

To avoid weed spread to new sites, it is essential that crop and pasture grain or forage is not contaminated with weeds. Vehicles, tillage implements and harvest equipment must be carefully cleaned to avoid transportation of weeds from infested areas. Animals also can be important in spreading some weeds, by seeds attaching to wool, hides or passing through the digestive tract. Careful quarantine to prevent weed spread to uninfested areas must underpin any successful weed management strategy.



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The roles of infrequent strategic burning and tillage are currently being reconsidered by farmers for infrequent use within the cropping rotation. Deep burial of herbicide-resistant *Lolium rigidum* using a mouldboard plough is an effective control tactic (Douglas and Peltzer 2004), and has been adopted recently by some farmers in SE Australia. Whatever the control option used, it is critical that it prevents replenishment of the weed seedbank. Innovations such as the Harrington Seed Destructor which pulverises weed seed at harvest also show potential (M. Walsh, *pers. comm*.).

## CONCLUSIONS

Weeds will continue to evolve to changing cropping systems and farmers will adapt their management strategies accordingly. Farmers will likely only adopt cost-effective, non-chemical control tactics (e.g. strategic tillage and burning) and crop competition for weed management as herbicide options disappear. Research is required to provide information on the percentage efficacy of new control tactics on the important weed species impacting on crop productivity. In the future, potential new strategies will need to be developed, such as the use of site-specific weed management, integration of bio-control, and utilising the benefits of biodiversity for weed seed predation.

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# COMPETITIVE HIERARCHY IN WEED SUPPRESSION BY BARLEY (*HORDEUM VULGARE*), CANOLA (*BRASSICA NAPUS*) AND WHEAT (*TRITICUM AESTIVUM*) CULTIVARS

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**ABSTRACT** One novel option of integrated weed management (IWM) to suppress weeds such as rigid ryegrass (*Lolium rigidum*) is to increase crop competitive ability by using competitive crop cultivars and manipulating seeding rate and row spacing. However, the extent of competitive ability of recently developed crop cultivars under weedy situations is not well known to growers. We examined the hierarchy in competitive ability of crop cultivars to suppress weeds in wheat, barley and canola crops grown under weedy conditions at two row spacing and seeding rates with or without herbicides at three locations over three seasons within the wheat-belt of Western Australia (WA). We measured biomass of crop and weed, crop canopy structures, weed seed head numbers, crop grain yield, and grain quality. Data were subjected to ANOVA and means were separated by LSD. We found that the competitive ability of cultivars of a single crop type (barley, canola or wheat) varied between seasons and sites and between vegetative and reproductive stages. Barley and wheat crops appeared to be more competitive to rigid ryegrass than canola crop and showed a greater ability to suppress the formation of rigid ryegrass heads. In weedy situations, some wheat cultivars such as Magenta had greater suppression of rigid ryegrass than the least competitive cultivars such as Wyalkatchem, clearly demonstrating that crop competition is an important IWM option. Some new competitive cultivars such as UA40 selected to have early vigour showed potential as genotypes for future breeding of competitive wheat varieties. However, ordering the competitive hierarchy of crop cultivars was unfair as the performance of cultivars was variable across sites and seasons. We conclude that although crop-competitive ability to suppress weeds varies between season and sites, competitive crops and crop cultivars are a non-chemical tool of IWM to suppress weeds and reduce pressure on herbicides.

***Keywords:*** Crop-weed competition, crop cultivars, biomass, canopy structure, grain yield, wheat, barley, canola.

## INTRODUCTION

In conservation cropping systems with no-till and full stubble retention, tillage is replaced by herbicides for weed control, thereby increasing the threat of herbicide resistance. Rigid ryegrass (*Lolium rigidum*) is one of the most important weeds in the temperate wheat-growing regions of southern Australia. It is estimated that this weed is causing the Australian farmers around $150 million (Jones *et al*. 2000) and more than $200 million every year (Young 2013) in lost grain yield.

Integrated weed management is an important component of Australian cropping systems to combat herbicide resistance in rigid ryegrass. Whilst herbicides are an essential part of



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managing weed emergence and seed set, crop management techniques such as competitive crops and high seeding rates can be used to suppress and manage weeds (Kirkland 2003; Scursoni and Satorre 2005; O'Donovan et al. 2007; Paynter 2009). Barley is generally considered to be more competitive against weeds like wild oats (*Avena fatua.*) and rigid ryegrass than wheat (O'Donovan et al. 2005).

It is very important to understand the hierarchy in the competitive ability of crop cultivars across seasons and sites to be able to recommend those for use a tool of Integrated Weed Management (IWM). Worthington *et al* (2013) observed that the development of wheat cultivars with superior competitive ability against Italian ryegrass Lo*lium multiflorum*) could play a role in maintaining acceptable yields and suppressing weed populations. Paynter and Hill (2009) found that barley cultivars Baudin, Flagship, and Hamelin were more competitive with rigid ryegrass than Buloke, Gairdner, and Vlamingh. Lemerle *et al* (1995) concluded that the order of decreasing competitive ability of different crop species in presence of 300 plants $m^{-2}$ of rigid ryegrass under New South Wales conditions was oats (*Avena sativa* L.)> cereal rye (*Secale cereale* L.) and triticale (×*Triticosecale*)> oilseed rape> spring wheat> spring barley> field pea (*Pisum sativum* L.)> lupin (*Lupinus angustifolius* L.). Crop competition is reduced with wider rows in conservation agriculture, so weed management relies more on herbicides and tillage with the associated risks and costs (Peltzer *et al* 2009).

In the situations where herbicide effectiveness is reduced and unreliable, use of competitive crop and cultivars may result in greater suppression of weed than herbicides alone. Several studies such as Lemerle *et al*. (1996) and Walker *et al*. (2002) showed that the efficacy of herbicides can be improved when combined with increased competitive ability in crops.

In the recent years, many growers in Western Australia (WA) have started using high seeding rates of wheat and barley to suppress weeds. However, the extent of competitive ability of the recently released crop cultivars under weedy situations is not well known to growers. The objective of this study was to examine the competitive hierarchy of crop species and cultivars to suppress weeds in wheat, barley and canola crops grown under weedy conditions with or without high seeding rate and herbicides.

## MATERIALS AND METHODS

A study was undertaken to conduct six trials in three locations over three seasons within WA Wheatbelt to examine the effect of crop species and crop cultivars on suppression of weed in conservation agriculture. In 2008, competitiveness of four cultivars (Table 1) of each crop (wheat, barley and canola) was examined under weedy conditions sown at normal seeding rates with or without herbicides at Meckering Western Australian No-till Farmers Association (WANTFA) research site and Wongan Hills Research Station of Department of Agriculture and Food WA (DAFWA). In 2009, effect of seeding rates (normal and double) of two selected cultivars per crop species (Table 1) on rigid ryegrass were examined under weedy conditions with or without herbicides at both locations.

In 2010, two trials were conducted on the on the interaction of eight cultivars (7 of wheat and 1 of barley) (Fig. 1) with herbicide (plus or minus herbicide) on rigid ryegrass at Eradu and Wongan Hills, WA.

**Measurement:** Canopy area (canopy height x width) and light interception through the crop canopy were measured at maximum tillering stage of wheat or barley and rosette stage of canola. The main weed species at all three sties was rigid ryegrass. Rigid ryegrass heads were counted at anthesis of wheat. Biomass of crop and weed was recorded in 2010. Grain yield was recorded at harvest.

**Statistical methods:** In 2008 and 2009, trials were conducted in a split-slit plot design with four replications. In 2008, crop type was assigned to the main plots, weed treatments in the sub-plots and cultivar in the sub-sub-plots. In 2009, crop type was assigned to the main plot, weed treatments in the sub-plot, seeding rate in the sub-sub-sub-plots and cultivars in the sub-



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sub-sub-sub-plots. In 2010, eight cultivars (7 of wheat and 1 of barley) (Fig. 1) and herbicide (plus or minus) were laid out in a randomised complete block design with four replications.

Data were subjected to ANOVA and means were separated by LSD. For grain yield of crops, ANOVA was performed for each crop separately to minimise the confounding effects of crop species in 2008 and 2009. For the ANOVA of other measurements such as crop canopy area and weed seed heads, crop species was used as the main factor. In 2010, data were analysed employing a randomised complete block design.

## RESULTS AND DISCUSSIONS

### 2008 and 2009 Trials

### Initial weed control

Initial in-crop weed control due to standard herbicides in each crop varied between crops, seasons and locations. Weed control was generally more effective in wheat and barley than in canola.

### Grain yield of crops

Interaction of weed control and cultivars influenced grain yield of wheat and barley in all trials except at Meckering in 2008. In canola, a significant interaction effect of weed control and cultivars was found at Meckering in 2008 and at Wongan Hills in 2009. These results did not conform to those of Lemerle *et al.* (1995) and O'Donovan *et a*l (2005).

In barley, Roe and Hindmarsh yielded better than Baudin and Buloke at both locations in 2008 regardless of weed control (Table 1). Under no in-crop weed control, Hindmarsh at Meckering and Baudin at Wongan Hills produced lower yield than under weed control.

In canola, Bravo and Thunder produced greater grain yield under weed control than under no weed control at Meckering in 2008. The other two cultivars did not respond to weed control at Meckering in 2008 (Table 1). In 2009, weed control significantly improved yield of Tanami and Bravo.

In wheat, Wyalkatchem yielded better under weed control than no weed control in two out of four trials and EGA Bonnie Rock yielded better under weed control than no weed control in three out of four trials. Other cultivars did not respond to weeding in either season or location.

Use of higher seeding rates in 2009 did not affect crop yield of canola and wheat at either location. Higher seeding rate of barley increased barley grain yield by 13% at Meckering in 2009 but not at Wongan Hills where ryegrass density was greater.

*Rigid ryegrass heads:* On average, barley and wheat cultivars had fewer rigid ryegrass heads at maturity than canola cultivars (Table 2). Rigid ryegrass head numbers were not recorded at Wongan Hills in 2008. In barley, herbicides reduced rigid ryegrass heads in Buloke at Meckering in 2008, Roe at Meckering and Wongan Hills in 2009, and Buadin barley at Wongan Hills in 2009. However, rigid ryegrass heads were not influenced by barley cultivars alone. In canola, herbicides reduced number of ryegrass heads but cultivar alone did not influence rigid ryegrass heads regardless of herbicide applications.

In wheat, herbicides reduced rigid ryegrass heads in most cultivars in all the trials compared to no herbicide. Wyalkatchem had more rigid ryegrass heads than other cultivars at Meckering in 2008 and at Wongan Hills in 2009 (Table 2).

### Canopy area

At maximum vegetative stage, barley had larger canopy area than wheat and wheat had larger canopy area than canola (data not shown). However, some cultivars of canola such as Thunder had greater light interception than wheat or barley probably due to large leaf size and wide leaf angle than other crops and cultivars. Regardless of crops, larger canopy area and greater light interception in most cultivars did not result in greater grain yield suggesting that there was a shift in the competiveness of cultivars of each crop between the vegetative and reproductive stages.



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**Table 1**. Grain yield (kg $ha^{-1}$) of different crop cultivars grown under in-crop weed control with herbicide and no weed control at Meckering and Wongan Hills in 2008 and 2009[a].

| Crops/ Cultivars | Meckering | | | | Wongan Hills | | | |
|---|---|---|---|---|---|---|---|---|
| | **2008** | | **2009** | | **2008** | | **2009** | |
| | Weed control | No weed control | Weed control | No weed control | Weed control | No weed control | Weed control | No weed control |
| | | | | **Barley** | | | | |
| Roe | 2474 | 2443 | 3323 | 2987 | 2444 | 2379 | 2500 | 2253 |
| Hindmarsh | 2405 | 2189 | – | – | 2500 | 2611 | – | – |
| Baudin | 1817 | 1686 | 3264 | 2741 | 2203 | 1951 | 2419 | 2083 |
| Buloke | 1928 | 1981 | – | – | 2145 | 2015 | – | – |
| LSD.05 | NS | | 453.5 | | 216.9 | | 187.9 | |
| | | | | **Canola** | | | | |
| CB™ Tanami | 960 | 789 | 595 | 595 | 1104 | 1063 | 980 | 571 |
| CB™ Boomer | 817 | 717 | – | – | 972 | 889 | | |
| Bravo | 863 | 540 | 618 | 530 | 1007 | 951 | 1057 | 637 |
| Thunder | 934 | 706 | – | – | 965 | 931 | – | – |
| LSD.05 | 211.8 | | NS | | NS | | 82.2 | |
| | | | | **Wheat** | | | | |
| Wyalkatchem | 1011 | 928 | 2441 | 1959 | 2396 | 2250 | 2319 | 1879 |
| Yitpi | 1323 | 1305 | – | – | 2194 | 2125 | – | – |
| Calingiri | 944 | 785 | – | – | 2326 | 2174 | – | – |
| EG Bonnie Rock | 1416 | 1409 | 2252 | 1902 | 2208 | 2424 | 2407 | 1960 |
| LSD.05 | NS | | 431.5 | | 150.4 | | 174.2 | |

[a] '–' = This cultivar was not used in 2009 trials

In barley, Roe produced higher grain yield both under herbicides and no herbicide applications than Buloke at Meckering and Wongan Hills in 2008. Buloke appeared to have larger canopy area at maximum tillering stage than Baudin. However, this did not result in higher grain yield of Buloke, suggesting that there was a shift in the competitiveness of this cultivar with age of plant (Hashem et al. 1998).

In canola, the ranking of cultivars (based on the canopy area and light interception recorded at the rosette stage) did not correspond to the final grain yield. However, the ranking of cultivar competitiveness at the flowering stage (visual assessment of the floristic composition of canola cultivars and weed density) appears to correspond more closely to the grain yield of canola.

**Table 2**. Effect of herbicide and different crop cultivars grown under in-crop weed control with herbicide and no weed control on rigid ryegrass heads (number $m^{-2}$) at Meckering and Wongan Hills in 2008 and 2009 seasons[a].

| Crops/Cultivars | Meckering | | | | Wongan Hills | |
|---|---|---|---|---|---|---|
| | **2008** | | **2009** | | **2009** | |
| | Weed control | No weed control | Weed control | No weed control | Weed control | No weed control |
| **Barley** | | | – | – | – | – |
| Roe | 1 | 11 | 16 | 40 | 36 | 145 |
| Hindmarsh | 3 | 9 | – | – | – | – |



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| | | | | | | |
|---|---|---|---|---|---|---|
| Baudin | 2 | 9 | 15 | 28 | 35 | 181 |
| Buloke | 1 | 19 | – | – | – | – |
| **Canola** | | | | | | |
| CB™ Tanami | 7 | 34 | 20 | 51 | 175 | 426 |
| CB™ Boomer | 18 | 37 | – | – | – | – |
| Bravo | 17 | 24 | 24 | 53 | 80 | 465 |
| Thunder | 16 | 35 | – | – | – | – |
| **Wheat** | | | | | | |
| Wyalkatchem | 1 | 25 | 18 | 47 | 32 | 204 |
| Yitpi | 3 | 11 | | | | |
| EG Bonnie Rock | 2 | 13 | 14 | 37 | 26 | 140 |
| Calingiri | 1 | 15 | | | | |
| LSD.05 | 11.5 | | 20.6 | | 111.9 | |

[a]Rigid ryegrass head number is not available for Wongan Hills in 2008. '–' = This cultivar was not used in 2009 trials.

In wheat, Bonnie Rock had the largest canopy area and light interception at maximum tillering stage but did not appear to produce greater grain yield and suppress more ryegrass heads than other cultivars except at Meckering in 2008. Of the remaining three cultivars, Calingiri showed higher canopy area and light interception at maximum tillering stage, but did not show a yield advantage in weedy or herbicide applied situations, suggesting that there was a shift in the competitiveness of this cultivar between vegetative and reproductive stages (Hashem *et al* 1998; Worthington *et al* 2013).

Seasonal and site conditions (such as time of sowing, rate and speed of crop emergence, herbicide efficacy, herbicide toxicity, emergence time and type of weed present) have a large influence on crop-weed dynamics. Even though there was not much difference in the suppression of rigid ryegrass by crop cultivars, measurement on the amount of rigid ryegrass seed production may indicate if cultivars affect ryegrass seed production as a consequence of competition (Worthington *et al* 2013).

A small change in the competitiveness of the crop of the weeds between the vegetative and reproductive stage of the crop may have a large impact on the competitive interaction between crops and weeds (Hashem *et al.* 1998). Such a shift in the competitiveness of crop cultivars with age of crop and weed plants needs to be understood to allow for improved predictions of crop yield due to weed competition.

In 2010, trials at Eradu and Wongan Hills showed that some new genotypes such as UA40 have greatly suppressed biomass of rigid ryegrass at both locations. Even some popular cultivars such as Magenta have suppressed rigid ryegrass at both locations to the same extent as UA40. These results clearly demonstrate that some cultivars of a given crop species are more competitive than others. Wheat genotypes such as UA40 have been selected for early vigour and stronger competitiveness and are to be used for breeding more competitive wheat cultivars.

These results lead to conclude that crop-weed competition is highly dynamic under field conditions probably due to variation between seasons and sites. Barley and wheat cultivars appear to be more competitive than canola leading to greater suppression of rigid ryegrass heads. These orders of competitive ability of crop species do not conform to that of Lemerle *et al* (1995) probably due to differences in cultivars, growing seasons and agro-ecological conditions of the area. Nevertheless, competitive crops and cultivars are cheap cultural IWM tool that should increase weed control efficacy by herbicide in situations where herbicide alone is less efficacious due to evolution of herbicide resistance.



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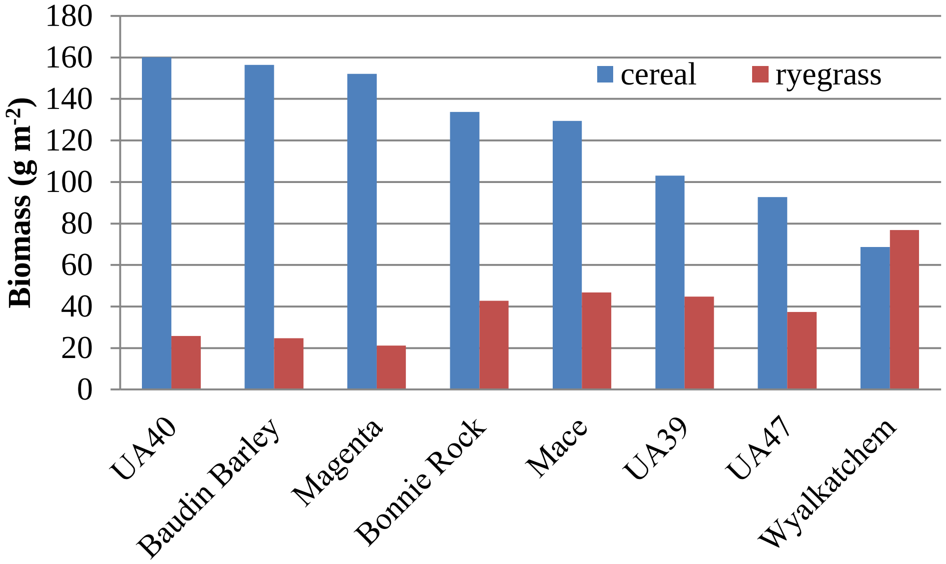


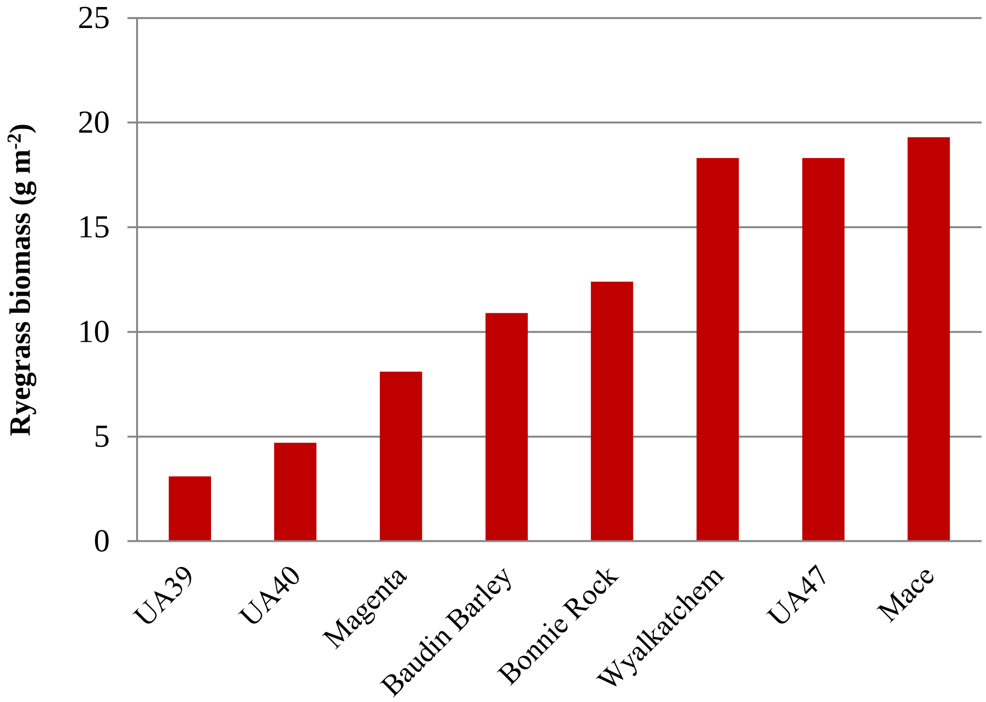


**Fig 1**. Biomass of cereal crop cultivars (wheat and barley) and rigid ryegrass at Eradu (above) and ryegrass biomass against different cereal cultivars (below) at Wongan Hills Western Australia in 2010 season.

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## *New Innovation in Weed Management*

# INNOVATIVE APPROACHES TO MANAGE GLYPHOSATE-RESISTANT WEEDS IN THE SUBTROPICAL GRAIN REGION OF AUSTRALIA

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**ABSTRACT** Broad-acre cropping in Australia is predominantly in the Mediterranean region of southern and western Australia, which consists mostly of winter crops grown under rain-fed conditions. This contrasts with the subtropical region in north-eastern Australia, which consists of a mix of summer and winter crops preceded by a 6-10 month zero-tilled fallow. In the subtropical region weed-free fallows are essential to conserve soil moisture to potentially maximise yields of the following crop. However following 2-3 decades of reliance on glyphosate for weed control in fallows, five weed species, *Echinochloa colona*, *Urochloa panicoides*, *Chloris truncata*, *Lolium rigidum* and *Conyza bonariensis*, have developed glyphosate resistance, threatening the viability of zero-till farming in the subtropical region. Recent surveys indicate that glyphosate-resistant populations of *E. colona* and *C. bonariensis* are now widespread problems across this region. Research on these problem weeds showed that they can be controlled effectively with a range of alternative chemical and non-chemical tactics that target different parts of the weeds' lifecycle. Strategic tillage and effective residual herbicides were effective in depleting the seed-bank. Seedling control was improved using non-glyphosate knockdown herbicides in the fallow, and changing crop rotation allowed use of selective post-emergent herbicides. As well, glyphosate still provided efficacy on some resistant populations if treated at the seedling stage under good growing conditions. Most importantly, the double-knock tactic was refined to ensure maximum control of sprayed survivors in fallow. Weed seeker technology used to apply follow-up treatment on low density populations of survivors greatly reduced replenishment of the seed-bank. As well, increased crop competition also reduced weed seed production of weeds in crop. These tactics, together with knowledge on seed-bank dynamics and increased focus on stopping seed production on survivors, are now the basis of new management strategies, which will be presented for glyphosate-resistant *E. colona* and *C. bonariensis*.

***Keywords:*** Glyphosate resistance; *Echinochloa*; *Conyza*; IWM; seed-bank.

## INTRODUCTION

Weeds have been estimated to cost Australian farmers around $1.5 billion a year in weed control activities and a further $2.5 billion a year in lost agricultural production (Australian Government 2012). A large part of this is due to the problems created by herbicide resistance, particularly glyphosate resistance, costing growers many hundreds of millions of dollars annually (WeedSmart 2013). If weeds are costing Australian agriculture more than $4 billion annually, then we need to ask the question whether our farming systems are in control of weeds, or are our weeds determining how we farm and our cropping viability? Innovative options are needed to improve this situation.

Successful weed management relies initially on the implementation of the best available agronomic practices to optimise crop production (McGillion and Storrie 2006). This involves selection of crop and rotation, and then maximising crop competition. These best farming



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practices need to be overlaid with best weed management practices, known as integrated weed management (IWM). This is a planned strategy consisting of a variety of chemical and non-chemical tactics that has the objectives of both reducing weed numbers and the size of the weed seed-bank in the soil.

IWM addresses the life cycles of problem weeds, and uses effective tactics for each phase of the weed life cycle within the different components of the crop rotation (McGillion and Storrie 2006). Best weed management practices, not only focus on controlling emerged seedlings, but take into account the seeds in the seed-bank, replenishment of the seed-bank and new incursions. A major weakness of current weed management is not taking into account depleting and minimising replenishment of the weed seed-bank in the soil. Emerged weeds are just the symptoms of the underlying cause, which is viable weed seeds within the surface soil and are the primary source of next flushes.

This paper briefly describes the current situation with glyphosate-resistant weeds in grain cropping systems in the sub-tropical region, and then outlines new approaches and tactics to manage two difficult-to-control weeds *Conyza bonariensis* (L.) Cronquist and *Echinochloa colona* L. (Link).

## MATERIALS AND METHODS

### Glyphosate resistance status

Once a weed population has been confirmed as glyphosate resistant, details are placed on the website of the Australian Glyphosate Sustainable Working Group (http://glyphosate resistance.org.au/).

### Weed ecology and seed-bank dynamics

Impact of burial depth on emergence was measured in a pot experiment sown with known quantities of viable seed of *C. bonariensis* and *E. colona* at 0, 2 and 10cm depths.

The impact of tillage and stubble on *E. colona* emergence patterns and seed persistence was measured in a field experiment that was densely infested with this weed. Soil cores (10 cm x 10cm) were collected to determine the number of viable seed (Taylor *et al*. 2005) at the beginning (September 2009) and two years later following the treatment implementation (Table 1). Emergence was measured over four years, and once all seedlings were counted, all were controlled to prevent replenishment of the seed-bank.

### Chemical control

A range of residual herbicides, knockdown herbicides and sequential applications of two different knockdown herbicides, known as the double knock, were assessed for glyphosate resistant *C. bonariensis* and *E. colona*. Much of this has been published recently (Werth *et al*., 2010; Walker *et al*. 2012; Widderick *et al*. 2013), but new data on herbicide effectiveness on *E. colona* from three field and two pot experiments is presented. In the field experiments, glyphosate-resistant populations were treated with either glyphosate or haloxyfop followed five days later by paraquat. In the pot experiments, glyphosate-resistant and glyphosate-susceptible populations were treated with either glyphosate or haloxyfop followed by paraquat at 1 to 21 days later.

### Non-chemical tactics

The impact of different tillage operations in reducing the subsequent emergence of *C. bonariensis* and *E. colona* was measured in a field experiment that was sown with seeds of the two species in 1 $m^2$ permanent quadrats. The four tillage treatments were a harrow, tynes, off-set disc and one-way disc, which had different levels of soil inversion, harrows with the least and one-way disc with the most. Emergence was compared to that in no-tilled plots for six months.



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The importance of crop competition was determined in a field experiment with a natural infestation of *C. bonariensis*. Wheat was sown in 25 and 50 cm rows at 50 and 100 plants/m$^2$, and the weed was left unsprayed. Prior to harvest, the number of seed heads was measured to determine the effect of crop competition on seed production.

## RESULTS AND DISCUSSION

### Glyphosate resistance status

Since the first glyphosate-resistant weed, *Lolium rigidum* Gaudin, was confirmed in Australia in 1996, 76 populations of *E. colona*, 57 populations of *C. bonariensis,* 10 populations of *Chloris truncate* R.Br. and 3 populations *Urochloa panicoides* P.Beauv. have been detected, the majority of which were in the sub-tropical region (Preston 2013). These populations have been found not only in broad-acre cropping, but in horticultural and non-agricultural situations, such as roadsides.

### Weed ecology and seed-bank dynamics

Following burial of 200 viable seeds in the pot experiment, 53, 1 and 0% of *C. bonariensis* and 11, 2 and 0% of *E. colona* emerged from depths of 0, 2 and 10cm respectively during the following year. Thus, these species are predominantly soil surface germinators, and suit infesting zero-tilled systems. This agrees with previous investigations by Wu *et al*. (2007) and Walker *et al*. (2010).

**Table 1**. Cumulative emergence of *E. colona* over four years following the implementation of various tillage and stubble treatments, and percentage of viable seed exhumed two years following no seed replenishment of the seed-bank, initially 20420 seeds/m$^2$. Each treatment was applied only once except the tyne tillage treatment, which was applied following each flush

| Treatment | Cumulative emergence (plants/m$^2$) over four years | Viable seed after two years as % of initial seed-bank |
|---|---|---|
| Zero till with no added stubble | 1910 | 1.0 |
| Harrow in Autumn | 1480 | 0.5 |
| Harrow in Spring | 1435 | 0.8 |
| Off-set disc | 825 | 2.8 |
| Tyne tillage | 925 | 0.9 |
| Zero till with added wheat stubble | 1520 | 4.0 |
| Zero till with added barley stubble | 1990 | 4.8 |
| Zero till with added sorghum stubble | 1725 | 1.3 |
| LSD ($p$=0.05) | 680 | 0.9 |

In the field experiment on seed-bank dynamics, the initial seed-bank was an average of 20420 seeds of *E. colona* per m$^2$, as measured in September 2009. Over four years, less than 10% emerged (Table 1), with the majority emerging during the first year (data not shown). The zero-till treatments, irrespective of addition of stubble, had the same levels of emergence.



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Tillage using off-set discs and regular use of tynes reduced emergence by an average of 55% compared with plots without tillage or stubble added. Within two years, approximately 99% of the seed had lost viability or germinated in most treatments. The off-set disc treatment resulting in seed burial increased seed persistence, which is consistent with that found when *E. colona* seed were buried at 10cm in previous research (Walker *et al*., 2010). Unexpectedly, the addition of wheat or barley stubble also increased seed persistence, but the reasons for this have not been elucidated.

**Chemical control**

Control of the three field populations of glyphosate-resistant *E. colona* with glyphosate (720 g/ha) was 10-97%, but was 94-99% with haloxyfop (156 g/ha) and 96-100% with clethodim (90 g/ha) (data not shown). These populations were at the tillering stage and growing under stress-free conditions. The sequential treatment, applied five days later with paraquat (500 g/ha), improved control for the glyphosate treatment to 99-100%, and for the haloxyfop and clethodim treatments to 100% consistently. This research is consistent with that found by Widderick *et al*. (2013) with glyphosate-resistant *E. colona* and Walker *et al*. (2010) with glyphosate-resistant *C. bonariensis*. In those investigations glyphosate alone was ineffective, but the double knock of paraquat following haloxyfop or glyphosate + 2,4-D was highly effective on *E. colona* and *C. bonariensis* respectively.

For both glyphosate-susceptible and glyphosate-resistant *E. colona* populations, the efficacy of the sequential application treatments was excellent (100%) with intervals from 1 to 14 days between the first application of glyphosate or haloxyfop followed by paraquat.

**Non-chemical tactics**

All tillage treatments reduced subsequent emergence, but the extent of reduction differed between the two species and equipment. In the zero-tilled plots, an average of 696 and 712 seedlings/m$^2$ emerged during the six months following sowing seeds of *C. bonariensis* and *E. colona* respectively. All tillage treatments reduced subsequent emergence of *C. bonariensis* by 91-99% and of *E. colona* by 58-81%, with the greatest reductions from the two disc treatments.

Crop competition reduced both emergence and seed head production of *C. bonariensis* in wheat. Growing wheat in narrower rows had a greater impact than increasing crop density. The combination of narrow rows of 25 cm and high crop density of 100 plants/m$^2$ resulted in 90% reduction in the number of *C. bonariensis* seed heads.

**Integrated weed management**

In recent years, growers have started to change their approach from short-term weed control, which focuses on minimising the economic impact on the current crop, to more long-term integrated weed management (IWM) with the aim to minimising the weed seed-bank. That is, there is a shift from treating the 'symptoms' to managing the 'cause'.

For an IWM approach to be successful, it requires a diverse range of chemical and non-chemical tactics to be applied to the different components of the weed's lifecycle. This needs prior planning to devise the different tactics and their timing. For glyphosate-resistant populations of *C. bonariensis* and *E. colona*, a range of effective tactics have been developed, and the combination of tactics targeting the seed-bank, controlling young plants and stopping seed production on survivors has been successfully implemented by many growers. As shown in our field experiments, two to three years of diligent practices to prevent replenishment of the seed-bank has driven the seed-bank down to less than 1%.

This research has been packaged and made widely available to growers to assist with implementing best management practices for glyphosate-resistant weeds, such as for *C. bonariensis* (Walker 2013).



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# PLANT PHENOMICS MAY HELP HERBICIDE RESEARCH AND DEVELOPMENT

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**ABSTRACT** Phenomics is a new omics which recently attracts wide interests from those working on genomics and crop breeding. A core element of phenomics is the non-destructive and rapid diagnosis of plant phenome for high throughput phenotyping (HTP), which can be used for crop breeding. Major technologies for phenomics include spectrum analysis of reflected light from plants and plant thermal image. For rapid diagnosis of herbicide resistance, similar approaches have been made by measuring chlorophyll fluorescence and plant temperature. Therefore, this study wasconducted to develop rapid bioassay and diagnosis methods based on phenomic technologies. Herbicides were sprayed to susceptible and resistant biotypes of *Echinochloa* species at a range of herbicide doses. Then, leaf chlorophyll fluorescence and leaf thermal image were measured using a chlorophyll fluorimeter (HandyPEA) and an infrared camera (FLIR T-420), respectively. Leaf chlorophyll fluorescence and leaf temperature behaved with increasing herbicide dose with clear discrimination between susceptible and resistant biotypes. Therefore, in this presentation, we will present perspectives of plant phenomic technologies for their potential application to herbicide bioassay and herbicide resistance diagnosis.

***Keywords:*** Phenomics, chlorophyll fluorescence, *Echinochloa*, herbicide resistance, bioassay.

## INTRODUCTION

Rapid and accurate bioassay of herbicidal molecules are an essential step for herbicide discovery. Early and accurate detection of herbicide-resistant weeds are also a key for decision-making in resistance management (Kim *et al.* 2000). Several herbicide bioassay and resistance detection methods have been developed; whole-plant bioassay, petri-dish assay, pollen germination assay, and stem node assay. Recently, we developed growth pouch method for herbicide bioassay and resistance diagnosis (Zhang *et al*. 2013). The methods for herbicide bioassay and resistance diagnosis should be rapid, accurate, cheap, reproducible and readily available, and should provide a steady result on herbicide efficacy or performance (Moss 1995). In herbicide development, bioassay of new molecules is an essential but expensive process. Many efforts have been made to develop high throughput screening (HTS) system. The HTS system mainly uses specific target sites, namely the enzymes and genes for screening but not the whole plant system. This limits the capability of HTS to screen other activities rather than the activity directly related to the selected target.

Plant phenomic technology has been used initially for crop breeding. Stress factors such as heat, cold, drought, and herbicidal damage, which could limit the photosynthetic capabilities of the plant, and this would therefore alter the chlorophyll fluorescence induction(Dayan and Zaccaro 2012). These stresses also limit plant transpiration and thus alter leaf temperature. Thermal and chlorophyll fluorescence imaging are powerful tools for the study of spatial and temporal heterogeneity of leaf transpiration and photosynthetic performance (Chaerle *et al.* 2006). Therefore, this imaging technology is a key for plant phenomics. In herbicide study, chlorophyll fluorescence measurement has been successfully applied to diagnose PS II-inhibiting herbicide resistance in several weed species (e.g. Norsworthy *et al.* 1998).

In this paper, we are going to introduce technology for plant phenomics and potential application of this technology for herbicide development and resistance diagnosis based on our results from studies conducted to develop a rapid non-destructive method by measuring leaf



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chlorophyll fluorescence and thermal temperature.

## MATERIALS AND METHODS

Resistant (R) and susceptible (S) biotypes of *Echinochloa crus-galli*, *E. oryzicola* (Im *et al.*, 2009) and *E. colona*(Kim *et al.*, 2000) were grown in pots placed in the glasshouse at Experimental Farm Station of Seoul National University, Suwon, Korea. The herbicides, cyhalofop-butyl, fenoxaprop-P-ethyl, penoxsulam, and propanil were applied to plants of *Echinochloa* spp. at a range of doses [*state the doses*] using a compressor pressurized belt-driven sprayer (R&D Sprayer, USA) equipped with an 8002E flat-fan nozzle (Spraying System Co. USA) adjusted to deliver 600 L $ha^{-1}$. All the treatments were replicated 3 times and arranged in a completely randomized design.

Following herbicide spray, leaf chlorophyll fluorescence and leaf temperature were measureduntil 10 days after herbicide treatment at a regular time interval. Measurements of chlorophyll fluorescence and thermal image were done with a portable chlorophyll fluorimeter (Handy PEA, Hansatech Instruments Ltd., UK) and an infrared camera (FLIR T420, FLIR systems Inc., Sweden), respectively. Twenty one days after herbicide treatment, the tested plants were harvested for their fresh weight. These fresh weight data were then compared with chlorophyll fluorescence and leaf temperature.

## RESULTS AND DISCUSSION

### Bioassay and resistance diagnosis using chlorophyll fluorescence

Leaf chlorophyll fluorescence ($F_v/F_m$ values)measured after herbicide treatmentshowed significant decrease ($p< 0.05$) with increasing herbicide dose regardless of herbicide modes of action (Fig. 1). The $I_{50}$ values for $F_v/F_m$ values of *E. colona* treated with propanilmeasured at 64 h after propanil treatment were 3100 g a.i. $ha^{-1}$and 1100 g a.i. $ha^{-1}$ for R and S biotypes, giving the R/S ratio of 2.8 (Fig. 1A). The parallel values for those *E. crus-galli* plants treated with cyhalofop-butyl were 1274.1 g and 512.9 g a.i. $ha^{-1}$ for R and S biotypes of Suwon when measured at 192 h after herbicide treatment, giving the R/S ratio of 2.5 (Fig. 1B). In the case of *E. oryzicola* treated with penoxsulam, the $I_{50}$ values for $F_v/F_m$ values measured at 192 h after herbicide treatment were 240.6 g and 68.7 g a.i. $ha^{-1}$ for R and S biotypes, respectively, giving the R/S ratio of 3.5 (Fig. 1C). Therefore, these results indicate that chlorophyll fluorescence may be used for herbicide bioassay and herbicide resistance diagnosis regardless of herbicide modes of action.

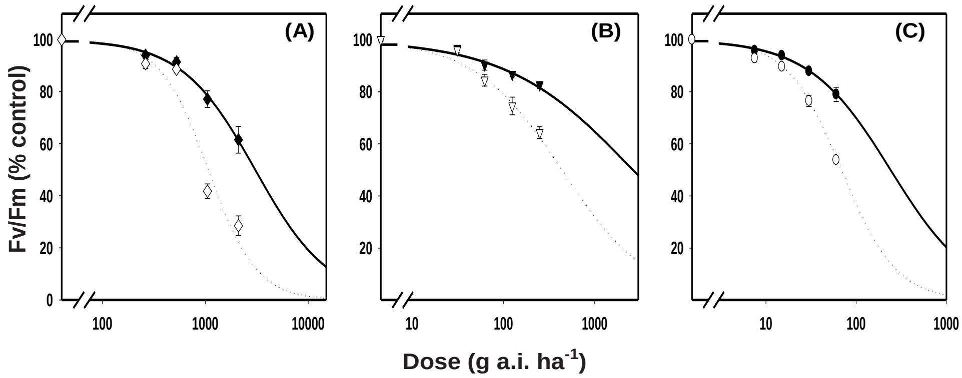


**Fig. 1.** Fv/Fm values in chlorophyll fluorescence of *E. colona* (R (◆), S (◇)) 64 h after propanil treatment (A), *E. crus-galli* (R (▼) and S (▽)) 192 h after cyhalofop-butyl treatment (B), and *E. oryzicola* (R (●) and S (○)) 192 h after penoxsulam treatment (C)

The $F_v/F_m$ values were also continuously decreased with time after herbicide treatment at the full recommended herbicide dose (Fig. 2). At the full propanil dose, Fv/Fm values were notably discriminated between R and S biotypes of *E. colona* even at 16 h after propanil treatment and reached the greatest discrimination between R and S biotypes at 64 h after



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treatment (Fig. 2A). In the cases of cyhalfop-butyl (Fig. 2B) and penoxsulam (Fig. 2C) require much longer time to show discriminative Fv/Fm values between R and S biotypes. This discrimination became notable from 144 h after herbicide treatment and significant between R and S biotypes at 168 h (Fig. 2B and 2C). Therefore, our results indicate that chlorophyll fluorescence may discriminate between R and S biotypes non-destructively in a relatively much shorter time than conventional whole plant bioassay. In this study, we used a portable chlorophyll fluorimeter, which requires a specific setting to measure chlorophyll fluorescence. If we can use fluorescence camera, acquisition of leaf chlorophyll fluorescence image is much easier and faster. This method can be incorporated into a HTS system in herbicide discovery and development.

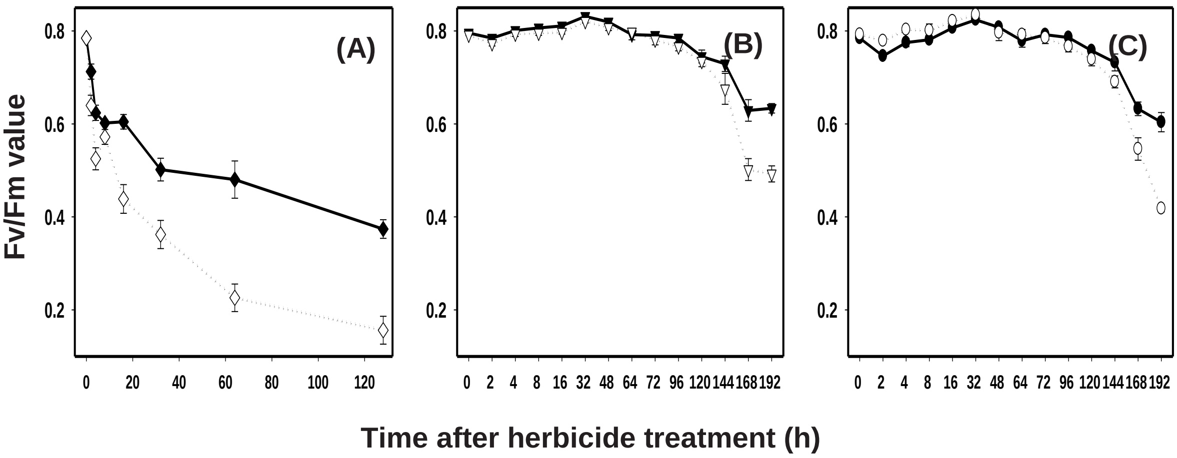


**Fig. 2.** Changes in Fv/Fm values in chlorophyll fluorescence with time at the full propanil dose for*E. colona* (R (◆) and S (◇)) (A), the full cyhalofop-butyl dose for*E. crus-galli* (R (▼) and S (▽)) (B), and the double penoxsulam dose for*E. oryzicola* (R (●) and S(○)) (C).

**Bioassay and resistance diagnosis using infra red thermal image**

Leaf temperaturemeasured 3 days after herbicide treatmentshowed significant increasewith increasing herbicide dose (Fig.3 and 4). When compared with no herbicide treatment, temperature increase was not observed in both R and S biotypes of *E. oryzicola* treated with 15 g a.i. $ha^{-1}$ penoxulam (Fig. 4). However, significant temperature increase was observed when increased penoxulam dose to 30 g a.i. $ha^{-1}$, indicating that leaf temperature may be a non-destructive parameter for herbicide bioassay, enabling to save time, space and cost for herbicide screening.

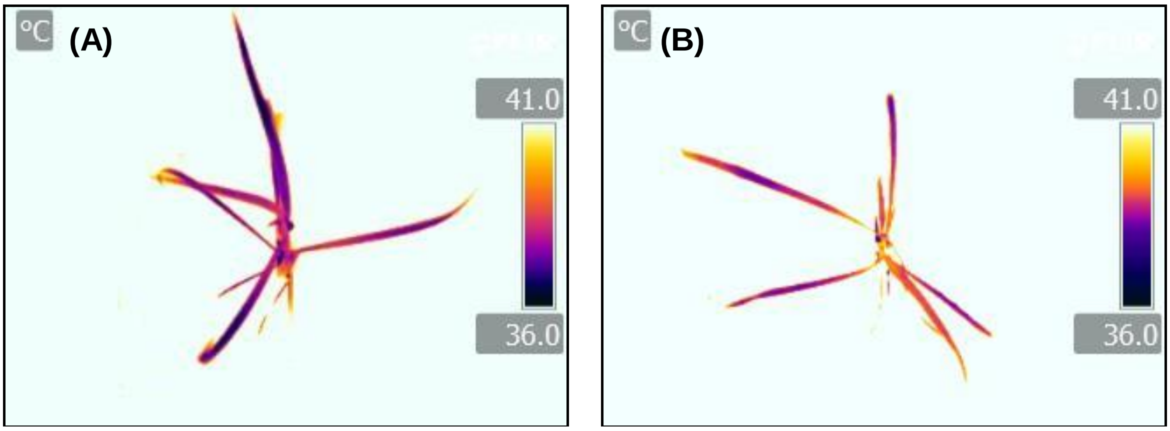


**Fig. 3.** Infra-red thermal image of *E. oryzicola*R (A) and S biotypes (B) treated with penoxsulam at 60 g a.i. $ha^{-1}$. The image was captured at 3 days after herbicide treatment.



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Difference in leaf temperature was also observed between R and S biotypes even at 3 days after herbicide treatment (Fig. 3 and 4). Greater temperature increase was observed in *E. oryzicola* S biotype as compared with R biotype when applied with 30 and 60 a.i. $ha^{-1}$ of penoxsulam. This result thus indicates that thermal image captured using infra red camera can be used to herbicide resistance diagnosis in a short period of time and non-destructive way.

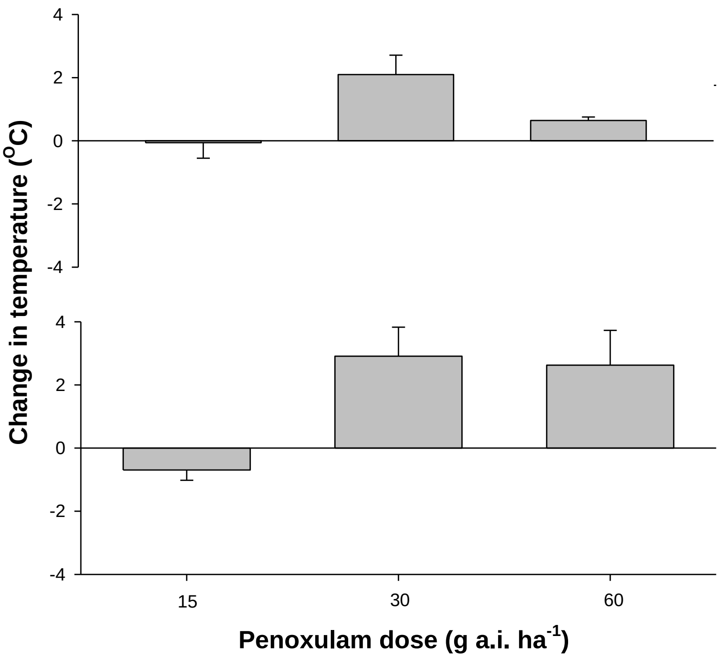


**Fig. 4.** Leaf temperature of *E. oryzicola*R (top) and S biotypes (bottom) treated with penoxsulam. The leaf temperature was measured at 3 days after herbicide treatment.

In conclusion, our findings demonstrate that chlorophyll fluorescence and leaf temperature measured non-destructively at early timing after herbicide treatment can be used for herbicide bioassay and resistance diagnosis. Therefore, these plant phenomic technologies can be applied for herbicide research and development. Other technologies associated with fluorescence and hyper-spectral image can also be considered for herbicide bioassay and resistance diagnosis.

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# IS THE GORSE POD MOTH AN EFFECTIVE BIOCONTROL AGENT OF GORSE IN NEW ZEALAND?

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**ABSTRACT** The gorse pod moth (*Cydia succedana* (Denis & Schiffermüller)) was released in New Zealand in 1992 as a biocontrol agent for gorse (*Ulex europaeus* L). Between August 2009 and April 2011 fifteen sites in the upper South Island and Lower North Island were monitored for gorse pod moth numbers and gorse seed production. Both factors varied among sites. However, overall only 27% of the gorse seeds produced were destroyed or were damaged to such an extent that they were not viable. Based on these numbers, gorse biocontrol by the gorse pod moth has not been successful, primarily because the gorse pod moth population and gorse flowering are not in synchrony.

***Keywords:*** Gorse flowering, gorse pod moth larvae, gorse seed production, phenology, seed destruction.

## INTRODUCTION

Gorse (*Ulex europaeus* L.) has been a noxious weed in New Zealand since 1900 (Moss 1960). While herbicide application has long been the major control method, reducing cost-effectiveness (Hill 1988) and environmental concerns have helped to drive the search for control alternatives, particularly bio-control. The first biocontol agent (BCA) for gorse *Exapion ulicis* Förster (gorse seed weevil – GSW) was released in New Zealand in 1931 (Kuschel, 1972). It has been reported to infest up to 90% of spring produced gorse seeds (Hill *et al* 1991), but because it is only active over 2-3 months in spring/summer, any gorse seeds produced in autumn escape predation. In New Zealand gorse is bivoltine (Sixtus, 2004).

In 1992 *Cydia succedana* (Denis & Schiffermüller) was released in New Zealand (Harman *et al.* 1996; Suckling *et al.* 1999) following confirmation that it was gorse-host specific (Hill *et al.* 1999). Since release, this BCA has been recorded throughout New Zealand, although its effectiveness in controlling gorse has not been recently assessed. In this study we monitored gorse pod moth (GPM) populations at fifteen sites in New Zealand and determined gorse seed damage as a result of GPM attack.

## MATERIALS AND METHODS

### Sample sites

The sites for this study were located throughout the upper South Island and lower North Island of New Zealand (Fig. 1). At each site, 15 gorse bushes were randomly selected and permanently identified for data collection over 12-18 months. The reproductive stage of the gorse was estimated each month (percentage bud, flower, green pod and black pod).



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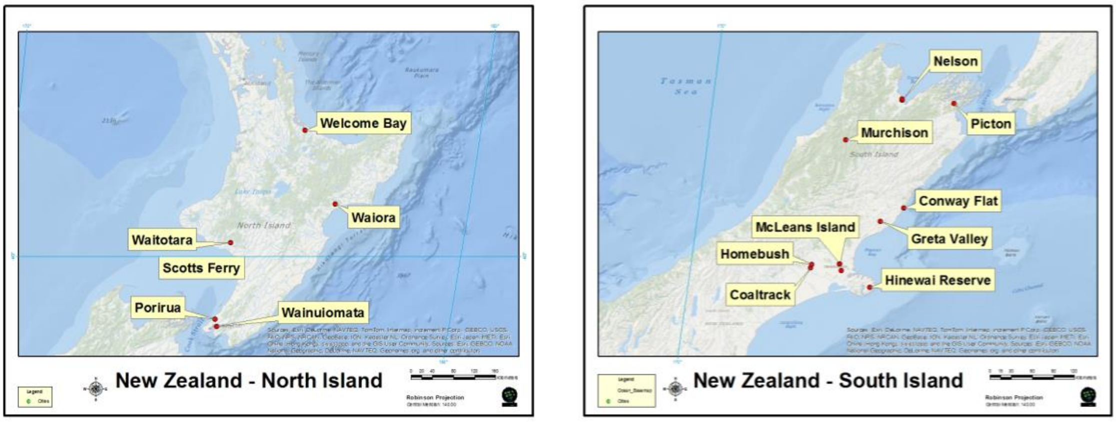


**Fig. 1**. Map of a) South Island, and b) North Island New Zealand showing locations of field sites.

Each month, provided there were sufficient black (= mature) pods present, samples of 30 pods were collected at random from the sample bush. Pods were opened by hand and sorted into intact pods (no evidence of damage), or damaged pods. The number of infected pods was counted: those damaged by GPM larvae or those damaged by GSW larvae. Seeds from GPM damaged pods were divided into four categories and counted: (i) seeds not damaged by larvae; (ii) seeds partly eaten by larvae; (iii) seeds eaten by larvae (as determined by the seed pedicels in the pod for which a seed was absent); and (iv) all contents eaten inside the pod by larvae, for which the count was assumed to be the same as for intact pods. For this study, data are presented as means per site (15 plants).

Moth population was monitored by placing pheromone traps containing (*E,E*)-8,10-dodecadien-1-yl acetate, which only attracted and trapped male GPM (Suckling *et al.* 1999). Five traps were placed at each site. The sticky bases were changed monthly when the reproductive stages of the gorse plants were monitored.

## RESULTS

Pod damage as a result of GPM attack varied markedly both within and among sites (Table 1). At five of the sites, all in the South Island, more pods were damaged by GSWthan GPM at the majority of assessment times (Table 1). Where GSW was present the mean percentage of pods/plant damaged by GPM was correspondingly low.

The percentage of pods damaged by GPM ranged from 3% at Coaltrack to 55% at Wairoa, with an overall mean of 21% (Table 1). Only at two North Island sites (Onepoto Bay and Wairoa) were more than 50% of the pods damaged. For the majority of sites most damage occurred in summer (Dec. – Feb.), but at other sites most damage was either in spring (Sept. – Nov.) or autumn (Mar. – May) (Table 1).

At most sites gorse flowering peaks were only poorly synchronised with GPM activity. Data for one South Island and one North Island site are presented in Fig. 2. At both Coaltrack and Waitotara, GPM numbers peaked in months when gorse was either not flowering or not flowering prolifically (Fig. 2).

## DISCUSSION

Both the GSW and GPM were present at all sites. GSW has been reported to attack up to 90% of spring produced gorse seeds in the central South Island (Hill *et al.* 1991) but less than 40% in the northern North Island (Cowley 1983). In the present study over 80% of gorse pods were damaged by GSW on at least one assessment date at Homebush, Coaltrack, Tasman and Wairoa, and over 50% of pods were damaged at three other sites. When GSW was active in late spring/early summer, pod damage was generally greater than that caused by GPM in the same time frame.



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**Table 1.** Percentage of gorse pods/plant damaged by gorse seed weevil and gorse pod moth, percentage of total gorse seeds plant damaged by gorse pod moth and month when highest damage occurred.

| Site[1] | % pods/plant damaged by GSW[2] | % pods/plant damaged by GPM[3] | % seeds/plant damaged or destroyed by GPM | Month of highest damage by GPM |
|---|---|---|---|---|
| Homebush | 33 | 16 | 18 | Sep 2009 |
| Coaltrack | 46 | 3 | 11 | Oct 2009 |
| Hinewai | 51 | 5 | 12 | Mar 2011 |
| Reserve | 4 | 9 | 7 | Dec 2010 |
| Greta Valley | 15 | 6 | 1 | Nov 2009 |
| Conway Flat | 62 | 23 | 61 | Jan 2010 |
| Murchison | 18 | 21 | 25 | Dec 2009 |
| Tasman | 11 | 30 | 39 | Dec 2010 |
| Picton | 0 | 23 | 22 | Mar 2010 |
| Wainuiomata | 5 | 54 | 55 | Sep 2010 |
| Onepoto Bay | 1 | 19 | 19 | Mar 2010 |
| Scotts Ferry | 4 | 18 | 18 | Dec 2009 |
| Waitotara | 33 | 55 | 65 | Jan 2011 |
| Wairoa | 16 | 16 | 18 | Dec 2009 |
| Welcome Bay | 21 | 21 | 27 | |
| Mean | | | | |

[1]No mature pods were produced at the McLeans Island site; [2]gorse seed weevil; [3]gorse pod moth

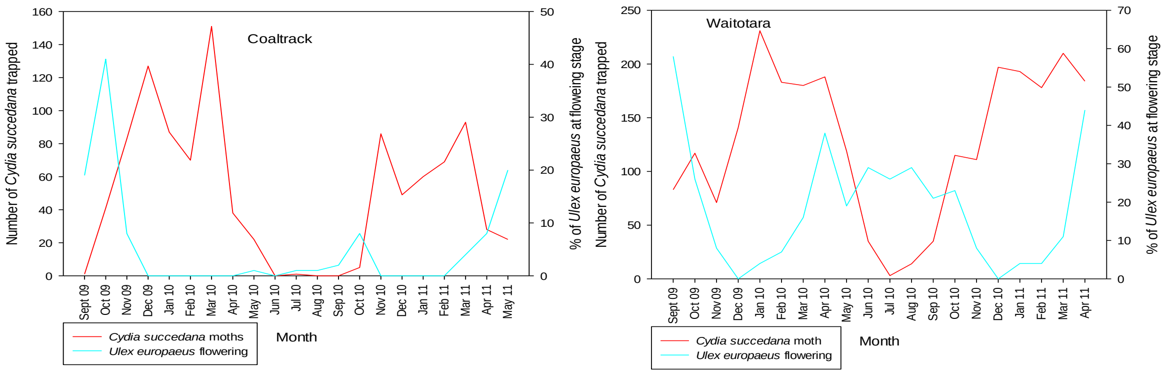


**Fig. 2.** The monthly mean number of male GPM trapped and the mean estimated percentage of gorseflowering during the period of the study for the Coaltrack and Waitotara sites.

GPM pod damage was greater at the North Island sites than for those in the South Island, but the greatest percentage of pods damaged at any individual assessment date was less than 20% at six sites, less than 60% at eight sites, but 90% at one assessment time at

Wainuiomata. Sixtus (2004) had previously reported GPM damage of only 10-20% of gorse pods at other South Island sites. The percentage of seeds damaged/destroyed by GPM varied markedly among the sites, ranging from 1 to 65%. Hill and Gourlay (2002) reported that a single GPM larva eats the seed in two-three gorse pods, but the high percentage of undamaged seeds (range 35-99%) illustrates how few larvae were present at the times seeds were being produced.

First-instar larvae of GPM enter the gorse plant through a fertilized flower, with the likelihood of then entering a green gorse pod being considered small (Hill and Gourlay 2002).



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GPM eggs hatch eight days after being laid , and the larval period is 6-7 weeks (Hill and Gourlay 2002). The ability for GPM to provide biocontrol therefore requires synchrony between the presence of GPM and flowering gorse. At most sites spring flowering (September – October) occurred 2-4 months before peak GPM populations (December – March), and at sites where flowers were present in autumn and winter, the GPM population was very low. The latter is likely to be temperature related. *Cydia* spp. appear to have a threshold temperature of around 10°C (Rock and Schaffer, 1983; Sixtus, 2004) and although the minimum temperature at which GPM becomes inactive has not been determined, minimum temperatures at all sites during winter were less than 10°C. Interestingly GSW has a threshold temperature of 7°C (Yeoh and Woodburn 2003), which may explain why, at the majority of the South Island sites, more gorse pod damage was caused by GSW than GPM.

In New Zealand, the land under and around gorse plants can receive 500-950 seeds/$m^2$ annually (Ivens, 1983). Even the best control offered by GPM would still leave a substantial number of seeds able to enter the soil seed bank. Both Crawley (1990) and Myers and Risley (2000) noted that seed-feeding biological control agents will be ineffective unless there are very high levels of seed predation. Unfortunately, in New Zealand, GPM does not damage or destroy enough gorse seeds to be a fully effective biological control agent.

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## *Tillage Practices in Weed Management Practices*

# THE EFFECT OF TILLAGE SYSTEMS AND TIME OF WEEDING ON THE GROWTH AND YIELD OF CORN (*ZEA MAYS* L.)

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**ABSTRACT** A field experiment to study the effect of tillage and time of weeding on the growth and yield of corn (*Zea mays* L.) was conducted at Lawang Subdistrict, Malang District, East Java Province, Indonesian from February 2011 until June 2011. The experiment was arranged in split plot design with three replications. Main plots were tillage systems consits of three levels., 1) no- tillage, 2) minimum tillage,3) maximum tillage and the subplots were time of weeding consist of four levels., 1) no weeding, 2) weeding at 21 days after planting (dap), 3) weeding at 21 and 42 days after planting, 4) weeding at 21,42 and 63 days after planting. The result showed that the dry weight of weed in no-tillage did not significantly different with minimum tillage and maximum tillage. The yield of corn was higher at no- tillage combined by weeding three times at 21, 42 and 63 days after planting.

***Keywords***: Corn, land preparation, weeding.

## INTRODUCTION

Corn is the second most important agricultural crop in Asia next to rice. A corn field is usually infested with a mixture of sedges, grasses and broadleaf weeds (Mercado, 1979). Weed reduce corn yield by competiting for moisture, nutrient, and light during growth season and interfere with harvest (Ferrel, *et al*, 2012). Akobundu (1983) stated that a primary consideration in no-tillage crop production is weed control. Efective weed control, practiced with little or no soil disturbance, is the key to modern no tillage system (Warren, 1983). Remison (1979) stated that two hand-weeding at 3 and 7 week after planting were found adequate for weed control. In Indonesia, hand weeding and mechanical control is most common weed control method employed in corn fields. No-tillage system is a new technology for land preparation in corn field.

## MATERIALS AND METHODS

The experiment was conducted at Lawang Subdistrict, Malang District, East Java Province, Indonesian from February 2011 until June 2011. The experiment was arranged in split plot design with three replications. The main plots were tillage systems consits of three levels., 1) no-tillage, 2) minimum tillage,3) maximum tillage and the subplots were time of weeding consist of four level., 1) no weeding, 2) weeding at 21 days after planting (dap), 3) weeding at 21 and 42 days after planting, 4) weeding at 21,42 and 63 days after planting. At maximum tillage plots were plowed by hoe to a depth of 20 – 25 cm two times with interval one weeks. At minimum tillage plots were plowed by hoe ones times. At no-tillage plots, plots were sprayed by glyphosate at dosage 1 l $ha^{-1}$. The maize variety Pioneer 21 was used. The corn seed was sown at planting distance 70 cm between row and 25 cm within row. Two seeds were sown per hole. Several plant parameters were measured at various growth stages. This included weed dry weight, plant height plant dry weight and grain yields. The data were analysed by analysis of variance, and tested for Least Significant Differences (LSD).



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## RESULTS AND DISCUSSION

**Weed dry weight**. Weed dry weight as affected by interaction between tillage system and time of weeding at 70 days after planting (dap) (Tabel 1). At tillage system treatment showed that weed dry weight was higher at no weeding treatment. At time of weeding treatment, weed dry weight was lower if weeding at 21,42 and 63 days after planting. Weed dry weight did not significantly different between no- tillage, minimum tillage or maximum tillage treatment if weeding was done at 21,42 and 63 days after planting.

**Plant height, plant dry weight and leaf area index .** Plant height, plant dry weight and leaf area index as affected by tillage system treatment (Tabel 2). At tillage system treatment, plant height, plant dry weight and leaf area index was higher at no- tillage. At time of weeding treatment, plant height and leaf area index did not significantly different between weeding at 21 and 42 dap with weeding at 21, 42, 63 dap.

**Corn yield.** Corn yield as affected by interaction between tillage system and time of weeding (Tabel 3). Corn yield was higher at no tillage treatment combined by weeding at 21,42 and 63 days after planting.

**Table 1**. Dry weight of weed (g $m^{-1}$) as affected by interaction between tillage system and time of weeding 70 days after planting (dap).

| Tillage systems | Time of weeding | | | |
|---|---|---|---|---|
| | No weeding | Weeding at 21 dap | Weeding at 21 and 42 dap | Weeding at 21,42 and 63 dap |
| No-tillage | 120.93 g | 48.93 d | 16.50 b | 6.43 a |
| Minimum tillage | 117.53 g | 84.80 f | 24.30 c | 7.73 a |
| Maximum tillage | 117.40 g | 79.60 e | 21.40 bc | 8.80 a |
| LSD 5 % | | 4.56 | | |

Notes: Numbers followed same letter did not significantly different at LSD test 5 %.

**Table 2.** Plant height and plant dry weight as affected by tillage system and time of at 70 days after planting (dap).

| Treatment | Plant height (cm) | Plant dry weight (g) | Leaf area index |
|---|---|---|---|
| Tillage system | | | |
| No-tillage | 190.35 b | 205.11 b | 2.26 b |
| Minimal tillage | 184.07 a | 197.03 a | 1.82 a |
| Maximum tillage | 181.88 a | 194.28 a | 1.84 a |
| LSD 5 % | 2.36 | 4.38 | |
| Time of weeding | | | |
| No weeding | 190.61 a | 196.62 | 1.82 a |
| Weeding at 21 dap | 194.55 b | 197.17 | 1.96 ab |
| Weeding at 21 and 42 dap | 199.38 c | 200.52 | 2.14 b |
| Weeding at 21, 42 and 63 dap | 197.18 c | 200.91 | 2.19 b |
| LSD 5 % | 2.32 | ns | 0.24 |

Notes: Numbers followed same letter are not significantly different at LSD test 5 %. ns. non significant.



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**Table 3.** Corn yield (t $ha^{-1}$) as affected by interaction between tillage system and time of weeding.

| Tillage system | Time of weeding | | | |
|---|---|---|---|---|
| | No weeding | Weeding at 21 dap | Weeding at 21 and 42 dap | Weeding at 21, 42 and 63 dap |
| No tillage | 5.85 ab | 5.90 ab | 6.11 c | 6.27 d |
| Minimum tillage | 5.84 ab | 5.77 a | 5.90 ab | 5.95 b |
| Maximum tillage | 5.83 ab | 5.88 ab | 5.94 b | 5.93 b |
| LSD 5 % | 0,15 | | | |

Notes: Numbers followed same letter are not significantly different at 5 % LSD test.

## CONCLUSIONS

The dry weight of weed in no-tillage did not significantly different with minimum tillage and maximum tillage. The yield of corn was higher at no- tillage combined by weeding three times at 21, 42 and 63 days after planting.

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# WEED CONTROL PRACTICES IN MAIZE (*ZEA MAYS* L.) UNDER CONVENTIONAL AND CONSERVATION TILLAGE PRACTICES

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**ABSTRACT** Weed infestation was a major constraint in maize production resulting in yield reduction of 24 - 83 %. Aggressive growth of broadleaved weeds dominated by several species of *Polygonum* (*P. pensylvanicum, P. persicaria, P. orientale*), *Stellaria media, Stellaria aquatica, Oldelandia diffusa, Oldenlandia umbellate, Physalis minima, Solanum niagrum,* cover the land rapidly and suppress the growth of maize seedlings. For tackling the weed problem the experiments were conducted to grow winter maize (Hybrid variety 900MGold) after rainy season rice crop under different crop establishment techniques (conventional and conservation tillage). 12 treatment combinations in which pre-emergence herbicides atrazine (1.0 kg/ha) and pendimethalin (0.50 kg/ha), post-emergence herbicide atrazine (1.0 kg/ha), weedy control and weed-free control treatments were tested for consecutive two years in Split Plot Design with three replications. Conservation tillage was performed by using 11 tynes zero tilled inclined plate metering seed drill within anchor residue (2.19 tonnes/ha with 20 cm height) of previous rice crop. The anchor residue in conservation tillage reduced weed growth of 46 to 50%. Integrated weed management practices composed by conservation tillage + atrazine as pre-emergence+atrazine as post-emergence registered highest weed control efficiency (95.75% to 98.04% at 30 days after sowing/DAS), lowest weed index (0.32% to 0.73% ) and higher grain yield (81.03 to 81.73 q/ha) statistically at par with weed-free control treatment (81.63 to 82.00 q/ha) in conservation tillage. 6.80 to 6.93 q/ha more grain yield was recorded than that of complete weed-free control plot of conventional tillage. Highest values of net return (Rupees 70,469 to 82,125/ha) and net return per rupee invested (2.70 to 2.74) were recorded by this treatment. Saving of Rupees 1549 to 1989/ha (1$=Rupees 60) as input cost was registered due to adaptation of conservation tillage.

***Keywords*:** Conservation tillage, conventional tillage, atrazine, pendimethalin.

## INTRODUCTION

*Polygonum spp.* (*Polygonum pensylvanicum, Polygonum persicaria, Polygonum orientale*), *Stellaria media, Oldelandia diffusa, , Physalis minima, Solanum nigrum* have been considered as one of the major constraints in maize production in *tarai* region of West Bengal. Profuse growth of several weeds with high invasive capacity coupled with poor fertility status often become limiting factor in maize cultivation in this region. Weed infestation is becoming a major constraint in maize production systems and it is reported that weeds reduced maize yield by 24-83% (Ashiq *et al.*, 2003; Dogan *et al.*, 2004). Control of weeds in maize is, therefore, very essential for obtaining a good crop harvest. The weeds also tend to shift with the change in tillage practices, management practices in term of irrigation, nutrient, and cropping system adopted, although there are other factors that govern the changes in the weed flora (Kamble *et al.* 2005). Increased weed problems and other irreversible damages that is breaking and imposing enforced dormancy of old and new seeds of weeds, loss of soil organic carbon, destruction of soil structure, creating hard pan at rhizoshpere zone, burning of fossil fuel, release of hydrocarbon in the atmosphere, rapid emergence and high growth rate of weeds especially at the initial phase of crop growth caused by the conventional soil turning tillage practice led to the need of exploring alternate crop establishment techniques for reducing damage on physicochemical and biological properties of soil, improving its resilience capacity and tackling weed problem by minimising chances of cyclic perpetuation of breaking and imposing enforced



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dormancy of weed seeds. Therefore, crop establishment through conservation tillage, which is characterized by minimum soil disturbance with residue management, was taken into consideration with the view of reducing soil turning process and harnessing the capacity of crop residue as mulch for inhibiting emergence and growth of weeds, conserving soil moisture and maintaining soil temperature. Keeping the above mentioned facts in view the experiments were conducted with objectives to study comparative efficiency of weed control practices and to work out economics in maize cultivation under conventional soil turning tillage and conservation tillage practices.

## MATERIALS AND METHODS

The field experiments were carried out during winter (*rabi*) seasons of 2011-12 and 2012-13 at the research farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal. The experiment was laid out in a split plot design with three replications in which tillage practices (Conventional soil turning tillage and conservation tillage) were assigned to main plots and weed control practices constituting 12 treatment combinations were assigned to sub-plots. The weed control treatments were atrazine 1.0 kg/ha as pre-emergence + hand weeding at 30 days after sowing (DAS), atrazine 1.0 kg/ha as pre-emergence + atrazine 1.0 kg/ha[1] post-emergence applied at 30 DAS, pendimethalin 0.50 kg/ha as pre-emergence + atrazine 1.0 kg/ha as post-emergence applied at 30 DAS, atrazine 1.0 kg/ha as post-emergence applied at 30 DAS, complete weed- free control, weedy control. The ultimate plot size was 15 x 3.5 $m^2$. The soil of the experimental field was sandy loam in texture having a pH ranging from 5.41 to 5.45, 0.59 % organic carbon content, low in available nitrogen (112.25 kg/ha), medium in available phosphorus (18.21 kg/ha) and low in available potassium (89.53 kg/ha). Maize variety DEKALB 900M Gold having 70 cm x 30 cm spacing was sown in 27$^{th}$ January and 2$^{nd}$ February and crop was harvested on 28$^{th}$ and 30$^{th}$ May in the year 2011-12 and 2012-13, respectively in both tillage practices. In conservation tillage glyphosate 1.5 kg/ha was applied seven days before sowing as pre-plant desiccators to kill the existing weed flora. The NPK ratio of 150:60:60 kg/ha was applied in both tillage practices. In case of conventional tillage nitrogen was applied in three equal splits each of 50 kg N/ha as basal, 30 days after sowing (early knee high stage) and 45 DAS (before tasseling stage). The entire dose of phosphorus and potassium (60 kg $P_2O_5$ and 60 kg $K_2O$/ha) were applied as basal along with nitrogen (50 kg N/ha) during final land preparation. In case of conservation tillage, a measured amount of granular fertilizer (NPK ratio 10:26:26) 231 kg/ha was applied during sowing and the amount (231 kg/ha) was measured on the basis of basal application of 60 kg $P_2O_5$ $ha^{-1}$ and 60 kg $K_2O$ $ha^{-1}$. Through the granular fertilizer 23 kg N/ha was applied during sowing and remaining 117 kg N/ha was applied in three equal split of each of 39 kg N/ha. 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ split were applied at 6 leaf stage of crop (22 DAS), 30 DAS and 45 DAS, respectively. The density and dry weight of weeds was recorded using quadrate (0.25 $m^2$) placed randomly at three places of each plot. Weed data were subjected to square root transformation ($\sqrt{X+0.5}$) before statistical analysis. The data of weed dry weight was recorded at 30 and 60 DAS.

## RESULTS AND DISCUSSION

### Effect on weeds

The weed flora observed in the experimental plot were *Polygonum pensylvanicum, Polygonum persicaria, Polygonum orientale, Oldelandia diffusa, Oldenlandia umbellate, Stellaria media, Stellaria aquatica, Physalis minima, Solanum nigrum, Chenopodium album, Cynodon dactylon, Cyperus rotundus* and *Digitaria sanguinalis*. Among these weeds *Polygonum spp, Oldenlandia diffusa, Cynodone dactylon* and *Digitaria sanguinalis* were dominant weeds in the conservation tillage plots.

Different crop establishment technique along with application of herbicides significantly influenced the weed dry weight and weed control efficiency at 30 DAS and 60 DAS in both the years. Among the weed control treatments combination of conservation tillage



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with atrazine as pre-emergence + atrazine as post-emergence recorded the lowest weed dry weight and highest weed control efficiency (92 to 96% at 30 DAS and 75 to 76% at 60 DAS) (Table 1). Highest dry weight and lowest weed control efficiency were found in conventional tillage with atrazine as post-emergence. Toxicity of atrazine on weeds in term of preventing germination of weed seeds resulted in higher weed control efficiency in comparison to pendimethalin which has recorded lower weed control efficiency values.


**Fig. 1.** Anchor residue in conservation tillage act as surface mulch for suppressing growth of weeds.

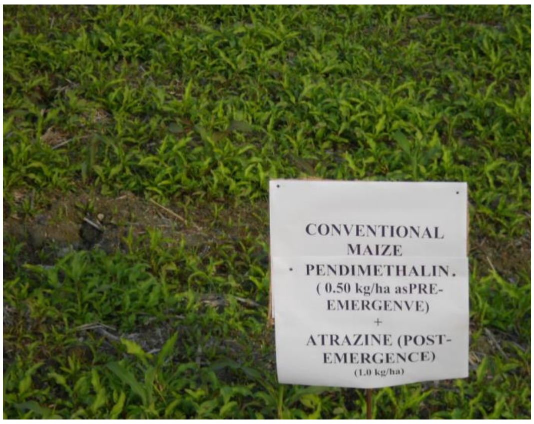


**Fig. 2.** Aggressive weed growth in conven-tional soil turning tillage.

Combination of conservation tillage and herbicide application gave comparatively higher weed control efficiency against the respective herbicides tested in conventional tillage. Conservation tillage alone because of its capacity to suppress the growth of the weed recorded the weed control efficiency of 46% to 48% at 30 DAS and almost 51% at 60 DAS (Table 2) in which weed dry weight value of weedy control plot of conservation tillage was compared against weedy control plot of conventional tillage. Weed smothering effect of conservation tillage amplified herbicidal effect on weed control and because of this reason lower weed control efficiency (22 to 24% at 30 DAS and 32-33% at 60 DAS) of pendimethalin in conventional tillage was increased to higher value of 81 to 82% in conservation tillage at 30 DAS and 71 to73% at 60 DAS (Table 2) when weed dry weight of pendimethalin treated plot under conservation tillage was compared against the weed dry weight of weedy control plot of conventional tillage (Ishyaya *et al.*, 2008). Atrazine alone as post-emergence treatment applied at 30 DAS showed lower weed control efficiency especially in conventional tillage and this is because of the fact that atrazine shows contact action on weeds at juvenile stage. Aggressive growth of weeds in conventional tillage reflected as higher weed dry weight at 30 DAS and that in turn reduced the contact activity of atrazine. However, its combined application with other herbicides improved its contact action leading to comparatively higher weed control efficiency value when it is applied in combination with pendimethalin and atrazine as pre-emergence. Even it was so much effective when post-emergence application of atrazine was made after its pre-emergence treatment in comparison to the treatment atrazine+hand weeding. Similar finding was reported by Kolage *et al.* (2004); Gopinath and Kundu (2008).



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**Fig. 3.** Weed control by atrazine (pre-emergence) + atrazine (post-emergence) in conservation tillage incontrol by atrazine (pre-emergence) + hand weeding incontrol by atrazine (pre-emergence)+hand weeding tillage.

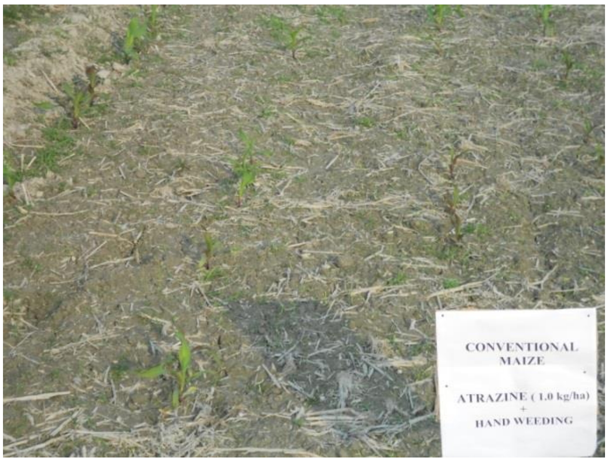


**Fig. 4.** Weed control with Atrazine (pre-emergence) + atrazine (post-emergence) in conventional tillage.

**Effects on crops**

All the weed control treatments of different crop establishment techniques significantly increased the yield attributing characters and yield of maize compared with weedy control treatment (Table 3). Selective nature of the treatment atrazine as pre-emergence and atrazine as post-emergence resulted in higher values of yield attributes and grain yield. It is important to mention that 9.2% more yield was obtained in weed-free control treatment under conservation tillage than the same treatment tested in conventional tillage. In addition to weed suppression capacity, conservation tillage also conserves soil moisture and maintains soil temperature. These altogether influence better nutrient absorption and root growth. Once the weed factor was eliminated the significant variation on yield performance between conservation tillage and conventional tillage was mainly due to the effect of conservation tillage on soil moisture conservation, less diurnal fluctuation of soil temperature and placement of fertilizer through zero tillage machine. These effects along with weed suppression capacity of conservation tillage resulted in higher grain yield in the treatments under conservation tillage which was significantly higher than respective treatment tested under conventional tillage. Integrated weed management practices including conservation tillage + atrazine 1.0 kg/ha as pre-emergence+atrazine 1.0 kg/ha as post-emergence registered higher grain yield (81.03 to 81.73 q/ha) which was statistically at par with weed-free control treatment (81.63 to 82.00 q/ha) in conservation tillage (Table 3) (Khajanji *et al.,* 2006). This treatment in conservation tillage registered 7.06 to 7.36 q/ha more grain yield than that of conventional soil turning tillage. Even the same treatment in conservation tillage registered 6.80 to 6.93 q/ha more grain yield than that of complete weed-free control plot of conventional tillage (Patel *et al.,* 2000 and Kolage *et al.,* 2004).

**Economics**

Adoption of conservation tillage saved the input cost of Rs. 1549 to 1989/ha in comparison to conventional tillage. Large variation on net return between two years was primarily due to difference on support price (Rs 980/q in 2011 and Rs 1175/q in 2012 Ishaya *et al.* (2008) observed a favourable net return in zero and minimum tillage over conventional tillage and concluded that these systems are economically sound, and hence acceptable to producers.



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The treatment atrazine (pre-emergence)+atrazine (post-emergence) under conservation tillage registered Rs 8,453 to Rs 10,846/ha more net return than its net return under conventional tillage (Table 4). This was mainly due to saving of input cost and more grain yield under conservation tillage in comparison to conventional tillage. The maximum net return of Rs. 70,469/ha to Rs. 82,125/ha was obtained in conservation tillage with atrazine (pre-emergence) + atrazine (post-emergence) treatment resulting in highest value of net return per rupee invested (Rs 2.70 to Rs. 2.74 per rupee invested) during both the year of experimentation (Singh *et al.,* 2009; Gopinath and Kundu, 2008). Lowest of net return per rupee invested was found in conventional tillage with pendimethaline as pre-emergence + atrazine as post-emergence treatment (Rs. 0.97 to Rs. 0.92 per rupee invested).

## CONCLUSIONS

Conservation tillage has the capacity to suppress the growth of weed. The anchor residue of previous rice crop alone conferred the weed control efficiency to the tune of 46 and 50% in comparison to weedy growth of conventional tillage. Integrated weed management practices including conservation tillage + atrazine 1.0 kg/ha as pre-emergence+atrazine 1.0 kg/ha as post-emergence registered highest weed control efficiency and that in turn reflected as higher grain yield statistically at per with complete weed free-condition in conservation tillage and significantly higher than that obtained in complete weed-free plot of conventional tillage. This was because of placement of fertilizer and conservation of moisture through surface mulching of anchor residue. Highest values of net return and net return per rupee invested were also recorded in conservation tillage+atrazine as pre-emergence+atrazine as post-emergence. Saving of input cost of Rs. 1549 to 1989/ha was recorded due to adoption of conservation tillage practices in comparison to conventional tillage.Therefore, it could be concluded that implication of weed management practices including atrazine 1.0 kg/ha as pre-emergence followed by atrazine 1.0 kg/ha as post-emergence (at 30 DAS) in maize cultivation with the adoption of crop establishment through conservation tillage became highly profitable over conventional soil turning tillage practice.

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**Table1.** Effect of treatment combinations on weed dry weight (g/m$^2$) and weed control efficiency (%)

| Treatment combinations | Weed dry weight at 30 DAS | | Weed dry weight at 60 DAS | | Weed control efficiency at 30 DAS | | Weed control efficiency at 60 DAS | |
|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 |
| **CNT+Atrazine as pre-emergence + hand weeding at 30 DAS** | 1.76(2.61) | 2.23(4.53) | 3.93(14.96) | 4.10(16.29) | 90.33 | 84.67 | 73.71 | 72.61 |
| **CNT+Atrazine as pre-emergence + Atrazine as post-emergence** | 1.59(2.06) | 2.30(4.79) | 3.81(14.04) | 3.87(14.49) | 92.38 | 83.77 | 75.34 | 75.64 |
| **CNT+ Pendimethalin as pre-emergence + Atrazine as post-emergence** | 4.56(20.40) | 4.84(22.98) | 6.23(38.42) | 6.42(40.76) | 24.40 | 22.15 | 32.49 | 31.47 |
| **CNT+ Atrazine as post-emergence** | 5.17(26.26) | 5.45(29.24) | 6.36(39.95) | 6.56(42.56) | 2.67 | 0.94 | 29.81 | 28.44 |
| **CNT+ Weed-free control** | 0.71(0.00) | 0.71(0.00) | 0.71(0.00) | 0.71(0.00) | 100.00 | 100.00 | 100.00 | 100.00 |
| **CNT+ Weedy control** | 5.24(26.48) | 5.48(29.52) | 7.57(51.91) | 7.74(59.48) | - | - | - | - |
| **CST+Atrazine as pre-emergence + hand weeding at 30 DAS** | 1.10(0.72) | 1.37(1.44) | 2.70(6.88) | 2.80(7.38) | 95.11 | 90.58 | 75.48 | 74.88 |
| **CST+Atrazine as pre-emergence + Atrazine as post-emergence** | 1.01(0.53) | 1.31(1.25) | 2.66(6.61) | 2.79(7.33) | 96.38 | 91.80 | 76.44 | 75.05 |
| **CST+Pendimethalin as pre-emergence + Atrazine as post-emergence** | 2.30(4.84) | 2.47(5.64) | 4.00(15.54) | 4.19(17.10) | 66.95 | 63.14 | 44.61 | 41.79 |
| **CST+Atrazine as post-emergence** | 3.84(19.29) | 3.96(15.19) | 4.05(15.94) | 4.29(17.96) | 2.44 | 0.65 | 43.17 | 38.88 |
| **CST+Weed-free control** | 0.71(0.00) | 0.71(0.00) | 0.71(0.00) | 0.71(0.00) | 100.00 | 100.00 | 100.00 | 100.00 |
| **CST+Weedy control** | 3.89(14.65) | 3.97(15.29) | 5.34(28.06) | 5.46(29.38) | _ | _ | _ | - |
| **CD (*P*=0.05)** | NS | 0.36 | 0.39 | 0.31 | - | - | - | - |

Data subjected to square root of transformation ($\sqrt{X+0.5}$). Figure in parenthesis indicates original value.
CNT-Conventional soil turning tillage, CST-Conservation tillage.



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**Table 2.** Interaction effect between tillage practices and weed control practices on weed control efficiency (%) based on weedy control treatment of conventional tillage practice at 30 DAS and 60 DAS

| **Treatment combinations** | **Weed control efficiency at 30 DAS** | | **Weed control efficiency at 60 DAS** | |
|---|---|---|---|---|
| | **2011** | **2012** | **2011** | **2012** |
| **CNT+Atrazine as pre-emergence + hand weeding at 30 DAS** | 90.33 | 84.67 | 73.71 | 72.61 |
| **CNT+Atrazine as pre-emergence + Atrazine as post-emergence** | 92.38 | 83.77 | 75.34 | 75.64 |
| **CNT+Pendimethalin as pre-emergence + Atrazine as post-emergence** | 24.40 | 22.15 | 32.49 | 31.47 |
| **CNT+Atrazine as post-emergence** | 2.67 | 0.94 | 29.81 | 28.44 |
| **CNT+Weed-free control** | 100.00 | 100.00 | 100.00 | 100.00 |
| **CNT+Weedy control** | - | - | - | - |
| **CST+Atrazine as pre-emergence + hand weeding at 30 DAS** | 97.34 | 95.12 | 87.91 | 87.59 |
| **CST+Atrazine as pre-emergence + Atrazine as post-emergence** | 98.04 | 95.75 | 88.39 | 87.68 |
| **CST+Pendimethalin as pre-emergence + Atrazine as post-emergence** | 82.06 | 80.91 | 72.69 | 71.24 |
| **CST+Atrazine as post-emergence** | 47.03 | 48.53 | 71.99 | 69.81 |
| **CST+Weed-free control** | 100.00 | 100.00 | 100.00 | 100.00 |
| **CST+Weedy control** | 45.71 | 48.19 | 50.70 | 50.60 |

CNT-Conventional soil turning tillage, CST-Conservation tillage



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**Table 3.** Effect of treatment combinations on yield attributes, grain yield, harvest index, stover yield and weed index.

| Treatment combinations | Cob length (cm) | | Girth of Cob (cm) | | 1000 seed weight (g) | | Grain yield (q ha$^{-1}$) | | Harvest index (%) | | Stover yield (q ha$^{-1}$) | | Shelling percentage | | Weed index (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **2011** | **2012** | **2011** | **2012** | **2011** | **2012** | **2011** | **2012** | **2011** | **2012** | **2011** | **2012** | **2011** | **2012** | **2011** | **2012** |
| **CNT+Atrazine as pre-emergence + hand weeding at 30 DAS** | 15.33 | 15.00 | 15.67 | 15.00 | 294.67 | 294.00 | 74.30 | 73.60 | 31.13 | 30.83 | 164.38 | 165.13 | 83.07 | 82.47 | 0.84 | 0.67 |
| **CNT+Atrazine as pre-emergence + Atrazine as post-emergence** | 15.67 | 15.33 | 16.00 | 15.67 | 295.67 | 294.67 | 74.67 | 73.67 | 31.13 | 30.70 | 165.24 | 166.33 | 83.33 | 82.70 | 0.35 | 0.58 |
| **CNT+Pendimethalin as pre-emergence + Atrazine as post-emergence** | 14.33 | 14.00 | 14.67 | 14.00 | 277.00 | 275.00 | 44.17 | 42.60 | 27.60 | 26.97 | 115.84 | 115.39 | 79.13 | 78.60 | 41.06 | 42.51 |
| **CNT+Atrazine as post-emergence** | 14.00 | 13.33 | 14.67 | 14.00 | 276.33 | 275.67 | 42.07 | 41.20 | 27.17 | 25.80 | 112.75 | 118.48 | 78.83 | 78.43 | 43.87 | 44.40 |
| **CNT+Weed-free control** | 16.00 | 15.67 | 16.00 | 16.00 | 296.67 | 296.00 | 74.93 | 74.10 | 31.70 | 31.30 | 161.47 | 162.68 | 83.93 | 83.27 | 0.00 | 0.00 |
| **CNT+Weedy control** | 12.33 | 12.00 | 14.67 | 13.00 | 270.33 | 269.00 | 25.77 | 24.17 | 26.27 | 25.30 | 72.37 | 71.36 | 78.17 | 76.93 | 65.61 | 67.39 |
| **CST+Atrazine as pre-emergence + hand weeding at 30 DAS** | 16.33 | 16.00 | 16.67 | 16.33 | 296.33 | 295.33 | 81.27 | 80.90 | 33.07 | 32.40 | 164.51 | 168.86 | 83.77 | 83.23 | 0.89 | 0.90 |
| **CST+Atrazine as pre-emergence + Atrazine as post-emergence** | 16.67 | 16.00 | 16.67 | 16.00 | 296.67 | 296.00 | 81.73 | 81.03 | 33.13 | 32.50 | 164.97 | 168.37 | 83.77 | 83.37 | 0.32 | 0.73 |
| **CST+Pendimethalin as pre-emergence + Atrazine as post-emergence** | 15.00 | 15.00 | 15.00 | 14.67 | 287.67 | 286.00 | 72.37 | 71.77 | 30.60 | 30.10 | 164.20 | 166.70 | 81.53 | 80.77 | 11.75 | 12.09 |
| **CST+Atrazine as post-emergence** | 14.67 | 13.67 | 14.67 | 14.67 | 282.67 | 282.00 | 70.53 | 69.80 | 30.77 | 30.30 | 158.73 | 160.56 | 80.73 | 80.07 | 13.98 | 14.50 |
| **CST+Weed-free control** | 17.00 | 16.67 | 16.67 | 16.67 | 297.33 | 297.00 | 82.00 | 81.63 | 33.87 | 32.90 | 160.14 | 166.51 | 84.00 | 83.53 | 0.00 | 0.00 |
| **CST+Weedy control** | 13.33 | 12.67 | 14.67 | 14.33 | 274.00 | 273.67 | 59.33 | 58.50 | 29.13 | 27.83 | 144.36 | 151.70 | 80.03 | 79.23 | 27.64 | 28.33 |
| **CD (*P*=0.05)** | 1.12 | 1.19 | 1.15 | 0.76 | 3.75 | 2.13 | 1.33 | 1.27 | - | - | 7.48 | 6.64 | - | - | - | - |

CNT-Conventional soil turning tillage, CST-Conservation tillage



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**Table 4.** Economics (Rs. ha$^{-1}$) of maize cultivation as influenced by different treatment combinations.

| Treatments | Common cost | | Treatment cost | | Total cost | | Grain yield | | Stover yield | | Gross return | | Net return | | Net return per rupee invested | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 |
| **CNT+Atrazine as pre-emergence + hand weeding at 30 DAS** | 25049 | 28624 | 4672 | 6652 | 29721 | 35276 | 74.30 | 73.60 | 164.35 | 165.10 | 89249.10 | 102990.27 | 59528.10 | 67714.27 | 2.00 | 1.92 |
| **CNT+Atrazine as pre-emergence + Atrazine as post-emergence** | 25049 | 28624 | 2624 | 3284 | 27673 | 31908 | 74.67 | 73.67 | 165.16 | 166.29 | 89689.54 | 103187.32 | 62016.54 | 71279.32 | 2.24 | 2.23 |
| **CNT+Pendimethalin as pre-emergence + Atrazine as post-emergence** | 25049 | 28624 | 2814 | 3474 | 27863 | 32098 | 44.17 | 42.60 | 115.86 | 115.37 | 54869.08 | 61592.28 | 27006.08 | 29494.28 | 0.97 | 0.92 |
| **CNT+Atrazine as post-emergence** | 25049 | 28624 | 1312 | 1642 | 26361 | 30266 | 42.07 | 41.20 | 112.78 | 118.49 | 52503.33 | 60258.99 | 26142.33 | 29992.99 | 0.99 | 0.99 |
| **CNT+Weedy control** | 25049 | 28624 | 0 | 0 | 25049 | 28624 | 25.77 | 24.17 | 72.33 | 71.35 | 32484.31 | 35531.21 | 7435.31 | 6907.21 | 0.30 | 0.24 |
| **CST+Atrazine as pre-emergence + hand weeding at 30 DAS** | 23500 | 26635 | 4672 | 6652 | 28172 | 33287 | 81.27 | 80.90 | 164.50 | 168.79 | 96091.28 | 111936.64 | 67919.28 | 78649.64 | 2.41 | 2.36 |
| **CST+Atrazine as pre-emergence + Atrazine as post-emergence** | 23500 | 26635 | 2624 | 3284 | 26124 | 29919 | 81.73 | 81.03 | 164.95 | 168.30 | 96593.34 | 112044.17 | 70469.34 | 82125.17 | 2.70 | 2.74 |
| **CST+Pendimethalin as pre-emergence + Atrazine as post-emergence** | 23500 | 26635 | 2814 | 3474 | 26314 | 30109 | 72.37 | 71.77 | 164.13 | 166.66 | 87331.90 | 100991.91 | 61017.90 | 70882.91 | 2.32 | 2.35 |
| **CST+Atrazine as post-emergence** | 23500 | 26635 | 1312 | 1642 | 24812 | 28277 | 70.53 | 69.80 | 158.72 | 160.56 | 84994.58 | 98071.30 | 60182.58 | 69794.30 | 2.43 | 2.47 |
| **CST+Weedy control** | 23500 | 26635 | 0 | 0 | 23500 | 26635 | 59.33 | 58.50 | 160.13 | 166.49 | 74159.27 | 85386.73 | 50659.27 | 58751.73 | 2.16 | 2.21 |

Support price of maize grain (2011)-Rs 980/q and (2012) Rs 1175/q. Price of stover Rs 100/q. (1$=Rupees 60)
CNT-Conventional soil turning tillage, CST-Conservation tillage.



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## *Fate of Herbicides and Herbicide Residues*

# SOIL PROPERTIES GOVERNING BIODEGRADATION OF THE HERBICIDE GLYPHOSATE IN AGRICULTURAL SOILS

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**ABSTRACT** The relationships between soil properties and glyphosate biodegradation in different agricultural soils was investigated in this study. Soils differ hugely in soil texture, soil organic matter content, pH, oxalate extractable $Al^{3+}$ and $Fe^{3+}$. The biodegradation experiments were conducted under test conditions: water tension of –15 kPa as soil moisture, a soil density of 1.3 g $cm^{-3}$ and at 20 °C in the dark. The biodegradation experiments showed that the mineralization of glyphosate in 21 agricultural soils greatly varied. Between 7.6 to 68.7 % of the applied $^{14}C$-glyphosate was mineralized to $^{14}CO_2$ in the 21 different soils within 32 days of incubation. The highest and lowest mineralized glyphosates were observed in Feldkirchen (68.7%) and Brejze soil sample (7.6%), respectively. Glyphosate was mineralized rapidly by the microorganisms in the soil solution and the highest mineralization rate was reached shortly after application. The mineralization of glyphosate in soils was individually regulated by exchangeable $H^+$, soil pH-$CaCl_2$, oxalate extractable Al $^{3+}$ and bacterial cell numbers at the end of the experiments, but it was collectively controlled by exchangeable $H^+$, $Ca^{2+}$ ions and plant available K. Moreover, soil textures, soil organic content, $P_2O_5$, $Cu^{2+}$, oxalate extractable $Fe^{3+}$ and CEC were found not to have any correlation with mineralization of glypohsate. The NaOH extractable residues were bioavailable for degradation whereas the bound residues of glyphosate in soils were mostly formed by microbial activity.

***Keywords*****:** Soil properties, herbicide, degradation, mineralization, glyphosate

## INTRODUCTION

Glyphosate (*N*-(phosphonomethyl)glycine) is the most widely used herbicide worldwide (Jensen et al., 2009) and classified as a relatively safe compound for human and environment because of it's less persistent, high biodegradability by micro-organisms (Wiren-Lehr et al., 1997; Sorensen et al., 2006). Glyphosate adsorbs rapidly and strongly into soils through hydrogen bonds in organic matter and by anion exchange processes in the clay minerals, iron and aluminium oxides, making it essentially immobile and unbioavailable (Hansen et al., 2004; Mamy et al., 2005; Borggaard and Gimsing, 2008). However, recently glyphosate and one of its metabolites (aminomethylphosphonicacid [AMPA]) have frequently been detected in Norwegian, Swedish, Danish and Netherlands surface and ground water, especially, AMPA has been detected more frequently than glyphosate with high concentration (Sorensen et al., 2006; Adriaanse et al., 2008; Rafiei Keshteli et al., 2011).

Glyphosate degradation in soils is dominated by microbiological processes, which are mediated principally by bacteria and fungi (Reddy et al., 2008; Laitinen, 2009). Pesticide degradation studies in soils are very important for assessing of the persistence of pesticides and



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their degradation products. Degradation of glyphosate varies greatly between soils and such variances have been ascribed to microbiological and chemical properties of the soils (Albers et al., 2009; Kim et al., 2011), e.g. general soil microbial parameters including soil microbial biomass (Wiren-Lehr et al., 1997), population size of *Pseudomonas* spp. bacteria in the soils (Gimsing et al., 2004a), soil respiration rate (Borggaard and Gimsing, 2008), the soil pH (Gimsing et al., 2004b; Borggaard and Gimsing, 2008; Jensen et al., 2009), soil organic carbons (Yu and Zhou., 2005; Zablotowicz et al., 2009; Albers et al., 2009; Rafiei Keshteli et al., 2011), clay content (Autio et al., 2004), phosphorous content (Jonge et al., 1999; Gimsing et al., 2004a; Jensen et al., 2009), Al/Fe-oxides (Moshier and Penner 1978), and $Cu^{2+}$ content (Getenga and Kengara 2004; Mamy et al., 2005).

Information concerning studies in adsorption and desorption of glypphosate in agricultural soils can be easily to find from the literature, but the degradation of glyphosate in agricultural soils and the impact of soil parameters on glyphosate degradation have not been studied much. In literature many authors have reported various correlations between glyphosate degradation and soil parameters. Since in most studies only a few soils were investigated it cannot be excluded, that the established correlations were probably found by chance. Therefore, to avoid accidental results and to establish a sound basis for resilient correlations, we conducted our glyphosate degradation study in a huge variety of 21 agricultural soils, differing in soil texture, soil organic matter content, pH, oxalate extractable $Al^{3+}$ and $Fe^{3+}$. Thus, the aim of the study was to elucidate the impact of soil parameters on glyphosate degradation, thereby explaining the different behaviour of glyphosate in different soils.

## MATERIALS AND METHODS

### Soil

The experiment was conducted using 21 agricultural soils. These 21 soils are typical for agricultural soils of Germany and Slovenia. There was a big variation in the different soil characteristics (soil textures, organic matter content, total N, C/N, plant available P, oxalate extractable $Al^{3+}$, $Fe^{3+}$, $Cu^{2+}$, CEC, pH-$CaCl_2$, water content at water potential of -15 kPa and heterotrophic bacteria). All soils were taken from the upper Ap layer of arable fields (0-30 cm), sieved (2 mm) after sampling, homogenized and stored at 4ºC in the dark before use. At the beginning of the experiments all soils were conditioned and moistened to a water potential close to -15 kPa at room temperature (20 ± 2 °C) for 2 weeks and compacted to the soil density of 1.3 g $cm^{-3}$ to equilibrate the microbial processes and to make sure that all soils have the comparable conditions at the start of the experiments. Some soil parameters are determined and presented in Tables 1 and 2.

### Chemicals

$^{14}C$-labelled glyphosate [N-(phosphonomethyl)glycine] was labelled on the phosphonomethyl group (PerkinElmer, USA). The purity of radioactive glyphosate was >97.0%. $^{14}C$-glyphosate was mixed with non labelled glyphosate which was purchased from Dr. Ehrenstorfer (Augsburg, Germany) resulting in a final specific radioactivity of 1.6 Bq $mg^{-1}$ (for degradation experiments). The purity of non labelled glyphosate was 98%. Aminomethylphosphonic acid (AMPA) which was also purchased from Dr. Ehrenstorfer (Augsburg, Germany) had the purity of 98 %. Sodium hydroxide (NaOH), monopotassium phosphate ($KH_2PO_4$), sodium chloride (NaCl), calcium chloride dihydrate ($CaCl_2x2H_2O$), $NH_4Cl$, $NH_4NO_3$, methanol ($CH_4O$), diatomaceous earth, water for chromatography were purchased from Sigma-Aldrich (Steinheim, Germany). Scintillation cocktails (Ultima Gold XR, Ultima Flo AF, Permaflour E) and Carbosorb E were obtained from Packard (Dreieich, Germany).

**Table 1.** Some characteristics of soil samples

| Name of soil (site of origin) | Sand [%] | Silt [%] | Clay [%] | Water content -15 kPa (%) | pH ($CaCl_2$) | Organic mattter [%] | C [%] | N [%] | $P_2O_5$ (mg $100g^{-1}$) | $K_2O$ (mg $100g^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|



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| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1 (Ada-A02)** | 62.5 | 27.4 | 10.1 | 21.9 | 5.7 | 2.9 | 1.7 | 0.2 | 17 | 4.1 |
| **2 (Apace-njiva)** | 66.4 | 31.2 | 2.4 | 20.7 | 7.0 | 2.6 | 1.5 | 0.2 | 4 | 22.6 |
| **3 (Berta-A02)** | 46.4 | 39.4 | 14.2 | 28.1 | 5.7 | 2.5 | 1.5 | 0.2 | 8 | 12.5 |
| **4 (Brezje)** | 8.3 | 73.2 | 18.5 | 32.7 | 5.2 | 2.8 | 1.6 | 0.2 | 5 | 21.1 |
| **5 (Dunja - A06)** | 62.4 | 25.9 | 11.7 | 17.4 | 5.4 | 2.2 | 1.3 | 0.2 | 11 | 13.2 |
| **6 (Feldkirchen)** | 34.8 | 47.0 | 18.2 | 28.2 | 7.0 | 3.4 | 2.0 | 0.3 | 39 | 9.4 |
| **7 (Grace - A13)** | 50.3 | 41.3 | 8.4 | 21.0 | 5.4 | 2.6 | 1.5 | 0.2 | 12 | 9.6 |
| **8 (Hanna - A15)** | 62.3 | 24.2 | 13.5 | 18.4 | 5.2 | 1.7 | 1.0 | 0.1 | 7 | 8.2 |
| **9 (Hohenwart)** | 67.2 | 20.5 | 12.3 | 22.4 | 6.2 | 1.7 | 1.0 | 0.1 | 21 | 21.1 |
| **10 (Joy -A19)** | 31.6 | 45.6 | 22.8 | 31.9 | 5.9 | 2.7 | 1.6 | 0.2 | 34 | 43.2 |
| **11 (Kelheim)** | 76.2 | 15.5 | 8.3 | 12.5 | 6.5 | 1.2 | 0.7 | 0.1 | 23 | 17.0 |
| **12 (Konjise)** | 33.8 | 60.2 | 6.0 | 34.6 | 6.9 | 4.5 | 2.6 | 0.2 | 4 | 7.9 |
| **13 (Lamanose)** | 10.3 | 69.6 | 20.1 | 35.8 | 5.8 | 4.3 | 2.5 | 0.3 | 5 | 18.7 |
| **14 (Lea -A18)** | 18.9 | 66.8 | 14.3 | 28.9 | 5.2 | 1.9 | 1.1 | 0.2 | 6 | 23.8 |
| **15 (Lomanose)** | 21.9 | 60.2 | 17.9 | 25.8 | 5.8 | 1.7 | 1.0 | 0.2 | 11 | 16.8 |
| **16 (Neumark)** | 85.5 | 8.8 | 5.7 | 12.6 | 5.2 | 1.6 | 0.9 | 0.1 | 11 | 12.2 |
| **17 (Pearl - A20)** | 29.3 | 51.8 | 18.9 | 28.3 | 5.0 | 2.3 | 1.3 | 0.2 | 12 | 31.7 |
| **18 (Scheyern Lysi)** | 17.2 | 62.6 | 20.2 | 30.1 | 5.5 | 2.7 | 1.6 | 0.2 | 20 | 5.3 |
| **19 (Skrinjar)** | 67.5 | 27.0 | 5.5 | 19.2 | 7.1 | 1.6 | 0.9 | 0.1 | 21 | 24.7 |
| **20 (Zepovci)** | 41.3 | 43.1 | 15.6 | 24.0 | 5.7 | 2.9 | 1.7 | 0.2 | 11 | 24.7 |
| **21 (Zepovci (Plitv.))** | 11.8 | 72.2 | 16.0 | 27.4 | 5.2 | 1.9 | 1.1 | 0.2 | 8 | 20.2 |

**Table 2.** Some characteristics of soil samples (cont.)

| **Name of soil (site of origin)** | **$Al_{Ox}$ (mg $100g^{-1}$)** | **$Fe_{Ox}$ (mg $100g^{-1}$)** | **$Cu^{2+}$ (mg $kg^{-1}$)** | **Ca** | **Mg** | **K** | **Na** | **H** | **CEC** | **Heterotrophic bacteria ($x10^7$ CFU $g^{-1}$)*** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | [$mmol_c$ $100g^{-1}$] | | | | | | |
| **1** | 63 | 198 | 4 | 8.5 | 0.8 | 1.0 | 0.04 | 5.7 | 16.0 | 0.3 |
| **2** | 62 | 248 | 4 | 11.1 | 2.3 | 0.1 | 0.04 | 1.5 | 15.0 | 0.5 |
| **3** | 76 | 265 | 3 | 9.0 | 1.0 | 0.6 | 0.04 | 5.3 | 15.9 | 0.4 |
| **4** | 187 | 518 | 2 | 7.2 | 0.9 | 0.6 | 0.07 | 11.1 | 19.8 | 0.1 |
| **5** | 80 | 211 | 62 | 7.0 | 0.6 | 0.6 | 0.04 | 5.3 | 13.6 | 0.3 |
| **6** | 139 | 310 | 12 | 26.4 | 2.5 | 0.5 | 0.05 | 3.5 | 32.9 | 1.1 |
| **7** | 106 | 259 | 3 | 8.7 | 0.7 | 0.6 | 0.04 | 7.4 | 17.5 | 0.6 |
| **8** | 83 | 215 | 2 | 7.2 | 0.5 | 0.2 | 0.04 | 5.7 | 13.6 | 0.5 |
| **9** | 75 | 206 | 4 | 5.5 | 1.2 | 0.4 | 0.05 | 3.9 | 11.1 | 1.6 |
| **10** | 101 | 320 | 39 | 13.1 | 1.8 | 0.7 | 0.06 | 6.7 | 22.4 | 0.9 |
| **11** | 44 | 132 | 8 | 5.5 | 1.2 | 0.3 | 0.05 | 2.0 | 9.1 | 0.8 |
| **12** | 88 | 381 | 7 | 10.8 | 4.6 | 0.1 | 0.06 | 3.2 | 18.8 | 0.1 |
| **13** | 134 | 456 | 4 | 16.4 | 3.6 | 0.3 | 0.06 | 9.2 | 29.6 | 0.4 |
| **14** | 107 | 345 | 3 | 6.4 | 0.8 | 0.4 | 0.07 | 6.9 | 14.6 | 0.3 |
| **15** | 72 | 252 | 3 | 9.5 | 1.8 | 0.3 | 0.09 | 5.4 | 17.0 | 0.7 |
| **16** | 88 | 110 | 1 | 2.6 | 0.4 | 0.2 | 0.05 | 4.3 | 7.5 | 0.7 |
| **17** | 125 | 319 | 4 | 6.9 | 0.8 | 0.5 | 0.05 | 8.8 | 17.0 | 0.8 |



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| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **18** | 102 | 349 | 10 | 9.1 | 1.6 | 0.6 | 0.06 | 7.1 | 18.4 | 0.9 |
| **19** | 57 | 257 | 4 | 10.8 | 0.5 | 0.1 | 0.06 | 1.5 | 13.0 | 0.5 |
| **20** | 165 | 476 | 4 | 7.8 | 0.7 | 0.9 | 0.05 | 10.6 | 20.0 | 0.3 |
| **21** | 147 | 430 | 2 | 4.4 | 0.5 | 0.7 | 0.10 | 9.4 | 15.1 | 0.1 |

* CFU = colony-forming unit at the start of degradation experiments

**Glyphosate mineralization experiments**

*Pesticide application procedure* All biodegradation experiments were performed in 4 replicates with 50 g soil (dry mass) for each replicate. $^{14}$C-glyphosate was dissolved in autoclaved and distilled water and mixed with non-labeled glyphosate which was also dissolved in sterilized distilled water. This mixture was regarded as the application standard solution with a concentration of a.i of 5.42 µg $\mu L^{-1}$ and a specific radioactivity of 166.70 Bq $\mu g^{-1}$. The application standard (0.089 mL) was applied to an oven dried, pulverized soil aliquot of 3.5 g (dry mass) in a glass beaker and carefully stirred for 1 minute with a spatula. After homogenous distribution the spiked aliquot was transferred to another glass beaker containing the rest of equilibrated soils (46.5 g dry mass) and mixed for another 2 min. The total concentration of glyphosate was 10µg $g^{-1}$ in each set corresponding to a total radioactivity of 83,000 Bq.

*Test system, experimental conditions and samplings* The spiked soils were transferred to 500 mL brown incubation flasks, compacted to a soil density of 1.3 g $cm^{-3}$ and soil water was adjusted to a water potential of -15 kPa. The flasks were covered with special rubber caps, and incubated at 20 ± 1°C in the dark for a period of 32 days. The soil humidity was controlled weekly. The rubber caps were equipped with an air inlet and outlet system as well as a facility to trap the evolved $CO_2$. The air exchange system should prevent unaerobiosis in the incubation flasks and consisted of a canal which was made of a stainless needle with a diameter of 1 mm. To eliminate $CO_2$ from the ambient air entering the flasks, a 12 mL plastic syringe (Latex FREE, Tuttlingen, Germany) filled with granular $CO_2$ absorber (soda lime) was connected to the canal at the top of the cap. Below the cap a small plastic beaker was placed containing 0.1 M NaOH solution (10 mL) to capture $^{14}CO_2$ released from glyphosate mineralization from the soil samples. The NaOH solution was exchanged three times per week and from the collected solution an aliquot of 2 mL was mixed with 3 mL of scintillation cocktail Ultima Flo AF to determine $^{14}CO_2$ in a liquid scintillation counter (Tricarb 1900 TR, Packard, Dreieich, Germany).

At the end of the experiment, 30 g of each soil sample (dry mass) were used for pore water extraction, 7 g of each soil sample were extracted with NaOH to determine the quantity and quality of the extractable residues as well as to quantify the non extractable residues, while 1 g of each soil sample was used for cell counts.

*NaOH extraction, clean up and HPLC analysis* For NaOH extraction, the method used by Gimsing et al. (2004a) was applied. At the end of the experiment, 7 g of soil (dry mass) was extracted with 28 mL 0.1 M NaOH by shaking on overhead shaker (Reax 2, Heidolph, Schwabach, Germany) for 17 hours. The supernatant was collected after centrifuging for 10 min at 3020 rcf. The supernatant was filtered through filter paper circles (No. 589/1, Whatman, Dassel, Germany). Radioactivity of the filtered supernatant was measured by scintillation counting using 100 µl of supernatant aliquot and 5 mL of scintillation cocktail Ultima Gold XR to quantify the NaOH extractable pesticide residues.

Subsequently, extracts were concentrated and cleaned up before injecting to HPLC. The concentration of the NaOH extracts was carried out on a Büchi Rotavapor R-114 which was connected to a Büchi water bath B-480 (Büchi, Flawil, Switzerland) at 30 °C to around 150 µl. The concentrated samples were filtered through centrifugal filters (modified Nylon 0.2 µm, 500 µl, VWR International GmbH, Darmstadt, Germany) in a table centrifuge (Biofuge Pico,



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Heraeus Instruments, Osterode, Germany) for 5 minutes at 9070 rcf. Purified samples were stored at -20 °C prior to HPLC analysis.

Twenty µl of each sample (NaOH extract) were injected via an Auto Sampler AS50 (Dionex, Idstein, Germany) to a HPLC system (GP50 Gradient Pump, Dionex, Idstein, Germany) that was connected with a Radioflow detector LB 509 (Berthold, Wildbad, Germany). The column, PRP-X400, 7µm, 4.6 x 250 mm (Hamilton, Reno, USA), was used at a flow velocity (isocratic) of 0.5 mL $min^{-1}$. 5mM $KH_2PO_4$ (pH 1.9) (A) and 5mM KOH (Regenerant-RG019) (Pickering Laboratories, Mountain View, CA 94043, U.S.A) (B) were used as mobile phases. The gradient program was: 1) 0-20 min: 100 % (A) and 0% B; 2) 21-25 min: 0% (A) and 100% (B). $^{14}C$-glyphosate and its metabolites (AMPA, sarcorsine, glycine, methylamine) were identified by comparison of their retention times with standard substances. After each analysis the column was regenerated with Regenerant-RG019 (Pickering Laboratories, Mountain View, CA 94043, U.S.A) at a flow velocity (isocratic) of 0.5 mL $min^{-1}$ for 30 min.

*Quantification of non-extractable $^{14}C$-labelled residues* After extraction with 0.1M NaOH, the rest of radioactivity remaining in the soil was considered as non-extractable residues. Soil material was intensively mixed and homogenized with 3.5 g diatomaceous earth (Sigma-Aldrich, Steinheim, Germany) for 2 min in a mortar. Four aliquots of each soil sample were weighed (aliquot mass varied between 0.1 and 0.3 g) in combustion cups and mixed with 8 drops of saturated aqueous sugar solution to accelerate and ensure a complete oxidation of the $^{14}C$. The oxidation step was done with an automatic sample-oxidizer 306 (Packard, Dreieich, Germany). $^{14}CO2$ from the combustion was trapped in Carbo-Sorb E and mixed with Permaflour E before scintillation counting. The extractable and non-extractable glyphosate residues were calculated after the combustion.

*Bacterial cell counts* Bacterial cell counts were performed to count the cultivable and heterotrophic bacteria in the different soils. The method for bacterial cell counts was adapted from Ngigi et al. (2011). Soil bacteria were extracted from the soil by mixing 1 g of soil (dry mass) with 99 mL of buffer solution containing (per L) 0.1 g NaCl, 0.02 g $CaCl \cdot 2H_2O$, 0.2 g $MgSO_4 \cdot 7H_2O$, 5.0 g Tween 80. Before use the buffer solution was autoclaved for 20 min at 121 °C. Soil and buffer solution were shaken vigorously for 1 hour on a shaker at 150 rpm. The soil particles were allowed to sediment for 10 min. Then 0.1 mL of the supernatant was transferred to 0.9 mL of sterilized buffer solution for further dilution steps. A total of 4 dilutions ($10^{-1}$ to $10^{-4}$) were established. Finally, 0.1 mL of each dilution was spread in triplicates on Lysogeny broth (LB) agar media (10 g trypton enzymatic digest from casein, 5 g yeast extract, 5 g NaCl, 15 g agar and 0.1 mg cycloheximide in 1 L of distilled water). This medium was also autoclaved for 20 min at 121 °C before use. The number of CFU was determined after three days of incubation at 25 °C by counting.

**Statistical analysis**

The data were statistically analysed using analysis of variance (ANOVA) and multiple regression analysis (SPSS 12.OG for Windows, USA).

## RESULTS AND DISCUSSION

In this study, 21 soil samples with huge variability of soil parameters were investigated to compare the biodegradation of glyphosate in different soils. The main aim in this part is to check the correlation between soil parameters and glyphosate mineralization. The selected soil parameters for correlation were exchangeable [$H^+$], silt, clay, soil organic matter, C, N, C/N, $P_2O_5$, $Cu^{2+}$, oxalate extractable $Al^{3+}$, oxalate extractable $Fe^{3+}$, $K_2O$, $CFU_{beginning}$ and $CFU_{end}$, $Ca^{2+}$, $Mg^{2+}$, $K^+$, $Na^+$, CEC, and Ph.



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**Mineralization of glyphosate**

After 33 days of incubation a big variance of cumulative mineralization can be observed. Between 7.6 to 68.7 % of the applied $^{14}C$-glyphosate was mineralized to $^{14}CO_2$ in the 21 different soil types (Table 3). Shortly after application, a high amount of glyphosate was mineralized. The lowest mineralization of $^{14}C$-glyphosate was identified in Brezje soil while the highest mineralization of $^{14}C$-glyphosate was obtained in Feldkirchen and Apace-njiva soils. Low mineralization of glyphosate was also observed in Zepovci, Zepovci(Plitv.) and Lamanose soils. In these 3 soils less than 30% of the initial glyphosate was mineralized after 32 days. In contrast, other soils had a higher mineralization activity and $^{14}CO_2$ production after 32 days reached 31.2-68.7% of the initial glyphosate. High variability of glyphosate degradation in laboratory experiments was also reported in the studies of Smith and Aubin (1993); Wiren-Lehr et al. (1997); Cheah et al. (1998); Gimsing et al. (2004a); Bonfleur et al. (2011). They showed that Dt50 lab values varied between 4 to 180 d (mean 49 d) at 20 ±1°C. A big difference in biomineralization of glyphosate among 21 soils indicates that agricultural soils have difference in ability to degrade glyphosate. The firstly rapid mineralization of glyphosate was observed for most soils during the first 4 days without a lag phase, but mineralization rates subsequently decreased over time, as found in other earlier studies (von Wiren-Lehr et al., 1997; Gimsing et al., 2004a). At the end of the biodegradation experiments, mass balances were established. Mass balances of $^{14}C$-glyphosate are presented in Table 3. In all soils, the $^{14}C$ mass balances were quite good: over 94 % of the totally applied $^{14}C$-glyphosate was recovered at the end of the biodegradation experiments.



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**Table 3**. Behavior of $^{14}$C-glyphosate in different soils

| Soil | Cum. Min (%)[a] (1) | NaOH extract. residues (%)[a)*] (2) | Non-extract. residues (%)[a] (3) | Total recovery** (%)[a] (4) | Quality of NaOH extract. residues | | |
|---|---|---|---|---|---|---|---|
| | | | | | Glyphosate (%) (5) | AMPA[d] (%) (6) | Unknown (%) (7) |
| **1** | 44.7 | 48.3 | 4.8 | 97.8 | 37.7 | 2.3 | 8.3 |
| **2** | 67.3 | 24.5 | 9.6 | 101.4 | 18.9 | 2.2 | 3.4 |
| **3** | 48.9 | 42.7 | 6.3 | 97.9 | 34.4 | 0.0 | 8.3 |
| **4** | 7.6 | 91.0 | 2.5 | 101.1 | 88.0 | 0.0 | 3.0 |
| **5** | 39.5 | 53.9 | 3.9 | 97.3 | 45.2 | 0.0 | 8.7 |
| **6** | 68.7 | 23.3 | 9.0 | 101.0 | 12.2 | 7.6 | 3.6 |
| **7** | 35.5 | 57.7 | 3.7 | 96.9 | 44.1 | 2.8 | 10.8 |
| **8** | 32.2 | 59.8 | 4.1 | 96.1 | 54.0 | 0.0 | 5.8 |
| **9** | 55.8 | 37.7 | 6.3 | 99.8 | 24.8 | 5.7 | 7.3 |
| **10** | 47.9 | 46.9 | 8.2 | 103.0 | 31.3 | 7.1 | 8.5 |
| **11** | 51.8 | 37.3 | 6.2 | 95.3 | 25.1 | 4.1 | 8.1 |
| **12** | 49.1 | 35.7 | 9.5 | 94.3 | 23.3 | 4.5 | 7.9 |
| **13** | 25.5 | 64.4 | 6.7 | 96.6 | 45.0 | 8.3 | 11.1 |
| **14** | 37.3 | 55.7 | 5.4 | 98.4 | 30.0 | 0.0 | 25.7 |
| **15** | 43.7 | 46.8 | 6.4 | 96.9 | 30.4 | 8.0 | 8.4 |
| **16** | 31.2 | 63.0 | 3.1 | 97.3 | 48.8 | 2.5 | 11.7 |
| **17** | 31.5 | 63.6 | 3.7 | 98.8 | 29.5 | 0.0 | 34.1 |
| **18** | 32.5 | 59.8 | 5.0 | 97.3 | 40.9 | 5.5 | 13.4 |
| **19** | 61.6 | 28.8 | 11.4 | 101.8 | 16.9 | 4.8 | 7.1 |
| **20** | 19.5 | 73.3 | 4.1 | 96.9 | 65.8 | 0.0 | 7.5 |
| **21** | 18.4 | 78.5 | 2.7 | 99.6 | 55.9 | 11.3 | 11.3 |

a) % of applied $^{14}$C-glyphosate after 32 days; the mean value is presented
b) Aminomethylphosphonic acid
* Total NaOH extractable residues (2) = (5) + (6) + (7)
** Total recovery (4) = (1) + (2) + (3)



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**Identification of the parameters governing mineralization of glyphosate**

In order to identify the factors which govern glyphosate mineralization in 21 agricultural soils, soil parameters, NaOH extractable residues, $^{14}C$-glyphosate residues, non-extractable residues and the mineralized glyphosate were compared at the end of the biodegradation experiments and several significant correlations could be discovered.

*Relationship between mineralized glyphosate and extractable acidity (extractable $H^+$ cations)*
As can be seen in Fig. 1, according to univariate correlation analysis there was highly significant and negative correlation between the cumulative mineralization glyphosate and extractable $H^+$ cations ($p = 0.000$). This illustrates that the extractable $H^+$ cations interfered the mineralization process in soils. Therefore, the assessment of extractable $H^+$ cations in soils appears suitable for ranking of soil according to the mineralization of the compound. This could be explained by the fact that a binding between the carboxylic (or phosphonic acid) groups of glyphosate and extractable $H^+$ cations is formed (Shoval and Yariv, 1979). Therefore, the bioavailability of glyphosate is reduced in soil with high extractable $H^+$ cations.

*Relationship between mineralized glyphosate and NaOH extractable residues* NaOH extractable residues of the 21 investigated soils were performed after 32 days. The results show that the NaOH extractable fraction in all soils was relatively high and very various. Approximately between 23 and 91 % of initial glyphosate after 32 days incubation was extracted with NaOH 0.1M (Table 3). Soils with higher mineralization had lower NaOH extractable fraction. A correlation was performed to check the relationship between mineralized glyphosate and NaOH extractable residues. Fig. 2 shows that there was a negative correlation between mineralized glyphosate within 32 days and NaOH extractable residues ($p = 0.0000$). This shows that NaOH extractable residues were non-available for microorganisms to be degraded. This result is not in accordance with the study of Gimsing et al. (2004a) who showed that glyphosate adsorbed to iron or aluminum oxides in the soils can be desorbed and subsequently mineralized. However, according to Stenrod et al. (2005) NaOH extraction could extract both bioavailable and non-bioavailable glyphosate in soils. However, the correlation between the mineralization of glyphosate and NaOH extractable residues has not been given in the literature.

*Relationship between mineralized glyphosate and $^{14}C$-glyphosate residues from extractable pool* $^{14}C$-glyphosate is the major component in the NaOH extract as compared to AMPA and unknown metabolites (Table 3). To test whether there is any relationship between the mineralized glyphosate and NaOH extractable residues, we calculated correlation between both values. There is exist significantly negative correlation between $^{14}C$-glyphosate residues from extractable pool and mineralized glyphosate ($p = 0.0000$; Fig. 3). This indicates that in soils with low mineralization glyphosate is present in a high amount and that this glyphosate could not be degraded / mineralized because it was adsorbed to Al- or Fe-oxides. Gimsing et al. (2004a) also found glyphosate as a major component in the NaOH extract. This might be a hint that adsorbed glyphosate by Al/Fe-oxides is slowly released to soil solution and as long as glyphosate is degraded to degradation products in soil solution by microorganism, the degradation products are quickly mineralized to $CO_2$. However, no literature showing the correlation between NaOH extractable residues and mineralized glyphosate has been found.



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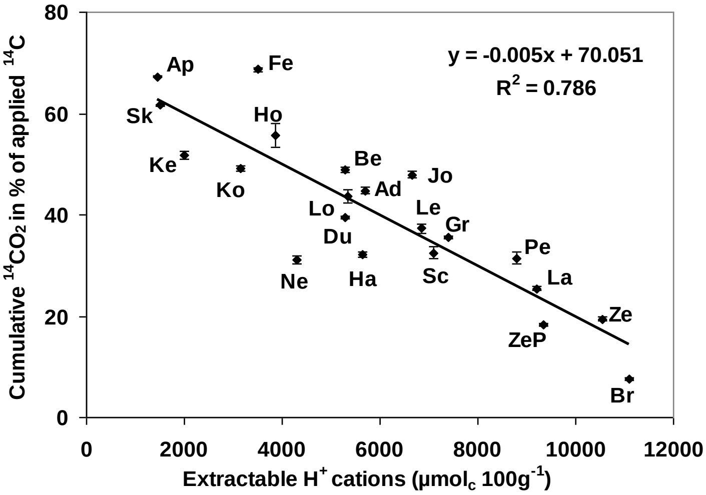


**Fig. 1.** Correlation between cumulative mineralization of glyphosate and extractable $H^+$ cations in soils (bars indicate standard deviation of 4 samples).

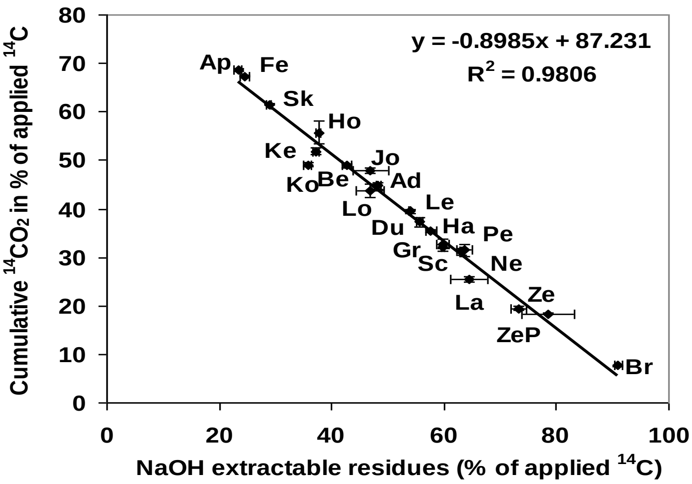


**Fig. 2.** Correlation between cumulative mineralization of glyphosate and NaOH extractable residues (bars indicate standard deviation of 4 samples).

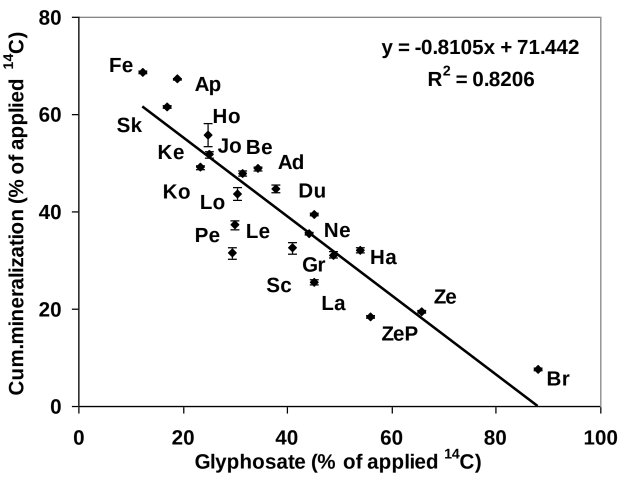


**Fig. 3.** Correlation between cumulative mineralization of glyphosate and glyphosate residues (from extractable residues) (bars indicate standard deviation of 4 samples).



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*Relationship between mineralized glyphosate and non-extractable residues* The amount of non-extractable residues was relatively low. It varied between 2.5 % and 11.4 % of the initial glyphosate (Table 3). The non-extractable residues and mineralized glyphosate were compared together to see whether there is any relationship between both parameters. As can be seen from Fig. 4, a significant and positive correlation between mineralized glyphosate and non-extractable residues (p = 0.0000) was found. The high mineralization of glyphosate in soils coincided with non-extractable residues at the end of the experiment. This could be an argument that the bound residues in the present study are partly formed by an incorporation of $^{14}C$-glyphosate into microbial biomass (Charnay et al., 2004), and that the microorganisms were able to utilize glyphosate for growth-related metabolism (Lancaster et al., 2010). Moreover, glyphosate and degradation products can form bound residues by themselves.

*Relationship between mineralized glyphosate and bacterial cell counts* There was a significantly positive correlation between mineralization of glyphosate and bacterial cell counts (p = 0.003; Fig. 5). This shows that the mineralization of glyphosate in soils is limited not only by availability of glyphosate and its degradation products, but also by the bacterial activity. This study result was in contrast with previous studies of Gimsing et al. (2004a) and Castillo et al. (2010) which have shown that no correlation between bacterial cell counts and mineralization of glyphosate in soils was found. However, they found a positive correlation between the mineralization of glyphosate and numbers of *Pseudomonas* sp.. Moreover, Castillo et al. (2010) also found that applying of glyphosate into soil stimulated the increase of population of *Pseudomonas sp*.. Therefore, it can be assumed that the bacterial cell numbers at the end of the experiment seemed to be the degrading microorganisms for glyphosate in soils and it was likely that microbes capable of degrading glyphosate aerobically exist in soils.

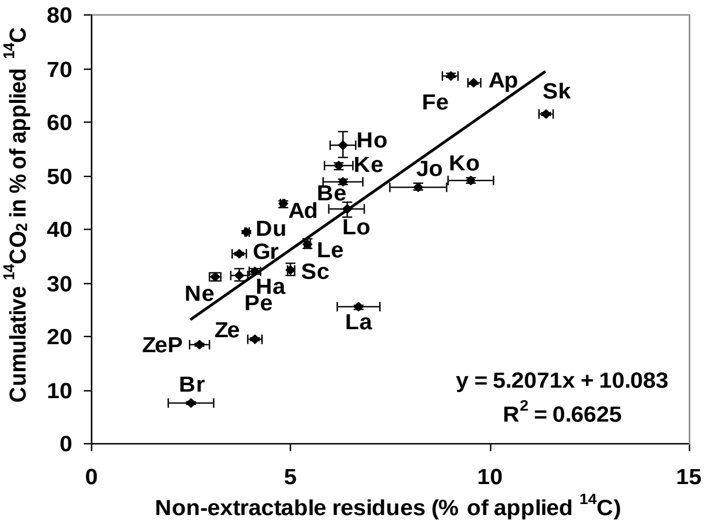


**Fig. 4.** Correlation between cumulative mineralization and non-extractable residues (bars indicate standard deviation of 4 samples).

*The interacting functions of the different soil parameters on mineralized glyphosate* In order to investigate the interacting functions of the different soil parameters on cumulative glyphosate mineralization, a multiple regression analysis was used. The input parameters were extractable $H^+$ cations, silt, clay, soil organic matter, C, N, C/N, plant available P, $Cu^{2+}$, oxalate extractable $Al^{3+}$, oxalate extractable $Fe^{3+}$, plant available K, $Ca^{2+}$, $Mg^{2+}$, $K^+$, $Na^+$, CEC, pH, CFUs at beginning and CFUs at the end of the experiments. The mineralized amount of glyphosate in the experiments was best described by the following model (n = 21):

$$Gly_{cum.min} [\%] = -0.005 \times [H^+_{Ext.}] + 1.025 \times [Ca^{2+}] + 0.332 \times [\text{plant available K}] + 56.338$$

(Adjusted $R^2$ = 0.90, p = 0.25x$10^{-9}$)



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$[H^{+}_{Ext.}]$ is extractable $H^+$ cations in soils (µmol$_c$ 100 $g^{-1}$ soil),
$[Ca^{2+}]$ is exchangeable $Ca^{2+}$ in soils (mmol$_c$ 100 $g^{-1}$ soil)

The result of multiple regression analysis reveals extractable $H^+$ cations, $Ca^{2+}$ and plant available K as key parameters governing glyphosate mineralization in the 21 tested soils and $Ca^{2+}$ and plant available K contributes additionally to extractable $H^+$ cations to the mineralization of glyphosate. In this multiple regression, extractable $H^+$ cations has a negative correlation with mineralization of glyphosate, whereas exchangeable $Ca^{2+}$ and plant available K have a positive correlation with cumulative mineralization of glyphosate. Once again, this result indicates that extractable $H^+$ cations is an important factor which reduces the bioavailability of glyphosate in soils, and as a consequence the mineralization of glyphosate is reduced. Regarding $Ca^{2+}$ and plant available K, cumulative mineralization was found to be positively correlated with exchangeable $Ca^{2+}$ and plant available K, respectively. This is not consistent with the result study of Caetano et al. (2012). In their study they showed that glyphosate formed a metallic complex with $Ca^{2+}$ in soil. But there is no information regarding the effect of plant available K on mineralization or adsorption of glyphosate in soils. Therefore, it is proposed in this study that a complexation between glyphosate with exchangeable $Ca^{2+}$/plant available K will not reduce the bioavailability and mineralization of glyphosate. In the contrary, $Ca^{2+}$-glyphosate complexes may be transported more efficiently across microbial cell walls than sole glyphosate compound as it has already been argued for $Cu^{2+}$ complexes in literature (Kools et al., 2005). However, these mechanisms have not been documented and should be clarified.

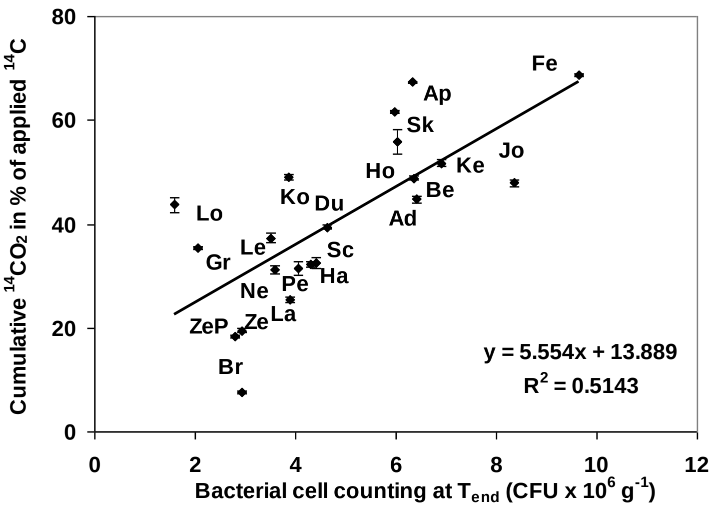


**Fig. 5.** Correlation between cumulative mineralization of glyphosate and bacterial cell counting (bars indicate standard deviation of 4 samples).

## CONCLUSIONS

Degradation of glyphosate in soils greatly varies, depending on soil properties. Mineralized glyphosate is affected by extractable acidity ($H^+$ cations) and bacterial cell counts. Sorption behavior and bioavailability of glyphosate in soil are important to regulate mineralization. extractable $H^+$ cations, $Ca^{2+}$ ions and plant available K have been identified as important soil parameters that collectively control the mineralization of glyphosate in soil. Glyphosate that is absorbed by Al/Fe-oxides and extractable $H^+$ cations can be extractable with NaOH 0.1 M, but it is not available for degradation by soil microorganisms. Non-extractable residues of glyphosate which have been identified as a result of microbial activity.



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# HERBICIDE DESORPTION IN ALFISOLS AND VERTISOLS OF ANDHRA PRADESH, INDIA

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**ABSTRACT** Adsorption–desorption is an important process for determining the ultimate fate of herbicides in soil. Adsorption-desorption studies of oxadiargyl and butachlor were conducted at AICRP on Weed Control, Acharya N G Ranga Agricultural University, Hyderabad during 2007-2008 by batch equilibrium technique in selected soils of Andhra Pradesh. Desorption isotherms showed a considerable hysteresis which was more prominent when desorption was carried out at higher concentrations of adsorbed oxadiargyl and butachlor. Desorption isotherms at lowest level of adsorbed herbicide concentration were close to adsorption isotherms. The cumulative desorption after five desorption steps was significantly different for all the initial concentrations of adsorbed herbicide and for all the soils. Desorption of these herbicides was higher in Alfisol than Vertisol. Desorption isotherms confirmed to Freundlich's equation and the Freundlich's constants increased with increasing initial concentration of adsorbed herbicide thus confirming the irreversible nature of the adsorption of oxadiargyl and butachlor on these soils.

***Keywords***: Oxadiargyl, butachlor, adsorption-desorption.

## INTRODUCTION

Soil applied herbicides undergo a number of consequential processes which determine their fate in soil environments. Adsorption–desorption or sorption dynamics and bound residues are important processes in determining the ultimate fate of herbicides in soils because detoxification mechanisms such as degradation, metabolism, microbial uptake and mobilization are operative only on the non-sorbed fractions of the chemical to the sites on soil mineral or organic surfaces. Adsorption decreases the concentration of chemical in solution and hence decrease bioavailability. Sorption influences mobility, persistence, degradation and volatility of pesticide in soil (Kalpana *et al.*, 2002). Adsorption and desorption are involved in determining the ease by which herbicides move through the soil profile and also their plant availability and microbial degradability thus persistence. Since oxadiargyl is a relatively new herbicide in use in India, there is little information on either its adsorption and desorption of oxadiargyl and butachlor in four soil types of Andhra Pradesh, hence this study was carried out for adsorption-desorption of oxadiargyl and butachlor.

## MATERIALS AND METHODS

Sorption experimental studies were conducted at All India Coordinated Research Programme on Weed Control, Acharya N G Ranga Agricultural University, Hyderabad, with Alfisols and Vertisols of Andhra Pradesh during 2007-2008. Representative soil samples were collected from the surface horizon with no background of oxadiargyl and butachlor application. The soils were air dried and passed through a 2 mm sieve. The physico-chemical properties of these soils were analysed by using standard procedures (Jackson, 1973). Adsorption studies were conducted using the batch equilibration technique by using technical grade oxadiargyl (98 % purity) and butachlor (86.87% purity).

The amount of herbicide desorbed was calculated as follows:

$$Co^{n} = Ce^{n-1} \times 15/20$$



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Where,

$Co^{n}$ = initial concentration of herbicide on $n^{th}$ day
$Ce^{n-1}$ = equilibrium concentration on $(n-1)^{th}$ day

The amount of herbicides desorbed on $n^{th}$ day is given by ($Ce^{n}$– $Co^{n}$) x 20. For example, the amount of oxadiargyl desorbed on the $5^{th}$ day is calculated as ($Ce^{5}$– $Co^{4}$ x 0.75 ) x 20. The data on desorption studies was subjected to mathematical analysis. Desorption was carried out on selected Vertisols and Alfisols of Andhra Pradesh over a period of 5 days by successive withdrawl and dilution of supernatant and reestablishment of equillibrium of soils on which the herbicide was previously absorbed. The most commonly used method to characterize adsorption – desorption has been Batch equilibrium technique (Amit Bist *et al.*, 2005).

## RESULTS AND DISCUSSION

**Desorption of oxadiargyl and butachlor from selected Vertisols and Alfisols**

The adsorption of these herbicides increased with their initial concentration of herbicide and this phenomenon was quite common in adsorption studies (Arvind *et al.,* 2000 and Nagamadhuri, 2003). The isotherms indicated that greater amount of herbicides were adsorbed in soils ($V_2 > A_8 > V_3 > A_4$). The higher adsorption on $V_2$ may be due to high organic carbon content. Similar observations were made for greater surface adsorption of butachlor in soils having high organic matter content. At low level of initial concentrations of the adsorbed herbicides the desorption isotherms were close to adsorption isotherms there by indicating an increase in the degree of irreversibility in adsorption–desorption. Desorption of these herbicides was higher in Alfisols (Fig 2 and 4) than Vertisols (Fig 1 and 3). Higher desorption in this soil may be attributed to low organic carbon and low clay content. The variation in percent desorbed may be due to heterogeneity involved in different soils, that vary widely in type and energy of bonding. In general, higher amounts of herbicide was desorbed during first washing and the amount progressively decreased with each subsequent washings. The per cent cumulative desorption revealed that the adsorption of these herbicides is almost irreversible indicating that the soil organic matter and clay content plays an important role in the adsorption – desorption of oxadiargyl and butachlor from soil solution affecting the bio availability of herbicides in soil. As observed from the adsorption-desorption data of oxadiargyl and butachlor, the desorption peaks exhibited pronounced hysterises in all the soils and for every level of initially adsorbed herbicide (Fig 1 to 4). The desorption was more pronounced when it was carried out at higher levels of adsorbed herbicide. At lower levels adsorption isotherms were very close to desorption isotherms, thus indicating irreversibility of adsorption-desorption mostly with increase in sorbed herbicide. The percent cumulative desorption in all the soils decreased with an increase in the amount initially adsorbed herbicide.

The data was subjected to mathematical analysis by Freundlich equation. The Freundlich constants $K_F$ values for desorption isotherms were found to be increased with increasing initial concentrations of the herbicides. Larger $K_F$ values for adsorption than for desorption indicate stronger binding of herbicides to the soils. The cumulative percentage of the herbicides adsorbed at the end of the desorption stages indicate that the percent desorbed in all the soils and for the herbicides used in the study decreased with increase in the amount initially adsorbed. The significant variations in the cumulative desorption between all soils and at different levels of initial concentration of herbicides can be due to the fact that the soil is a heterogeneous entity with sorption sites that vary widely in type and energy of binding. With the increasing amount of adsorbed herbicide, not only the surface sites are occupied but the herbicide may find its way into soil incorporation caused by clay and structural complex polymer net work.



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The extent of herbicides was more pronounced as the incubation time increased and that the herbicide recovery decreased with incubation time. The main cause of hysteresis during desorption could be the presence of a number of heterogeneous adsorbing sites of varying energy levels on soils. In addition some modifications could be taking place in the soil itself during adsorption desorption process.

**Table 1**. Physico-chemical and textural characteristics of four selected soils of Andhra Pradesh.

| **Soil No. & Name** | **pH** | **EC(dS $m^{-1}$)** | **OC (g/kg)** | **Texture (%)** | | |
|---|---|---|---|---|---|---|
| **Vertisols** | | | | Sand | Silt | Clay |
| $V_2$ : RARS, Lam | 7.82 | 0.22 | 0.85 | 55.8 | 11.0 | 33.20 |
| $V_3$ : Kurnool | 8.65 | 0.29 | 0.45 | 84.4 | 2.00 | 13.60 |
| ***ALFISOLS*** | | | | | | |
| $A_4$ : ARS, Ananthapur | 7.71 | 0.24 | 0.67 | 74.4 | 3.00 | 12.60 |
| $A_8$ : Karimnagar | 6.57 | 0.16 | 1.06 | 76.0 | 0.4 | 23.60 |

**Table 2.** Freundlich constants ($K_F$ and n) for desorption of oxadiargyl

| **Soil No. & name** | **Initial Concentration (ug/mL)** | | | | | | | | **% cumulative desorbed** |
|---|---|---|---|---|---|---|---|---|---|
| | **20** | | **40** | | **60** | | **80** | | |
| | **$K_F$** | **n** | **$K_F$** | **n** | **$K_F$** | **n** | **$K_F$** | **n** | |
| $V_2$ : RARS, Lam | 0.62 | 0.625 | 0.4 | 0.55 | 0.82 | 0.75 | 0.3 | 0.9 | 42.48 |
| $V_3$ : Kurnool | 0.28 | 0.45 | 0.36 | 0.44 | 1.12 | 0.3 | 0.66 | 0.5 | 36.25 |
| $A_4$ : ARS, Ananthapur | 0.22 | 0.4 | 0.42 | 0.88 | 0.38 | 1.01 | 0.18 | 0.66 | 43.58 |
| $A_8$ : Karimnagar | 0.58 | 0.3 | 0.74 | 0.50 | 0.52 | 0.37 | 1.12 | 0.75 | 52.54 |

**$K_F$ & n :** Freundlich constants

**Table 3.** Freundlich constants ($K_F$ and n ) for desorption of butachlor

| **Soil No. & Name** | **Initial Concentration(ug/mL)** | | | | | | | | **% cumulative desorbed** |
|---|---|---|---|---|---|---|---|---|---|
| | **20** | | **40** | | **60** | | **80** | | |
| | **$K_F$** | **n** | **$K_F$** | **n** | **$K_F$** | **N** | **$K_F$** | **n** | |
| $V_2$ : RARS, Lam | 0.62 | 0.62 | 0.40 | 0.55 | 0.82 | 0.75 | 0.30 | 0.90 | 38.56 |
| $V_3$ : Kurnool | 0.28 | 0.45 | 0.36 | 0.44 | 1.12 | 0.30 | 0.66 | 0.50 | 42.84 |
| $A_4$ : ARS, Ananthapur | 0.22 | 0.40 | 0.42 | 0.88 | 0.38 | 1.01 | 0.18 | 0.66 | 45.32 |
| $A_8$ : Karimnagar | 0.58 | 0.30 | 0.74 | 0.50 | 0.52 | 0.37 | 1.12 | 0.75 | 48.65 |

**$K_F$ & n :** Freundlich constants



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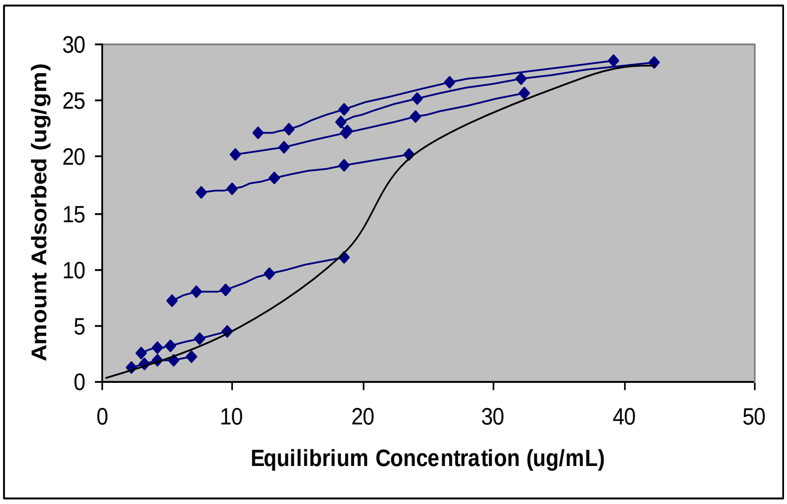


**Fig. 1.** Sorption isotherms of oxadiargyl in Vertisol -2

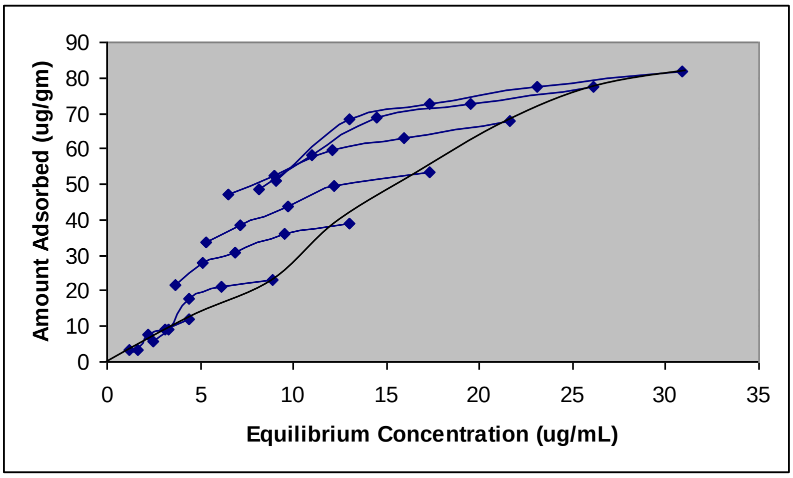


**Fig. 2.** Sorption isotherms of oxadiargyl in Alfisol -4

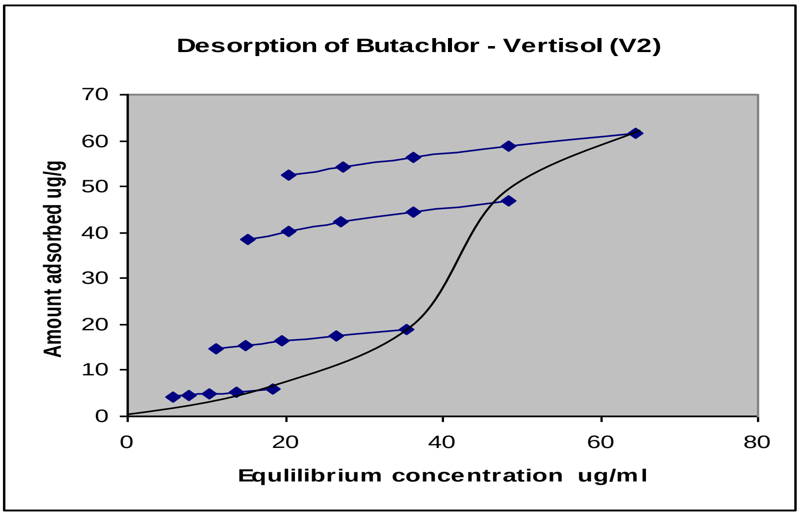


**Fig. 3.** Sorption isotherms of butachlor in Vertisol - 2



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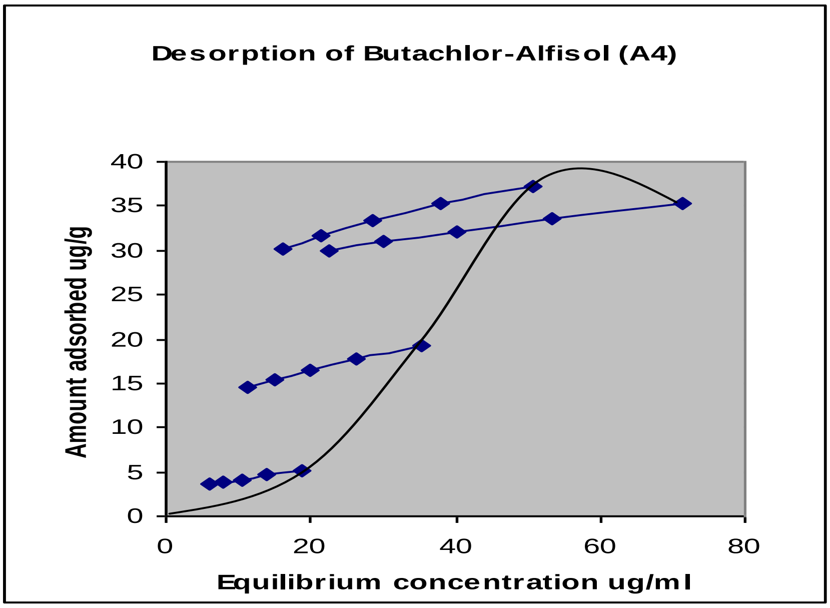


**Fig. 4.** Sorption isotherms of butachlor in Alfisol 4

## CONCLUSIONS

Desorption isotherms of soil adsorbed herbicides showed hysteresis, which was more when the desorption was carried out from higher levels of initial concentrations of herbicide solution. At low levels of initial concentrations of herbicide the desorption isotherms were very close to the adsorption isotherms, thereby indicating the degree of irreversibility in adsorption – desorption. Hysteresis was more pronounced at higher initial concentrations of herbicides. The cumulative percent of herbicide desorbed at end of time successive desorption stages showed a decrease with increase in the amount initially adsorbed. With increase in initial concentration of herbicide solution from lower to higher concentration, the $K_f$ values increased for all soils.

The variation in percent desorbed may be due to heterogeneity involved in different soils, that vary widely in type and energy of bonding. In general, higher amounts of herbicide was desorbed during first washing and the amount progressively decreased with each subsequent washings. The per cent cumulative desorption revealed that the adsorption of these herbicides is almost irreversible indicating that the soil organic matter and clay content plays an important role in the adsorption – desorption of oxadiargyl and butachlor from soil solution affecting the bioavailability of herbicides in soil.

## ACKNOWLEDGEMENTS

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# CHANGES IN CHEMICAL AND BIOLOGICAL CHARACTERISTICS OF SOIL UNDER LONG TERM APPLICATION OF HERBICIDES IN RICE –RICE SYSTEM

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**ABSTRACT** With a view to assess the long term impact of popular rice herbicides viz., butachlor, pretilachlor and 2,4-D in a laterite soil under Ultisol order, a "Long term herbicide trial in rice – rice system" was started in 2001 under All India Coordinated Research Programme on Weed Control, at the Agricultural Research Station, Mannuthy. The herbicides were applied twice a year at the rate of 1.25( butachlor), 0.45 ( pretilachlor) and 1.00 ( 2,4-D) kg $ha^{-1}$ as per the treatments . Surface soil samples ( 0-15 cm depth) were taken from the treatments during the period 2001- 2012. Soil chemical changes were assessed by analyzing organic carbon, pH, available P and K of the samples collected during the month of March. Earth worm count and microbial population were estimated from the samples taken at different times during the crop period. The impact of herbicides was elucidated by statistical analysis of the data: cumulative changes over the years for soil parameters and their relative changes in comparison to hand weeding. The magnitude of changes were more in the case of biological than chemical parameters. Among the biological parameters, soil fungi showed greatest variation, their population increased considerably. Bacterial population declined in all the treatments, the cumulative change being -89.3 to 90.3%. The earth worm population in different treatments remained similar to hand weeding. Rainfall pattern of the year played greater role in determining the population of microflora and earthworm. No adverse change in chemical properties was observed over a period of 10years. Soil pH was the least affected parameter. It is thus evident that the long term application of these herbicides at the recommended rates do not pose any serious threat to the rice ecosystem.

***Keywords***: Earthworm, microflora, progressive changes , soil pH.

## INTRODUCTION

The herbicides, butachlor, pretilachlor and 2,4-D are recommended in wet seeded rice in Kerala at the rate of 1.25 , 0.45 and 1.00 kg $ha^{-1}$ respectively at 6-9 , 3-5 and 20 days after sowing (KAU, 2007). At this rate of application, if these herbicides are assumed to be evenly distributed in the top 15 cm soil, the resulting herbicide concentration would be approximately 0.25- 0.60 mg/kg or even lower. However, there is a serious concern among the public about their adverse effect on the soil. Long term experiments are the best means to study the changes in chemical and biological properties of soil. Positive and negative effects of herbicides on the growth and activities of microorganisms in soils had already been reported (Gianfreda *et al*., 1995; Devi *et al*., 2008). With higher and more frequent inputs of pesticides, lower values of available N, EC, and TDS were observed. Changes in these properties were also attributed to the adverse effects of herbicides on soil microorganisms as shown in many research works (Fantroussi *et al*., 1999; Haney *et al*., 2002). Earthworms are also good bioindicators of soil quality. Earthworm population reflect the structural, microclimatic, nutritive and toxic status of soils (More, 2010).



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In view of the above, the present study was conducted to assess the long term impact of the above three herbicides on the chemical and biological properties of a laterite soil coming under Ultisol order in the Kerala state, India.

## MATERIALS AND METHODS

The trial was started in 2001 under All India Coordinated Research Programme on Weed Control, at Agricultural Research Station, Mannuthy of Kerala Agricultural University. . The plots have been receiving herbicide application twice in a year as per the treatments (Table 1)

**Table 1. Treatments**

| Treat. No. | Rice (1st crop) | Rice (2nd crop) |
|---|---|---|
| **T1** | HW-Twice (25 & 40 DAS) | HW-Twice (25 & 40 DAS) |
| **T2** | Butachlor + 2,4-D (100% NPK) | Butachlor + 2,4-D(.100% NPK) |
| **T3** | Butachlor + 2,4-D (100%NPK) | Pretilachlor + 2,4-D (100%NPK) |
| **T4** | Butachlor + 2,4-D (75%NPK+25% through FYM) | Pretilachlor + 2,4-D (75% NPK+ 25% throughFYM) |
| **T5** | Butachlor (rotated with pretilachlor between years) + 2,4-D (100%NPK) | Butachlor (rotated with pretilachlor, between years) + 2,4-D (100%NPK) |
| **T6** | Butachlor (rotated with pretilachlor between years)+ 2,4-D (75%NPK+25% through FYM) | Butachlor (rotated with pretilachlor between years ) + 2,4-D (75%NPK + 25% through FYM) |

*Butachlor @1.25kg/ha., Pretilachlor @ 0.75 kg/ha, 2,4-D @ 1.0 kg/ha.
NPK 100%: 70:35:35 kg/ha

Short duration rice varieties (Hraswa / Onam ) were raised in puddled field during the first and second crop seasons from 2001.The treatments were applied in large sized plots of 100 $m^2$ each. The bunds were kept permanent so as to avoid any mixing with the soil from outside the plot during land preparation. The fertilizers N, P and K were applied through urea/ ammonium phosphate, rock phosphate and muriate of potash respectively as per the treatment requirements.

Soil chemical changes were assessed by analyzing the major chemical properties viz., organic carbon, pH, available P and K of soil samples collected at 0-15 cm depth from each treatment during the month of March (two months after the second crop) every year from 2002 to 2012. The chemical analysis was performed as per standard procedures (Jackson, 1958., Hesse, 1972). Biological properties viz., earth worm count and microbial population were estimated from the soil samples taken at different intervals during the crop period (2007 to 2012 for earthworm and 2001- 2006 for soil microflora). Quantitative estimation of microflora was done by the method suggested by Rangaswami (1988). Herbicide residues in soil was estimated at different sampling intervals as per the standard procedures. From 2007 to 2012, the residues were estimated in soil, grain and straw immediately after harvest of the crop. The impact of herbicides on soil properties was elucidated by statistical analysis of the data *viz.*, i) cumulative change over the years for the major soil parameters and (ii) relative changes in soil properties in comparison to handweeding.

## RESULTS AND DISCUSSION

The changes in chemical characteristics after the harvest of rice crop in 2002 and 2012 are presented in Tables 2 and 3. Biological characteristics are presented in Tables 4, 5, 6, 7 and 8. In order to calculate the relative and cumulative changes in properties with respect to hand weeding, the herbicide treatments were set apart into three groups, *viz.*, hand weeding , herbicides without FYM( T2, T3 and T5 were pooled) and herbicides with FYM ( T4 and T6 were combined).



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**Effect of treatments on chemical properties of soil**

Among the different chemical properties, the relative changes with respect to hand weeding was maximum in the case of available K (- 9.5 to + 29 %) followed by organic carbon (-4.5 to + 23.6%). The relative changes in available K in the treatment without FYM were -9.5 and + 18.0 % for the year 2002 and 2012 respectively (Table 3) while that of treatment with FYM were – 8.1 and + 29.0 % respectively. In the case of FYM applied treatments, the relative change in available P was + 6.42 % in 2002 and + 19.1 % in 2012. At the same time, the treatment without FYM recorded changes of + 2.3 and - 0.3%, respectively.

During 2002, organic carbon changes (Table 2) were in the range of – 4.5 (with FYM ) to+ 23.6 ( without FYM) and that of 2012 were in the range of + 6.25 ( without FYM) and + 9.38 ( with FYM) . Greater relative changes in organic carbon in the treatment without FYM in the year 2002 could be attributed to the higher population of *Echinochloa* which would have played a key role in improving the organic carbon content of soil. The importance of FYM in improving the adsorption of herbicides and enhancing the efficacy for controlling *Echinochloa* population had been reported ( Devi and Abraham , 2010).

Soil pH was the least affected parameter. The relative changes in pH was zero in the treatment without FYM during the years 2002 and 2012. The FYM treatment recorded a change in pH of +1.9 % in both the years.

**Table 2.** Changes in pH and organic carbon over the period of 2002-2012

| Treatments | pH | | | Organic carbon( %) | | |
|---|---|---|---|---|---|---|
| | 2002 | 2012 | Change over the period (%) | 2002 | 2012 | Cumulative change over the period ( %) |
| Hand weeding (100% NPK) | 5.3 ( 0) | 5.3 ( 0) | 0 | 0.89 ( 0) | 0.64 ( 0) | -28.1 |
| Herbicides with 100 % NPK | 5.3 (0)) | 5.3 ( 0) | 0 | 0.98 ( +23.6) | 0.68 ( +6.25) | - 30.6 |
| Herbicides with 75 % NPK + FYM | 5.4 ( +1.9) | 5.4 ( +1.9) | 0 | 0.85 ( - 4.5) | 0.70 ( +9.38) | - 17.6 |

*Values in parentheses indicate relative changes with respect to hand weeding (%)

The cumulative changes in chemical properties (except pH) over a period of 10 years were higher than the relative changes over hand weeding. The cumulative changes in the different treatments ranged from -17.6 to - 30.6 %( organic carbon), +11.0 to +27.6 % (available P) and +9.2 to +53.4% (available K). Herbicides with FYM gave higher P and K changes than the other treatments. Positive effect of FYM on available P as well as K in the soil had been reported (Chaudhary *et al.*, 1981).



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**Effect of treatments on biological properties**

Observations on microbial population at different sampling intervals in the first crop season of 2001 indicated that butachlor residues in soil at 1 and 7 days after spraying inhibited the bacterial growth significantly. More detailed microbial analysis was conducted in the year 2002. Effect of herbicides on the population of bacteria and fungi studied at 1, 7, 15, 30 and 45 DAS and at the time of harvest showed that there was reduction in the population of soil microflora up to 30 days after spraying in all the herbicide applied plots. After 30 days, their population increased and tended to reach the original level by the time of harvest. At the time of harvest, no significant differences were noticed in bacterial and fungal population (Tables 4 and 5). The changes in biological properties are presented in Tables 6, 7 and 8. The cumulative changes were more pronounced than relative changes. The results also showed such magnitude of cumulative changes were more in the case of biological parameters than chemical parameters.

**Table 3.** Changes in available P and K over the period 2002-2012

| Treatments | Available P ( kg/ha) | | | Available K( kg/ha) | | |
|---|---|---|---|---|---|---|
| | 2002 | 2012 | Change over the period (%) | 2002 | 2012 | Cumulative change over the period (%) |
| Hand weeding (100% NPK) | 25.7 ( 0) | 29.3 ( 0) | + 14.0 | 128.4 ( 0) | 140.3 ( 0) | + 9.2 |
| Herbicides with 100 % NPK | 26.3 ( +2.3) | 29.2 ( - 0.3) | + 11.0 | 116.2 ( - 9.5) | 165.6 (+ 18.0) | + 42.5 |
| Herbicides with 75 % NPK +FYM | 27.35 ( +6.42) | 34.9 ( +19.1) | + 27.6 | 118.0 ( - 8.1) | 181.0 ( +29.0) | + 53.4 |

*Values in parentheses indicate relative changes with respect to hand weeding( %)

**Table 4.** Population of soil bacteria (No x $10^6$/g of soil) during the first crop, 2002

| Treatments | Before spraying | 1 DAS | 7 DAS | 15 DAS | 30 DAS | 45 DAS | Harvest |
|---|---|---|---|---|---|---|---|
| $T_1$ | 8.0 (2.86)* | 26.0 (5.14) | 14.6 (3.35) | 10.6 (3.32) | 8.0 (2.90) | 6.6 (2.67) | 10.0 (3.23) |
| $T_2$ | 12.6 (3.61) | 6.0 (2.53) | 0.6 (0.10) | 1.3 (1.27) | 3.3 (1.73) | 2.6 (1.64) | 6.3 (2.60) |
| $T_3$ | 14.0 (3.80) | 12.3 (3.41) | 1.3 (1.18) | 2.0 (1.47) | 0.6 (0.10) | 2.0 (1.47) | 12.0 (3.50) |
| $T_4$ | 22.6 (4.81) | 5.6 (2.46) | 19.0 (4.35) | 7.3 (2.73) | 3.6 (2.04) | 6.6 (2,64) | 12.6 (3.54) |
| $T_5$ | 10.0 (3.04) | 8.0 (2.89) | 1.0 (1.17) | 0.6 (0.10) | 2.3 (1.64) | 5.6 (2.44) | 11.3 (3.37) |
| $T_6$ | 16.6 (4.02) | 2.0 (1.56) | 1.6 (1.35) | 5.3 (2.35) | 3.3 (1.93) | 6.6 (2.67) | 10.3 (3.20) |
| CD (0.05) | NS | 1.146 | 2.052 | 1.103 | 1.023 | NS | NS |

*Values in parentheses indicate □x+0.5 transformed values



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**Table 5.** Population of soil fungi (No x $10^4$/g of soil) during the first crop season of 2002

| Treatments | Before spraying | 1 DAS | 7 DAS | 15 DAS | 30 DAS | 45 DAS | Harvest |
|---|---|---|---|---|---|---|---|
| $T_1$ | 6.3 (2.58)* | 5.0 (2.34) | 4.6 (2.25) | 3.0 (1.86) | 4.3 (2.20) | 7.3 (2.79) | 8.0 (2.90) |
| $T_2$ | 3.3 (1.93) | 3.0 (1.86) | 1.3 (1.27) | 0.6 (1.05) | 0.6 (1.05) | 5.0 (2.32) | 5.5 (2.62) |
| $T_3$ | 8.3 (2.93) | 4.3 (2.15) | 6.0 (2.54) | 3.6 (2.03) | 3.6 (2.03) | 7.6 (2.85) | 7.3 (2.04) |
| $T_4$ | 4.0 (2.11) | 1.6 (1.46) | 2.0 (1.56) | 3.0 (1.86) | 1.6 (1.39) | 5.0 (2.34) | 6.3 (2.59) |
| $T_5$ | 4.0 (2.11) | 1.6 (1.46) | 2.0 (1.56) | 3.0 (1.86) | 1.6 (1.39) | 5.0 (2.34) | 6.3 (2.59) |
| $T_6$ | 9.0 (3.08) | 3.6 (2.0) | 4.6 (2.02) | 6.0 (2.53) | 3.6 (2.04) | 7.0 (2.68) | 10.0 (3.20) |
| CD (0.05) | NS | NS | NS | 0.590 | 0.634 | NS | NS |
| CV % | 20.59 | 22.80 | 32.53 | 18.98 | 21.66 | 14.79 | 20.90 |

*Values in parentheses indicate $\sqrt{x+0.5}$ transformed values

**Table 6.** Changes in the population of soil bacteria over the period 2001- 2006

| Treatments | Population of bacteria ( No. X $10^4$ ) per gram soil | | Cumulative change over the period ( %) |
|---|---|---|---|
| | Before first crop 2001 | Before first crop 2006 | |
| Hand weeding (100% NPK) | 460.0 ( 0) | 44.7 ( 0) | 90.3 |
| Herbicides with 100 % NPK | 363.3 ( - 21.05) | 37.3 ( -16.42) | 89.7 |
| Herbicides with 75 % NPK + FYM | 400.0 ( -13.04) | 42.84 ( -4.1) | 89.3 |

*Values in parentheses indicate the relative changes with respect tohand weeding ( %)

Among the boiological parameters, soil fungi showed greatest variation over a period of 5 years from first crop 2001 to first crop 2006 , The cumulative changes showed by the different treatments ranged from + 420.0 to +1203.9 % , the higher positive effects were shown by the herbicide treated plots with FYM. In the case of bacteria, cumulative changes in population ranged from -89.3 to -90.3% , which indicated that herbicide application is not the factor responsible for variations in the population of soil microflora. The same trend was noticed over the period 2002- 2006 -87.4 to -94.5%).Other factors like climate especially rainfall, continuous application of fertilizers, etc. would have significant effect on the soil microflora.

The data on earthworm population during the first crop 2007 and 2012 showed that earth worm population was more at 1 month after spraying compared to two months after spraying. This was mainly due to the higher moisture content of the soil at 1 month after sparaying.The cumulative changes in earth worm population over the period of first crop (30DAS) 2007 to first crop (30 DAS) 2012 in the herbicide treated plots were similar to hand weeding treatment, the changes being -88.5(HW), -88.1(herbicide without FYM) and -74.9 % (herbicide with FYM). Compared to cumulative changes, the magnitude of relative changes was less. It was seen that organic matter and moisture content are the major factors affecting the growth of earth worm.



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**Table 7**. Changes in the population of soil fungi over the period 2001-2006

| Treatments | Population of fungi ( No x $10^3$ ) per gram soil | | Cumulative change over the period (%) |
|---|---|---|---|
| | Before first crop 2001 | Before first crop 2006 | |
| Hand weeding (100% NPK) | 10.0<br>( 0) | 52.0<br>( 0) | ( + 420.0) |
| Herbicides with 100 % NPK | 5.9<br>( -40.67) | 68.1<br>( + 30.99) | ( + 1049.6) |

*Values in parentheses indicate the relative changes with respect to hand weeding (%).

**Table 8.** Changes in the population of earthworm over the period 2007 to 2012.

| Treatments | Population of earthworm (No../ kg soil) in the first crop (30 DAS ) | | Cumulative change over the period (%) |
|---|---|---|---|
| | 2007 | 2012 | |
| Hand weeding (100% NPK) | 75.5<br>( 0) | 8.66<br>( 0) | -88.5 |
| Herbicides with 100 % NPK | 48.5<br>( - 35.8) | 5.78<br>( - 33.3) | -88.1 |
| Herbicides with 75 % NPK + FYM | 49.3<br>( - 34.7) | 12.35<br>( + 42.6) | -74.9 |

**Butachlor, pretilachlor and 2,4-D residues in soil, grain and straw**

Analytical data on herbicide residues indicated that all the three herbicides dissipated from the soil very fast and reached levels below the detectable level by the time of harvest. Grain and straw samples also did not show any detectable amount of herbicide residues. In order to see whether there is any build up of residues due to continuous application of herbicides, a comparison of the residue levels at 30 DAS over the years 2002 and 2006 was made. There was an increase in the content of all the three herbicides in soil (compared to the data obtained in 2002) in the FYM applied plots. This could be the reason for better weed control achieved in those treatments. However, the residues dissipated and reached below the detectable level of 0.01ppm by the time of harvest.

The present investigation made it clear that the long term application of herbicides at the recommended rates do not pose any serious threat to the rice ecosystem.

**Table 9.** Changes in residue levels between the years.

| Treat ments | First crop | | | | | | Second crop | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Buta(µg/g) | | Pretil(µg/g) | | 2,4-D(µg/g) | | Buta(µg/g) | | Pretil(µg/g) | | 2,4-D(µg/g) | |
| | 2002 | 2006 | 2002 | 2006 | 2002 | 2006 | 2002 | 2006 | 2002 | 2006 | 2002 | 2006 |
| T1 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| T2 | 0.024 | 0.029 | NA | NA | 0.020 | 0.015 | 0.020 | 0.045 | ND | NA | 0.016 | 0.019 |
| T3 | 0.041 | 0.032 | NA | NA | 0.035 | 0.014 | NA | NA | 0.030 | 0.025 | 0.015 | 0.028 |
| T4 | 0.020 | 0.028 | NA | NA | 0.015 | 0.023 | NA | NA | 0.010 | 0.030 | 0.005 | 0.025 |
| T5 | NA | NA | 0.0205 | 0.017 | 0.015 | 0.010 | NA | NA | 0.025 | 0.020 | 0.010 | 0.010 |
| T6 | NA | NA | 0.0130 | 0.019 | 0.010 | 0.034 | NA | NA | 0.010 | 0.040 | 0.005 | 0.030 |

*NA: Not analysed (the particular herbicide is not applied)



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## *Allelopathy and Allelochemicals*

# ISOLATION AND CHARACTERIZATION OF ALLELOPATHIC SUBSTANCE FROM *LEUCAS ASPERA*

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**ABSTRACT** *Leucas aspera* (Willd.) L., a medicinal herb is well known for many pharmacological and toxicological properties, but very little is known about its allelopathic activity. Hence, to investigate the allelopathic potential of *L. aspera*, the aqueous methanol extract of this plant at four different concentrations were tested against nine test plant species, *viz.* cress (*Lepidum sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), rapeseed (*Brassica napus L.*), Italian ryegrass (*Lolium multiflorum* Lam.), crabgrass (*Digitaria sanguinalis* L. scop.), barnyard grass (*Echinochloa crus-galli* L. Beauv.), jungle rice (*Echinochloa colonum* L. Link) and timothy (*Phleum pratense* L.). The aqueous methanol extract of *L. aspera* significantly inhibited the seedling growth of all test plant species at concentrations ≥30 mg dry weight equivalent extract/mL. The inhibitory activity of the *L. aspera* extract was concentration and test-plant-species dependent. Based on $I_{50}$ values, the hypocotyl of cress and the root of crabgrass were the most sensitive to the extract, whereas coleoptile of jungle rice and the root of rapeseed were the least sensitive. These results indicate that aqueous methanol extract of *L. aspera* have allelopathic properties to suppress the growth of other plant species, and motivated us to further work with this extract for isolation and characterization of those allelopathic compounds. The extract was partitioned into ethyl acetate and aqueous fractions, and stronger inhibitory activity was observed on ethyl acetate fraction. Then the ethyl acetate fraction was purified by column of silica gel, Sephadex LH-20, reverse phase $C_{18}$ cartridge and HPLC. An active allelopathic compound was determined through spectral data. This compound could be responsible for the inhibitory activity of *L. aspera* on several test plant species used in our research. Furthermore, it might lead to the development of new natural herbicide to control major crop weeds especially barnyard grass in more sustainable way.

***Keywords:*** Allelopathy, medicinal plants, Lamiaceae, natural herbicide, sustainable agriculture

## INTRODUCTION

Current agricultural practices are mainly relying on heavy use of synthetic herbicides to control weeds, which may create a number of environmental hazards. Researchers are now searching for new alternatives of synthetic herbicides which are bio-degradable and environment friendly. In this backdrop, allelopathic medicinal plants could play a crucial role. Allelopathy is known as the harmful or beneficial effects of one plant on another through the release of allelochemicals (Molísch 1937). The allelochemicals are released into the environment through exudation, decomposition, leaching and/or volatilization, and may be toxic or stimulatory to the plant itself or other plant species (Scognamiglio et al. 2012; Islam and Kato-Noguchi 2013). Isolation and identification of active allelochemicals from allelopathic medicinal plants could be helpful to design new natural herbicide as a substitute of synthetic herbicides.



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*Leucas aspera* (Willd.) Linn. is an erect, small, herbaceous, ethno-botanically important medicinal plant belonging to Lamiaceae family. It is found in the high land crop fields, roadsides, homesteads and fallow lands of south Asia. Though the plant is considered as deleterious weed in the highlands of some areas, it has many pharmacological and/or insecticidal properties (Mangathayaru et al. 2006; Islam and Kato-Noguchi 2012). Since the first phytochemical study of *L. aspera* was reported long ago (Shirazi 1947), there have been no reports so far on the isolation of allelopathic compounds from this plant. Furthermore, to the best of our knowledge the first preliminary study on the allelopathic activity of *L. aspera* was done by Islam and Kato-Noguchi (2012). Hence, the current research was conducted to isolate and characterize the active allelochemicals from *L. aspera*.

## MATERIALS AND METHODS

The whole plant parts (leaves, stem and roots) of dried *L. aspera* were extracted with 70% (v/v) aqueous methanol and filtered. The residue was re-extracted with methanol and filtered. Two filtrates were combined and an aliquot of the extract (final assay concentration @ 3, 10, 30 and 100 mg dry weight [DW] equivalent extract/mL) was added to a sheet of filter paper (No. 2) in a 28 mm Petri dish. After evaporation of the methanol, 0.6 mL of 0.05% Tween 20 solution was added into the Petri dish. The biological activity of the extract was determined against cress (*Lepidum sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), rapeseed (*Brassica napus L.*), Italian ryegrass (*Lolium multiflorum* Lam.), crabgrass (*Digitaria sanguinalis* L. scop.), barnyard grass (*Echinochloa crus-galli* L. Beauv.), jungle rice (*Echinochloa colonum* L. Link) and timothy (*Phleum pratense* L.). Those test plants were sown on the filter paper in the Petri dish. The hypocotyl/coleoptile and root growth of the test plant species were determined at 48 h after incubation in darkness at 25 ºC. The bioassays were conducted according to Islam and Kato-Noguchi (2012) with some modification.

Experimental data were analyzed using predictive analytics software (PASW) statistics17.0 (SPSS Inc., Chicago, Illinois, USA) and GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, California, USA).

## RESULTS AND DISCUSSION

### Allelopathic activity

The aqueous methanol extract of *L. aspera* significantly inhibited the seedlings growth of all test plant species at concentrations ≥30 mg DW equivalent extract/mL. The inhibitory activity of *L. aspera* plant extract was concentration and test-plant-species dependent. In addition, the root growth of test plant species was more sensitive to extract than the hypocotyl/coleoptile growth. The hypocotyl/coleoptile growth of cress, lettuce, alfalfa, rapeseed, Italian ryegrass and timothy showed >80% inhibition at a concentration of 100 mg DW equivalent extract/mL, whereas that of barnyard grass and jungle rice shown >65% inhibition. At the same concentration, the root growth of timothy, jungle rice and barnyard grass was completely inhibited and that of cress, lettuce, alfalfa, rapeseed and Italian ryegrass shown more than 90% inhibition. The concentration required for 50% inhibition (defined as $I_{50}$) of the hypocotyl/coleoptile and root growth of those nine test plant species ranges from 7.5−60.4 and 2.2−17.1 mg DW equivalent extract/mL, respectively. The hypocotyl growth of cress and the root growth of crabgrass were the most sensitive to the extract, whereas coleoptile growth of jungle rice and the root growth of rapeseed were the least sensitive. These results suggest that *L. aspera* plant extract may have allelopathic properties and, thus contain allelochemicals.

### Isolation and characterization

The aqueous residue was adjusted to pH 7.0 with 1M phosphate buffer, and partitioned into ethyl acetate and aqueous fractions. The isolation process of active allelochemicals was carried out using the ethyl acetate fraction, as the fraction showed stronger inhibitory activity than the aqueous fraction. The fraction was then purified by column of silica gel, Sephadex LH-20,



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reverse phase $C_{18}$ cartridge and HPLC. Cress seedling was used as an indicator plants to check the biological activity in every step of purification. Finally, an active allelochemical was determined through spectral data.

Results showed that this allelochemical could be responsible for the growth retarding activity of *L. aspera* on different test plant species used in our research, and may provide the chemicals basis for the development of new natural herbicide to control major crop weeds especially barnyard grass. The species dependent inhibitory activity of *L. aspera* might be helpful to develop selective herbicides. Furthermore, the crude extract and/or residue of that plant could also be recommended to apply directly as bio-herbicide.

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# ALLELOPHATIC ACTIVITY OF LANTANA LEAF EXTRACT (*LANTANA CAMARA*) ON THE WEED INTEA (*CAMELLIA SINENSIS*)

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**ABSTRACT** Weed is one of the important constraints of tea production world wide, which could inflict loss up to 20% in average. Weeds are commonly controlled using synthetic-chemical herbicides. Besides demanding a high cost, the use of synthetic-chemical herbicides can also inflict various negative impacts, such as residue problem, which recently has become a critical issue in world-tea trade. Therefore, an economic and safe alternative method of control is *i.e.* biological control needed to be explored and used in controlling weeds in tea. A study to evaluate the allelophatic activity of Lantana leaf extract was conducted at Pasir Sarongge Experimental Garden, Research Institute for Tea and Cinchona, from June up to December 2009. Results showed that Lantana leaf extract could suppress the growth of weed in tea plantation. Lantana leaf extract at 10% concentration obtained a better suppression and significantly different compared to check synthetic herbicide as well as mechanical weeding.

***Keywords***: Allelophatic activity, Lantana leaf extract, weed suppression, tea plant.

## INTRODUCTION

Weeds are a problem chiefly in new clearings and pruned fields and the need for their control persists till the bushes cover the ground. Several species of grasses, broadleaved, ferns and sedges weeds invest the tea field under different agroclimatic conditions of the tea growing countries in the world.

It was estimated that the losses of crop do to weeds bellow the potential maximum are 9, 6.6 and 6.8 percent in Asia, Africa and South America respectively (Cramer, 1967 *in* Hajra, 2001). Magnitude losses for weed infestation in tea are quite high. In tea, broadleaved weeds reduce the yield to the extent of 12 percent whereas 21 percent losses are because of grasses in south India (Hudson, *et. al.*, 1997). But the losses of tea crop due to weeds could be insignificant with high quality of management (Willson, 1999).

Plant architecture plays an important role in determining the losses caused by weeds. The China hybrid tea plants bear many stems arising from collar level, which develop the soil pockets providing shelter to weeds. As such the weeds growing in these pockets escape various manual and chemical treatments for weed control. The problem is further aggravated incase of perennial grass as the same is difficult to uproot along with underground propagules. Further, the grass can not be dug out as it may damage stems of tea. Also application of herbicides over bush frame is risky operation (Singh, 1999).

The grass weed problem is greatest in the clearing and in mature tea during first two years of the pruning cycle. In open situations, with poor or no shade grass weeds become prominent. Soon after the grass is killed, it can be noticed that there is invasion of broad leave weeds flora (Sanusi, 1977). *Cyperus rotundus* Linn. is one of the predominant sedges weed in tea fields in Indonesia (Soedarsan *et. al.,* 1977). A thorough knowledge of weed flora, seed dormancy, critical period of weed growth are essential for proper weed management. Various methods of weed control are practiced.

In tea, hand weeding, sickling, forking and mulching are the conventional methods of weed control. Hand weeding is carried out in the nurseries and the newly planted tea areas where application of any chemical herbicide is hazardous for the plants. Hand weeding is the most efficient method, but it is time-consuming and rather expensive.



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Controlling weeds manually has become increasingly expensive and there is often a shortage of labor during peak cropping season. To cope up with the problems, herbicides were introduced in tea industry with satisfactory results. The benefits of chemical weed control in terms of tea yield were demonstrated by Rahman (1991). Though chemical weed control initially is more expensive than mechanical or manual control but in the long run, rational application of herbicides can only optimize weed control. On the other hand, the potential for undesirable environmental contamination from herbicides is relatively high, and there is a need for environmentally safe herbicide that are equally or more effective and selective than currently available synthetic herbicides.

Allelopathic compounds released by crops, weeds, or their residues may offer solutions to some of these needs (Putnam, 1988). Most of the natural products that cause allelopathy are a subset of the array of secondary compounds synthesized by plants. In general, allelopathic chemical groups useful or potentially useful in agriculture are including flavonoids, polyacetylenes, quinones and terpenes. Many of these compounds and their derivatives are believed to serve as models for new herbicide. Further, attention for exploring the feasibility of using allelopathic properties of certain potential weed species in tea weed management is also needed.

*Lantana camara* is an important weed species in tea field in Indonesia. Phenolic compounds found in the leaf of *L. camara* are effective against several weed species in upland rice such as Panicum *psilopodium* and *Digitaria sanguinalis* (Bansal, 1998). To investigate the allelopathic effect of *Lantana camara* on the germination and growth of weeds in tea plantation, a study was conducted in Indonesia Research Institute for Tea and Cinchona (RITC).

## MATERIALS AND METHODS

### Materials

### Extraction of Lantana Leaf

One kilogram of lantana leaves was homogenized with one liter of isopropyl alcohol using a blender. The extract was filtered and transferred into glass vials. One liter of distilled water was added to the extract. To evaporate the isopropyl alcohol, the vials were kept in a vacuum oven for about 24 hours. After evaporation of isopropyl alcohol, the final stock solution was stored in the refrigerator until the treatments were imposed.

### Methodology

**Experiment 1**. **Effects of lantana leaf extract on the germination of several weed species.** The experiment was laid out in a Completely Randomized Design with four replications. The design had the following treatments: 1). Lantana leaf extract at 1% concentration, 2). Lantana leaf extract at 5% concentration, 3). Lantana leaf extract at 10% concentration, 4). Lantana leaf extract at 20% concentration, 5). Synthetic pre-emergence herbicide Oxyfluorfen at 0.5% concentration, 6). Control (distilled water).

Lantana leaf extract 1, 5, 10 and 20 percent concentration were prepared by dissolving 5, 25, 50 and 100 ml of stock solution to 495, 475, 450 and 400 ml of distilled water, respectively. Fifty seeds of *Bidens pilosa* was tested for the germination in petri dishes lined with "Whatman No. 1" filter paper saturated with 5 ml of the extracts. Two sets of petri dishes for each treatment replicated four times were then placed at room temperature under laboratory condition. The filter papers were constantly moistened with the appropriate extract using a pipette every five days until the germination test was finished.

Germination percentage of weed species were determined by counting the number of seeds germinated in each petri dish at 14 days after sowing. The percentage of germination was determined using the formula: % germination = (number of seed germinated divided by number of seed sown) x 100%.



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**Experiment 2. Effects of lantana leaf extract on the growth of weed in young tea fields.**
The experiment was designed in a Randomized Completely Block (RCB) with four replications. The design had the following treatments: 1). Lantana leaf extract at 1% concentration, 2). Lantana leaf extract at 5% concentration, 3). Lantana leaf extract at 10% concentration, 4). Lantana leaf extract at 20% concentration, 5). Synthetic pre-emergence herbicide Oxyfluorfen at 0.5% concentration, 6). Mechanical weed control. The Lantana leaf extract as well as Oxyfluorfen were applied in high volume at 400 l/ha by a conventional knapsack sprayer. Weed control was evaluated by the dry weight of weed biomass sampled from two 50 by 50 cm quadrants in each plot at 1, 2 and 3 months after treatment.

## RESULTS AND DISCUSSION

**Experiment1. Effects of lantana leaf extract on the germination of *B. pilosa.***
The percent germinations of *B. pilosa* under laboratory condition at 14 days after sowing were significantly influenced when treated with the lantana leaf extracts at different concentration (Table 1). The seed of *B. pilosa* treated with distilled water as control produced the highest percent germination (98.25%) which was significantly higher compared to those seeds treated with 1% concentration of lantana leaf extract (59.50%). With further increase in the concentration of lantana leaf extract to 5, 10, and 20%, the percent germination of *B. pilosa* seeds were significantly decreased further to 43.50%, 30.25%, 31.75%, respectively. In the case of the application of synthetic pre-emergence herbicide Oxyfluorfen at 0.5% concentration, this treatment gave the lowest percent germinations of *B. pilosa* (27.00%).

**Table 1.** Percent germination of seeds of *B, pilosa* at varying concentration of lantana leaf extracts at 14 days after sowing.

| Treatment | Percent germination*) |
|---|---|
| Lantana leaf extract at 1% concentration | 59.50 c |
| Lantana leaf extract at 5% concentration | 43.50 b |
| Lantana leaf extract at 10% concentration | 30.25 a |
| Lantana leaf extract at 20% concentration | 31.75 a |
| Synthetic herbicide Oxyfluorfen at 0.5 % concentration | 27.00 a |
| Control (distilled water) | 98.25 d |

* Figures followed with the same letters are not significantly different according to the Duncan multiple range test at $p<0.05$.

**Experiment 2. Effects of lantana leaf extract on the growth of weed at young tea field.**
The growths of weeds at young tea field were highly affected when treated with the lantana leaf extracts at different concentration (Table 2). Since these were affected, the over-all effects were translated to the dry weights of weed biomass. Dry weight of weed biomass indicates their response to the application lantana leaf extracts.



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**Table 2**. The average of dry weight of weed biomass (g/0.25 $m^2$) at every observation

| Treatment | 1 MAT | 2 MAT | 3 MAT |
|---|---|---|---|
| Lantana leaf extract at 1% concentration | 5.67 d | 14.70 b | 27.73 cd |
| Lantana leaf extract at 5% concentration | 4.90 d | 14.97 b | 27.37 bc |
| Lantana leaf extract at 10% concentration | 4.30 c | 10.83 ab | 23.87 b |
| Lantana leaf extract at 20% concentration | 3.63 b | 9.63 a | 18.13 a |
| Oxyfluorfen herbicide at 0.5 % concentration | 0.73 a | 14.03 b | 32.47 cd |
| Control (mechanical weeding) | 6.60 e | 21.63 c | 37.17 d |

* MAT = month after treatment

** Figures followed with the same letters are not significantly different according to the Duncan multiple range test at $p$ <0.05.

The dry weight of weed biomass at young tea filed was markedly influenced by the application of lantana leaf extracts at any concentration. Dry weights of weed biomass at varying concentrations of lantana leaf extracts indicate significant differences due to concentration of extract. At the observations of one and two months after treatment, the application of lantana leaf extracts at any concentration obtained lower dry weight of weed biomass and significantly different compared to control treatment. Different result was obtained at three months observation, where the dry weights of weed biomass taken from the treatment of lantana leaf extracts at 1% concentration was comparable with that of the control treatment.

In terms of the application of synthetic pre-emergence herbicide Oxyfluorfen at 0.5% concentration, this treatment gave the lowest dry weight of weed biomass especially at one month observation. Different results were obtained at two and three months observations. At two months observation, this treatment was comparable to the treatment lantana leaf extracts at 1, 5 and 10% concentrations but higher and significantly different compared to lantana leaf extracts at 20% concentration. Meanwhile, at three months observation, this treatment was comparable only to the treatment lantana leaf extracts at 1 and 5% concentrations but higher and significantly different compared to lantana leaf extracts at 10 and 20% concentrations.

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# EFFECT OF DIFFERENT AGRONOMIC PRACTICES ON *VERNONIA ZEYLANICA* (L.) PLANT POPULATION CHANGES AND SEEDLING EMERGENCE PATTERN IN COCONUT PLANTATIONS IN SRI LANKA

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**ABSTRACT** *Vernonia zeylanica* (L.) belongs to the family Asteraceae is a prominent and dominant weed species in coconut plantations in Sri Lanka. This study reports the results of a field study conducted to determine the effects of selected agronomic practices on plant population changes and seedling emergence pattern of *Vernonia zeylanica*in coconut plantation in the low country dry zone of Sri Lanka. Treatments imposed were application of glyphosate (N-(phosphonomethyl)-glycine) ($T_1$), cover cropping with *Pueraria phaseoloides* ($T_2$), tractor harrowing ($T_3$), tractor slashing ($T_4$) and hand weeding ($T_5$). All treatments were applied twice a year except for the ($T_2$). Based on the results of reduction in biomass of *Vernonia zeylanica*biomass, application of glyphosate ($T_1$), cover cropping ($T_2$) and hand weeding ($T_5$) practices were very efficient treatments to reduce the *Vernonia zeylanica* population. Chemical weeding and cover cropping were the best methods to reduce *Vernonia zeylanica*seedling emergence density in the field. The effectiveness of slashing and harrowing practices in reducing *Vernonia zeylanica*seedling emergence density was lower than cover cropping and chemical weeding treatments. The seedling emergence densities were almost similar in slashed and harrowed plots. The seedling emerged depth of *Vernonia zeylanica*was very high in harrowed treatment when compared to other treatments. This indicated that loosening the soil creates more favorable environment for the germination of *Vernonia zeylanica* seeds buried in soil. Therefore, it can be suggested that the elimination of *Vernonia zeylanica* seeds in the top 2- 4cm depth in the soil seed bank by any means is likely to reduce the level of weed infestation by about 60% to 95%.

***Keywords:*** Cover crop, glyphosate, harrowing, slashing, seed germination

## INTRODUCTION

The growth habit and canopy structure of the coconut palm requires a wide spacing between palms, which permits abundant sunlight to the ground vegetation. Thus, a wide range of perennial and annual weed species invades unutilized space beneath the plantation (Senarathne et al. 2003). Such weeds invariably compete with coconut for soil moisture and nutrients, affecting growth and yield and obstructing routine management practices (Senarathne et al. 2003). *Vernonia zeylanica* (L) is an herbaceous, perennial, broad leaved, erect, strongly aromatic and deep rooted herb belonging to the family Asteraceae, and is one of the major endemic weed species present in coconut plantations in Sri Lanka. It competes for soil moisture, nutrients and light especially when palms are at the seedling stage. Jayaweera (1982) stated that it is under shrub with many straggling, divaricated cylindrical branches finely tomentone when young. Therefore, it grows vigorously by covering the ground of coconut plantations in both moist and dry conditions. Additionally it interferes and causes inconvenienced to estate management practices such as manuring, harvesting and collection of nuts. Initially this weed species spreads by seeds and stem cuttings, it can also re-grow after slashing. Therefore, there is an acute need to introduce effective and economically viable weed control strategies for coconut growers in Sri Lanka.

In integrated weed management, all available strategies are used to reduce weed seeds in the soil, to prevent weed emergence from the seed bank and to minimise competition from weeds (Holt, 1991; Swanton and Weise 1991; Thill et al. 1991; Liebman and Dyck 1993). This



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implies that any successful long term weed management programme requires the control of seedlings arising from the soil. Further, unpredictable emergence of weed seedlings from dormant seeds makes weed management a more difficult and costly operation in arable lands (Carves and Benoit 1989). Therefore, manipulation of environmental conditions to reduce emergence of undesirable species in the planted crop or at least to optimize the establishment of desirable species is an effective and economical strategy for weed management. In order to develop a sustainable integrated weed management strategy, a detailed understanding of the seed bank is required, incorporating germination characteristics of weed seeds and factors that regulate emergence and establishment of seedlings in the field. Although there are many studies on weed biology, weed competition and herbicide technology, little attention has been paid to investigate the regulation of weed seedling emergence in coconut lands, which is the focus of this study. Therefore, the objective of this study was to evaluate the effect of different practices for management of *Vernonia zeylanica* on the seedling emerging pattern and emerged seedling population under field conditions.

## MATERIALS AND METHODS

This experiment was carried out at the Nimalka Estate, Welipanna, in the Low country Dry Zone of North Western province of Sri Lanka from June 2010 to December 2012. The area is characterized by bi-modal pattern of rainfall with an annual mean precipitation of >1100mm. Approximately 65% of the annual rainfall is received from September to December (Maha rain season). The soil at the site is a predominantly well-drained Red Yellow Podzolic (RYP) soil with soft or hard laterite (70-90%) (De Alwis and Panabokke 1972). Surface soil is brown in colour with a sandy loam texture. Structure development is moderate due to presence of sand in the surface soil. Sub surface soil is dark to yellowish brown in colour with prominent mottles. Texture of the subsoil is sandy loam to sandy clay loam. Reaction of the soil is strongly acidic (pH 5.0 – 5.5). Base saturation of the subsurface soil is greater than 35%. Organic carbon content in the surface soil is generally less than 1% under natural conditions (Mapa et al. 2005). The experimental design was a Randomized Complete Block design with three replicates and plot size was four coconut squares (the spacing of the square planting system of coconut is 8.2m x 8.2m).

**Treatments:**

Five different weed management treatments

$T_1$. Chemical weeding (Application of Glyphosate 1.44 kg a.i. per hectare)
$T_2$. Establishment of cover crop (*Puereria phasioloides*)
T3. Tractor harrowing (once in six month) (0cm - 15cm depth)
$T_4$. Tractor slashing (once in six month)
$T_5$. Hand weeding (once in six months)
$T_6$. Unweeded (Control)

The different weed management systems were applied to control *Vernonia zeylanica*according to the schedule. In the chemically controlled plots glyphosate (1.44 ai kg /ha) was applied at 6 monthly intervals, at the latter part of the rainy season using a knapsack sprayer in the morning. Generally there was no rain for five to six hours after applying glyphosate. The cover crop was established to control *Vernonia zeylanica*and the over grown conditions of cover crop was managed to overcome competition by harrowing once a year. Tractor harrowing, slashing and hand weeding were done at the latter part of the rain season at six monthly intervals.



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**Data collection:**

**Weed biomass.** The *Vernonia zeylanica*biomass within 1m x 1m quadrates was collected from four random points per plot. Plant samples were dried at 80 $C^0$ for five days and weighed. The dry weight of *Vernonia zeylanica*was measured separately every two months from June 2010 to December 2012.

**Emergence of *Vernonia zeylanica*seedlings in the field.** Four permanent quadrates (1m x 1m) were fixed randomly in each plot to monitor emergence of *Vernonia zeylanica*seedlings. The emerging seedling count was taken before and after applying all treatments. The weeds which emerged around a 30cm border area outside each quadrate were removed frequently while the remaining area had free weed growth. The emerged *Vernonia zeylanica*seedlings within each quadrate were identified, counted and removed weekly for 12 weeks after applying the treatments. Estimation of average seedling density was obtained by summing the seedling count over the experimental period from June 2010 to December 2012.

**The seed depth of emerging weed seedlings in the field.** This study was done in 2010 Yala rain season (April – June), 2010 Maha rain season (September – October), 2011 Yala rain season (April – June), 2011 Maha rain season (September – October), 2012 Yala rain season (May – June) and 2012 Maha rain season (September – October). The germination and emergence of *Vernonia zeylanica*seeds at different depths in the soil was measured in the field. The study used 30 *Vernonia zeylanica* seedlings. The seedlings were marked at ground level with Indian ink and each seedling was excavated to the depth of its caryopsis and the length for the caryopsis to the ink mark of each seedling was measured as described by Witharama (1998). Estimation of average seedling emergence depth was calculated by measuring the seedling emergence depth over the four rain seasons.

**Data analysis:** Data were analysed statistically by the Procedure of Analysis of Variance and means were separated using the Least Significant Difference test at the 0.05 significance level. Statistical program was the Statistical Analysis System (SAS 1999).

## RESULTS AND DISCUSSION

### Effect of different agronomic practices on *Vernonia zeylanica*biomass

The lowest *Vernonia zeylanica*biomass was recorded in glyphosate applied plots (chemical weeding $T_1$) at a concentration of 1.44 kg a.i. /ha, in plots with a *Pueraria phaseoloides* cover crop ($T_2$) and hand weeding plots ($T_5$) (Fig. 1). When glyphosate was applied, the *Vernonia zeylanica*biomass was reduced and weed seeds in the soil seed bank initiated germination with the onset of rainy season.

Initially *Pueraria phaseoloides* took several months to establish a good ground cover. The biomass of *Vernonia zeylanica*was very high at the initial stages in cover cropped plots which gradually declined after December 2010. *Pueraria* regenerated with seeds with time and formed a good ground cover, thereby suppressing ground weed populations (Fig. 1). However, with time, cover crop management was essential to avoid possible competition between coconut palms and cover crops which suppressed the growth of *Vernonia zeylanica*and other weed species.

The two mechanical weeding treatments, tractor harrowing ($T_3$) and tractor slashing ($T_4$)) suppressed *Vernonia zeylanica*growth initially, but rapid re-growth was observed in the *Vernonia zeylanica*plants during the experimental period when compared to the other dicotyledonous weeds. Generally slashing damaged the aerial parts of the weeds but with no damage to the root system or underground plant parts such as stolons and rhizomes of the grass weed species. During favorable weather conditions, underground plant parts produced new shoots or new flushes. For example, the monocotyledonous weeds *Imperata cylindrica*,



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*Panicum maximum* and *Cynodon dactylon* and several dicotyledonous weeds *Lantana camara* and *Chromolaena odorata*produced a new flush within a few weeks of slashing. Tractor harrowing at six month intervals reduced the weed biomass significantly when compared to slashing. Harrowing was helpful to bury weed seeds in deep layers, and thus reduce the growth of weed population on the surface. However, this practice loosens the soil and which would create a suitable environment for the germination of some weed species seeds (Senarathne *et al.* 2003).

***Vernonia zeylanica* seedling emergence density in the field**

The numbers of *Vernonia zeylanica*seedlings gradually decreased with time in all weeding treatments except in the control ($T_6$), harrowing ($T_3$) and slashing plots ($T_4$) where a high *Vernonia zeylanica*seedling density was observed on the surface)(Fig. 2). The seedlingwere applied in July 2010, January 2011, July 2011, January 2012 and June 2012.

Emergence density was significantly lower ($P<0.05$) in chemical ($T_1$), hand weeding ($T_5$) and cover cropping ($T_2$) treatments compared to others. The densities of emerged Vernonia zeylanica seedling was almost similar in the chemically treated, hand weeded and cover cropped plots. The use of herbicides can also influence the species composition of the seed bank and seedling emergence density depending on the chemicals used (Ball 1992) and it can also cause specific shifts in weed populations (Roberts 1968). The *Vernonia zeylanica* seedling emergence densities were almost similar in slashed ($T_5$) and harrowed plots ($T_3$) (Fig. 2). Some weed species invaded a higher intensity of emergence in the no tillage planting than in the conventional tillage. The presence of seeds at the superficial layer of the soil and frequent cultivation, are factors that reduce the seed bank rapidly. This situation can facilitate seed loss by exposing seeds to variations in temperature and humidity, and breaking dormancy and finally reducing the seedling density in the field (Simpson et al. 1989). In the present study, chemical weeding and cover cropping were the best methods to reduce weed seedling emergence density. This may depend on several factors, including the pattern of rain fall and the time of germination at a site, the timing of seed input (seed rain) into the seed bank and different agronomic practices (Coffin and Lavenroth 1989) and seed and seed losses due to predators (Hodgkinson et al. 1980; Rice 1989). However, different weeding methods over the experimental period in different treatment plots produced dense stands of weeds and the seed rain from these plants probably caused the seed bank changes observed in subsequent sampling occasions.

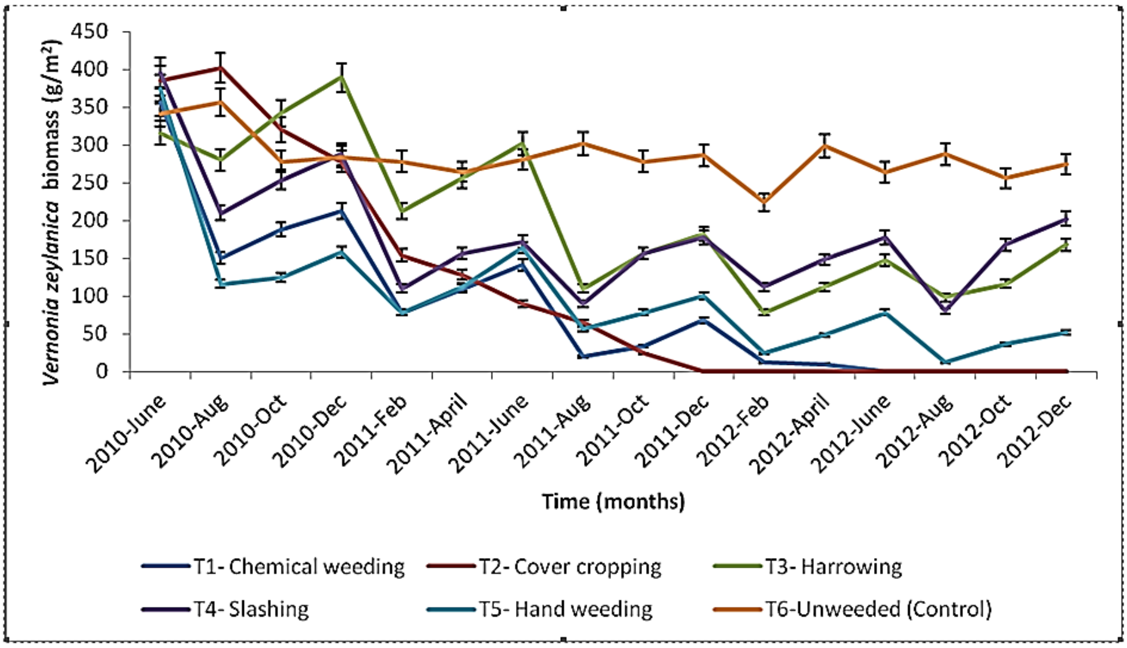




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**Fig. 1.** Effect of different agronomic practices on total biomass of *Vernonia zeylanica*from June 2010 to December 2012. Vertical bars indicate + SE of the mean, Treatments were applied in July 2010, January 2011, July 2011, January 2012 and June 2012.

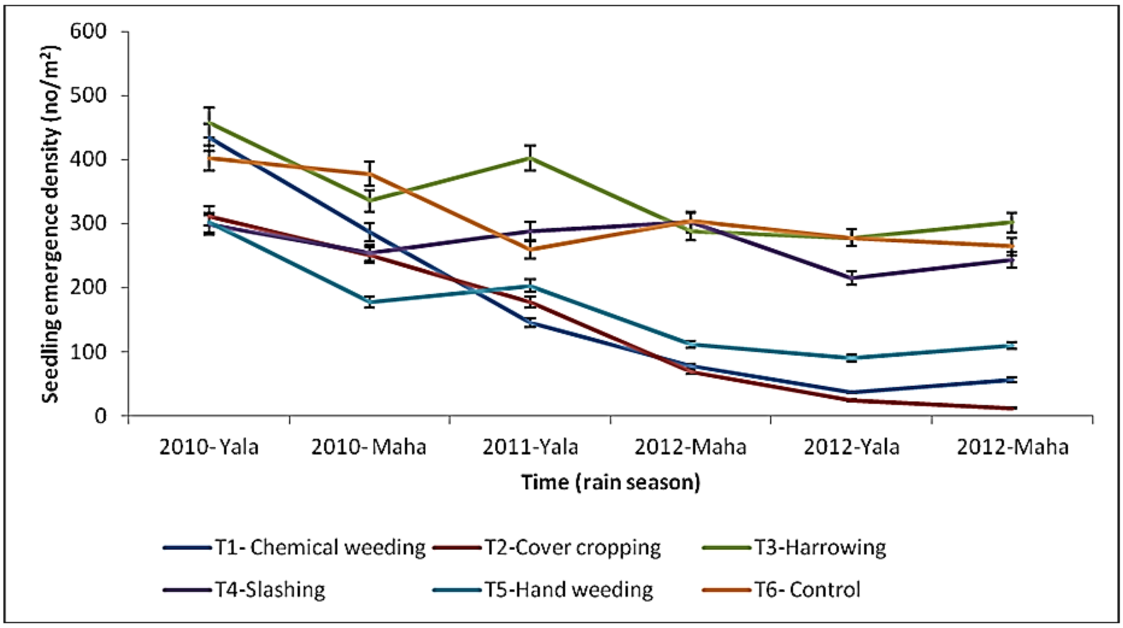


**Fig. 2.** Effect of different weeding treatments on average *Vernonia zeylanica* seedling emergence density from 2010 to 2012**.** Vertical bars indicate + SE of the mean, Treatments.

### The seed depth of emerged *Vernonia zeylanica* seedlings in the field

The average depths of *Vernonia zeylanica*seedlings emerged in the field are presented in Table 1. The seed depth of emerged seedling of *Vernonia zeylanica*was very high in harrowed weeding treatment when compared to other weeding treatments. Seventy six percent of the*Vernonia zeylanica* seedlings emerged from the surface to 2cm soil and 24% seedlings emerged at 2-4cm soil level indicating that more small seeds germinate in top soil layers. The highest depth from which *Vernonia zeylanica* seedlings emerged was 3.32cm in harrowed plots.

**Table 1.** The average number of *Vernonia zeylanica* seedlings which emerged at different soil depths in different weeding treatments

| Species / treatments | Depths of emergence (cm) | | | | Means emerged depth (mm) |
|---|---|---|---|---|---|
| | 0-2cm | 2- 4cm | 4- 6cm | 6-8 cm | |
| $T_1$. Chemical weeding | 28 | 2 | - | - | 05.95 d* |
| $T_2$. Cover cropping | 18 | 12 | - | - | 17.23 b |
| $T_3$. Harrowing | 13 | 17 | - | - | 21.13 a |
| $T_4$. Slashing | 25 | 5 | - | - | 12.01 b |
| $T_5$. Hand weeding | 26 | 4 | - | - | 09.26 c |
| $T_6$. Control | 27 | 3 | - | - | 03.79 d |
| **Total** | **137 (76%)** | **43 (24%)** | - | - | |

*Values followed by the same letters are not different at P<0.05 in each treatment

This indicated that loosening the soil creates more favorable environment for germinating of weed seeds buried in soil layers. Therefore, it can be argued that the elimination of weed seeds in the top 2cm or 4cm in the soil seed bank by any means is likely to reduce level of weed infestation by 60% and 95% respectively. The results of a similar study conducted by Naylor (1970) showed that 90% of the emerged population of *Alopecurus myosuroides* was



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derived from seeds in the top 2.5cm of the soil. However, The optimum depths for the emergence of seeds vary with different species. By compiling data for 31 species, King (1966) showed that optimum depth ranged from 0.05cm to 2.5cm and was roughly in proportion to the 1000 seed weight. Roberts and Feast (1973) examined the depth of seed burial in undisturbed and cultivated soil and found seedling emergence was greatest from cultivated soil at shallow depths of burial.

## CONCLUSIONS

Application of glyphosate (1.44 kg a.i./ha), hand weeding and cover cropping (*Pueraria phaseoloides*) are very effective methods to reduce *Vernonia zeylanica*biomass and weed seedling emergence density when compared to other mechanical weeding methods such as harrowing and slashing. Hand weeding is labor intensive method to manage weeds in coconut plantations. However, an integrated approach, application of glyphosate followed by establishment of leguminous creeping cover crops is very effective in controlling *Vernonia zeylanica*. Considering the soil seed bank, the results of this study have provided useful information on timing, emergence density and composition of *Vernonia zeylanica*population that are likely to emerge under different types of agronomic practices in relation to the seed bank. However the depth of weed seedling emergence was very high in harrowing treatment plots when compared to the other weeding methods. This indicated that loosening the soil creates more favorable environment for germinate weed seeds buried in deep soil layers.

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# SOME STUDIES ON ALLELOPHATIC POTENTIAL OF *CYPERUS ROTUNDUS* L

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**ABSTRACT** *Cyperus rotundus* (sedge weed) that exhibit allelophatic behaviour represent potential options for sustainable weed management. Previous study has shown that application of mulch from the weed suppressed broadleaved weed. Hence, in this study we carried out a series of experiments to elaborate whether the weed has an allelophatic potential for broadleaved weed control. Consistent with the previous study, the result of a field experiment in this study show that biomass aplication of *C. rotundus* as mulch, compost and soil ameliorant suppressed broad leved weed in soybean cultivation. However, a green house experiment show that biomass aplication had no negative effect on the growth and biomass production of 3 common broadleaved weeds, *Asystasia ganggetica*, *Mimosa pigra* and *Borreria alata*, and soybean. Study on the effect of the concentration of water extract of *C. rotundus* (0.5 – 4.5 kg/L) show that up to 1.0 kg/l concentration significantly decreased (more than 60%) seed germination of the three common broadleaf weeds in upland; but had no effect on seed germination of soybean. Analysis of allelochemical componds indicated that phenolic compounds from *C. rotundus*, cyperene and culmorin were specific compounds that only found in fresh *C. rotundus* with aquadest sovent. The study indicates that *C. rotundus* may be used as an option for seed germination control of broad leaf weeds.

***Keywords*:** Allelochemicals, bioherbicide, weed management, *Asystasia gangetica, Borreriaalata, Mimosa pigra, Cyperus rotundus*

## INTRODUCTION

There is a growing interest in allelophatic study especially on their potential ability to support sustainable agriculture system (Junaedi *et al* 2006). Commonly, allelophaty form as secondary metabolites on several plant organs such as roots, stems, leaves, flowers and seeds. Allelophaty of crops and weeds can be expressed in the form of exudates from roots, pollens, decomposition of plant organs, volatiles from leaf, stem and root, and also through the leaching of plant organs.

Nut sedge *(Cyperusrotundus* L.) is important weed in the world that distributed widely in all tropical and sub-tropical area. Holm*et al.* (1977) reported that *C. rotundus* is the member of the worst weeds, had become a serious problem in 90 countries on more than 50 kind of crops. This weed can cause serious problem because of its ability to suppress several crop production significantly and its difficulty to be controlled. This suppression is caused by the high competition to get resources, allelochemical of *C. rotundus*, and the combination of both factor.

Allelophaty of *C. rotundus* is not only to suppress crop growth and production, but also to suppress several weeds growth. Some literatures reported that allelophaty of *C. rotundus* is able to suppress the growth of crop or other plant including weeds (Izah, 2009;; Elrokiek, 2010; Palapa, 2009). However, specific and systematic studies regarding the use of allophaty of *C. rotundus* as agent for controllong weeds growth in an environmentally friendly agricultural system is still lacking.

This study was aimed at studying the potency of allelophaty of *C. rotundus* as biological controll of weeds in environmentally friendly crops production system.



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## MATERIALS AND METHODS

In order toassess the potency and prospective of *C. rotundus* allelophaty in weed control, a series of studies was done at Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Bogor, Indonesia, including field trials, greenhouse trials and laboratorium experiments.

**Experiment 1**. This experiment was a preliminary study that has been carried out in the field to identify several important prospective candidates that can be used to suppress weed growth and development in soybean production. The experiment was designed block design. The treatment was different mulches developed from several kind of weed that was applied in soybean production. The mulches were developed from paddy (*Oryza sativa*), cogongrass (*Imperatacylindrica*), nut sedge (*C. rotundus*), and waterhyacinth (*Eichorniacrassipes*). Black plastic mulch and no-mulch were used as control. Vegetation analysis was done at three and sixth week after planting to determine the growing weeds species and their growth and development.

**Experiment 2.** This field experiment was to know the effects of teki as organic material to weed growth and development on soybean field. The experiment was designed in a block design using three replications. The treatment was the formula of organic matter from C. rotundus as follow: (1) fresh of *C. rotundus* as mulch (2) dried *C. rotundus* as mulch, (3) fresh of *C. rotundus* incorporated with soil, (4) dried *C. rotundus* incorporated with soil, (5) composted *C. rotundus*. In addition (6) manuallyweeding and (7) non-weeding beds were used as controls. Vegetation analysis was done using quadran methods at fourth and eight weeks after planting.

**Experiment 3.** This experiment was a greenhouse experiment that was aimed at determining the effects of teki organic matter to growth and development of weeds, and to biomass production of several broad leaf weeds. This experiment was designed using complete randomized design. The treatment was several forms of organic materials from C. rotundus applied to three kind of broad leaf weeds and soybean planted in a polybag under greenhouse condition. The organic matters from *C. rotundus*(CR) were: (1) fresh CR incorporated with soil , (2) dried CR incorporatedwith soil, (3) fresh CR as mulch, (4) dried CR as mulch, (5) composted CR (6) powder of CR (7) extract of CR, (8) control. Three important weeds species were used as trial plant, those were *Asystasia gangetica*, *Borreria alata*, *Mimosa pigra*, dan soybean (*Glycine max*). Growth and development of plant were observed until generative stage, then were harvested to measure the biomass of each plant sample.

**Experiment 4.** This experiment was conducted at laboratory to study the effect of C. rotundus extract to the germination rate of broad leaf weeds and soybean seeds. The experiment was conducted using a complete randomized design with three replications. The treatment used in this experiment was the concentration of C. rotundus extract (using water as the solvent) ranging from 0.0 – 4.5 kg fresh teki/liter water with interval of 0.5 kg/liter. *Asystasia gangetica*, *Borreria alata*, *Mimosa pigra*, dan soybean (*Glycine max*) were used as object plants. Teki extract treatment was done to the 50 seeds of weeds, which have already broken for their dormancy and soybean on petridish in an incubator. The observation was made on the number of germinating seeds, plumule length, radicule length, and speed of germination periodically until 30 days old.

**Experiment 5.** This experiment is to analyze the allelochemical content of *C. rotundus*. The analysis was conducted on fresh and dried of *C. rotundus, C. rotundus* powder and *C. rotundus* compost. Analysis was done at Health Laboratorium using GC-MS analysis. Every sample was analyzed duplo.



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## RESULTS AND DISCUSSION

### Experiment 1. The effect of weed mulches to the growth and development of weeds in a soybean field

This experiment showed that generally the weed mulches able to increase the growth and production of soybean. Besides that, all mulches can suppress the growth of weeds significantly. There was an indication that *C. rotundus* mulch can suppress broad leaf weeds more effectively than paddy mulches, waterhyacinth mulches, and cogongrass mulches (Table 1). At second time of observation (six week after transplanting), weed biomass on teki mulch treatment was 16.18 g, the lowest compare to that of paddy mulch (45.55g), waterhyacinth mulch (34.35g) and cogongrass mulch (26.25 g). From this study, it is known that the production of soybean using weed mulches is lower than that using black plastic mulch (data not shown). The production of soybean using C. rotundus mulch was 158.90 g/plot, significantly lower than that of black plastic mulch (1023.00 g/plot), but still higher compare to that of control (without mulch/no weeding) (99.23 g/plot).

This results strengthen the hypothesis that teki has allelophatic effects to broad leaf plant. Negative effects of teki to broad leaf plants has been reported before (Izah, 2009; Fitria*et al*. 2011).

### Experiment 2. The effects of several organic matter of *C. rotundus* to the growth of weeds in a soybean cultivation

Weed biomass of *C. rotundus* can be used as mulch, compost, or soil ameliorant material in crops productions. This also has been shown in Experiment 2. The result of this experiment shows that *C. rotundus* that was applied in different formulas can be used to suppress the growth and development of broad leaf weeds. Table 2 show that weeds biomass in several treatments of *C. rotundus* were significantly lower than that in control beds. At 8 weeks after planting, broad leaf weeds biomass harvested inboth fresh and dried *C. rotundus* as mulches, both fresh and dried *C. rotundus* as soil amelorant and compost were 8.7, 6.7, 2.6, 10.5 and 4.4 g per plot, respectively, in which significantly lower than that on control (302.3 g/plot).



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**Table 1.**Weed growth on several weed organic mulch treatments

| Mulch resources | Time (WAT) | Number of weed species | | | Summed Dominance Ratio (SDR) (%) | | | Biomass (gram) | | | Total biomass (gram) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S | G | BL | S | G | BL | S | G | BL | |
| Rice straw | 3 | 1 | 8 | 15 | 1.49 | 59.04 | 39.46 | 0.50 | 73.91 | 29.21 | 103.61 |
| | 6 | 1 | 6 | 9 | 6.54 | 50.35 | 43.11 | 7.40 | 42.09 | 45.44 | 95.04 |
| Waterhyacinth | 3 | 0 | 5 | 9 | 7.81 | 58.46 | 33.73 | 11.10 | 41.88 | 32.05 | 85.03 |
| | 6 | 1 | 6 | 8 | 6.30 | 72.65 | 21.04 | 4.70 | 91.96 | 34.35 | 113.25 |
| Black polythylene | 3 | 1 | 4 | 3 | 0.00 | 60.94 | 39.06 | 0.00 | 6.63 | 5.87 | 12.50 |
| | 6 | 0 | 4 | 4 | 0.00 | 69.44 | 30.56 | 0.00 | 32.85 | 21.80 | 54.65 |
| Cogongrass | 3 | 0 | 5 | 8 | 0.00 | 67.76 | 32.23 | 0.00 | 40.94 | 20.73 | 61.67 |
| | 6 | 1 | 7 | 9 | 6.87 | 70.46 | 22.66 | 3.40 | 92.18 | 26.25 | 121.83 |
| Cyperus rotundus | 3 | 1 | 5 | 9 | 3.17 | 67.96 | 28.86 | 0.90 | 69.48 | 22.13 | 92.51 |
| | 6 | 1 | 7 | 6 | 4.88 | 72.18 | 22.92 | 1.28 | 53.68 | 16.18 | 71.14 |
| No mulch | 3 | 1 | 5 | 9 | 2.56 | 67.31 | 30.03 | 1.70 | 72.08 | 34.35 | 108.13 |
| | 6 | 0 | 5 | 8 | 0.00 | 65.78 | 34.21 | 0.00 | 137.30 | 33.00 | 170.30 |

Notes

S : Sedges

G : Grasses

BL : Broadleave



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However, contrary with Experiment 1 and other studies, the result of this study showed that the addition of teki organic matter did not produce significant effects on vegetative development of soybean, except at early growth stages. The highest increase was found on fresh teki plots. The possible explanation of this is that organic material treatment as full coverage can function optimally as mulches.

The result of this experiment strengthen the hypothesis that *C. rotundus* has allelophatic potential to suppress the growth of broad leaf weeds. In its application, teki can be applied through several formulas, such as as mulches and compost.

**Experiment 3. The effects of organic materials from teki to the growth of broad leaf weeds and soybean (under greenhouse condition)**

There was no negative effect of *C. rotundus* to the growth of weeds, except the application of *C. rotundus* extract 1 kg/L that can suppress *Borreriaalata* (Table 3). Even the application of *C. rotundus*organic materials or extract of *C. rotundus*can increase the growth and biomass of soybean.

**Table 2.** Growth of weeds on several treatment of *C. rotundus* organic matter

| Treatment of CR organic matter | Time (WAT) | Number of weed species | | | Summed Dominance Ratio (SDR) (%) | | | Biomass (g/0.25 $m^2$) | | | Total biomass (g/0.25 $m^2$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S | G | BL | S | G | BL | T | R | BL | |
| Manual weeding | 4 | 1 | 4 | 6 | 18.90 | 27.60 | 53.50 | 34.30 | 43.50 | 174.50 | 252.50 |
| | 8 | 1 | 1 | 8 | 4.50 | 6.70 | 88.90 | 1.00 | 1.20 | 28.20 | 30.40 |
| No weeding | 4 | 1 | 3 | 6 | 9.50 | 13.20 | 77.40 | 11.60 | 20.80 | 239.70 | 272.00 |
| | 8 | 0 | 1 | 5 | 0.00 | 4.70 | 95.30 | 0.00 | 3.20 | 302.40 | 305.60 |
| Fresh of CR as mulch | 4 | 1 | 6 | 7 | 20.10 | 26.50 | 53.40 | 35.40 | 57.80 | 104.50 | 197.70 |
| | 8 | 1 | 2 | 7 | 8.60 | 24.10 | 67.40 | 0.60 | 4.10 | 8.70 | 13.40 |
| Dry of CR as mulch | 4 | 0 | 5 | 10 | 0.00 | 37.10 | 63.00 | 0.00 | 36.10 | 103.60 | 139.70 |
| | 8 | 1 | 4 | 6 | 4.90 | 40.90 | 54.30 | 0.20 | 7.40 | 6.70 | 13.40 |
| Fresh of CR incorporated in soil | 4 | 1 | 4 | 7 | 45.10 | 23.50 | 31.40 | 125.50 | 69.00 | 77.20 | 271.70 |
| | 8 | 1 | 2 | 3 | 22.00 | 47.60 | 30.40 | 0.90 | 6.60 | 2.60 | 10.10 |
| Dry of CR incorporated in soil | 4 | 1 | 5 | 8 | 41.00 | 21.60 | 37.40 | 120.30 | 44.10 | 54.30 | 218.70 |
| | 8 | 1 | 3 | 5 | 4.50 | 32.40 | 67.60 | 0.50 | 5.00 | 10.50 | 16.00 |
| Composted CR | 4 | 0 | 3 | 7 | 0.00 | 31.50 | 68.50 | 0.00 | 96.50 | 172.20 | 268.70 |
| | 8 | 0 | 3 | 4 | 0.00 | 53.20 | 46.80 | 0.00 | 6.00 | 4.40 | 10.40 |

Notes CR *: C. rotundus*
S : Sedges
G : Grasses
BL : Broadleaves

These results are in line with the result of Experiment 2 that show that organic matter from *C. rotundus* does not have negative effect to the seedling. The low population of broad leaf weeds in soybean cultivation (Experiment 1 and 2) probably caused by the effects of *C. rotundus* allelophaty to their germination. Therefore, the mechanism of *C. rotundus* suppression to the broad leaf weeds might be expressed during germination periods.



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**Table 3**. Biomass of weeds *A. gangetica*, *B. alata, M. pigra* and soybean on different organic matter treatment of C. Rotundus*

| | Biomass (g) | | | |
|---|---|---|---|---|
| Organic matter treatment of *C. rotundus* | *A.gangetica* | *M. pigra* | *B. alata* | Soybean |
| Control | 34.41a | 10.53a | 12.28dc | 19.23d |
| Extract of C. rotundus (1kg/1L) | 46.38a | 8.28a | 6.85d | 30.72bc |
| Fresh CR incorporated with soil | 41.66a | 8.11a | 22.07ab | 37.19ab |
| Dried CR incorporated with soil | 44.35a | 13.97a | 14.78bc | 25.71cd |
| Fresh CR as mulch | 48.24a | 13.04a | 29.15a | 39.60a |
| Dried CR as mulch | 51.88a | 8.71a | 18.57bc | 30.66bc |
| Composted CR | 32.12a | 15.86a | 22.31ab | 25.68cd |
| Powder of CR | 53.96a | 15.58a | 21.19b | 23.58cd |

Notes: CR: *C. rotundus*

*Value with different letters in each column indicate significant differenceamong sectors by DMRT *p*< 0,05

**Experiment 4. The effect of *Cyperusrotundus*extract to the germination of broad leaf weeds and soybean**

Extract of *C. rotundus* treatment, concentration 0 – 4.5 kg/l, significantly affected seed germination, speed of germination, plumule length, radicule length of broad leaf weeds: *Asystasia gigangtea, Mimosa pigra dan Boreria alata*, but does not affect soybean. The effect of teki extract to the germination of *Asystasia gigangtea*and*Boreria alata* can be seen on Table 4 and Table 5.

Table 4 shows that the *C. rotundus* extract, concentration 0.5 kg/l, can suppress the germination percentage as 42.67% and germination speed as 12.53%, but does not significantly decrease the length of its plumule and radicule. On higher concentration (1 kg/l), teki extract can suppress the germination rate as 69.33%, while on concentration 1.5 kg/l, it can decrease the germination rate as 92.67%. The increase of extract concentration from 2 kg/l to 4.5 kg/l can caused the seed of weed failed to germinate.

**Table 4.** Effects of *Cyperus rotundus* extract to the germination percentage (%), speed of germination (% normal seedling/etmal), length of plumule (cm) and length of radicule of *A. gangetica*

| *Cyperus rotundus* extract (kg/L) | Germination Percentage | Speed of germination | Length of plumule | Length of radicule |
|---|---|---|---|---|
| 0 (kontrol) | 97.33a | 18.26a | 1.50a | 1.76a |
| 0.5 | 54.66b | 5.73b | 1.36ab | 1.83a |
| 1.0 | 28.00c | 2.36c | 1.13ab | 2.33a |
| 1.5 | 6.66d | 0.43d | 0.83bc | 0.76ab |
| 2.0 | 0.00d | 0.00d | 0.00d | 0.00b |
| 2.5 | 0.00d | 0.00d | 0.00d | 0.00b |
| 3.0 | 6.66d | 0.33d | 0.43dc | 0.86b |
| 3.5 | 0.00d | 0.00d | 0.00d | 0.00b |
| 4.0 | 0.00d | 0.00d | 0.00d | 0.00b |
| 4.5 | 0.00d | 0.00d | 0.00d | 0.00b |

Notes: value with different letters in each column indicate significant differenceamong sectors by DMRT p<0.05



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Table 5 shows that the responses of *Boreria alata*seed to the teki extract treatment are similar with those of *A.gangetica.* The number of *B. alata* that successfully germinated on concentration 0.5 kg/l and 1 kg/l are 52.00% and 32.00%. Different with *A.gangetica,*plumule and radicule length of *B. alata* were significantly decreased by application of C. rotundus extract. Plumule length of this species on *C. rotundus* extract concentration 1 kg/l was 0.86 cm, significantly different with control (1.63 cm); while its radicule length, on concentration 1 kg/L was 0.86 cm, significantly lower than that of control (2.33 cm). Although *C. rotundus* extract significantly suppress the germination of those three species of broad leaf weeds, there was no effect on seed germination of soybean. On all treatment (0.0 – 4.5 kg/l), the number of soybean seed that germinated were not-significantly ranging from 78.66 – 96.00%.It is known from this experiment that in line with the result of experiment 3 that the hypothesis that suppression mechanism of teki to broad leaf weeds (*A. gangetica, B. alata, M. pigra*) is operated on the germination stages. Another information from this experiment, similar with Weston (1996), is that allelophaty has specific or selective effects.

**Table 5**. The effects of *Cyperusrotundus* extract on the germination percentage (%), speed of germination (% normal seedling/etmal), length of plumule (cm) and length of radicule of *Borreria alata**.

| | Germination Percentage | Speed of germination | Length of plumule | Length of radicule |
|---|---|---|---|---|
| 0 (control) | 96.00a | 15.63a | 1.63a | 2.33a |
| 0.5 | 52.00b | 6.33b | 0.96b | 0.60c |
| 1.0 | 32.00c | 2.73c | 0.86b | 0.86b |
| 1.5 | 14.66d | 1.06d | 1.03b | 0.7bc |
| 2.0 | 0.00f | 0.00e | 0.00d | 0.00d |
| 2.5 | 1.33ef | 0.03e | 0.10d | 0.13d |
| 3.0 | 5.33ef | 0.23e | 0.56c | 0.53c |
| 3.5 | 0.00f | 0.00e | 0.00d | 0.00d |
| 4.0 | 5.33ef | 0.23e | 1.06b | 0.76cd |
| 4.5 | 9.33ed | 0.40e | 1.03b | 0.53c |

*Notes: Value with different letters in each column indicate significant difference among sectors by DMRT $p<0.05$

**Experiment 5. Analysis of allelochemical componds of *C. rotundus***

GC-MS analysis using aquadest as solvent was able to detect 16 compoundson fresh *C. rotundus*, while using etanol as solvent was able to detect 10 compouds on fresh *C. rotundus*, 12 compounds on dried *C. rotundus*, 19 compounds on compost of *C. rotundus*, and 3 compounds on *C. rotundus*powder. The difference of the number of compounds detected might be caused by the difference in the processing of the sample. The processing step such as drying and powdering could possible cause the loss and formation of some compounds.

**Table 6**. Analysis of allelochemical componds of *C. rotundus*

| | Aquadest | etanol 96 % | | | |
|---|---|---|---|---|---|
| | fresh | fresh | dried | compost | powder |
| Content of*C. Rotundus* | …………………… %................................. | | | | |
| *4-vinyl-2-methoxy-phenol* | 1.88 | 1.39 | - | - | - |
| *Cedranone* | - | - | - | 1.61 | - |
| *Choles-5-en-3-ol (3.beta)-, propanoate(CAS)* | - | - | - | - | 2.91 |



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| | | | | | |
|---|---|---|---|---|---|
| *Culmorin* | 1.81 | - | - | - | - |
| *Cyperene* | 0.73 | - | - | - | - |
| *Furanmethanol (CAS) fulfuryl alcohol* | - | 3.06 | - | - | - |
| *Ethylcholest-5-en-3.beta,-ol, Cholest-5-en* | 5.7 | - | - | 12.69 | - |
| *Hexadecanoic acid* | 29.53 | - | 6.31 | 12.13 | - |
| Total number of identified compounds | 16 | 10 | 12 | 19 | 3 |

From this analysis, it is known that cyperene and culmorin only can be identified on fresh *C. rotundus* using aquadest as solvent, and cannot be detected on other formula of *C. rotundus*.Lawal&Oyedeji (2009); Elrokiek (2010) have reported that *C. rotundus* contains phenolic compounds such as cyperene and culmorin. Phenolic compounds with high solubility in water have reported to have low allelophaty activities (Seigler 1996). Therefore, although teki extract could be very effective to suppress broad leaf weeds germination, for its application in the field as bioherbicide, further studies to solve these issues are needed.

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# APPLICATION OF ALLELOPATHY FOR WEED MANAGEMENT AND GROWTH PROMOTION IN WHEAT

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**ABSTRACT** Harmful effects of synthetic herbicides, emergence of herbicide resistant weed biotypes and the growing demand for toxic free foods has pushed the scientists to search environment friendly approaches to control weeds. In the light of these demands a natural phenomenon in plants Allelopathy has been proved to suppress weeds. In this study, mixtures of allelopathic water extracts of sorghum+sunflower and sorghum+sunflower+mulberry were sprayed each at 12, 15, 18 and 21 L $ha^{-1}$ at 40 and 55 days after sowing in wheat for wild oats (*Avena fatua* L.), little seed canary grass (*Phalaris minor* L.), lambs quarters (*Chenopodium album* L.) and swine cress (*Coronopus didymus* L.) control. A synthetic herbicide Affinity 50 WP (Carfentrazone-ethyl 0.75%+Isoproturon 50% applied at 1000 g a.i. $ha^{-1}$) and a weedy check were kept as control treatments. The increasing order of water extracts dose 12-21 L $ha^{-1}$ showed substantial decrease in total weed density and their dry biomass production of both narrow and broad leaved weeds in all the treatment combinations. The most effective treatment, which contributed to increase grain yield up to 19.5% by decreasing total weed dry matter by 87.14% over control, was the mixture of sorghum+sunflower+mulberry water extracts each at 18 L $ha^{-1}$ at 40+55 DAS after synthetic herbicide. It is concluded that the use of allelopathic water extracts may act as a potential weed control strategy in wheat.

***Keywords*:** Herbicide, weed management, wheat, allelopathy, water extracts

## INTRODUCTION

The need for safe food production has developed steadily in the previous years as a result of consciousness about food quality and environmental concerns due to agrochemicals used in agriculture. Worldwide huge crop losses have been found due to heavy weed infestations. The losses associated to weeds in Pakistan exceed beyond Rs. 120 billion on national level (Anonymous, 2005). The weeds loses in major cereal crops exist in the range of Rs. 40, 30 and 4 billion for rice, wheat and maize, respectively (Anonymous, 2005). Farmers normally relay on quick and effective control measures by using synthetic herbicides which produces several environmental tribulations for human health due their indiscriminate use (Kohli *et al*., 1998; Xuan *et al*., 2004).

To search for promising weed control methods scientists are investigating on various aspects of allelopathy as a means of weed suppression. Crops/plants release chemicals i.e. “allelochemicals” which could be utilized for managing weeds (Putnam and Defrank, 1979). Brassicas has been discussed as potential alternative to synthetic herbicides for weed control (Grossman, 1993). Different doses of sorghum water extract applied as single and multiple foliar sprays at different days after sowing (DAS) suppressed the total weed density at 120 DAS up to 48% (Cheema *et al.*, 1997). Grain yield of wheat was increased by 21% at 1:10 w/v ratio of sorghum water extract applied twice at 30 and 60 DAS with decrease in weed density and dry weights of 33.6 and 19.92% respectively (Cheema *et al.*, 2000). They also reported that there was no difference among single, double and triple applications of sorghum water extract in terms of weed control and wheat grain yield. sorghum water extract (100%) sprayed at 30 DAS significantly suppressed the density of weed species such as broad leaf dock 36%, swine cress 23%, lambsquarters 38%, fumitory 61% and the total biomass reduction of 53% was obtained with 100% sorghum water extracts with a corresponding yield increase in wheat of



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14% over control (Cheema *et al.,* 1997). It was also observed that sunflower water extracts (100%) inhibited these weeds by 24, 61, 31 and 21% respectively, total weed dry weight reduced by 51% and increased wheat yield by 7% over control. The mulberry extracts inhibited the seedling growth of bermuda grass more than wheat seedling and interestingly, its foliar spray at (100%) significantly inhibited the growth of Bermuda grass and promoted wheat growth (Haq *et al.,* 2010).

The facts mentioned above indicate that when sorghum, sunflower and mulberry water extracts are used separately inhibits the weeds up to 20-50% which is less than the standard weed control i.e. 80% or above; while it is quite possible when various extracts are tank mixed, these may depict cumulative effect.

## MATERIALS AND METHODS

This study designed for exploring weed control with different crop aqueous extracts in wheat was conducted at Agronomic Research Farm University of Agriculture, Faisalabad (31.25˚ N and 73.09˚ E), and Punjab, Pakistan during the winter of 2008-9. The experimental soil belongs to Lyallpur soil series (Aridisol-fine-silty, mixed, hyperthermic Ustalfic, Haplargid in USDA classification and Haplic Yermosols in FAO classification scheme.

The wheat variety Sehar-2006 was sown on a cultivated seed bed in 22cm spaced rows using a single row hand drill on Dec 4, 2008 using seed at 125 kg $ha^{-1}$. Fertilizers in the form of urea and diammonium phosphate were applied at 100 and 60 kg $ha^{-1}$ respectively. Half of the nitrogen and full dose of phosphorous were applied at the time of sowing, while the remaining half of nitrogen was applied with the first irrigation. The first irrigation was given 20 days after sowing and subsequent irrigations were adjusted according to the climatic conditions and need of the crop.

For weed control, mixture of sorghum, sunflower and mulberry water extracts were applied each at 12, 15, 18 L $ha^{-1}$. Synthetic herbicide Affinity 50 WP (carfentrazone-ethyl 0.75%+isoproturon 50% applied at 1000 g a.i. $ha^{-1}$) and a weedy check was maintained as control. The water extracts were prepared following the procedure by Cheema and Khaliq. (2000) and were applied twice at 40 and 55 days after sowing (DAS). The label dose of Affinity 50 WP was applied at 30 DAS. The volume of the spray (340 L $ha^{-1}$) was determined after calibration by following the Ross and Lembi (1985) procedure. The knapsack hand-sprayer fitted with a flat fan nozzle by maintaining a pressure of 207 kpa.

Data pertaining to individual and total weed density and biomass in a unit area was recorded 60 DAS with the help of quadrate measuring 0.25 m x 0.25 m randomly placed at two places in each experimental unit. Weeds were oven dried at 70 $^{0}$C to get the dry weight for 72 hours. Wheat crop was harvested and threshed manually in third week of April, 2009 from individual treatment plots; grain yield was weighed in kilograms and expressed as mega gram per hectare (Mg $ha^{-1}$). The other yield traits were recorded by standard sampling techniques.

Data obtained were analyzed statistically by using statistical package MSTAT-C (Freed and Scott, 1986). Analysis of variance (ANOVA) was performed by using Fisher's analysis of variance technique while multiple comparisons among treatment means was made using least significant difference (LSD) test at $P<0.05$ (Steel *et al.*, 1997).

## RESULTS

The pre-dominant weeds flora in the field was wild oat, little seed canary grass, swine cress, lambsquarters and few plants of field bind weed. All of the weed control treatments substantially inhibited density of wild oats as compared to weedy check (Table 1). Maximum inhibition of both narrow and broad leaf weeds was obtained with synthetic herbicide. It was followed by allelopathic water extracts (WE) treatment viz. sorghum + sunflower + mulberry each at 21 L $ha^{-1}$ at 40+55 DAS and sorghum + sunflower + mulberry water extracts each at 18 L $ha^{-1}$ at 40+55 DAS (Table 1). These WE treatments (WE 15-12 L $ha^{-1}$) in case of wild oat density were statistically at par with each other while in case of little seed canary grass only the



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treatment (sorghum + sunflower + mulberry at 18-21 L $ha^{-1}$) was followed by synthetic herbicide. The weed dry biomass inhibition similar as observed in case of their densities (Table 1). Both of the broad leaf weeds lambs quarters and swine cress were effectively inhibited by synthetic herbicide by 95% and 96.3% respectively. The density of lambs quarters by WE treatments was almost similar in the treatment combination of sorghum + sunflower + mulberry at 15-21 L $ha^{-1}$ as well as in water extract combination of (sorghum + sunflower at 15-21 L $ha^{-1}$. But maximum density inhibition in lambs quarters 94% and Swine cress 91% was achieved by sorghum + sunflower + mulberry each at 21 L $ha^{-1}$. Weed dry biomass production was inhibited in the similar way as density inhibition by water extracts treatments (Table 2). Maximum dry biomass inhibition 2.66g (i.e. 94.8%) over control was observed in herbicide as compared to control treatment. It was followed by WE treatment of sorghum + sunflower + mulberry at 21 L $ha^{-1}$ (6.11g) with 88% inhibition over control treatment (51.18g). The WE treatment of sorghum + sunflower at 15-21 L $ha^{-1}$ and sorghum + sunflower + mulberry at 21 L $ha^{-1}$ were statistically at par with each other to inhibit dry biomass production by 77-87% (Table 2). The total weed density and dry biomass production was also effectively inhibited by WE treatments to enhance grain yield. Highest grain yield 3.78 t $ha^{-1}$ with 29% increase over control was obtained from herbicide treatment. Among the (WE) maximum increase in grain yield (3.5 t $ha^{-1}$ with 19.5% increase over control) was obtained from treatment combination of sorghum+sunflower+mulberry each at 18 L $ha^{-1}$ at 40-55 DAS. The WE treatments sorghum+sunflower each at 18 L $ha^{-1}$ and sorghum+sunflower+mulberry each at 21 L $ha^{-1}$ at 40+55 DAS were statistically at par with each other to increase grain yield (16%) over control. Other water extracts though increased yield but none significantly.

All the treatments produced higher net benefits over control (weedy check) (Table 3). Highest net benefits (PKR 79180) were obtained from synthetic herbicide. It was followed by the treatment sorghum+sunflower+mulberry each at 18 L $ha^{-1}$ at 40+55 DAS. The marginal analysis revealed that highest marginal rate of return (1629.76%) was obtained from the WE treatment sorghum+sunflower each at 12 L $ha^{-1}$ at 40+55 DAS (Table 4). It was followed by treatment sorghum+sunflower+mulberry each at 18 L $ha^{-1}$ at 40+55 DAS and label dose of herbicide with 816 and 681 MRR%, while all other treatments were uneconomical due to higher cost that vary.

## DISCUSSION

Several plant species produce a number of secondary metabolites, and some of these compounds play an imperative role by acting as protection machinery in the plant rhizosphere as allelopathic substances (Duke *et al*. 2000). Aqueous extract treatments significantly inhibited the density and dry weight of the weed species present at the experimental site. Density and dry biomass of wild oats, little seed canary grass, swine cress and lambsquarters was significantly reduced by all the combinations of sorghum extract with either of the extracts as with sunflower alone or sunflower+mulberry (Table 1). Inhibition in density and dry weight of both grassy and broad leaved weeds substantially increased with increasing the rate of WE dose from 12 to 21 L $ha^{-1}$. Greater weed control at higher WE rates was may be due to increased concentration of allelopathic compounds present in sorghum such as m-coumaric acid, caffeic acids, gallic acid, protocateuic acid, syringic acid, vanillic acid, p-hydroxybenzoic acid, p-coumaric acid, benzoic acid, ferulic acid (Haskins & Gorz, 1985; Netzly & Butler, 1986; Nimbal *et al*., 1996)., in sunflower as α-naphthol, scopolin and annuionones (Wilson & Rice, 1968; Macias *et al*., 1998 & 2002; Anjum & Bajwa, 2005) and in mulberry as palmatic acid, ascorbic acid, Galic acid and vanilic acid (Haq *et al*., 2010). The weed density and dry matter production was reduced at parallel basis with increasing (WE) dose and also combining three (WE) i.e sorghum+sunflower+mulberry instead of sorghum+ sunflower (Table 1). These findings are supported by Cheema *et al*. (1997) who reported reduction in weed dry weight with sorghum and sunflower extracts by foliar spraying. Greater decrease in the total weed dry mass (WE) treatments with increasing dose showed that higher concentration of allelochemicals may be



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more effective for weed biomass reduction. The dry matter accumulation reflects the growth behavior of a weed and gives the better indication of weed crop competition than the weed density. Greater weed dry weight reflects more utilization of soil and environmental resources by the weed at the expenses of the crop growth. This relationship has been proved by increase in grain yield with decreasing dry biomass. A similar set of results have also been reported by Cheema and Khaliq, (2000) that 21% at 1:10 w/v ratio of sorghum water extract applied twice at 30 and 60 DAS increase wheat grain yield by 21% with decreases in weed density and dry weights of 44 and 49% over control, respectively. The maximum increase in grain yield over control in water extracts treatments was by sorghum+sunflower+mulberry each at 18 instead of 21 L $ha^{-1}$ showed that at certain range of concentration the allelochemicals may have negative effect on wheat yield. This assumption could be seen within this study in which sorghum+sunflower+mulberry each at 12 L $ha^{-1}$ produced similar amount of grain yield over control as obtained by sorghum+sunflower each at 21 L $ha^{-1}$ (Table 2). The net benefits obtained with (WE) treatment combination of sorghum, sunflower and mulberry each at 18 L $ha^{-1}$ gave fairly good net benefits (PKR 74002.5) and was followed by sorghum and sunflower (WE) each 12 L $ha^{-1}$ with PKR 72098 (Table 4). Although herbicide label dose gave highest net returns PKR 79150/ $ha^{-1}$. Yet, in terms of the harmful effects of synthetic chemicals on the environment and context of organic agriculture (weed control) the net benefits with water extracts are a land mark in the promotion of organic farming.

A combination of potent allelopathic water extracts like sorghum, sunflower, and mulberry each at 18 L $ha^{-1}$ can be used as weed management strategy in modern agriculture with 87% weed control and 19.5% increase in wheat grain yield.

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**Table 1.** Phytotoxic effect of allelopathic water extracts on density and dry biomass production of common wheat weeds.

| Treatments | | Grassy weeds | | | | Broad Leaves weeds | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Avena fetua* L. | | *Phalaris minor* L | | *Chenopodium album* L. | | *Coronopus didimus* L. | |
| | Rate at ($ha^{-1}$) | Density | Dry weight | Density | Dry weight | Density | Dry weight | Density | Dry weight |
| Weedy check (weedy check) | - - | 14.00a | 13.09a | 18.67a | 23.52a | 9a | 4.05a | 13a | 8.45a |
| Sorghum+Sunflower each | 12 L at 40+55 DAS | 4.50b | 4.00b | 5.53b | 8.62b | 2.67b | 0.97b | 4.67b | 3.03b |
| Sorghum+Sunflower+mulberry each | 12 L at 40+55 DAS | 3.83bc | 3.26bc | 4.80bc | 7.30bc | 2.17bc | 0.97b | 3.33c | 2.16bc |
| Sorghum+Sunflower each | 15 L at 40+55 DAS | 2.83bcd | 2.81bc | 4.67bcd | 6.86bcd | 1.5cd | 0.67bc | 3.83bc | 2.49b |
| Sorghum+Sunflower+mulberry each | 15 L at 40+55 DAS | 3.00bcd | 2.67bcd | 3.83cde | 5.70bcd | 1.17de | 0.62bc | 3.17c | 1.41cd |
| Sorghum+Sunflower each | 18 L at 40+55 DAS | 3.00bcd | 2.67bcd | 4.00cde | 6.61bcd | 1.17de | 0.22c | 2.83cd | 1.19d |
| Sorghum+Sunflower+mulberry each | 18 L at 40+55 DAS | 2.33bcd | 1.78cd | 3.00ef | 5.39bcd | 0.67de | 0.27c | 2de | 1.19d |
| Sorghum+Sunflower each | 21 L at 40+55 DAS | 2.67bcd | 2.37bcd | 3.33de | 5.63bcd | 0.67de | 0.29c | 2de | 1.08d |
| Sorghum+Sunflower+mulberry each | 21 L at 40+55 DAS | 1.83 d | 1.63cd | 2.83ef | 4.90cd | 0.5e | 0.22c | 1.17ef | 0.76d |
| Herbicide Affinity | 1000 g a.i. | 1.67d | 0.89d | 1.67f | 3.37d | 0.42e | 0.17c | 0.83f | 0.76d |
| LSD values at 5% probability | | 2.469 | 1.863 | 1.420 | 3.604 | 0.978 | 0.581 | 1.147 | 0.908 |



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**Table 2.** The percent increase in wheat grain yield and decrease in total weed dry mass as influenced by different allelopathic water extracts.

| Treatments | | Total Weeds Dry weight inhibition | Grain yield t $ha^{-1}$ | Decrease in total weeds dry weight | Increase in grain yield |
|---|---|---|---|---|---|
| | Rate at $ha^{-1}$ | | | % | % |
| Weedy check (weedy check) | - - | 51.18a | 2.93d | - - | - - |
| Sorghum+Sunflower each | 12 L at 40+55 DAS | 14.31b | 3.33bcd | 72.0 | 13.7 |
| Sorghum+Sunflower+mulberry each | 12 L at 40+55 DAS | 11.60bc | 3.27bcd | 77.3 | 11.6 |
| Sorghum+Sunflower each | 15 L at 40+55 DAS | 11.79bc | 3.03cd | 77.0 | 3.4 |
| Sorghum+Sunflower+mulberry each | 15 L at 40+55 DAS | 9.14cd | 3.27bcd | 82.1 | 11.6 |
| Sorghum+Sunflower each | 18 L at 40+55 DAS | 9.68cd | 3.40abc | 81.1 | 16 |
| Sorghum+Sunflower+mulberry each | 18 L at 40+55 DAS | 6.58d | 3.5ab | 87.1 | 19.5 |
| Sorghum+Sunflower each | 21 L at 40+55 DAS | 7.75d | 3.27bcd | 84.9 | 11.6 |
| Sorghum+Sunflower+mulberry each | 21 L at 40+55 DAS | 6.11de | 3.40abc | 88.1 | 16 |
| Herbicide Affinity 50 WP | 1000 g a.i. | 2.66e | 3.78a | 94.8 | 29 |
| LSD values at 5% probability | | 3.782 | 0.424 | | |



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**Table 3. Economic analysis**

| | Treatments | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameters | A | B | C | D | E | F | G | H | I | J | Remarks |
| Grain yield | 2.93 | 3.33 | 3.27 | 3.03 | 3.27 | 3.40 | 3.5 | 3.27 | 3.40 | 3.78 | Mg $ha^{-1}$ |
| Adjusted yield | 2.64 | 3.06 | 2.94 | 2.73 | 2.94 | 3.06 | 3.15 | 2.94 | 3.06 | 3.40 | Mg $ha^{-1}$ (10% reduction to bring at farmers level) |
| Gross income | 62700 | 72675 | 69825 | 64837.5 | 69825 | 72675 | 74812.5 | 69825 | 72675 | 80750 | Wheat price at PKR 23750 $Mg^{-1}$ |
| Cost of herbicide | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1375 | Affinity 50 WP (Carfentrazone-ethyl + isoproturon) (PKR 550/800 g) |
| Cost of extracts | 0 | 186.67 | 280 | 233.33 | 350 | 280 | 420 | 326.67 | 490 | 0 | Expenditure on preparation of extracts (70/18 L) Naeem *et al*., 2010 |
| Spray rent | 0 | 130 | 130 | 130 | 130 | 130 | 130 | 130 | 130 | 65 | PKR 65 $spray^{-1}$ |
| Spray application cost | 0 | 260 | 260 | 260 | 260 | 260 | 260 | 260 | 260 | 130 | PKR 130 $man^{-1}day^{-1}$ (one man-day $ha^{-1}$) |
| Total cost that vary | 0 | 576.67 | 670 | 623.33 | 740 | 670 | 810 | 716.67 | 880 | 1570 | PKR |
| Net benefits | 62700 | 72098.33 | 69155 | 64214.17 | 69085 | 72005 | 74002.5 | 69108.33 | 71795 | 79180 | PKR $ha^{-1}$ |
| Increase in net benefits (%) | - | 15 | 10.3 | 2.4 | 10.2 | 14.8 | 18 | 10.2 | 14.5 | 26.3 | Compared with control |

A = control (weedy check)
B = Sorghum+sunflower water extracts each at 12 L $ha^{-1}$ at 40+55 DAS
C = Sorghum+sunflower+mulberry water extracts each at 12 L $ha^{-1}$ at 40+55 DAS
D = Sorghum+sunflower water extracts each at 15 L $ha^{-1}$ at 40+55 DAS
E = Sorghum+sunflower+mulberry water extracts each at 15 L $ha^{-1}$ at 40+55 DAS
F = Sorghum+sunflower water extracts each at 18 L $ha^{-1}$ at 40+55 DAS
G = Sorghum+sunflower+mulberry water extracts each at 18 L $ha^{-1}$ at 40+55 DAS
H = Sorghum+sunflower water extracts each at 21 L $ha^{-1}$ at 40+55 DAS
I = Sorghum+sunflower+mulberry water extracts each at 21 L $ha^{-1}$ at 40+55 DAS
J = Herbicide Affininty 50 WP (Carfentrazone-ethyl 0.75%+Isoproturon 50%) at 1000 g a.i. $ha^{-1}$
PKR = Pakistani rupee (1 US dollar = 85 PKR); DAS = days after sowing; a.i = active ingredient.



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**Table 4. Marginal analysis**

| | Extrat/ haerbicide | Rate ($ha^{-1}$) | Total cost that vary (PKR $ha^{-1}$) | Net benefits (PKR $ha^{-1}$) | Marginal cost | Marginal net benefits | Marginal rate of returns (%) |
|---|---|---|---|---|---|---|---|
| A | control (weedy check) | - | 0 | 62700 | 0 | 0 | 0 |
| B | Sorghum+sunflower water extracts | each at 12 L $ha^{-1}$ at 40+55 DAS | 576.67 | 72098.33 | 576.67 | 9398.33 | 1629.76 |
| D | Sorghum+sunflower water extracts | each at 15 L $ha^{-1}$ at 40+55 DAS | 623.33 | 64214.17 | 0 | 0 | D* |
| C | Sorghum+sunflower+mulberry water extracts | each at 12 L $ha^{-1}$ at 40+55 DAS | 670 | 69155 | 0 | 0 | D |
| F | Sorghum+sunflower water extracts | each at 18 L $ha^{-1}$ at 40+55 DAS | 670 | 72005 | 0 | 0 | D |
| H | Sorghum+sunflower water extracts | each at 21 L $ha^{-1}$ at 40+55 DAS | 716.67 | 69108.33 | 0 | 0 | D |
| E | Sorghum+sunflower+mulberry water extracts | each at 15 L $ha^{-1}$ at 40+55 DAS | 740 | 69085 | 0 | 0 | D |
| G | Sorghum+sunflower+mulberry water extracts | each at 18 L $ha^{-1}$ at 40+55 DAS | 810 | 74002.5 | 233.33 | 1904.17 | 816.08 |
| I | Sorghum+sunflower+mulberry water extracts | each at 21 L $ha^{-1}$ at 40+55 DAS | 880 | 71795 | 0 | 0 | D |
| J | Herbicide Affininty 50 WP (Carfentrazone-ethyl 0.75%+ Isoproturon 50%) | at 1000 g a.i. $ha^{-1}$ | 1570 | 79180 | 760 | 5177.5 | 681.25 |

PKR = Pakistani rupee (1 US dollar = 85 PKR); DAS = days after sowing; a.i = active ingredient.
D* = Dominated due to less benefits or higher cost that vary than the preceding treatment.

$$\text{Formula of Marginal rate of return (MRR\%)} = \frac{\text{Change in net benefits}}{\text{Change in cost}} \times 100$$



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# POTENTIAL APPLICATION OF NUTSEDGE (*Cyperus rotundus* L.) EXTRACTS FOR WEED SUPPRESSION AND IDENTIFICATION OF ALLELOCHEMICALS

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**ABSTRACT** Productivity enhancement at the expense of synthetic chemicals to control weeds and pests has a negative impact on the environment quality and human health. Further, the resistance of weeds to synthetic herbicides is also a cause for concern. Due to the repercussions associated with the use of chemical herbicides, new plant products that are biodegradable have to be identified. In order to identify plants with biologically active natural products, selection of allelopathic plants is a good and commonly used approach. Laboratory experiments were conducted to determine the allelopathic effects of purple nutsedge (*Cyperus rotundus* L.) extracts on common upland weeds of Kerala like *Chromolaena odorata* (L.), *Synedrella nodiflora* and *Gomphrena decumbens* with a view to explore its weed seed inhibition potential. Aqueous nutsedge extracts inhibited germination and growth of *Gomphrena decumbens* while it had no significant influence on the germination of other two weed seeds. In *Gomphrena*, nutsedge extracts taken after flowering caused greater growth inhibition compared to extract taken before flowering indicating differential inhibition at various growth stages of nutsedge as well as increased allelochemical production after flowering stage. Suppression of plumule growth and significant reduction in radicle growth and vigour index was observed for all the three weed seeds tested. The study revealed greater inhibition rate for extracts collected after flowering, which indicated that allelochemical production is more after flowering. The tuber extracts of nutsedge as identified by HPLC technique revealed the presence of phenolic compounds *viz.* p-hydroxybenzoic acid and p-coumaric acid.

***Keywords:*** Allelopathic potential, bioassay, nutsedge extract, phenolic compounds, weeds seed inhibition

## INTRODUCTION

Productivity enhancement at the expense of synthetic chemicals to control weeds and pests has a negative impact on the environment quality and human health. Further, the resistance of weeds to synthetic herbicides is also a cause for concern. Due to the repercussions associated with the use of chemical herbicides, it is desirable to find new plant products that are biodegradable ,exhibit structural diversity and complexity and rarely contain halogenated atoms constitute one such class of chemicals (Ashrafi *et al.* 2007). In order to identify plants with biologically active natural products, selection of allelopathic plants is a good and commonly used approach. The allelopathic potentiality of nutsedge (Beiber 1967) and its effects on crop plants through inhibition of germination, growth or metabolism (Del Moral and Cates 1971) has received significant attention. Allelochemicals refer mostly to the secondary metabolites produced by the plants and are bye-products of primary metabolic processes. These secondary metabolites play greater role in reduction as well as enhancement of germination, establishment, growth, development and final biomass production of various species (Lal and Oudhia 1999). The effect of allelochemicals on metabolic changes of receiver plant include effect on cell division, elongation, membrane permeability, mineral uptake, stomatal movement, pigment synthesis, enzyme activity, photosynthesis and plant water relations (Wink and Twardowski 1992). The use of natural plant products particularly the allelochemicals for the management of weeds is a logical strategy and has been suggested by some earlier workers (Duke *et al.* 2000). Keeping this in view, an attempt has been made to study the allelopathic effect of nutsedge on some of the most prominent seed propagated upland weeds of Kerala. It is also envisaged to identify



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the allelochemicals present in purple nutsedge plant to explore its allelopathic potential for bio herbicide formulation.

## MATERIALS AND METHODS

### Preparation of nutsedge extracts

Laboratory experiments were undertaken to examine the allelopathic influence of nutsedge extracts on some of the important seed propagated annual weeds and the test plants were *Chromolaena odorata* (L.), *Synedrella nodiflora* and *Gomphrena decumbens.* The design of the experiment was Completely Randomised Design with 6 treatments in 4 replication. The treatments of the study consisted of aqueous extract of dry whole plant taken before ($T_1$) and after flowering ($T_2$) along with ethanol extract of whole plant taken before ($T_3$) and after flowering ($T_4$). Distilled water ($T_5$) and Ethanol ($T_6$) were taken as control treatments. For preparing the extract, purple nutsedge (*Cyperus rotundus* L.) plant samples were collected from infested fields at the respective growth stage. The plants were then cleaned off dirt and soil. It was then shade dried for one week. One hundred gram shade dried plant samples were immersed in 200 ml distilled water separately and kept at room temperature for 48 h. There after, it was stirred manually for few minutes and filtered through whatman no.1 filter paper. It was considered as a leachate of 50 per cent concentration and was further diluted with distilled water to 10 per cent concentration. Ethanol extract was prepared in the same way with ethanol as the extractant. Glass petri dishes (9 cm diameter) were sterilized in auto clave at an atmospheric pressure of 15 lb inch$^{-2}$ for one hour and later dried in hot air oven at $120^0$C. Seeds of test plants were sterilized by dipping in 0.1 per cent $Hg\ Cl_2$ solution for five minutes followed by repeated washing under tap water to remove residues of $Hg\ Cl_2$ and dried on in folds of ordinary filter paper. In each petri plate a Whatman No.1 filter paper was kept at bottom and there after 50 seeds each of test crop were arranged in circles on the top of the filter paper. Then 3 ml of the aqueous extract and ethanol extract or distilled water was added in each petri plate as per the treatments. Thereafter 2 ml solution of extract or distilled water was added uniformly as and when required till the end of the trial. As the weed seed endosperms were too little to support seedling growth in the petri dishes, the period of observation was limited to 7-10 days. The data on germination and seedling growth were recorded and the data were analysed statistically.

### Extraction and identification of inhibitory compounds

Nutsedge extract was prepared as per the procedure suggested by (Leela 1995) and the allelochemicals present in the tubers were identified by High Performance Liquid Chromatography (HPLC). One hundred and seventy five gram dried tubers of nutsedge were finely ground in a blender and soaked in 500 ml of methanol for 30 minutes. Then it was filtered through a muslin cloth. The final volume was made to 1000 ml. The filtrate was concentrated on a vacuum flash evaporator. The residue (10 ml) was diluted in 50 ml water to which 2.5 g of NaCl is also added. This is extracted thrice with 25 ml ethyl acetate each time. The ethyl acetate extracts combined, concentrated and the residue was hydrolysed with 2N NaOH. Then the pH was adjusted to 2.0 using 2N HCl. This was again extracted with ethyl acetate three times and evaporated to dryness. The dried residue was treated with 0.1 N $NaHCO_3$ solution and the pH was adjusted to 2.0. This was re-extracted with ethyl acetate three times and washed with distilled water to remove last traces of HCl. This was evaporated to dryness. The residue was dissolved in 25 ml ethyl acetate and phenols present were analysed with High Performance Liquid Chromatography (HPLC).



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## RESULTS AND DISCUSSION

Of the four treatments tried, ethanol extract at 10 per cent concentration taken as control killed all the weed seeds completely, while aqueous extracts caused inhibition of growth of some of the weed seeds tested. Aqueous nutsedge extracts inhibited germination and growth of *Gomphrena decumbene* while it had no significant influence on germination of *Synedrella nodiflora* and *Chromolaena odorata*. In *Gomphrena decumbens*, an annual dicotyledenous upland weed, nutsedge extract taken after flowering caused greatest (68 per cent) reduction in seed germination while nutsedge extract taken before flowering caused a reduction percentage of 47. Suppression of plumule growth was observed in all the weed seeds tested and nutsedge extracts taken after flowering inhibited *Gomphrena decumbens* and *Chromolaena odorata* while extract taken before flowering inhibited *Synedrella nodiflora*. Significant reduction in radicle growth was caused by aqueous extract of nutsedge in all the three weed seeds. Presence of coumarins in *Cyperus* extract could interfere with root cell elongation, water relations and photosynthesis in plant (Lal and Oudhia 1999).

**Table 1.** Allelopathic influence of purple nutsedge extracts on germination and growth of *Chromolaena odorata*

| Treatments | Germination percentage | Plumule length, cm | Radicle length, cm | Dry weight, mg $plant^{-1}$ | Vigour index |
|---|---|---|---|---|---|
| $T_1$ | 27.50 (31.53) | 0.55 | 0.10 | 0.18 | 17.87 (4.20) |
| $T_2$ | 20.00 (26.18) | 0.23 | 0.10 | 0.08 | 6.47 (2.51) |
| $T_3$ | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) |
| $T_4$ | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) |
| $T_5$ | 47.50 (44.28) | 1.57 | 0.75 | 0.20 | 112.75 (10.25) |
| $T_6$ | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) |
| SE | 5.66 | 0.04 | 0.04 | 0.02 | 0.95 |
| CD(0.05) | NS | 0.14 | 0.12 | 0.07 | 3.05 |

Figure in parenthesis indicate angular and square root transformed values

The dry matter production is one of the deciding factors of plant vigour and it is a function of growth of both root and shoot. Dry weight of *Gomphrena decumbense* and *Chromolaena odorata* were significantly affected by nutsedge extracts. This reduction in dry weight is consequent to the earlier reduction in growth parameters like plumule and radicle growth. Vigour index was drastically reduced for all the weed plants. The reduction in vigour index was observed under both types of plant extracts. Inhibition of radicle growth, which in turn resulted in poor nutrient absorption and consequent poor biomass accumulation, could be the reason for such a drastic reduction in seedling vigour.

**Table 2.** Allelopathic influence of purple nutsedge extracts on germination and growth of *Synedrella nodiflora*

| Treatments | Germination percentage 10 DAS | Plumule length, cm | Radicle length, cm | Dry weight, mg $plant^{-1}$ | Vigour index |
|---|---|---|---|---|---|
| $T_1$ | 52.50 (50.30) | 1.05 | 0.13 | 0.18 | 58.87 (7.49) |
| $T_2$ | 47.50 (48.71) | 1.25 | 0.16 | 0.14 | 68.50 (7.70) |
| $T_3$ | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) |
| $T_4$ | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) |
| $T_5$ | 80.00 (70.43) | 3.93 | 0.31 | 0.20 | 344.42(18.27) |
| $T_6$ | 0.00 (0.00) | 0.00 | 0.00 | 0.00 | 0.00 (0.00) |
| SE | 13.54 | 0.16 | 2.21 | 2.73 | 1.57 |



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| | | | | | |
|---|---|---|---|---|---|
| CD(0.05) | NS | 0.52 | 7.08 | NS | 5.03 |

Figure in parenthesis indicate angular and square root transformed values
NS- Non significant

**Table 3.** Allelopathic influence of purple nutsedge extracts on germination and growth of *Gomphrena decumbens*

| Treatments | Germination percentage 7 DAS | Plumule length, cm | Radicle length, cm | Dry weight, mg $plant^{-1}$ | Vigour index |
|---|---|---|---|---|---|
| $T_1$ | 45.00 (42.03) | 1.37 | 0.23(0.47) | 0.25 | 72.75 (8.43) |
| $T_2$ | 27.50 (31.01) | 1.15 | 0.28(0.51) | 0.49 | 39.75 (6.12) |
| $T_3$ | 0.00 (0.00) | 0.00 | 0.00(0.00) | 0.00 | 0.00 (0.00) |
| $T_4$ | 0.00 (0.00) | 0.00 | 0.00(0.00) | 0.00 | 0.00 (0.00) |
| $T_5$ | 85.00 (70.05) | 2.35 | 1.03(1.00) | 0.54 | 289.00(16.93) |
| $T_6$ | 0.00 (0.00) | 0.00 | 0.00(0.00) | 0.00 | 0.00 (0.00) |
| SE | 5.09 | 0.07 | 0.06 | 0.04 | 0.83 |
| CD(0.05) | 16.31 | 0.22 | 0.20 | 0.13 | 2.65 |

Figure in parenthesis indicate angular and square root transformed values

The study revealed greater inhibition rate for extracts collected after flowering, which indicated that allelochemical production is more after flowering. Differential inhibition by nutsedge extracts taken at different stages of growth can be explained by the fact that production of allelochemicals is regulated by the stage of the plant and is modified by environmental stresses like temperature extreme, nutrient moisture variables, insects and diseases and radiation (Einhellig 1995). Inhibition obtained at post flowering stage may be due to the higher level of allelochemical production after flowering in nutsedge (Jha and Sen 1982). Presence of p-hydroxy benzoic acid, caffeic acid, o-coumaric acid and ferulic acid in *Cyperus rotundus* reported earlier by Leela (1995*)*. Allelopathy even though considered as an undesirable property these characters can be profitably exploited. Collectively, these results showed differential toxicity of the allelopathic chemicals among the sp depending upon the stage and method of application. The use of water extracts of allelopathic crops particularly nutsedge alone or in combination with other water extracts will provide an economical, environmentally safe and effective weed control technique as an alternative for herbicides. Hence it is probable that these chemicals would be useful as applied herbicides and can be effectively utilized for the management of other weeds.

In the present study, the tuber extracts of nutsedge as identified by HPLC technique revealed the presence of phenolic compounds viz. p-hydroxybenzoic acid and p-coumaric acid (Table 4). The readily visible effects of these allelochemicals include inhibition or retardation of germination, reduced radicle or coleoptile extension, curling of the root axis, discolouration, increased number of seminal roots, reduced dry weight accumulation, and lowered reproductive capacity (Turk et al. 2002; Weir et al. 2004). These gross morphological effects may be secondary manifestations of primary events caused by a variety of more specific effects acting at the cellular or molecular levels in receiver plants (Rice 1970). Presence of p-hydroxy benzoic acid, caffeic acid, o-coumaric acid and ferulic acid in tuber extracts of Cyperus rotundus is reported earlier (Leela 1995). Reduction in germination and growth of weed seeds observed in present study corroborate eariler findings (Rice 1970 ; Leela 1995)



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**Table 4.** Identification of allelochemicals in the tuber extract of purple nutsedge (*Cyperus rotundus* L.)

| Sl. No. | Reference compounds | Retention time, min | Retention of peaks in sample, min |
|---|---|---|---|
| 1 | Gallic acid | 1.98 | |
| 2. | 3,4-dihydoxy phenyl acetic acid | 3.08 | |
| 3. | p-hydroxy benzoic acid | 4.40 | 4.40 |
| 4. | Caffeic acid | 4.84 | |
| 5. | Vanillic acid | 5.28 | |
| 6. | Gentisic acid | 5.73 | 5.88,6.07,6.79,7.88 |
| 7. | p-coumaric acid | 8.58 | 8.71 |
| 8. | Ferulic acid | 11.45 | |
| 9 | m-coumaric acid | 13.43 | 13.31,14.61 |
| 10. | o-coumaric acid | 20.69 | |
| 11. | Salicylic acid | 27.96 | |

Allelochemicals are present in virtually all plant tissues including leaves, flowers, fruits, stems, roots, rhizomes, seeds and pollen. They may be released from plants into the environment by means of four ecological processes *viz*., volatilization, leaching, decomposition and through root exudates. The inhibition in germination and growth of test crops observed in bioassay was marginal. This could presumably be due to the lower concentration of allelochemicals released from the tubers to the soil from which only a portion could be trapped by the adsorbent. Considering the uncontrolled growth in the field situation and a relatively high proportion of live and dormant tuber remaining in the soil coupled with the unfavourable stress conditions to which the soil will be exposed the growth of adjacent crops can expected to be more pronounced in the field situation than the conditions prevailing in the present study. Tens of thousands of secondary substances are known today, but only a limited number has been implicated as allelochemicals. The chemistry of the bioactive compounds in allelopathy is of fundamental importance for the understanding of interactions between plants. However, our present knowledge remains extremely unlimited because of the difficulties in dealing with the recovery of trace organic compounds by conventional solvent extraction methods. The present work suggests that exploration of the composition of a cluster of allelochemicals, which are simple in structure, possess various biological activities is a promising alternative for developing new herbicides from individual plant allelochemicals.

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# HERBICIDAL ACTIVITY OF PORGANIC™, APPLICATION AND ITS POTENTIAL USED AS NATURAL POST-EMERGENCE HERBICIDE IN PADDY RICE

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**ABSTRACT** This study was undertaken to explore the potential of natural herbicide from *Aglaia odorata* Lour. (PORGANIC™) use as selective post-emergence herbicide for rice field weeds control. The PORGANIC™ (30% ai) foliar spray at the rates of 10 and 20 kg (ai) $ha^{-1}$ was tested and compared with control (unweeded), check (commercial herbicide of pyribenzoxim at recommendation rate of 31.25 g (ai) $ha^{-1}$) and mixture of half rate of PORGANIC™+ pyribenzoxim. Rice field plot size was 4x4 $m^2$. Germinated rice cv. Pathumtanee 1 was uniformly sawn using sawing machine at 62.5 kg $ha^{-1}$ and forliar treatments were applied at 15 days after sowing (DAS). The three major weeds were found in the experimental field [barnyardgrass (*Echinochloa crus-galli* Beauv.), gooseweed (*Sphenoclea zeylanica* Gaertn.) and tall fringer rush (*Fimbristylis miliacea* (L.) Vahl.)]. Weed density was evaluated at 30 days after treatments and rice yield was evaluated 120 days after sawing. The results showed that foliar spray of PORGANIC™ at the rate of 10 kg (ai) $ha^{-1}$ had a significant inhibitory effect on *E. cruss-galli* and *S. zeylanica* density and their density was reduced to 42.31 and 29.93% of control but less effect than pyribenzoxim while density of *F. miliacea* was increased to 13.19% over control. PORGANIC™ at the rate of 10 kg (ai) $ha^{-1}$ mixed with pyribenzoxim at the dose of 15.63 g (ai) $ha^{-1}$ had a non-significant inhibitory effect on density of all of the weed species compared with pyribenzoxim applied alone. The dry weight of the weeds was reduced in a similar manner to density. Rice density, dry biomass and yield did not affect by application of PORGANIC™ at the rate of 10 kg (ai) $ha^{-1}$ and more interestingly applied of PORGANIC™ at the rate of 10 kg (ai) $ha^{-1}$ mixed with pyribenzoxim at the dose of 15.63 g (ai) $ha^{-1}$ increased in rice density, biomass and grain yield over pyribenzoxim commercial recommendation rate.

***Keywords*: ***Aglaia odorata*, natural herbicide, reduced herbicide dose, rice

## INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food of more than 3 billion people worldwide, it is an important commodity in Asia serving daily as a source of carbohydrate, proteins, lipids, vitamins and minerals (Walter et al. 2008). Therefore, rice production should maintain its trend in order to support the constantly growing consumption demand. Weed problems such as barnyardgrass (*Echinochloa crus-galli* Beauv.), one of the greatest yield limiting weeds in rice cultivation systems, will increase because rice and barnyardgrass often emerge together in the field (Ahn and Chung 2000). It is better adapted for growth under dry rather than wet conditions and is expected to become a greater problem in dry, direct-seeded rice (Im et al.1993). Manual weeding has been commonly used in rice fields to minimise yield losses from weed competition. However, declining labour availability for agriculture, increasing labour costs related to reduced labour supply, and water scarcity have required rice producers to look for



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alternative weed-control treatments. Selective herbicides have been extensively used, given their ease of application, high efficacy, and low cost relative to alternative methods (Pingali et al. 1997). However, the use of herbicides has been accompanied globally by the potential build up of herbicide resistant weeds, weed species population shifts, and concerns about environmental contamination and impacts on human health (Johnson and Mortimer 2005). Ecological control through the application of naturally occurring allelopathic substances to agriculture practice has been an important and useful method to control weed (Macias 1995). Thus, the best way to control barnyardgrass in an environmentally acceptable and sustainable approach is to develop natural compounds like allelochemicals. The genus Aglaia (Meliaceae) is distributed mainly in the tropical rain forest of South-East Asia. Aglaia has attracted considerable attention as a possible new source of unique and natural integrated pest management products (Greger et al. 2001). Chinese rice flower (*Aglaia odorata* Lour.) is a tree widely distributed in central and southern Thailand. The aqueous extract from dried leaves and twig residues of *A. odorata* inhibit weed seed germination and have the potential for organic weed control (Laosinwattana et al. 2009). However, a study on the herbicidal effects of an *A. odorata* herbicide in suspension concentrate (SC) on rice weed control has not been conducted.

The objectives of this research were to evaluate the natural herbicide from *A. odolata* Lour. (PORGANIC™) to control barnyardgrass and adverse effects on growth and yield in wet seeded rice conditions.

## MATERIALS AND METHODS

### Plant materials and preparation of PORGANIC™

Mature leaves of three-year old Chinese rice flower (*A. odorata* Lour.) plants growing at the experimental field of King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand were collected, cleaned several times with tap water, and dried in a hot-air oven at 45°C for 72 h. They were then cut into small pieces, ground into a powder in an electric blender, and sieved through a 40 mesh (420 μm) sieve. Dry leaf powder was extracted with ethanol:water (75:25 v/v) at room temperature. After 24 hrs of extraction, the brown supernatants were filtered through four layers of cheesecloth and re-filtered through Whatman no. 1 filter paper (Whatman Inc. Clifton, NI, USA.). Following filtration, the brown supernatants were dried by evaporation of the solvent using a rotary evaporator (BUCHI Rotavapor R255), BUCHI, Lausanne, Switzerland), under a partial vacuum at 45°C until constant crude extract weight was reached. After that each dry leaf powder residue was re-extracted 2 times with the same extraction solvent as the same condition of the first extraction procedure, and then crude extract of the extraction number 1, 2 and 3 were pooled. The natural herbicide from *A. odorata* (PORGANIC™) was prepared by mixing 30% *A. odorata* crude extract with 4% surfactant and 66% of solvent.

### Greenhouse experiment:

An experiment was performed to explore the effect of PORGANIC™ on the toxicity and growth of barnyardgrass *(Echinochloa crus-galli*) and rice (*Oryza sativa* L.) under pot conditions in an experimental greenhouse. For this, soil was collected from upper 0–10 cm soil horizon in farmers' paddy rice fields where rice was grown in the previous season. Seeds of barnyardgrass were collected from paddy rice field and rice cultivar Pathumtanee 1 were obtained from commercial seed lots that were available for purchase by growers. Barnyardgrass and rice seed were soaked in water for 12 hrs and each twenty seed was planted in individual 15-cm diameter plastic pots containing a paddy rice soil. Pots were maintained in a greenhouse with natural condition. Pots were sub-irrigated with tap water to field capacity to imbibe seed and initiate germination for 3 days. After emergence, barnyardgrass and rice plants were sub-irrigation to maintained 2-cm water level with tap water. An experiment was conducted to evaluate the effect of PORGANIC™ natural herbicide at the rates of 5, 10 and 20 kg (ai) $ha^{-1}$ applied at 5, 10 and 15 days after sawing (DAS) with spray volume of 500 L/ha. The pots were arranged in a



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completely randomized design and sub-irrigated daily with tap water to maintained 2-cm water level. Visual ratings for weed and rice injury were recorded at 21 DAT, Percentage of weed and rice injury ratings were based on a scale of 0-100, with 0 being no control or no injury and 100 being complete control or rice death. Plant height and dry weight of above ground part of both weed and rice were harvested at 21 DAT.

**Field experiment:**

To evaluate the potential use of PORGANIC™ as a weed suppressant under field conditions, experiments were performed in a farmer's field selected in Nong Chok district, Bangkok, Thailand during in 2013. In the selected fields, plots of 4 x 4 m size were prepared and these plots received no prior herbicidal/special treatment. *O. sativa* was sown in using seeds at 62.5 kg (ai) ha$^{-1}$. The experimental design was randomized complete block with four replications. Treatments included untreated control, the recommendation rate of pyribenzoxim (Pyranchore$^{TM}$) alone (31.25 g(ai)ha$^{-1}$), three PORGANIC™ rates (5, 10 and 20 kg (ai) ha$^{-1}$) and the combination of reduced rated of pyribenzoxim+ PORGANIC™ (15.63 g + 10 kg (ai) ha$^{-1}$). Foliar spray was applied as post-emergence spray at 14 DAS using a hand spray. The spray volume (500 L ha$^{-1}$) was determined by calibration. All other conventional practices (fertilizers: 187 kg (ai) ha$^{-1}$ of base fertilizer (16-20-0) were applied 20 DAS, 62.5 kg (ai) ha$^{-1}$ of urea (46-0-0) was applied 40 DAS, and 62.5 kg (ai) ha$^{-1}$ of base fertilizer (15-15-15) was applied 60 DAS; watering; but no weeding/herbicide treatment. Weed species, density and rice plant stands were counted at 30 days after treatments (DAT) from four replicated in each treatment. At each sampling, two quadrates of 1x1 m$^2$ were placed in each plot to determine the weed species, density and rice plant. At crop harvest, only above ground part of weed dry weight was determined as weed density was difficult to evaluate. The dry weight was measured after drying the samples at 60°C for 72 hrs. Rice plant dry weight and grain yield were determined by randomly sampling area of two quadrates of 1x1 m$^2$ from each plot. Plant dry weight and grain yield were converted to percentage of control (untreated) at 14% moisture content.

**Statistical analysis**

Analysis of variance was calculated for all data and comparisons between treatments were made at probability level $p \leq 0.05$ using Tukey's Studentized Range Test.

## RESULTS AND DISCUSSION

**Effect of PORGANIC™ natural herbicide on injury, plant height and dry weight of barnyardgrass and rice in pot tested under greenhouse condition.**

Post-emergence application of PORGANIC™ natural herbicide was applied at doses of 5, 10 and 20 kg (ai) ha$^{-1}$ when barnyardgrass and rice seedling growth stages were 5, 10 and 15-day old plants. The results of PORGANIC™ injury are characterized by chlorosis of the leaves followed by necrosis of the affected tissues. Overall, visual barnyardgrass injury increased as PORGANIC™ dose ranged from 5 to 20 kg (ai) ha$^{-1}$ and decreased from 15 to 5 day old of seedling growth stages (Table 1.). In 5 and 10 days old, evaluations conducted at 21 DAT demonstrated completely injury when the highest dose of PORGANIC™ was applied. Treatments containing the lowest dose of PORGANIC™ displayed barnyardgrass injury 20% and 12.5% when applied at 5 and 10 days old, respectively. In the same evaluation, treatments containing 5, 10 and 20 kg (ai) ha$^{-1}$ applied at 5, 10 and 15 day old of rice seedlings (Table 2.) resulted in 0 to 35% injury, which was significantly lower than the injury observed in barnyardgrass, excepted for at the highest dose of 20 kg (ai) ha$^{-1}$ applied at 5 days old resulted in completely rice injury. For plant high and dry weight of above ground parts of both barnyardgrass and rice plants evaluated at 21 DAT. Similar to the results of plant injury visual observation, plant height and dry weigh of both barnyardgrass and rice decreased when increased of PORGANIC™ dose and decreased when applied from 15 to 5-day old plants.



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However, overall plant injury, height and dry weight of rice had less effect than those of barnyardgrass.

**Table 1.** Effect of PORGANIC™ natural herbicide applied as post emergence herbicide on plant injury, plant height and dry weight of barnyardgrass (*Echinochloa crus-galli* Beauv.) in pot tested at 21 DAT*.

| Plant growth stages (DAS) | Application dose (kg (ai) ha$^{-1}$) | Plant injury | Plant height | Dry weight |
|---|---|---|---|---|
| | | ------------------Inhibition (% of control) --------------- | | |
| | Control (Untreated) | 0.00c | 0.00c | 0.00c |
| 5 | 5 | 20.00c | 4.05c | -13.33c |
| 10 | 5 | 12.50c | -4.79c | -11.11c |
| 15 | 5 | 0.00c | 1.69c | -13.21c |
| 5 | 10 | 77.50ab | 75.80b | 80.13ab |
| 10 | 10 | 67.50b | 78.63ab | 66.11b |
| 15 | 10 | 12.50c | 13.81c | 3.64c |
| 5 | 20 | 100.00a | 100.00a | 100.00a |
| 10 | 20 | 100.00a | 100.00a | 100.00a |
| 15 | 20 | 70.00ab | 77.38ab | 72.25b |

*Note: Means in the same column with the same letters are not significantly different, according to Tukey's test at p = 0.05

**Effect of PORGANIC™ natural herbicide and pyribenzoxim herbicide treatments on weed control and rice in experimental paddy rice field condition.**

The dominate weed species in the experiment were barnyardgrass (*Echinochloa crus-galli* Beauv.), gooseweed (*Sphenoclea zeylanica* Gaertn.) and finger rush (*Fimbristylis miliacea* (L.) Vahl.).

**Table 2.** Effect of PORGANIC™ natural herbicide applied as post emergence herbicide on plant injury, plant height and dry weight of rice (*Oryza sativa* L.) in pot tested at 21 DAT.

| Plant growth stages (DAS) | Application dose (kg (ai) ha$^{-1}$) | Plant injury | Plant height | Dry weight |
|---|---|---|---|---|
| | | ------------------Inhibition (% of control) --------------- | | |
| | Control (Untreated) | 0.00d | 0.00e | 0.00d |
| 5 | 5 | 7.50d | 6.51e | 12.50c |
| 10 | 5 | 5.00d | -2.17e | 1.49d |
| 15 | 5 | 0.00d | 0.13e | -1.93d |
| 5 | 10 | 35.00b | 35.75b | 8.47cd |
| 10 | 10 | 22.50c | 23.06cd | 6.25cd |
| 15 | 10 | 5.00d | 10.50d | 1.76d |
| 5 | 20 | 100.00a | 100.00a | 100.00a |
| 10 | 20 | 32.50bc | 40.06b | 22.39b |
| 15 | 20 | 22.50c | 30.75bc | 16.37bc |

*Note: Means in the same column with the same letters are not significantly different, according to Tukey's test at $p = 0.05$

The application of PORGANIC™ natural herbicide, pryribenzoxim herbicide and theirs combination showed significant difference resulted on density, plant dry weight of weed and rice and rice grain yield. Results indicated that all treatments significantly reduced the densities and dry weight of barnyardgrass and gooseweed weeds but, had no significantly effect on finger rush weed species (Table. 3). These results indicated that barnyardgrass and goosegrass were



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sensitive to PORGANIC™ natural herbicide, while finger rush was tolerance. Application of PORGANIC™ at the dose of 20 kg (ai) $ha^{-1}$ had weed density of 22.33, 20.37 and 116.67% over control in barnyardgrass, gooseweed and finger rush, respectively. Weed dry weight of all weed species was also similar to the results of weed density. The plot which received pryibenzoxim herbicide at dose of 31.25 g (ai) $ha^{-1}$ had completely control of all weed species. The treatment combination of PORGANIC™ at the dose of 10 kg (ai) $ha^{-1}$ with half dose of pyribenzoxim (15.63 g (ai) $ha^{-1}$), weed density and dry weight of all weed species was nearly the same results in the pyribenzoxin herbicide alone at full dose plots.

For rice, the results (Table 4) showed that all PORGANIC™ and pyribenzoxim herbicide treatments significantly reduced rice density and plant dry weight compared with control. The plots, which received PORGANIC™ treated at 20 kg (ai) $ha^{-1}$, caused as much as 48.78% of rice plant density compared with control (untreated). In the same evaluation, treatment containing of pyribenzoxim herbicide at 31.25 g (ai) $ha^{-1}$ resulted in 78.38% of rice plant density. Therefore, pyribenzoxim herbicide applied at 31.25 g (ai) $ha^{-1}$ resulted in decreasing rice plant density lower than PORGANIC™ treated at 20 kg (ai) $ha^{-1}$. Interestingly, the combination treatment of PORGANIC™ at the dose of 10 kg (ai) $ha^{-1}$ with half dose of pyribenzoxim (15.63 g (ai) $ha^{-1}$) provided 98.1% of rice plant density. These results indicated that PORGANIC™ applied alone at the dose of 20 kg (ai) $ha^{-1}$ and pyribenzoxim herbicide applied alone at 31.25 g (ai) $ha^{-1}$ significantly decreased rice plant density compared with untreated treatment, while the combination of half dose of PORGANIC™ + pyribenzoxim had no effect on rice density. More interestingly, the treatment combination of PORGANIC™ at 10 kg (ai) $ha^{-1}$ with reduced dose of pyribenzoxim at 15.63 g (ai) $ha^{-1}$ produced a maximum rice grain yield with a 204.17% over control, and it was non-significantly compared with full dose of pyribenzoxim at 31.25 g (ai) $ha^{-1}$ treatment. Our results indicated that PORGANIC™ natural herbicide had specific compatibility for certain herbicide (pyribenzoxim). Morevoer, these findings suggest that herbicide doses can be decreased considerably (50%) when the dose used in combination with PORGANIC™ natural herbicide. Similar Jabran et al. (2010) and Farooq et al. (2011) reported that dose of metribuzin+phenoxaprop used for effective weed control of wheat can be reduced by more than 70% if the herbicide id use mixed together with sorghum and sunflower water extracts.

**Table 3.** Effect of PORGANIC™ natural herbicide applied as a post-emergent herbicide on weed density and dry biomass under field conditions at 30 DAT*.

| Weed | Untreated Control | PORGANIC™ ( kg (ai) $ha^{-1}$) | | Pyribenzoxim g (ai) $ha^{-1}$ | PORGANIC™ + Pyribenzoxim kg (ai) $ha^{-1}$ + g (ai) $ha^{-1}$ |
|---|---|---|---|---|---|
| | | 10 | 20 | 31.25 | 10+15.63 |
| **Barnyardgrass** | | ---------------------------------% of control--------------------------------- | | | |
| Density | 100.00a | 45.81b | 22.33c | 1.28d | 7.69cd |
| Dry weight | 100.00a | 45.46b | 27.67bc | 6.06d | 13.59bc |
| **Gooseweed** | | | | | |
| Density | 100.00a | 48.15b | 20.37c | 0.00c | 0.00c |
| Dry weight | 100.00a | 38.78b | 18.37bc | 0.00c | 0.00c |
| **Finger rush** | | | | | |
| Density | 100.00a | 113.19a | 116.67a | 0.00b | 3.13b |
| Dry weight | 100.00b | 159.37a | 147.88a | 0.00c | 3.23c |

*Note: Means in the same row with the same letters are not significantly different, according to Tukey's test at $p = 0.05$



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**Table 4.** Effect of PORGANIC™ natural herbicide applied as a post-emergent herbicide on density dry biomass and grain yield of rice under field conditions*.

| | Untreated Control | PORGANIC™ (kg (ai) ha$^{-1}$) | | Pyribenzoxim 31.25 g (ai) ha$^{-1}$ | PORGANIC™ 10 kg(ai) ha$^{-1}$ and Pyribenzoxim 15.63 g (ai) ha$^{-1}$ |
|---|---|---|---|---|---|
| | 0 | 10 | 20 | | |
| | ---------% of control--------- | | | | |
| Density | 100.00a | 79.76b | 48.78c | 78.38b | 98.14a |
| Dry biomass | 100.00a | 89.91a | 53.74b | 81.32a | 93.76a |
| Yield | 100.00c | 156.32b | 115.37c | 201.58a | 204.17a |

*Note: Means in the same row with the same letters are not significantly different, according to Tukey's test at p = 0.05

## CONCLUSIONS

Pyribenzoxim was generally used as early-post emergence herbicide for weed control in direct seeded rice. These researches showed that the use of PORGANIC™ natural herbicide in mixture with pyribenzoxim is one of the most effective combination herbicide for control grass weed and sedge. Rice grain yield from PORGANIC™ natural herbicide (half dose) in mixture with pyribenzoxim (half dose) plot had no significant difference from the plot received pyribenzoxim at full dose application. These results provide a reasonable for suggesting that combination of PORGANIC™ natural herbicide together with half dose of pyribenzoxim will increase environmental safety by reducing reliance on synthetic herbicides.

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# HERBICIDAL ACTIVITY OF PORGANIC™, PHYTOTOXIC EFFECTS AND ITS PHYSIOLOGICAL MECHANISMS ON BIOASSAY PLANTS

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**ABSTRACT** The natural herbicide from *Aglaia odorata* Lour. was prepared by mixing crude ethanol extract from *A. odorata* leaves with adjuvants at the ratio of 30 : 70 to obtain soluble concentrate formulation (PORGANIC™ 30% a.i.). In this work, studies on the physiological effects induced by PORGANIC™ onto wild pea *(Phaseolus lathyroides* L.) and barnyardgrass (*Echinochloa crus-galli* Beauv.) were performed by observing changes in leaf membrane integrity, leaf photosynthetic pigment (i.e. chlorophyll a, chlorophyll b and carotenoid) content and leaf malondialdehyde (MDA) content as an indicator of lipid peroxidation. PORGANIC™ at the concentrations of 2.5, 5, 10 and 20 kg a.i. $ha^{-1}$ by foliar spraying was tested on both bioassay plants at growth stage of 30 days after planting and the data was collected at 1, 3, 5 and 7 days after treatment. Results showed that the electrolyte leakage increased as increasing the concentrations of PORGANIC™ in both test weeds indicating membrane disruption and loss of integrity. PORGANIC™ decreased chlorophyll a, chlorophyll b and carotenoid content when compared with the control indicating the deficiency of absorbed light in the PORGANIC™ treated leaf and interference of photosynthetic process. Moreover, treated leaves exhibited an increase in MDA content, suggesting lipid peroxidation.

***Keywords*:** *Aglaia odorata* Lour., Natural herbicide, Total chlorophyll and carotenoid content, electrolyte leakage

## INTRODUCTION

From our previous investigation, *Aglaia odorata* Lour. plant has been reported to show allelopathic effect in laboratory and field conditions (Laosinwattan et al. 2012; Teerarak et al. 2012). These findings imply that the allelochemicals and other phytotoxic constituents of *A. odorata* have highly phytotoxic potential as organic herbicide enough to act the management of weeds in field crop. In this work, studies on the physiological effects induced by the natural herbicide from *A. odorata* PORGANIC™ onto wild pea *(Phaseolus lathyroides* L.) and barnyardgrass (*Echinochloa crus-galli* Beauv.) were performed by observing changes in leaf photosynthetic pigment content, leaf membrane integrity and leaf malondialdehyde (MDA) content. Chlorophyll pigments are essential functional and structural cofactors in all photosynthetic pigment-proteins involved in photosynthesis (Fiedor et al. 2008). Carotenoid pigments absorb light at wavelength between 400 and 550nm and transfer it to the chlorophylls (Siefermann-Harms 1987), protect the photosynthetic apparatus by quenching triplet chlorophyll, singlet oxygen and other free radicals (Oelmüller 1989) and carry out the photosystem assembly and the stability of light harvesting complex proteins as well as thylakoid membrane stabilization (Niyogi et al. 2001). Electrolytes are contained within the membranes of plant cells that support numerous biochemical and biophysical reactions (Leshem 1992). Under environmental stresses, plant membranes are often associated with increases in permeability and loss of membrane integrity and cell compartmentation (Campos et al. 2003).



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Unstressed or undamaged plant cells maintain electrolytes within the membrane. The measurement of electrolyte leakage from plant tissues is a classical method to estimate membrane integrity by comparing the conductivity of the leaked contents from injured and uninjured tissues in water (Mattsson 1996). High solute losses by leakage have been reported for corn leaf after herbicide fluridone treatment (Kim et al. 2004). MDA is one of the end products of the reaction of oxygen with unsaturated fatty acids (Leshem 1987), and has been considered to be a toxic process leading to decomposition of phospholipids in membranes. Therefore, present investigation was undertaken to evaluate mechanisms of phytotoxicity of PORGANIC™ on wild pea for representative broad leaf and barnyardgrass for representative narrow leaf weeds.

## MATERIALS AND METHOD

### Extraction and preparation of natural herbicide from *A. odorata*

Allelopathic plant, *A. odorata*, were collected at maturity leaves well cleaned with tap water, chopped into 1-cm-long pieces, oven-dried at 45°C for 5 days and ground into powder in a blender. Dried leaves of *A. odorata* were extracted with ethanol to obtain crude ethanol extract. This crude extract was used for preparation of natural herbicide from *A. odorata*. The natural herbicide from *A. odorata* L. was prepared by mixing crude ethanol extract from *Aglaia odorata* L. leaves with adjuvants at the ratio of 30 : 70 to obtain soluble concentrate formulation (PORGANIC™ 30% ai.).

### Plant bioassays and treatment with PORGANIC™

For bioassay plant, wild pea seeds were collected from an upland field. Wild pea hard seed coats were scrubbed with sandpaper to break their dormancy. For another, barnyardgrass seeds were placed in the shade at room temperature for 3 months, and then incubated at 60°C in a hot-air oven for 48 h to break their dormancy. Empty and undeveloped seeds were discarded by floating in tap water. PORGANIC™ at the concentrations of 2.5, 5, 10 and 20 kg a.i $ha^{-1}$ by foliar spraying was tested on both bioassay plants at growth stage of 30 days after planting and the data of membrane integrity, leaf photosynthetic pigment (i.e. chlorophyll a, chlorophyll b and carotenoid) content and leaf malondialdehyde content was collected at 1, 3, 5 and 7 days after treatment.

### Photosynthetic pigment content

Five leaf disks (0.6 cm diameter) from barnyardgrass and wild pea were cut into uniform size and sequentially extracted with aqueous 80% acetone in a mortar using a pestle and the suspension was filtered through a Whatman filter paper No. 1. Chlorophyll and carotenoid contents were determined spectrophotometrically using spectronic GENESYS 20 spectrophotometer (Thermo Electron Corporation, USA) at 3 wavelengths: 663 nm for chlorophyll a, 647 nm for chlorophyll b and 470 nm for carotenoids. Photosynthetic pigment contents were computed using Lichtenthaler's equation (Lichtenthaler 1987).

### Electrolyte leakage

Fifteen freshly cut leaf discs (0.6 cm diameter each) from barnyardgrass and wild pea were floated on 15 mL of distilled water. The electrolyte leakage in the solution was measured after 0.5, 1, 1.5 and 2 h of floating at room temperature using a digital conductivity meter (Consort C860, Belgium).

### Lipid peroxidation

Lipid peroxidation was measured in terms of the production of MDA, an end product of lipid peroxidation, was estimated based on thiobarbituric acid-reactivity, according to Hodges et al. (1999). Barnyardgrass and wild pea leaf materials (nearly 0.1 g FW each) were homogenized in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA) solution. The homogenate was centrifuged at



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10,000 × *g* for 10 min and 1 ml of the supernatant was added to 4 ml of 0.5% (w/v) TBA in 20% TCA. The mixture was heated at 95 ◦C for 30 min, and the reaction was stopped by transferring it to an ice bath for 10 min. Following centrifugation at 10,000×g for 5 min, the supernatant was taken and the absorbance was determined at 532 and 600 nm. The concentration of TBA reacting substances (TBARS) was calculated from the absorbance at 532 nm (a correction was performed subtracting the absorbance at 600 nm for unspecific turbidity) by using a molar extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$.

**Statistical analysis**

Data were analyzed using analysis of variant (ANOVA). Whenever ANOVA indicated significant effects ($p < 0.05$), a pair wise comparison of means by Tukey's studentized range test was carried out.

## RESULTS

Photosynthetic pigment contents, considered an indicator of photosynthetic efficacy of barnyardgrass and wild pea leaves were evaluated at 1, 3, 5 and 7 days after treatment (Table 1 and 2). PORGANIC™ foliar application caused a reduction in chlorophyll a, b and carotenoid levels of both bioassay plants. PORGANIC™ at 5, 10 and 20 kg a.i. $ha^{-1}$ markedly reduced photosynthetic pigment contents in the leaves of barnyardgrass and wild pea within 1 day after treatment. At the highest PORGANIC™ concentration (20 kg a.i. $ha^{-1}$) and longest evaluation time of the 7 days after treatment the barnyardgrass leaf had contents of chlorophyll a, b and carotenoid with, 7.15, 2.97 and 1.86 µg/$cm^2$, respectively, while the control had pigment contents with 29.16, 14.13 and 9.97 µg/$cm^2$, respectively. At the highest PORGANIC™ concentration and longest evaluation time in wild pea, leaf had chlorophyll a, b and carotenoid with, 12.65, 5.26 and 3.10 µg/$cm^2$, respectively, while the control had pigment contents with 40.59, 22.09 and 16.32 µg/$cm^2$, respectively.

The levels of electrolyte conductivity an indicator of membrane leakage of barnyardgrass and wild pea leaves were measured using conductivity meter (Fig. 1 and 2). Leakage values were similar in control and PORGANIC™ treated leaf at concentration of 2.5 kg a.i $ha^{-1}$ by foliar spraying. After treatment with PORGANIC™ treated leaves of both plants at higher concentrations (5-20 kg a.i $ha^{-1}$) strongly increased electrolyte leakage. Leakage values of both plants increased dose-dependently as increasing concentrations of PORGANIC™ and soaking time.



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**Table 1.** Effects of foliar applied PORGANIC™ on chlorophyll a, b and carotenoid content of barnyardgrass young leaf at 1, 3, 5 and 7 days after treatment (DAT).

| PORGANIC™ rate (kg a.i. ha$^{-1}$) | Chlorophyll a (µg/cm$^2$) | | | | Chlorophyll b (µg/cm$^2$) | | | | Carotenoid (µg/cm$^2$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 DAT | 3 DAT | 5 DAT | 7 DAT | 1 DAT | 3 DAT | 5 DAT | 7 DAT | 1 DAT | 3 DAT | 5 DAT | 7 DAT |
| 0 | 27.34 | 28.18 | 28.93 | 29.16 | 12.20 | 13.04 | 13.94 | 14.13 | 8.79 | 9.20 | 9.47 | 9.97 |
| 2.5 | 25.39 | 24.16 | 18.29 | 15.34 | 10.54 | 9.94 | 7.61 | 5.29 | 7.45 | 6.55 | 6.14 | 4.91 |
| 5 | 23.00 | 19.79 | 17.65 | 13.03 | 9.34 | 8.20 | 6.69 | 4.97 | 6.49 | 5.58 | 4.45 | 3.52 |
| 10 | 16.12 | 14.77 | 13.27 | 9.45 | 7.44 | 6.51 | 5.90 | 3.64 | 5.51 | 4.23 | 3.23 | 2.97 |
| 20 | 14.35 | 12.36 | 11.27 | 7.15 | 6.76 | 5.75 | 4.11 | 2.97 | 4.90 | 3.59 | 2.90 | 1.86 |

**Table 2.** Effects of foliar applied PORGANIC™ on chlorophyll a, b and carotenoid content of wild pea young leaf at 1 at 1, 3, 5 and 7 days after treatment (DAT).

| PORGANIC™ rate (kg a.i./ha) | Chlorophyll a (µg/cm$^2$) | | | | Chlorophyll b (µg/cm$^2$) | | | | Carotenoid (µg/cm$^2$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 DAT | 3 DAT | 5 DAT | 7 DAT | 1 DAT | 3 DAT | 5 DAT | 7 DAT | 1 DAT | 3 DAT | 5 DAT | 7 DAT |
| 0 | 37.23 | 38.60 | 39.63 | 40.59 | 20.28 | 20.97 | 21.63 | 22.09 | 15.26 | 15.70 | 16.05 | 16.32 |
| 2.5 | 35.89 | 34.88 | 33.08 | 31.59 | 15.94 | 14.03 | 13.85 | 12.46 | 12.98 | 11.75 | 10.67 | 10.91 |
| 5 | 30.59 | 28.09 | 26.13 | 24.31 | 14.61 | 11.75 | 10.07 | 9.64 | 10.33 | 9.45 | 8.95 | 7.25 |
| 10 | 25.83 | 22.13 | 20.93 | 16.50 | 11.51 | 9.71 | 8.61 | 7.68 | 9.22 | 7.65 | 6.07 | 5.62 |
| 20 | 22.53 | 19.39 | 16.41 | 12.65 | 9.36 | 7.56 | 6.13 | 5.26 | 7.95 | 6.84 | 4.38 | 3.10 |

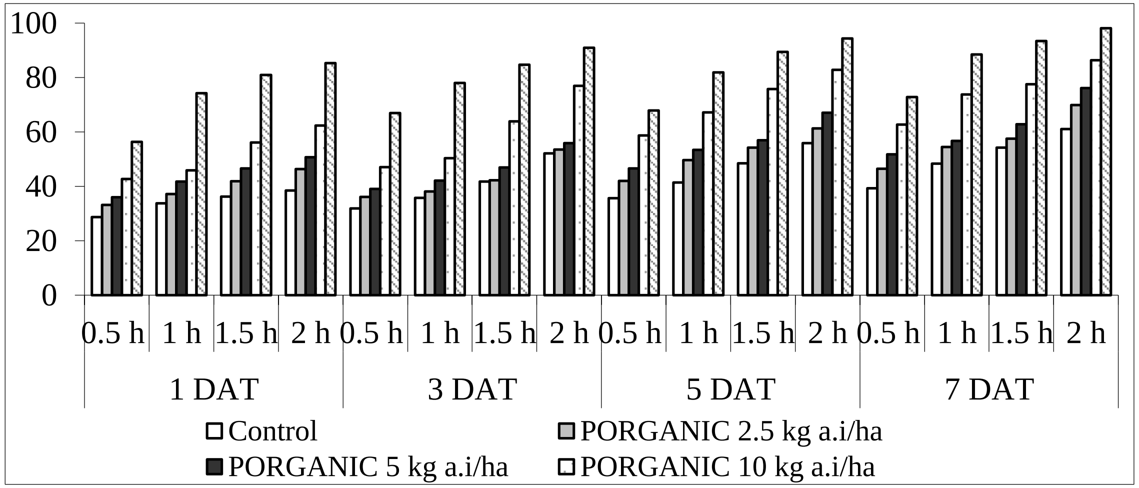


**Fig. 1.** Conductivity values (µS/cm) in leaf discs of barnyardgrass after treatment with PORGANIC™ for 1, 3, 5 and 7 days.



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The levels of MDA, an indicator of lipid peroxidation, were measured using the TBARS-assay in leaves. Parallel to electrolyte leakage, the amount of MDA increased in response PORGANIC™compared to control (Fig. 3 and 4). MDA content was similar in control and PORGANIC™ treated leaves of both plants at concentrations of 2.5 and 5 kg a.i $ha^{-1}$, being significant only after 5 days of treatment. In response to higher treatment (10, 20 kg a.i $ha^{-1}$), MDA accumulation in barnyardgrass and wild pea leaves was significantly higher than in control at the beginning of treatment.

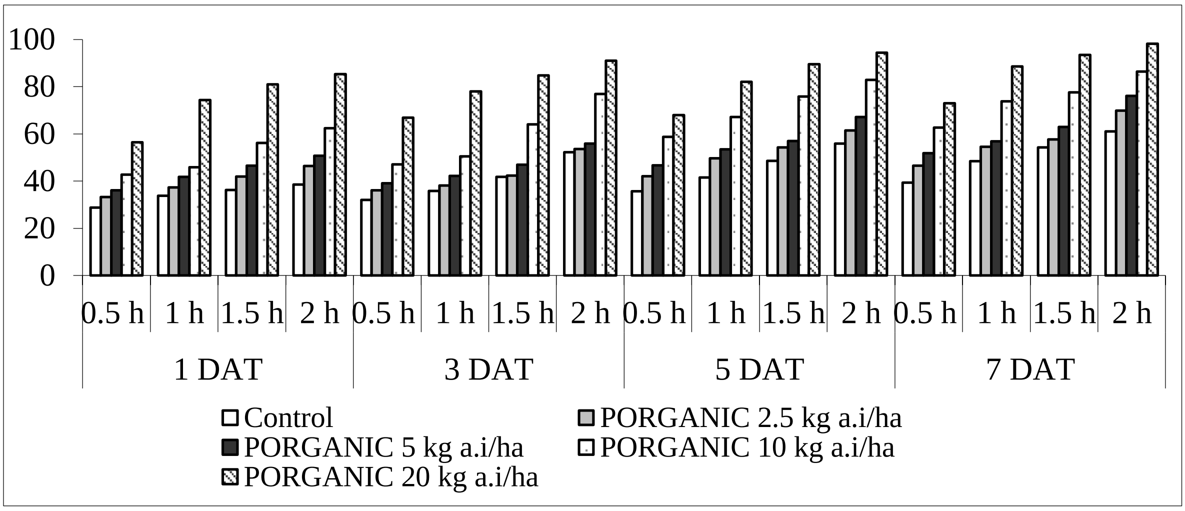


**Fig. 2.** Conductivity values (μS/cm) in leaf discs of wild pea after treatment with PORGANIC™ for 1, 3, 5 and 7 days**.**

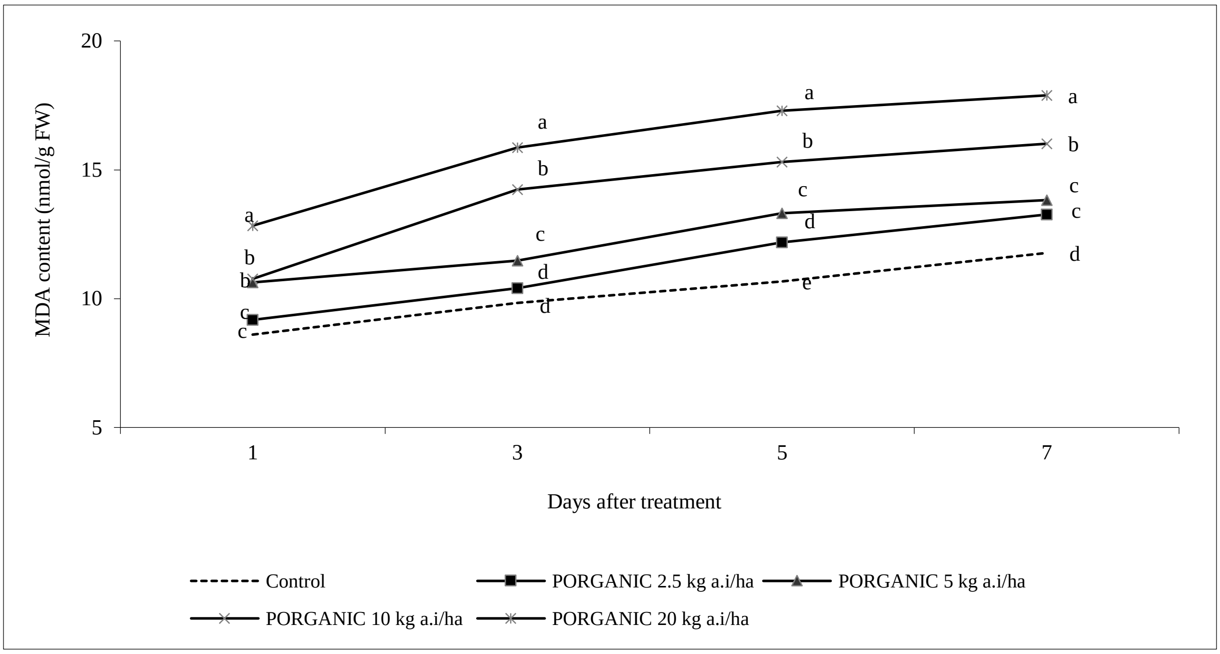


**Fig. 3.** MDA content in barnyardgrass leaf after treatment with PORGANIC™ for 1, 3, 5 and 7 days**.**



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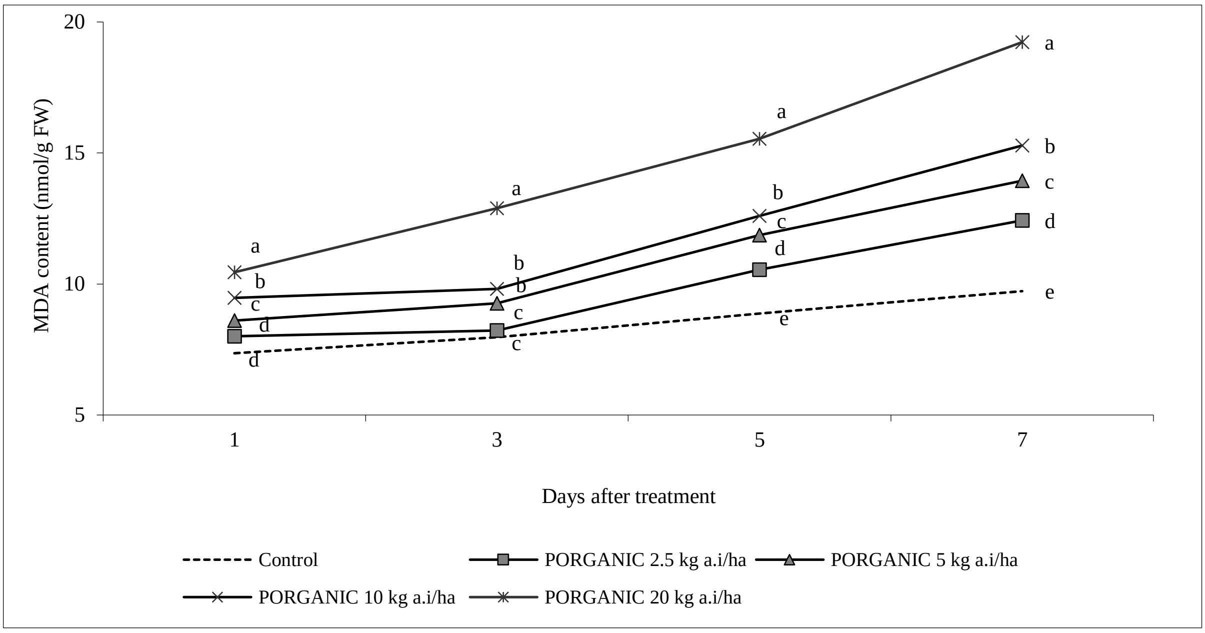


**Fig. 4.** MDA content in wild pea leaf after treatment with PORGANIC™ for 1, 3, 5 and 7 days**.**

## DISCUSSION

Barnyardgrass and wild pea leaves showed a consistent increase in electrolyte leakage and lipid peroxidation, and a decrease in photosynthetic pigment contents, indicating phytotoxicity of PORGANIC™. Our work showed that the effect of PORGANIC™ action on photosynthesis is a reduction in the content of photosynthetic pigments, chlorophyll a, b and carotenoids (Table 1 and 2). A similar decrease in the pigment level can be observed in barley (*Hordeum vulgare* L.) leaves after treatment with herbicide clomazone (Kaňa et al. 2009). Carotenoids (carotenes and xanthophylls) protect the photosynthetic apparatus by quenching triplet chlorophyll, singlet oxygen and other free radicals (Oelmüller 1989). This means that the deficiency of absorbed light in the leaf of PORGANIC™ treated wild pea and the decrease of the chlorophylls could be a result of a carotenoid deficiency-induced photooxidation of the chlorophylls. Moreover, carotenoid pigmemts are important for the photosystem (PS) assembly and the stability of light harvesting complex proteins as well as thylakoid membrane stabilization (Niyogi et al. 2001). Consequently, these phenomena lead to lower the formation of pigment–protein complexes in thylakoid membranes of chloroplasts and negative effect on photosynthesis. Increasing levels of leaf electrolyte leakage were depended on duration time after treatment (Fig. 1 and 2). This was evident that barnyardgrass and wild pea leaves treated with PORGANIC™ enhanced solute leakage and resulted in a loss of membrane stability which negatively affected biochemical functions and cell viability (Jaspers and Kangasjärvi 2010). Kim et al. (2004) have shown similar in a loss of membrane stability of corn leaf after herbicide fluridone treatment. Parallel to electrolyte leakage, the amount of MDA increased in response PORGANIC™compared to control (Fig. 3 and 4). Membranes, lipid physical structures, are composed of phospholipid bilayer in which protein is embedded, but these are disrupted when lipids oxidize and lose structural organization. Membranes become leaky or stiffen, either way losing functionality when component phospholipids oxidize in living tissues (Campos et al., 2003). This phenomenon might indicate that barnyardgrass and wild pea may experience oxidative damage in leaves because of increasing of activated oxygen species (ROS). Activated oxygen species such as $O^-_2$ or $H_2O_2$ and their interaction product, hydroxyl radical (OH), react with and degrade proteins, lipids and nucleic acids (Arora et al. 2002). Similar reports have also been published in mung bean (*Phaseolus aureus*) in response to 2-Benzoxazolinone (BOA) treatment (Batish et al. 2006) and in pea (*Pisum sativum* L., cv. sugar snap boys) after treatment with imazethapyr, an imidazolinone herbicide (Zabalza et al. 2007).



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## CONCLUSIONS

The present study showed that PORGANIC™ foliar application displayed strong phytotoxic activity against barnyardgrass and wild pea. The herbicidal actions of PORGANIC™ revealed that the electrolyte leakage increased as increasing the concentrations of PORGANIC™ in both test weeds indicating membrane disruption and loss of integrity. PORGANIC™ decreased chlorophyll a, chlorophyll b and carotenoid content when compared with the control indicating the deficiency of absorbed light in the PORGANIC™ treated leaf and interference of photosynthetic process. Moreover, treated leaves exhibited an increase in MDA content, suggesting lipid peroxidation.

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# ALLELOPATHIC POTENTIAL OF *TAGETES ERECTA* LINN; OPTIMAL EXTRACTION SOLVENT AND ITS PARTIAL SEPARATION OF ACTIVE COMPOUNDS

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**ABSTRACT** Recently, the allelopathy has been introduced as a viable option for alternative weed management under sustainable agriculture. Marigold (*Tagetes erecta* L.) plant growing around the experiment field at the King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand was used for all experiments. Allelopathic effects of stem, leaf, flower and root aqueous extract of *T. erecta* were assayed on seed germination and seedling growth of bioassay plants. The results showed that the degrees of growth inhibition can be classified in order of decreasing inhibition as leaf > root > flower > stem extract. To study the optimization of solvent extraction of crude extract from *T. erecta* leaf, 5 different solvent mixtures of ethanol containing of water (100, 75, 50, 25 and 0%) were tested. The quantity of crude extracted materials was found to bee dependent on solvent proportion. The recovery of crude extraction increased with increase in the water concntrations. A mixture of ethanol with water at 75:25 (v/v) was the most effective and gave the greatest quantity of crude extract from *T. erecta*. In the second phase, extraction residue of each solvent mixture was repeated using the same ethnaol containing different volumes of water for 3 times. The result showed that recovery of crude extract was 45-60% in the first number of extraction. These results indicated that extraction of crude extract from *T. erecta* with ethanol:water mixtures must be repeated at least two times of extraction. Crude ethanol:water (75:25 (v/v)) was separted by acid-base solvent partitioning into acidic fraction (AE), neutral fraction (NE), hydrolyze fraction and aqueous fraction (AQ). AE fraction showed the greatest inhibitory effect on bioassay plant, followed by crude ethanol, NE fraction and AQ fraction, respectively.

***Keywords:*** *Tagetes erecta*, Marigold, allelopathy, solvent separation

## INTRODUCTION

Marigold is in the family Asteracaea Genus Tagetes, a widespread ornamental plant and is commonly known as marigold which bears bright yellow and orange flowers. It is available in many parts of the world. It is annual aromatic plant widely grown in Thailand and South East Asia for ornamental plant. The species *Tagetes erecta* (Rhama and Madhavan 2011), *T. minuta* (Tereschuk et al. 1997), *T. patula* (Szarka et al. 2010) are most common, other species referred to are often specific to a region *T. lucida* (Capunzo et al. 2003), *T. mendocina* (Lima et al. 2009). This plant exhibits nematocidal, fungicidal, and insecticidal activity (Vasudevan et al. 1997; Xu et al. 2011). It has been used as cover crop to protect plant-parasite nematodes, inhibition of growth of microorganism and pesticide an attractive crop to natural enemies for long times (Silveira et al. 2009; Hooks et al. 2010). Allelopathy is a plant-plant or plant-microorganism biochemical interaction (Rice 1984). Allelopathic compounds have received considerable attention as potential sources of novel natural herbicides. *Tagetes* species had been vastly reported. Aqueous extracts from *T. minuta* significantly inhibited seed germination and seedling growth of *Lotus corniculata* var. japonicus and *Lactuca sativa* (Kil et al. 2002). Aqueous extract and essential oil of *T. minuta* inhibited the induction of callus and growth of *Oryza sativa*, *Brassica campestris*, *Raphanus sativus* and *Sesamum indicum* (Lee et al. 2002). *T. minuta* leaf powder at 1 and 2 t $ha^{-1}$ significantly reduced weed emergence and dry weight in



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rice field, and also significantly increased rice yield (Batish et al. 2007). It not only possesses excellent pesticidal properties but also has strong antioxidant properties (Maity et al. 2011).

This research was studied on allelopathic and herbicidal potential of *T. erecta* on seed germination and seedling growth of wild pea (*Phaseolus lathyroides* L.).

## MATERIALS AND METHODS

### Plant materials

Whole plants (leaves, stem, flower and root) of *Tagetes erecta* L. plants growing at the experimental field at the King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand were collected at 45 days after planting. The plants were cleaned from soil immediately with running tap water, dried-up in a hot-air oven at 45°C for 3 days and ground to powder (100 mesh) in an electrical blender. Seed of wild pea (*Phaseolus lathyroides* L.) collected from paddy field in the Ladkrabang district, Thailand. Hard seed coats of *P. lathyroides* were scrubbed with No.0 sandpaper to break their dormancy. These species were selected for the experiment due to big seeds and tolerate allelochemicals.

### Aqueous extracts bioassay

Aqueous extracts were prepared from leaf, stem, flower and root materials from each *T. erecta* L. by dissolving 10 grams of each powered material in 100 mL of distilled water at 8°C for 72 h, followed by filtration though three layer of cheesecloth to remove any debris. The supernatant was then filtered through Whatman No.1 filter paper (Whatman Inc. Clifton, NI, USA) to a concentration of 100 mg/mL of dried plant material and stored in a refriferator at 4°C until bioassay. Dilutions of each *T. erecta* L. extract of 12.5, 25, 50 and 100 mg/mL were prepard in distilled water. Five milliliters was added in each 9 cm diameter Petri dish, lined with filter paper and twenty healthy seeds of *P. lathyroides* were placed in each Petri dish. Four replicates were maintained per treatment in a completely randomized manner in a growth chamber with a temperature of 25–32°C, a 12/12 hour dark/light photoperiod, with light intensity (cool White 840) of 100 µmol $m^{-2}$ $s^{-1}$ and relative humidity of around 80%. Treatment with distilled water was used as the control. Germination was deemed to have occurred only after the radicle had protruded beyond the seed coat by at least the dimension of the seed at seven days after treatment. Seedling growth was measured as the root and shoot lengths at seven days after treatment.

### Effect of solvent extraction on the crude extracts yield and bioassay

Ten grams of 100 mesh of *T. erecta* L. leaf power were extracted (ratio 20g: 200 mL) with a different solvent system at room temperature for 48 hours. Solvent systems used were absolute ethanol containing different volumes of distilled water (75%, 50%, 25% and 0%) and distilled water. After 48 hours of extraction, the brown supernatants were filtered through three layers of cheeseclothes and re-filtered through Whatman No.1 filter paper. Following filtration, the brown supernaytants were dried by evaporation of the solvent using a rotary evaporator (BUCHI Rotavapor R255), BUCHI, Lausanne, Switzerland) under a partial vacuum at 45°C until constant crude extract weight was reached. After that each residue was re-extracted 3 times with the same extraction solvent as the same condotion of the first extraction procedure, and then crude extract number 1, 2 and 3 were combined. Stock solution of each crude extract generated by ethanol in water at 100%, 75%, 50%, 25% and 0% (v/v) was prepared by dissolvoing each sticky crude extract with acetone in a motar jar and wettable power (bentonite:anionic surfactant; 95:5 (w/v)), was added in to motar jar in a 3:7 ratio (crude fraction: wettable power). The mixture was slowly pulverized until completely dried; acetone was added three times and kept in the dark at a low temperature until used. Each concentration of crude extracts (100%, 75%, 50%, 25% and 0% ethnaol in water) was performed in wettable power by dissolved in distilled water to contain four concentrations ranging from 1250 to 10000 ppm. Bioassays on seed germination and seedling growth were tested as previously described.



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**Solvent partitioning of active compounds and bioassay**

The crude extract was prepared from *T. erecta* L. leaf power by extraction with 75% ethanol in distilled water. After filtration using Whatman No.1 filter paper, the residue was repeatedly extracted 3 times with 75% ethanol for 72 hours at 25 °C and filtered. The filtrates were combined and evaporation in the rotary evaporator at 45°C, leaving a sticky residue (original crude fraction; OR fraction). This residue was then diluted with 500 mL of distilled water and stirred vigorously on a magnetic stirrer at 45°C for 20 min, resulting in an aqueous solution which was acidified to pH 3 by 6N HCl. The filtrate was extracted with ethyl acetate three times (0.5 L x 3). After adjusting the pH to neutral, the aqueous phase was dried up by reducing presure at 45°C, and an aqueous residue was obtained (AQ fraction). The ethyl acetate solutions were combined and then treated with anhydrous magnesium sulfate. This solution were divied to 2 parts, the first was evaporated to dryness obtained EtOAc fraction, another was concentrated to about 0.5 L., and then extracted three times with saturated aqueous $NaHCO_3$ (0.5 L x 3). The ethyl acetate phase was dried over with anhydrous magnesium sulfate and concentrated by reducing pressure, and an ethyl acetate-soluble neutral fraction was obtained (NE fraction). The combined sodium bicarbonate phase was evaporated to about 1 L, adjusted to pH 7 by 6 HCl, and then extracted with ethyl acetate (0.5 L x 3). The ethyl acetate solutions were combined, dried over $MgSO_4$, and then evaporated to obtain the ethyl acetate-soluble acidic fraction (AE fraction), and the remains of the aqueous phase were discarded. The inhibitory activities from each fractions (OR, AQ, NE and AE fraction), were prepared as same as previously described (Fig. 1). Each fraction of OR, AQ, NE and AE fraction was prepared to contain four concentrations from 2000 to 16000 ppm. Bioassays on seed germination and seedling growth were tested as previously described.

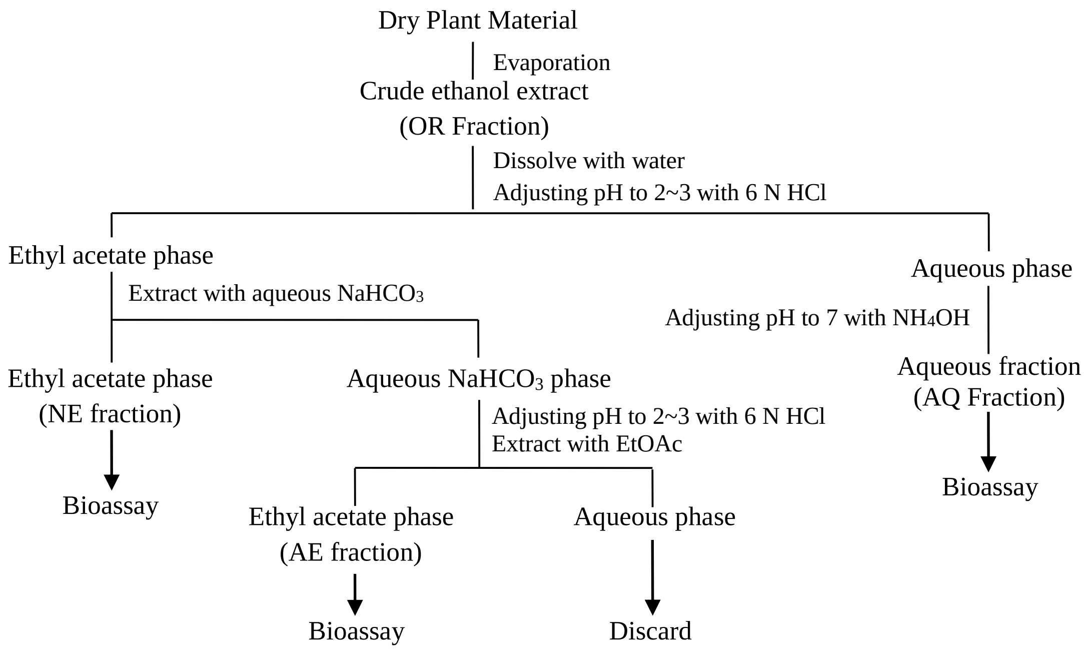


**Fig. 1.** Flow chart for acid-base solvent partitioning from marigold (Laosinwattana et al. 2007)

**Statistical analysis**

Data are analyzed using analysis of variant (ANOVA). Whenever ANOVA indicated significant effects ($p < 0.05$), a pairwise comparison of means by Tukey's studentized range test is carried.



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## RESULTS AND DISCUSSION

### Aqueous extracts bioassay

Inhibition effects of stem, leaf, flower and root aqueous extracts of *T. erecta* were assayed at concentrations of 12.5, 25, 50 and 100 mg/mL for their effect on seed germination and seedling growth of *P. lathyroides* compared with that of distilled water control. The results indicated that degree of inhibitory was significantly different depending on difference concentration and plant parts extract being tested. The degree of inhibition increased with increasing concentration. Among plant parts, leaf aqueous extracts was the most inhibition effect on germination, shoot length and root length of *P. lathyroides*. The leaf showed the greatest inhibitory effect on bioassay plant, followed by root, flower, and stem extract, respectively (Fig. 2). It was similar results to Laosinwattana et al. (2009), who reported that leaf and branches aqueous extracts of *Aglaia odorata* inhibitory effects of barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) and wild pea (*Phaseolus lathyroides* L.) Lin et al. (2006), who reported that the inhibitory effects of Saururaceae (*Houttuynia cordata* Thunb.) varied with the weed species.

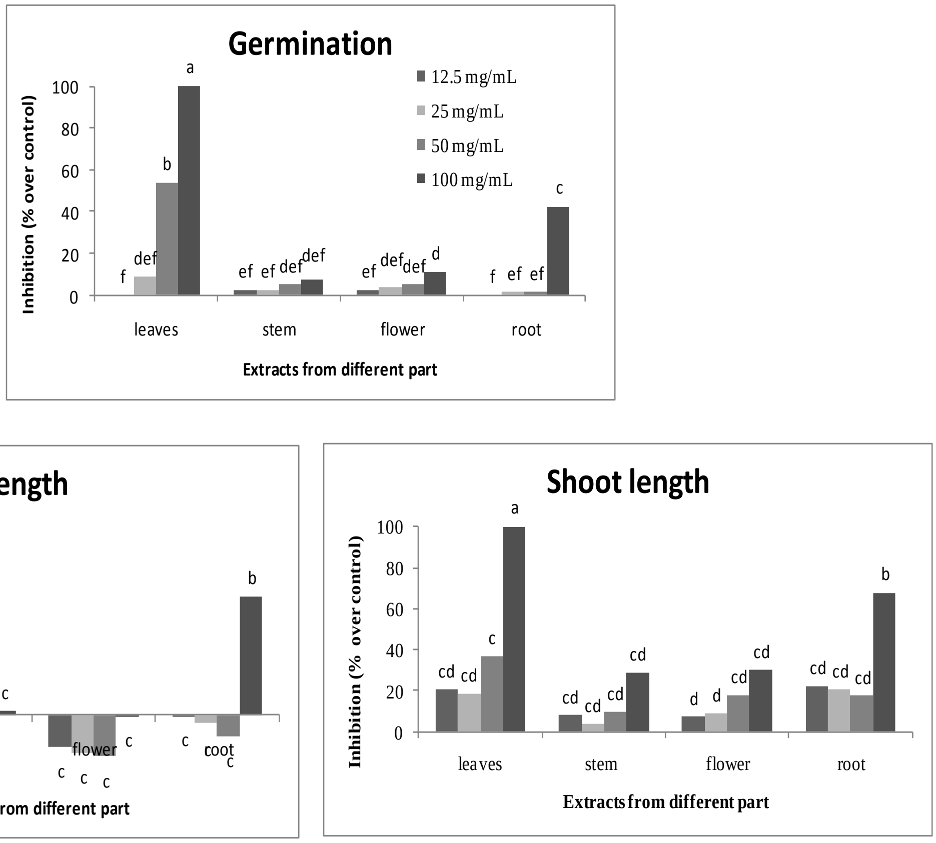


**Fig. 2.** Effects of aqueous extracts of from different *Tagetes erecta* L. plant parts on germination of *P. lathyroides* seeds at 7 days after treatment. Means followed by the same letter(s) are not significantly different by Tukey's (p=0.05)

### Effect of solvent extraction on the crude extracts yield.

The results in Fig. 3 show that the dried leaf of *T. erecta* L. extracted by various solvent systems showed different inhibitory influence on *P. lathyroides* seed germination and seedling growth in various ways. The degree of inhibitory was different depending on source of crude extract and concentration being tested. At 10000 ppm concentration, *P. lathyroides* seed germination was reduced by 7%, 12%, 5%, 5%, and 5% over control (distilled water) when treated with crude extract obtained from 100%, 75%, 50%, 25% and 0% ethanol in water, respectively. Thus, it can be seen that extracts prepared by different solvents carried varying degrees of inhibitory activities. These results indicate that selective extraction from natural sources by appropriate solvent systems is important for obtaining fractions with high allelopathic potential



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and high crude extraction yield. It was significant difference results to Poonpaiboonpipat et al. (2011), who reported that 50% ethanol in water demonstrated the highest potential extraction activity produced by *Jasminum sambac*. This finding is supported by Li et al. (2006); Luthria et al. (2007); Garcia et al. (2010), who reported that different solvent systems have been used for the extraction of secondary metabolites from plant materials because their extraction efficacy depends on their chemical nature.

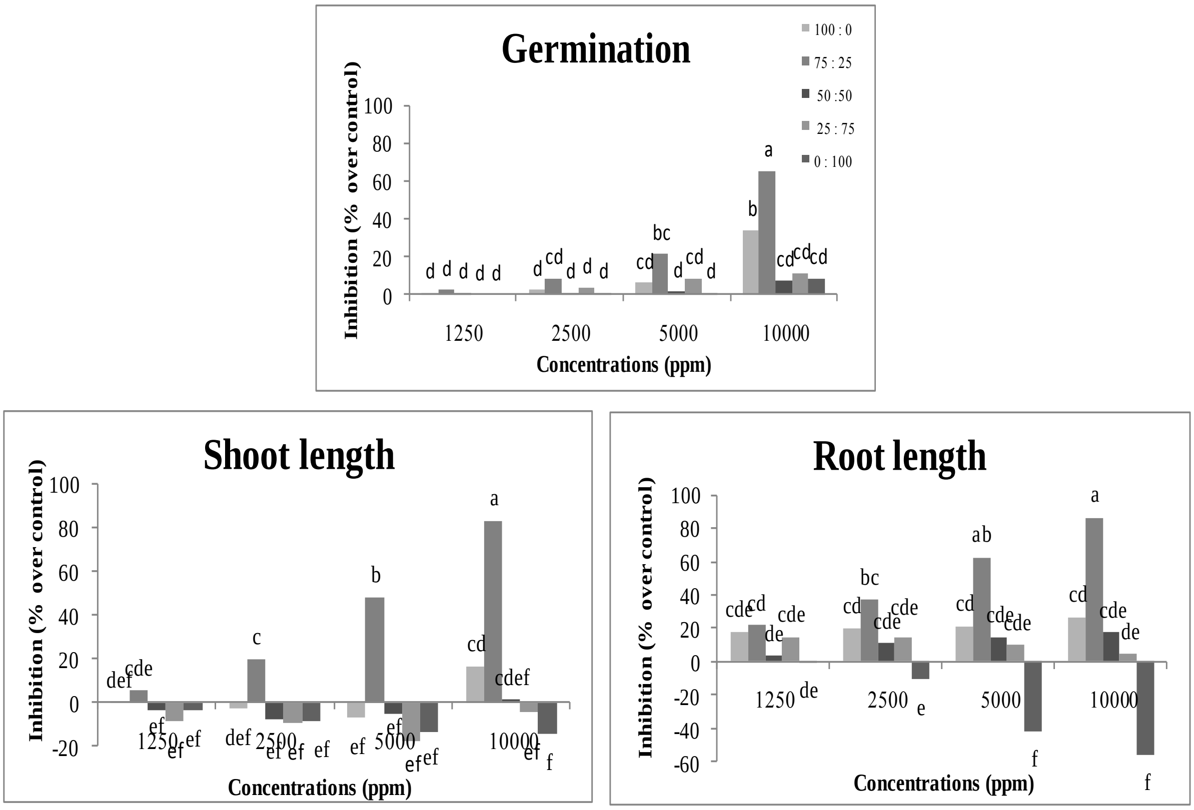


**Fig. 3.** The inhibitory effect of crude extraction obtained by different ethanol percentages in water from from *T. erecta* on germination, shoot length and root length of *P. lathyroides* seeds at 7 days after treatment. Means followed by the same letter(s) are not significantly different by Tukey's (p=0.05)

**Solvent partitioning of active compounds**

An aqueous-ethanol crude extract fraction (OR) from *T. erecta* was separated into three fractions: the aqueous (AQ) fraction, neutral compound (NE) fraction, and acidic compound (AE) fraction. The growth inhibitory activities of AQ, NE, AE fractions and OR at the concentration of 2000 to 16000 ppm were evaluated on seed germination and seedling growth of *P. lathyroides*. The results showed that (Fig. 4) OR fraction showed strong activity, giving 88.75%, 100% and 100% inhibition of *P. lathyroides* germination, shoot length and root length, respectively at the concentration of 16000 ppm. After solvent partitioning, at this step, relative inhibition increased, as compared to the OR fraction. The AE fraction showed the greatest activity fraction with complete inhibition of germination, shoot length and root length of *P. lathyroides* at the concentration of 16000 ppm. The NE and AQ fractions showed a weaker inhibition effect on *P. lathyroides* germination shoot length and root length when compared with the OR fraction. These results indicated that most of phytotoxic compounds produced by *T. erecta* could be presented in AE fraction. It was similar results to Poonpaiboonpipat et al. (2011), who reported that AE fraction was the most allelochemical compounds produced by *Jasminum sambac* and Teerarak et al. (2010), reported that a secoiridoid glucoside named oleuropine which identified as an allelopathic compound from AE fraction of a related *Jasminum officinale* var. *grandiforum.* The importance of allelochemicals mixtures is



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recognized both in herbicide research and exploring plant allelochemicals (Inderjit *et al*., 2002). It is suggested that the mixture compound in AE fractions gave significantly inhibited the tested weed species.

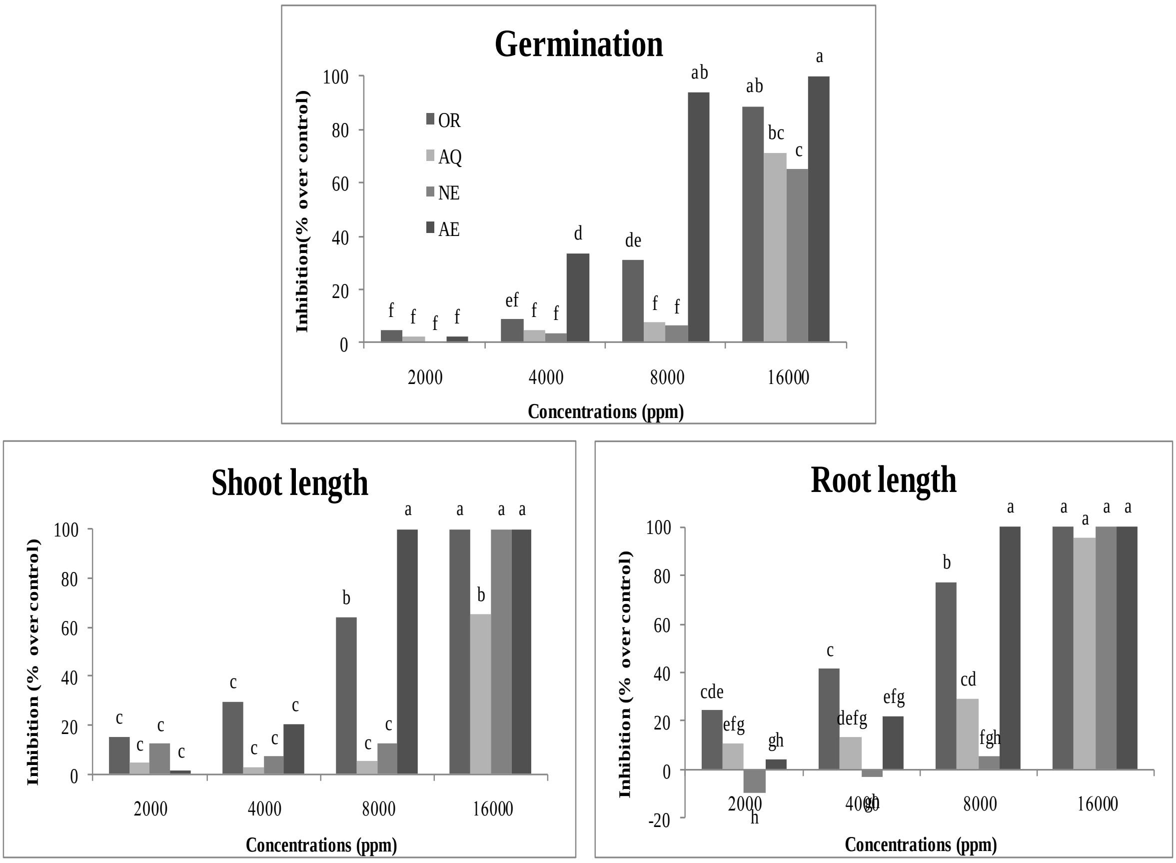


**Fig. 4.** Effect of aqueous-ethanol (OR), aqueous (AQ), neutral (NE) and acidic (AE) fractions at the concentrations of 2,000 to 16,000 ppm on germination, shoot length and root length of *P. lathyroides* seed.

## CONCLUSIONS

Allelopathic effects of stem, leaf, flower, and root aqueous extract of *T. erecta* were assayed at concentrations of 12.5, 25, 50 and 100 mg/mL for their effects on seed germination and seedling growth of *P. lathyroides*. Aqueous extracts from leaf extract had a greater inhibitory effect on seed germination and seedling growth of *P. lathyroides* more than root, flower, and stem extract, respectively. The crude ethanol: water (75:25 (v/v)) fraction (OR) was separated by solvent partitioning into aqueous fraction (AQ), neutral compound fraction (NE), and acidic compound fraction (AE). AE fraction showed the greatest inhibitory effect on P. lathyroides, followed by NE fraction, AQ fraction and OR fraction, respectively.

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# INFLUENCE OF RICE RESIDUE MANAGEMENT PRACTICES AND HERBICIDES ON WEED GROWTH AND YIELD IN WHEAT (*TRITICUM AESTIVUM*)

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**ABSTRACT** In Eastern Indo Gangetic Plains (EIGPs) of India, rice-wheat cropping system is an important yearly crop sequence. Traditionally, Farmers remove entire rice residue before wheat sowing leaving enough intra - row space for weeds to grow and compete with wheat crop. The present study was conducted for two years to assess the effect of three rice residue management practices- residue retention up to 30cm., residue incorporation before sowing and residue mulch at 4.0 ton per ha. in combination with Post Emergence herbicide treatments on weed dynamics and growth and yield in wheat. In both the years of study, *Chenopodium album* L., *Rumex denticulate* L., *Melilotus alba* L., *Anagallis arvensis* L., *Phalaris minor* Retz.and *Cyperus rotundus* L. were major weeds in wheat. The density and dry weight of weeds was significantly less in rice residue mulch than rice residue retention and incorporation treatments. Herbicide mixture of sulfosulfuron + metsulfuron-methyl (25g + 4.0g a.i./ha.) was most effective in reducing weed growth. Wheat yield attributing characters and yield were maximum in combination of rice residue mulch with sulfosulfuron + metsulfuron-methyl and minimum in unweeded control without mulch.

***Keywords***: Rice residue, herbicides, weed suppression, wheat yield

## INTRODUCTION

Among various wheat based cropping system, rice-wheat is major one, occupying about 10.0 million hectare in India and worldwide this system occupies about 24.0 million hectare area (Ladha *et al*., 2000: Timsina and Conor, 2001). Weeds are a major problem in the productivity of rice based cropping systems .In India, narrow leaf weeds viz. canary grass ( *P. minor )*and broad leaved weed sour dock (*R. denticulate)* are of major concern in irrigated wheat under rice-wheat cropping system (Balyan and Malik, 2000).

In the system, residue management problem has been accentuated by combine harvesting of crops, which leaves stubble of about 30-40 cm in height and spread most of the straw in the field. Most farmers burn or incorporate the residue before sowing of wheat crop. Although ploughing in of residues has been shown to increase herbicide efficiency ( Rules, 1991) the long term buildup of crop residue can reduce and also ash reduces herbicide efficiency (Bowerman, 1995). Crop residues retained on the surface has multi benefits like moisture conservation, suppress weed seed germination and or seedling growth and of complement the effects herbicide in succeeding crop (Rodder *et al.*1998, Kimsoon *et al.* 1994, Dastgheib 2006).

The mulch are stable from previous crop present at the time of application of pre emergence herbicides not only intercept herbicides but act as physical barrier to prevent herbicide contact to weed seed (Banks and Robinson, 1982). Thus, post emergence treatments accord better weed control efficiency when crop residues are present on surface. Alone application of herbicides either control narrow leaf or broad leaf weeds, while herbicide mixtures, may besides, providing broad spectrum weed control also be one of the options for management or delay of cross resistance development against herbicides (Yadav and Malik, 2005; Dhawan *et al.*, 2009). Keeping in the view the above facts, an investigation was planned to study on the response of herbicides under rice residue management practices in wheat.



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## MATERIALS AND METHODS

The present study was conducted during winter season of 2009-10 and 2010-11 at the Agricultural Research Farm, Institute of Agricultural Sciences of Banaras Hindu University, Varanasi, India situated at $25.2^{0\prime}$ N latitude, $83.0^{0\prime}$ E longitude and 75.7 m above Mean sea level. The soil was sandy clay loam in texture (etnisol) having pH 7.8, 0.43% organic carbon, 205.14 kg $ha^{-1}$ available N., 17.46 kg $ha^{-}$1available P and 237.34 kg $ha^{-1}$ available K.

The treatment comprised of three rice residue management practices viz. Rice residue retained to a height of 30cm ($R_0$), Rice residue mulches @ 4t/ha in crop rows just after sowing($R_{1)}$ and Rice residue incorporation before sowing in main plots and five weed control treatments, viz. $W_0$- weedy check, $W_1$- weed free, $W_2$- sulfosulfuron 25 g/ha alone, $W_3$- sulfosulfuron 25 g/ha + metsulfuron 4 g/ha and $W_4$- isoproturon 1.0 kg/ha + metsulfuron 4 g/ha in sub plots. The experiment was laid out in spilt plot design (SPD) with four replications. Existing weed species were destroyed by application of glyphosate at 2 kg $ha^{-1}$ (3 days before sowing) in all the residue treatments. Wheat cultivar HUW 234 was sown with the help of zero-till seed drill on December 2&3 during respective years. Crop received a uniform nutrients dose of 120-60-60 kg N-P-K Kg $ha^{-1}$. One–third nitrogen and full dose of phosphorus and potassium was applied at sowing and remaining two-thirds was applied in two equal splits at crown root initiation and tillering stages**.** Irrigation was given at critical stages as per need of the crop. Herbicides were applied as post emergence at 32 days after sowing (DAS) with the help of hand operated knapsack sprayer, fitted with flat fan nozzle with water as carrier at 600 liters $ha^{-1}$. Weed count was recorded randomly from two places in each plot using 50cmx50cm quadrate. Weeds were cut at ground level, washed with tap water, sun dried and thereafter oven dried at $65^oC$ for 48 hrs and dry weight was recorded in g $m^{-2}$ at 60DAS.Weed indices such as weed control efficiency and weed index were calculated using weed dry weight recorded at 60 days after sowing. Grain yield and its attributes were also recorded during the course of investigation. The data recorded on weeds were subjected to square root transformation ($\sqrt{}x+0.5$).

## RESULTS AND DISCUSSION

### Effect on weed

Crop was infested with broad leaved weeds viz. common lambsquarters (*Chenopodium album* L.) sour dock (*Rumex denticulate* L.), white swwet clover (*Melilotus alba),* Scarlet pimpernel *(Anagallis arvensis* L.*)* and narrow leafweeds like canary grass (*P. minor* Retz) and purple nut sedge( *Cyperus rotundus* L. ) during the present study. Relative composition weeds flora revealed that average weed population of broad leaved species was more in comparison to narrow leaved weed species during both years. Relatively more number of broad leaved weeds has also been observed under zero- till system (Chhokar *et al.* 2007).

Rice residue mulch practices significantly reduced weed density of weeds in comparison to rice residue retained and it was at par with rice residue incorporated (Table 1). Among the varying rice residue management, rice residue mulch had the maximum weed control efficiency followed by rice residue incorporated. Placement of rice residues on the soil surface creates improper growing conditions which do not allow the weed seeds to germinate and as a result reduced dry weight of weeds is observed under residue placement treatments (Rahman *et al.,* 2005).

Herbicidal treatments caused significant suppression of weed growth compared with untreated crop. Tank mix application of sulfosulfuron + metsulfuron at 25 + 4 g/ha.was most effective in reducing density of important weed species infesting the crop and also total weed density. It was at par with isoproturon + metsulfuron at 1.0 Kg+4 g/ha and sulfosulfuron alone at 25 g/ha.

Significant reduction in dry matter accumulation by weeds was observed due to control of important weed species in the crop. In reducing N, P, and K depletion by weeds, rice residue mulches and rice residue incorporation treatments recorded significantly lower depletion than rice residue retained. Higher N, P, and K uptake by weeds in rice residue retained treatment



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might be due to more weed dry matter accumulation in this treatment. Herbicidal treatments recordedsignificantly less nutrient depletion by weeds than unweeded (control) treatment. The effect was more pronounced in sulfosulfuron 25 g/ha + metsulfuron 4 g/ha treated crop as compared to isoproturon 1.0 kg/ha + metsulfuron 4 g/ha and sulfosulfuron 25 g/ha treatments (Table 2). HigherDry matter in weeds accumulate more nutrient content and leads to more nutrients depletion by weeds (Panday *et al*. 2000 ).

**Table 1.** Density of weed species as influenced by rice residue and weed management treatments at 45 DAS (2 years pooled data).

| Treatments | Rate ($g/m^2$ ) | Weed density (No./ $m^2$) | | | | |
|---|---|---|---|---|---|---|
| | | *P.minor* | *C.album* | *R.denticulate* | *C. rotundus* | Total |
| Residue management | | | | | | |
| $R_0$ - Rice Residue Retained | | 6.49 (41.7) | 6.22 (38.2) | 6.1 (36.77) | 6.12 (37.05) | 17.93 (300.62) |
| $R_1$ -Rice Residue Mulches | | 5.81 (33.35) | 5.56 (30.45) | 5.53 (30.15) | 5.59 (30.82) | 15.55 (242.30) |
| $R_2$ -Rice Residue Incorporated | | 5.89 (34.30) | 5.73 (32.37) | 5.69 (31.97) | 5.7 (32.00) | 15.93 (154.55) |
| CD ($p$=0.05) | | 0.46 | 0.29 | 0.31 | 0.31 | 11.32 |
| Herbicides | | | | | | |
| $W_0$ - Weedy Check | | 7.09 (49.75) | 7.4 (54.25) | 6.78 (44.5) | 6.69 (44.29) | 19.16 (367.87) |
| $W_1$ - Weed free | | 0.71 (0.00) | 0.71 (0.00) | 0.71 (0.00) | 0.71 (0.00) | 0.71 (0.00) |
| $W_2$ - Sulfosulfuron | 25 | 6.75 (45.170 | 6.41 (40.66) | 6.44 (41.05) | 6.48 (41.54) | 18.16 (330.92) |
| $W_3$ - Sulfosulfuron + metsulfuron | 25+4 | 6.57 (42.67) | 5.99 (35.5) | 6.26 (38.75) | 6.32 (39.54) | 17.53 (308.29) |
| $W_4$ - Isoproturon + metsulfuron | 1000+4 | 6.72 (44.67) | 6.2 (37.95) | 6.32 (39.54) | 6.45 (41.08) | 17.92 (322.04) |
| CD (P=0.05) | | 0.23 | 0.22 | 0.21 | 0.20 | 9.32 |

**Table 2.** Weeds dry weight and nutrient depletion by weeds as influenced by rice residue and weed management treatments (2 years pooled data).

| Treatments | Rate (g/ha) | Weeds dry weight ($g/m^2$) | Nutrient depletion (kg/ha) | | |
|---|---|---|---|---|---|
| | | | Nitrogen | Phosphorus | Potassium |
| Residue management | | | | | |
| $R_0$ - Rice Residue Retained | | 4.39 (20.06) | 2.34 (5.00) | 1.42 (1.52) | 2.67 (6.63) |
| $R_1$ -Rice Residue Mulches | | 4.34 (18.29) | 2.04 (3.68) | 1.22 (0.99) | 2.44 (5.44) |
| $R_2$ -Rice Residue Incorporated | | 4.38 (18.75) | 2.23 (4.46) | 1.31 (1.22) | 2.48 (5.65) |
| CD (P=0.05) | | NS | 0.10 | 0.07 | 0.12 |



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| Herbicides | | | | | |
|---|---|---|---|---|---|
| $W_0$ - Weedy Check | | 6.85 (46.52) | 4.03 (15.79) | 2.33 (4.97) | 4.92 (23.63) |
| $W_1$ - Weed free | | 0.71 (0.00) | 0.71 (0.00) | 0.71 (0.00) | 0.71 (0.00) |
| $W_2$ - Sulfosulfuron | 25 | 4.19 (17.10) | 1.66 (2.25) | 1.00 (0.50) | 1.65 (2.25) |
| $W_3$ - Sulfosulfuron + metsulfuron | 25+4 | 3.91 (14.88) | 1.52 (1.83) | 0.92 (0.35) | 1.49 (1.70) |
| $W_4$ - Isoproturon + metsulfuron | 1000+4 | 4.16 (16.84) | 1.59 (2.04) | 0.94 (0.38) | 1.56 (1.93) |
| CD (P=0.05) | | 0.18 | 0.095 | 0.06 | 0.09 |

**Effect on crop**

The efficiency of herbicide treatments in enhancing grain yield of wheat was significantly higher in rice residue mulch treatment than rice residue incorporated and rice residue retained treatments. Combination of sulfosulfuron 25 g/ha + metsulfuron 4 g/ha recorded the highest grain yield which was significantly superior to isoproturon 1000 g/ha + metsulfuron 4.0 g/ha and alone application of sulfosulfuron 25 g/ha, irrespective of residue management treatments (Table 3).

Higher grain yield with the application of herbicides under rice residue mulch could be ascribed to reduction in weed intensity which ultimately helped the crop to utilize nutrients, moisture, light and space more efficiently and hence increased the grain yield The presence of rice residue might have physically impede seedling growth or inhibits germination and growth of weeds by allelopathy, thus improving overall performance of applied herbicide treatments compared with residue retention and incorporation treatments (Crutchfield *et al*. 1986 ).

**Table 3**. Crop yield and nutrients uptake as influenced by rice residue and weed managementtreatments at 45 DAS (2 years pooled data).

| Treatments | Rate ($g/m^2$ ) | Yield (kg/ha) | | Nutrient uptake (kg/ha) | | |
|---|---|---|---|---|---|---|
| | | Grain | Straw | Nitrogen | Phosphorus | Potassium |
| Residue management | | | | | | |
| $R_0$ - Rice Residue Retained | | 3732.5 | 5713.8 | 43.77 | 9.91 | 41.37 |
| $R_1$ -Rice Residue Mulches | | 4457.2 | 6486.5 | 56.51 | 12.20 | 49.17 |
| $R_2$ -Rice Residue Incorporated | | 4012.7 | 6040.5 | 48.60 | 10.62 | 44.50 |
| CD (P=0.05) | | 248.9 | 4936.2 | 4.85 | 0.99 | 3.16 |
| Herbicides | | | | | | |
| $W_0$ - Weedy Check | | 2939.1 | 6669.6 | 31.84 | 7.38 | 31.18 |
| $W_1$ - Weed free | | 4668.8 | 6003.0 | 60.38 | 12.98 | 53.93 |
| $W_2$ - Sulfosulfuron | 25 | 4023.7 | 6446.2 | 48.455 | 10.55 | 42.74 |
| $W_3$ - Sulfosulfuron + metsulfuron | 25+4 | 4410.8 | 6346.2 | 54.78 | 12.05 | 49.33 |
| $W_4$ - Isoproturon + metsulfuron | 1000+4 | 4295.0 | 6669.6 | 52.68 | 11.57 | 47.90 |
| CD (P=0.05) | | 144.1 | 180.6 | 3.61 | 0.64 | 1.94 |



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Nutrients (N, P &K ) uptake by crop was maximum in rice residue mulch treatment and minimum in rice residue retained treatment. Relatively weed free condition under rice residue mulch favoured crop growth and led to better development of agronomic characters and finally crop yield and nutrient uptake by crop. Similarly, sulfosulfuron + metsulfuron at 25 + 4 g/ha recorded higher uptake of N, P and K by grain and straw and minimum uptake was recorded under weedy treatment. Singh *et al.* (2009) reported that reduction in nutrient depletion by weeds due to herbicide application led to higher nutrients uptake by crop in herbicides treated crop.

It is concluded that for productivity of wheat, higher nutrient uptake in crop and weed, use of rice residue mulches and control weed density and weed dry matter through application of sulfosulfuron 25 g/ha + metsulfuron methyl 4 g/ha.

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# ISOLATION AND IDENTIFICATION OF ALLELOPATHIC SUBSTANCES FROM THE LITTER OF JAPANESE RED PINE

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**ABSTRACT** The vegetation under Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) forests is sparse although sunlight intensity under the forests is sufficient for herbaceous plants to grow. The sparse vegetation of the red pine forest floors indicates that the red pine has strong allelopathic effect. Allelopathic substances are probably released by either leaching from needles or degradation of the litter. Several putative allelopathic substances have been isolated from red pine needles. However, the information of allelopathic effects of the litter of pine forest floors is limited. In this study, the allelopathic potential of red pine litter was determined and putative allelopathic substance was isolated and characterized. Aqueous methanol extracts of litter inhibited the growth of roots and shoots of cress, lettuce, alfalfa, timothy, crabgrass, barnyard grass and ryegrass, and increasing the extract concentration increased the inhibition, suggesting that the red pine litter may contain growth inhibitory substances. The extract of the litter was purified, and a growth inhibitory substance was isolated by silica gel column, Sephadex LH-20 column, $C_{18}$ cartridge and HPLC. The growth inhibitory substance was then characterized by $^{1}$H-NMR and $^{13}$C-NMR as abscisic acid-β-D-glucopyranosyl ester. The concentrations required for 50 % growth inhibition on the roots and shoots of cress in the assay were 0.23 and 0.61 μM, respectively, and on those of barnyard grass were 1.1 and 2.8 μM, respectively. Abscisic acid-β-D-glucopyranosyl ester may contribute to the growth inhibitory effect of the pine and may play an important role in the allelopathy of red pine.

***Keywords*:** Abscisic acid-β-D-glucopyranosyl ester, allelopathy, litter, *pinus denshiflora*.

## INTRODUCTION

Allelopathy is the chemical interaction in local plant communities and affects plant development including plant growth and germination (Rice 1984). Allelopathic substances are present in all parts of the allelopathic plant species. During the decomposition of plant materials through physical, chemical and biological processes in the soil, some allelopathic substances are activated and released to the environment (Makino et al. 1996; McCalla and Haskins 1964). Therefore, it is considered that litter is important one of allelopathic sources in the environment.

Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) belonging to Pinaceae family and is widely distributed in Japan, Korea and China. The pine is one of the pioneer plants that can grow in relatively nutrients-poor soils (Nakaji et al. 2001). The vegetation under the red pine forests is sparse, although sunlight intensity under the red pine forests is sufficient for these herbaceous plants to grow (Rice 1984). The phenomenon could be due to the accumulation of allelopathic substances in forest floors leached from red pine and to the inhibition of the growth of neighboring plants. Allelopathic activity of pine extracts had already been determined and some allelopathic substances are isolated from the pine needles (Kato-Noguchi et al. 2009; 2011). Although allelopathy of pine forest floors litter is also very important to elucidate the reason for the formation of sparse forest floors under red pines, the information of allelopathy of red pine litter is limited. This study aimed to determine the allelopathic activity of red pine litter and to isolate and identify the allelopathic substances from litter.



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## MATERIAL AND METHODS

### Extraction

Litter of red pine (*Pinus densiflora* Sieb.et Zucc.) was randomly collected from the top layer of forest floors below 20 trees (young and old trees) in area of 10 × 10 $m^2$, from mountainous terrain in Takamatsu, Japan during June, 2011. The collected litter (45 g dry weight) were extracted with 300 mL of 80% (v/v) of aqueous methanol for 2 days. After filtration using filter paper (No. 2; Toyo Ltd., Tokyo), the residue was extract again with 300 mL of cold methanol for 2 days and filtered. The two filtrates were combined and evaporated with a rotary evaporator at 40°C to produce an aqueous residue.

### Bioassay

Dicotyledonous plants, cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.) and alfalfa (*Medicago sativa* L.), and monocotyledonous plants, timothy (*Phleum pretense* L.), crabgrass (*Digitaria sanguinalis* Scop.), barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) and ryegrass (*Lolium multiflorum* Lam.) were chosen for bioassay as test plant species

Sample for bioassay was evaporated to dryness and dissolved in methanol, added to the filter paper in 2.8 cm Petri dishes, and the methanol was evaporated. Then the filter paper was moistened with 0.6 mL of 0.05% (v/v) Tween 20. Ten seeds of cress, lettuce, alfalfa, or 10 germinated seeds of timothy, crabgrass, barnyard grass or ryegrass were arranged on the filter paper in Petri dishes. The monocotyledonous test plants were germinated in dark at 25°C for 48 h (timothy/barnyard grass), 72 h (ryegrass) and 120 h (crabgrass). The hypocotyl/coleoptile and root lengths of cress, lettuce and alfalfa, timothy, crabgrass, barnyard grass and ryegrass were measured at 48 h after incubation in dark at 25°C. Control seeds were also arranged on the filter paper moistened with the aqueous solution of Tween 20 without the extract.

### Isolation and identification of allelopathic substance

The aqueous residue was then adjusted to pH 7.0 with 1 M phosphate buffer, partitioned three times against an equal volume of ethyl acetate. The ethyl acetate fraction was evaporated to dryness and chromatographed on a column of silica gel, Sephadex LH-20, reverse phase $C_{18}$ cartridges and HPLC. In every step of purification, biological activity was detected by cress bioassay according to the aforementioned procedure. Finally, an active substance was characterized by $^{1}H$- and $^{13}C$-NMR and mass spectra.

## RESULTS AND DISCUSSION

Aqueous methanol extracts of red pine litter inhibited the hypocotyl/coleoptile and root growth of cress, lettuce, alfalfa, timothy, crabgrass, barnyard grass and ryegrass, and the inhibitory activity was concentration dependent. Comparing the extract concentrations required for 50 % inhibition ($I_{50}$), the hypocotyl and root growth of cress were most sensitive to extract, whereas those of crabgrass were least sensitive. These results suggest that the red pine litter may contain growth inhibitory substances.

A growth inhibitory substance in the litter extract was isolated and characterized by spectral data as abscisic acid-β-D-glucopyranosyl ester. $I_{50}$ values of abscisic acid-β-D-glucopyranosyl ester on hypocotyls and roots of cress were 0.61 and 0.23 μM, respectively, and those of barnyard grass were 2.8 and 1.1 μM, respectively. Abscisic acid-β-D-glucopyranosyl ester was isolated and characterized from Japanese red pine needles, and the endogenous concentration of this substance was 4.1-21.5 μmol /kg pine needles (Kato-Noguchi et al. 2011). The present result indicates that abscisic acid-β-D-glucopyranosyl ester remains in pine litter. Considering the presence of this ester in both needles and litter and its inhibitory activity, acid-β-D-glucopyranosyl ester may be considered to play an important role of allelopathy of Japanese red pine through the inhibition of the growth of neighbouring plant species. Even though most plant tissues contain potential allelochemicals, only those released to the environment can inhibit the germination and growth of neighbouring plant species and may act



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as allelochemicals in natural ecosystems (Bais et al. 2004; Putnam and Tang 1986). The natural products those are found in root exudates or soil have higher allelopathic potential than those simply identified in plant tissues (Neimeyer and Perez 1995; Perez and Ormeno-Nunez 1991).

The present results suggest that abscisic acid-β-D-glucopyranosyl ester may play an important role in red pine allelopathy since the active substance has the strong inhibitory activity and present in both red pine needles and litter. This allelopathic active substance may also be one of the factors responsible for the formation of sparse forest floors vegetation.

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# COMPARATIVE ALLELOPATHIC EFFECTS OF C*HROMOLAENA ODORATA* (L.) KING & ROBINSON AND *MIKANIA MICRANTHA* H.B.K. ON *AGERATUM CONYZOIDES*

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**ABSTRACT** The allelopathic effects of the aqueous leaf extract and leaf debris of *Chromolaena odorata* and *Mikania micrantha* incorporated into the soil were tested on *Ageratum conyzoides* in the laboratory and greenhouse. The experiments were conducted to determine the effects of the aqueous leaf extract on seed germination indices and seedling growth and fresh weight and the effects of leaf debris incorporated into the soil on the germination and seedling growth on *Ageratum conyzoides*. Three concentrations each of the aqueous leaf extract (12.5, 25.0 and 50.0 g/L) and leaf debris (2.5, 5.0 and 10.0 g/500 g soil), *viz.*were used in the experiments. The aqueous leaf extract and leaf debris of *C. odorata* and *M. micrantha* incorporated into the soil showed significant effects on total germination, germination indices and seedling growth of *Ageratum conyzoides* both in the laboratory and greenhouse. The *C. odorata* leaf extract caused greater reduction in total germination, germination indices and seedling growths of *A. conyzoides* in the laboratory experiments. However, the *M. micrantha* leaf debris caused more retardation on total germination and seedling growth of *A. conyzoide s*in the greenhouse. Therefore, the inhibitory effects of *C. odorata* and *M. micrantha* on weed growth parameters of *A. conyzoides* were found to be concentration and species-dependant.

***Keywords:*** Allelopathy, aqueous leaf extract, leaf debris, *Chromolaena odorata*, *Mikania micrantha*

## INTRODUCTION

The invasive nature of weeds is thought to be due to their ability to displace other species by means of allelopathy. The use of allelopathic plants in weed management strategies had been currently getting great interest at national and international levels (Weston 1996). These allelopathic chemicals could be used for weed management directly or their chemical content could be used to develop new herbicides (Khan et al 2005). Therefore, the use of the allelopathic plant properties of *Chromolaena odorata* and *Mikania micrantha* undoubtedly has potential to be developed further to complement existing chemical pesticides.

*Chromolaena odorata* (Family: Asteracea) is also known as the Siam weed in Malaysia. It is self propagated by seed dispersion and the seeds germinate mainly after the rainy season and the seedlings need sunlight and partial shade to survive (Holm et al. 1977).Ambika and Jayachandra (1980) reported that the laboratory and field studies of the leachates and extracts inhibited crop growth. Studies found that phenolics, alkaloid and amino acids were the main allelochemicals in this weed (Ambika and Jayachandra 1984).

*Mikania micrantha* (Family: Asteraceae) is also known as ‘selaput tunggul or‘‘ulam tikus’ in Malaysia. It is a problematic weed in cacao, rubber and oil palm plantations because it has a very fast growing habit and can suppresses the growth of other plants by reducing the light availability through rapidly forming a dense cover over the host plant and secreting allelochemicals in the surrounding area (Tripathi et al. 2012). Ismail and Chong (2002) had identified caffiec acid, P-hydroxybenzaldehyde, resorcinol and vanillic acid as allelochemicals in the leaf extracts of *M. micrantha.* Shao et al. (2005) had isolated sesquiterpenoids and these compounds inhibited seed germination and seedling growth of the test species.



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However, there are no reports yet on the comparative allelopathic effects of the leaves of *C. odorata* and *M. micrantha* on germination indices and seedling growth of weed species. Thus, a study was conducted to determine whether *C. odorata* and *M. micrantha* are allelopathic to growth of *A. conyzoides* which occurs commonly in oil palm and rubber plantations of Malaysia. Thus, the objectives of the study are (1) to determine the effects of the aqueous leaf extract on seed germination indices and seedling growth and (2) to determine the effects of the leaf debris incorporated into the soil on germination and seedling growth.

## MATERIALS AND METHODS

### Plant Materials

*Chromolaena odorata* and *Mikania micrantha* plants were collected from Bangi; located at (N 02$^{0}$ 53.362', E 101$^{0}$ 44.382'). The leaves were collected from March until December 2011. The leaves were washed and oven dried at 30 $^{0}$C for 72 hours, ground by a commercial blender and kept in the laboratory at room temperature. Weed seeds of *Ageratum conyzoide*s (goat weed) were collected from around the greenhouse of Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia.

### Effect of the Aqueous Leaf Extracts on the Bioassay Species

Approximately 10 g each of dried *Chromolaena odorata* and *Mikania micrantha* leaves were soaked separately in 200 mL distilled water and agitated for 48 hours by an orbital shaker (120 rpm; Firstek Scientific Model S102, Hsin Chuang, Taiwan) at room temperature (28 ± 3$^{o}$C). The extracts were filtered through a layer of plastic filter and further centrifuged (D-78532, Hettich Zentrifugen, Germany) at 6000 rpm for 15 min. The supernatant of each species was collected and filtered through one layer of 0.2 µm cellulose membrane filter (Whatman International Ltd., Maidstone, England) using a filter pump. The extracts were kept in a refrigerator at 4°C until time of use. Three concentrations of the aqueous extracts used in the experiment were 12.5 g/L, 25.0 g/L and 50.0 g/L. Exactly 5 mL of each aqueous extract or distilled water (for control) were used to wet the filter paper. Three were three replicates for each concentration. The petri dishes were incubated at 28$^{0}$C (12 hour photoperiod) and checked daily. Seeds were considered germinated when the radicle length was more than 2 mm. The seed germination indices and seedling growth, i.e shoot length, radicle length and fresh weight were observed and recorded after seven days (Ismail and Siddique 2011).These indices were calculated as in Table 1.

### Effect of Leaf Debris on Bioassay Species

The dried leaves of *Chromolaena odorata* and *Mikania micrantha* were ground separately by a commercial blender and stored at 4$^{0}$C until they time of use. Three concentrations of leaf debris used in the experiment were 2.5 g, 5.0 g, and 10.0 g per 500 g soil. The debris was incorporated separately into the soil (83% sand, 10% clay, 7% silt; 1.3% organic matters, pH 5.68) and placed in black polybags (height 19.5 cm x diameter 10.5 cm). Similar polybags were filled with soil without the debris for the control. Twenty seeds of the weed species were sown in separate bags and watered regularly. The polybags were kept in the greenhouse (temperature: 25-30$^{0}$C, light density: 780±250 µEm$^{-2}$s$^{-2}$ and relative humidity: 84%). After 14 days, the seedlings were thinned into 10 seedlings per polybag. Germination and seedling growth i.e shoot length; fresh and dry weights were observed and recorded after four weeks (Ismail and Siddique 2011).

### Statistical analysis

All experiments were conducted using the Completely Randomised Design (CRD) with three replications and were conducted twice. The experimental data was subjected to the analysis of variance (one-way ANOVA) and means were compared using the Duncan Multiple range test at the 5% level of significance. The statistical analysis was done using the SPSS version 17.0 software (SPSS Inc., Chicago, USA).



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**Table 1.**Formulae from calculating germination indices

| Germination index | Formulae | | References |
|---|---|---|---|
| Total germination (Final germination percentage) ($G_T$) | $G_T = \frac{N_T \times 100}{N}$ | $N_T$: proportion of germinated seeds in each treatment for the final measurement<br>N: Number of seeds used in bioassay | Widely used |
| Speed of germination (S) | $S = (N_1 x 1) + (N_2-N_1) x 1/2 + (N_3-N_2) x 1/3 + .................. - N_{n-1}) x 1/n$ | ($N_n$ $N_1$, $N_2$, $N_3$, $N_{n-1}$, $N_n$: Proportion of germinated seeds observed at first, second, third......(n-1), (n) days or hours | Bradbeer 1988 |
| Speed of accumulated germination (AS) | $AS = [N_1/1 + N_2/2 + N_3/3 ... + N_n/n$ | $N_1$, $N_2$, $N_3$, $N_n$: cumulative number of seeds which germinate on time 1,2,3......,N | Bradbeer 1988 |

## RESULTS AND DISCUSSION

### Effect of the Aqueous Leaf Extract on the Bioassay Species

The results (Table 2) showed that different concentrations of the aqueous extract of *C. odorata* and *M. micrantha* significantly reduced shoot length, radicle length and fresh weight of *A. conyzoides* with the exception of shoot length at 12.5 g/L of *C. odorata* extract. All seedling growth parameters of *A. conyzoides* showed more retardation with the *C. odorata* than with the *M. micrantha* leaf extract. In addition, the results showed that the retardation or inhibition was concentration dependant because there were increments in inhibition percentage of *A. conyzoides* with increasing concentration of the leaf extracts. This is accordance to previous report by Fariba et al. (2007), whereby allelochemicals were found to stimulate or inhibit plant growth depending on their concentration. Besides, both extracts also had more inhibitory effects on radicle than on shoot length of the bioassay species. This is because the root is the first organ that absorbs allelochemicals from the environment. Besides, root tissue has greater permeability compared to shoot tissue (Nishida et al. 2005).

The inhibitory effect of both the aqueous extracts on $G_T$, S and AS depended on the extract concentration and plant species (Table 3). $G_T$, S and AS of *A. conyzoides* were completely inhibited by the *M. micrantha* extract at all concentrations. However, the *C. odorata* extract showed higher inhibition percentage control on $G_T$, S and AS compared to that of the *M. micrantha* leaf extract. In the present study, multiple indices were used in order to validate the findings of Bewley and Black (1985). $G_T$ of *A. conyzoides* at the 12.5 g/L concentration of the *C. odorata* extract was not significant but the S and AS indices of *A. conyzoides* were significant at the same concentration. This showed that the $G_T$ was not sensitive enough at the lowest concentration of *C. odorata* to express allelopathic activity.

### Effect of the Leaf Debris on the Bioassay Species

From the data (Table 4) it could be seen that the germination and seedling growth of *A. conyzoides* were significantly reduced with increasing amounts of *M. micrantha* leaf debris incorporated into the soil. In addition, incorporated *M. micrantha* leaf debris caused greater reduction in germination and seedling growth of *A. conyzoides* than that by the *C. odorata* leaf debris. There were no significant effects in germination percentage of *A. conyzoides* observed for the different concentrations used. This may be because of the high amount of allelochemical-induced inhibition of nutrient uptake in the presence in of *M. micrantha* (Ismail and Chong 2002).This also indicates that *M. micrantha* may contain higher amount of phytotoxins compared to *C. odorata*. Phytotoxins are water soluble substances bound in the



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tissues and released into the soil during the decomposition process by soil microorganisms (Alsaadawi and Salih 2009). In addition, several studies found that numerous allelopathic compounds are water soluble which can release easily into the soil and affect the target plant (Patrick 1971).

**Table 2**. Effect of the aqueous leaf extracts of *C. odorata* and *M. micrantha* on seedling growth (% of the control) of *A. conyzoides.*

| Leaf extract conc. (g/L) | Shoot length | | Radicle length | | Fresh weight | |
|---|---|---|---|---|---|---|
| | C | M | C | M | C | M |
| 0 (control) | 100.0[a] | 100.0[a] | 100.0[a] | 100.0[a] | 100.0[a] | 100.0[a] |
| 12.5 | 113.8[a] | 77.2[b] | 14.7[b] | 22.1[b] | 44.6[b] | 33.4[b] |
| 25.0 | 17.7[b] | 25.8[c] | 2.1[c] | 4.5[c] | 15.7[c] | 30.9[b] |
| 50.0 | 0.0[c] | 1.77[d] | 0.0[c] | 0.3[c] | 0.0[d] | 0.0[c] |

*Note: Means within rows followed by same alphabet are not significantly different ($p$<0.05) according to Duncan Multiple range test .C= *C. odorata*, M= *M. micrantha*

It can be concluded that the aqueous leaf extract and leaf debris of *Chromolaena odorata* and *Mikania micrantha* incorporated into the soil have significant effect on the total germination, germination indices and seedling growth of *Ageratum conyzoides* both in the laboratory and greenhouse experiments. The *Chromolaena odorata* leaf extract caused greater reduction in total germination, germination indices and seedling growth of *A. conyzoides* in the laboratory experiments. However, the *M. micrantha* leaf debris caused more retardation on total germination and seedling growth of *A. conyzoides*in the greenhouse experiments. Therefore, the inhibitory effects of *C. odorata* and *M. micrantha* on weed growth parameters of *A. conyzoides* were found to be concentration and species-dependant.

**Table 3.** Effect of the aqueous leaf extract of *C. odorata* and *M. micrantha* on seedling germination indices (% of the control) of *A. conyzoides*

| Leaf extract conc. (g/L) | $G_T$ | | S | | AS | |
|---|---|---|---|---|---|---|
| | C | M | C | M | C | M |
| 0 (control) | 98.3[a] | 98.3[a] | 3.37[a] | 3.37[a] | 10.69[a] | 10.69[a] |
| 12.5 | 80.0[a] | 73.3[b] | 2.11[b] | 1.55[b] | 6.38[b] | 4.05[b] |
| 25.0 | 23.3[b] | 31.7[c] | 0.40[c] | 0.53[c] | 0.80[c] | 0.95[c] |
| 50.0 | 0.0[b] | 1.7[d] | 0.00[c] | 0.02[d] | 0.00[c] | 0.02[c] |

*Note: [a] Means within rows followed by same alphabet are not significantly different ($p$<0.05) according to Duncan Multiple range test .C= *C. odorata*, M= *M. micrantha*
[b] $G_T$ (total germination), S (speed of germination) and AS (speed of accumulated germination).

**Table 4.** Effect of leaf debris of *C. odorata* and *M. micrantha* incorporated into soil on the germination and seedling growth (% of the control) of *A. conyzoides.*

| Leaf debris conc. (g/500 g soil) | Germination | | Shoot length | | Fresh weight | | Dry weight | |
|---|---|---|---|---|---|---|---|---|
| | C | M | C | M | C | M | C | M |
| 0 (control) | 100.0[a] | 100.0[a] | 100.0[a] | 100.0[a] | 100.0[a] | 100.0[a] | 100.0[a] | 100.0[a] |
| 2.5 | 100.0[a] | 41.7[b] | 71.2[b] | 31.7[b] | 69.2[b] | 31.8[b] | 52.9[b] | 26.3[b] |
| 5.0 | 86.7[a] | 33.3[b] | 59.1[c] | 30.0[b] | 90.9[a] | 36.7[b] | 62.7[b] | 21.9[b] |
| 10.0 | 83.3[a] | 15.0[c] | 60.2[c] | 13.0[c] | 71.0[b] | 25.5[b] | 52.8[b] | 9.6[c] |



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*Note: Means within rows followed by same alphabet are not significantly different ($p$<0.05) according to Duncan Multiple range test .C= *C. odorata*, M= *M. micrantha*

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# ALLELOPATHIC EFFECTS OF DIFFERENT WEEDS ON THE SEED GERMINATION OF WHEAT AND CHICKPEA.

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**ABSTRACT** Allelopathic extracts of weeds have the potential to create and maintain eco friendly environment in various crops worldwide. Synthetic herbicides pose health risk for human and livestock. This study investigated the allelopathic effects of some weeds (*Convolvulus arvensis* L., *Parthenium hysterophorus* Linn and *Cyperus rotundus* L.) at various concentrations on the seed germination of wheat and chickpea. Completely Randomized (CR) design with factorial arrangements was used for the conducted of experiment and the trial was comprised on three replications and consequently it was repeated three times. The weed concentrations were 0, 25, 50, 75 g $L^{-1}$. The trial was run under the controlled environment at the Weed Science Department laboratory The University of Agriculture Peshawar, Pakistan during November, 2012. The data was recorded on germination %. Weeds and their concentration showed inhibitory effects on the tested crops. *P. hysterophorus* showed more inhibitory effects (18.88 and 23.33%) germination at 75 g $L^{-1}$ in wheat and chickpea respectively compared with untreated check (96.66 and 92.22%). The remaining two concentrations of weeds also showed inhibitory effects on the seed germination. *C. rotundus* and *C. arvensis* were also noticed to be inhibitory in nature and effected the germination of the tested crops. It was concluded that the weed species under the study having strong inhibitory effects on the tested crops and these could be manage in proper time.

***Keywords*****:** Allelopathy, germination, crops.

## INTRODUCTION

The word allelopathy is the combination of two Greek words “allelo” and “pathy” which means, the reciprocal suffering of two organisms especially plants. The direct or indirect effect which may either inhibitory or stimulatory effect of one plant on other through chemical release (allelochemicals) that escapes into the environment (Rice, 1984) Allelochemical may release into environment either from above ground parts (flower, stem, leaves) or through root exudates (Ferguson *et al.,* 2003).

Weeds species have inhibitory effect on crops but still some weeds also contain stimulatory effect on seed germination (Narwal, 2004). Generally competition exists among crop and weeds. The allelopathic effect of weeds on crops germination and seedling growth differ from weed to weed (Hamayun *et al*., 2005). Different parts of weeds have different effects on other plants and varies for its allelopathic potential. Soil become pollutes through the release of water soluble chemical compounds in the soil, and adversely effect the crop plants (Batish *et al*., 2007).

*Parthenium hysterophorus* is invasive plant known as Parthenium (Mahadevappa 1997 and Navie *et al*., 1996). It is spreading abruptly aroundly the country (Pakistan), moreover, it has also allelopathic properties (Kohli *et al.,* 2006). It can effect both plants and human health (Wiesner *et al*., 2007).

Purple nut sedge (*Cyperus rotundus*) is thought to be one of the world worst weed which is distributed in 52 different crops and 92 countries including subtropical and tropical areas (Rao, 2000). In Pakistan (*Cyperus rotundus*) is the most common and noxious weed of rice. *Cyperus rotundus* causes 23-29% losses in different crops (Okafor *et al,*. 1976).



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*Convolvulus arvensis* (*Convolvulaceae*), a climbing or creeping perennial (0.5–2 m) tall. *C. arvensis* is considered as one of the most dangerous weeds in the world. It may cause 20- 70% crop yield loss and makes problems in harvesting.

Chickpea (*legumanaceae*) and important pulse legume around the world. This pulse crop provides an important source of dietary protein for human consumption; and also plays important role in soil fertility due to nitrogen-fixing ability (Maiti, 2001).

Chickpea crop faces competition mainly from annual broad-leaf weeds due to identical growth pattern of chickpea and weeds, and Severity also increases with advance in growth. Weeds compete with crop plants mainly for sunlight and space, moisture, nutrients etc. They also reduce chickpea quality and affect market value rate (Saxena, 1979). It is reported in literature that allelopathic effect of many different weeds affect wheat and chickpea and other beans crops (Singh *et al*., 2005). Wheat is used as main food source in many countries of the world (Richards, 2000). Weed cause serious problem in wheat crop. In Pakistan the average loss in wheat crop due to weeds is 25-35% (Nayyar *et al*., 1994).

Keeping in view the allelopathic potential of *C. arvensis*, *C. rotundus* L., and *P. hysterophorus* L., and their possible negative effect on several crops and weed seeds, an experiment was conducted to test the water extract of these weeds on seed germination of wheat and chickpea.

**Objectives:**

1. To test phytotoxic effect of different weeds on seed germination of wheat and chickpea.
2. To compare the toxicity level of the tested weeds at various concentration.

## MATERIALS AND METHODS

A laboratory based experiment entitled "Allelopathic effects of different weeds on the seed germination of wheat and chickpea" was conducted at the University of Agriculture Peshawar during November 2012. Completely Randomized Design (CRD) with factorial arrangement having three replications was used for the used for the trial under controlled evnvironment.

### Weeds collection

The weeds include *Cyperus rotundus* L., *Convolvulus arvensis* and *Parthenium hysterophorus* were collected from the research fields of New Developmental Research Farm of The University of Agriculture Peshawar, Pakistan. Fresh and healthy plants were cut at the base above ground with the help of sickle. After collection plants specimens were washed under running tap water to clean from soil and other particles. After washing the whole plants were kept in oven for 48 hours at 65 C˚. After drying the plants were chopped and ground individaully with the help of grinder. The final ground samples were kept in plastic bags for further use in the experiment.

### Extract preparations

The ground samples of C. *rotundus,* C. *arvensis*, and *P. hysterophorus* were weighed. Three concentrations of each sample were made @ 25, 50, 75 and 0g $L^{-1}$. After weighing the samples were mixed in clear filtered water at room temperature for 24 hours. After 24 hours water extracts were obtained by filtering the solution through muslin cloth. Extract of each weed and their respective concentration were bottled and tagged for further use.

### Experimantal Setup

Ten seeds each of chickpea and wheat were put separately in petri dishes (9 cm dia) containing three layers of Whatman No.1 filter paper. The petri dish were replicated three times. Three mL of the extract as per treatment were added to each petri dish and tape water was used as a control treatment for comparison. During the period of 10 days the petri dishes were observed daily and an equal amount of extract was added to each petri dish as needed to prevent seeds or



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seedlings from drying out. The germination data was recorded on daily basis for a week by using the following formula.

$$\text{Germination \%} = \frac{\text{Germinated seed}}{\text{Total seed}} \times 100$$

**Statistical Analysis**

The recorded data for each extract and their respective concentrations was subjected individually to the ANOVA technique by using MSTATC computer software (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

**Chickpea germination (%)**

The statistical analysis of the data (Fig.1) showed that extract of different weeds species significantly affected the seed germination of chickpea at P value 0.002 (Fig.1). The weed mean showed that maximum (83.88%) germination was recorded in *C. rotundus.* While minimum (55.27%) germination was noted in *P. hysterophorus.* In case of concentration maximum (94.44 %) seed germination was observed under the control treatment, where only distal water was applied. While the minimum (44.44 %) seed germination was calculated under the extract concentration of 75 g $L^{-1}$ (Fig.1). Further the result for interaction between weeds and their extracts concentraions showed that high value (96.66 %) germination was found under the combination of *C. rotundus* x 0 g $L^{-1}$ Similarly the minimum (18.88 %) germination was noted for *P. hysterophorus* x 75 *g*$L^{-1}$.

In addition, the higher concentration of parthenium leaf extracts have greatly reduced the seed germination, shoot length, shoot weight, root length and root weight of soybean (*Glycine max* L.), mungbean (*Vigna radiata* L.) and maize (Khan *et al*., 2011).

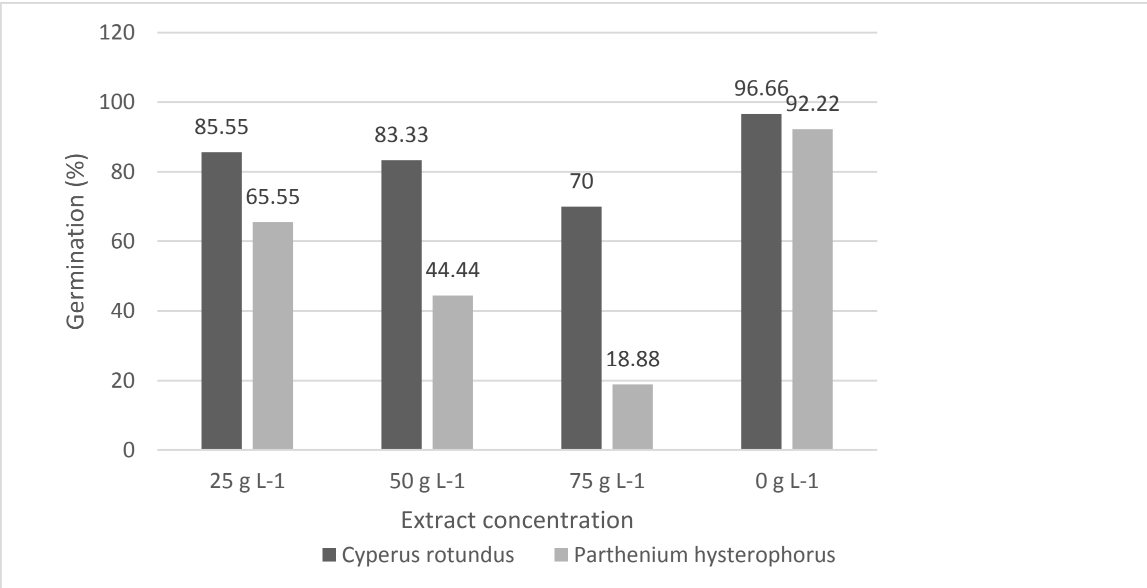


**Fig. 1.** Allelopathic effect of *Cyperus rotundus* and *Parthenium hysterophorus* x extract concentrations on seed germination of chickpea.

**Wheat germination (%)**

Analysis of variance of the data (Fig.2) showed that weeds (*C. arvensis*, *P. hysterophoru)* and their concentrations (0, 25, 50, 75 g $L^{-1}$) had the significant effects on the seed germination of wheat at *P* value 0.002. The mean data for weed depicted that maximum (82.50%) germination



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was recorded for *C. arvensis,* while minimum (74.95%) germination was observed for *P. hysterophorus* (Fig.2). Among the concentrations maximum (89.44%) germination of wheat was recorded under 0 g $L^{-1}$ concentration while minimum (43.33%) germination was recorded in 75 g $L^{-1}$, (Table-2). The interaction mean showed that maximum (92.22%) germination of wheat seed was recorded in *C. arvensis* x 0 g $L^{-1}$ and the minimum (23.33%) germination was noted under the concentration of *P. hysterophorus* x (75 g $L^{-1}$). The effect of the above mentioned weeds and their concentrations on the seed germination of wheat pertaining to the facts that *P. hysterophorus* and *C. arvensis* both have the negative effects on the wheat seed germination. Moreover with the increasing concentration of extract the germination % was decreased. They reported the germination inhibition in wheat and chickpea under different weeds extract and their concentrations. Maharjan *et al*. (2007) have also found parthenium leaf extract has the inhibitoriest effect to the seed germination of wheat and other crops. He recorded considerable reduction in the biomass of wheat and other cereal crops seedlings when treated with the vegetative parts of parthenium.

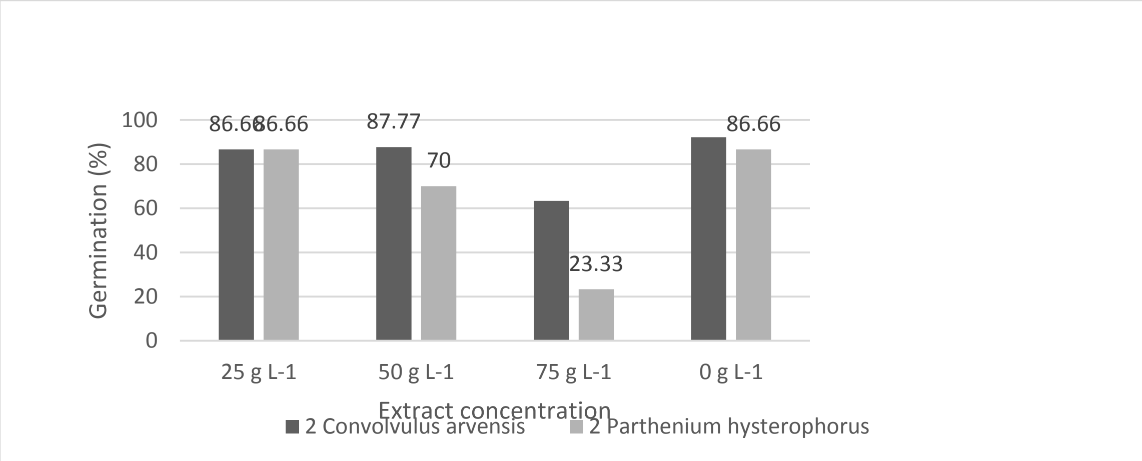


**Fig. 2**. Allelopathic effect of *Convolvulus arvensis* and *Parthenium hysterophorus* x extract concentrations on seed germination of wheat.

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# ALLELOCHEMICALS IN *CUSCUTA CAMPESTRIS* YUNCKER

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**ABSTRACT** Golden dodder (*Cuscuta campestris* Yuncker), commonly known as Rumput Emas in Malaysia is a parasitic weed infesting many crops and weed species alike. A phytochemical study on the chemical constituents of *C. campestris* was carried out. Six compounds were isolated through chromatographic method and identified as sitosterol**1**, pinoresinol**2**, arbutin**3**, kaempferol**4**, quercetin**5**, and astragalin**6**. The ethanolic extract of *C. camprestris* displayed inhibitory allelopathic effects at doses 500 ppm and above by inhibiting seeds germination and seedling growth of lettuce, radish and weedy rice as the test plants. The allelopathic potential of three compounds isolated, *viz.* kaempferol**4**, sitosterol**1** and pinoresinol**2** were investigated. All the three compoundsat doses of 1-100µM showed stimulatory effects on plant growth of radish, lettuce and weedy rice seedlings. The response of all assayed species was dose-dependent.

***Keywords:*** *Cuscuta campestris*, seed germination, seedling growth, flavonoids, sitosterol, pinoresinol, arbutin.

## INTRODUCTION

Synthetic herbicides when used repetitively not only are likely to persist in the environment as bound residues and damage other organisms, but also may lead to the emergence of resistantweed species (Adam *et al.* 2010).The release of bioactive compounds or allelochemicals from natural sources has the ability to suppress the growth of weeds (Baki *et al.* 2009). Natural products are a source of compounds that might be used as herbicide directly or as lead structure for herbicide discovery (Duke *et al*., 2000). Our preliminary screening study showed that the crude aquous extracts of *C. campestris* as a parasitic weed with special features – the haustoria infesting and growing on different types of weeds and plant crops alike, possess some potent bioactive constituents that are herbicidal in nature, and strongly inhibited the growth of lettuce and radish seedlings (Othman *et al*. 2012).

*Cuscuta campestris* Yuncker from the family Cuscutaceae (Convolvulaceae) is an annual obligate angiosperm parasite with golden yellow colour (Shen *et al.* 2005). This parasite twines on other plants and attaches to the above-ground parts of a wide range of host plants. A single plant of *C. campestris* may attack varieties of host plants at a time and the host can be weeds or crops (Bungard *et al.*, 1999; Baki *et al*. 2009, Othman *et al.* 2012). It grows in abandoned area like shrubs and bushes on roadsides and open spaces in Malaysia (Baki *et al.* 2009).

In this communication we investigated the allelopathic activities of *C. campestris* and assess its potential as a natural herbicide, primarily focusing on its bioactive chemical constituents and chemical bioassay.

## MATERIALS AND METHODS

### Extraction, Isolation and Separation

The *Cuscuta* samples were separated from the hosts after collection from Johore, Malaysia in October–December 2010. All the samples were dried in the oven for 24 - 48 hours at 60°C.About 200g of the dried samples of *Cuscuta* was dismembered and extracted with ethanol at room temperature for three times. The ethanol extract was freed from the solvent by rotary evaporator



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and freeze dried. About 5g of the crude was subjected to column chromatography (CC) with silica gel as the adsorbent. Gradient elutions with three solvents were employed as the mobile phase; hexane, ethyl acetate (EA), and methanol. Every fraction was collected in 200 mL of eluents. A total of 28 fractions were collected from the first column. Each fraction was tested on TLC to check for purity. Fraction II (Hexane: EA; 100: 0), VI (Hexane: EA; 80: 20), and X (Hexane: EA; 20: 80) were further subjected into CC in order to obtain single spot on TLC.

Fraction II (245.7mg) was fractionated in hexane-ethyl acetate. Of 38 fractions that were tested with TLC and NMR, fractions 19-23 gavesitosterol**1** (1.3mg).Fraction VI (71.4mg) was subjected to CC using hexane: ethyl acetate solvent system to give two compounds; pinoresinol **2**(3.5mg, 50:50) and kaempferol**4** (5.6mg, 0:100). From fraction X, 27 sub fractions were obtained and sub fraction 10 was subjected again to CC with dichloromethane and ethyl acetate as solvent systems. From this fraction, eluted by a mixture of dichloromethane-ethyl acetate (50: 50), was purified quercetin**5** (0.5mg), while Fraction XII was further purified using HPLC to give arbutin**3** (2.1mg) and kaempferol-3-*O*-glucoside **6**(1.3mg). All the structures were identified by comparing their NMR and UV spectra with those reported in the literature.

**Bioassays**

Bioassays based on seed germination, shoot and root growths of radish (*Raphanus sativus*), lettuce (*Lactuca sativa*) and weedy rice (*Oryza sativa*) were used to study the allelopathic potential of *C. campestris*.

About 1 ml of ethanol extract of dried *C. campestris* was dissolved in 200 ml deionized water with 1 % of ethanol. The extract was set as original concentration (5000 ppm) and diluted into 100 ppm, 200 ppm, 500 ppm, and 1,000 ppm. An 8 ml aliquot of the extract was pipette in to petri dish that was lined with filter paper and previously sown with 20 seeds of radish (*R. sativus*). The control used was deionized water with 1% of ethanol. These petri-dishes were placed in Precision Plant Growth Chamber Model 818 (230 V, 860 watts) for 7 days. Three replicates were prepared for each treatment. The plants were frozen seven days after treatment in order to avoid subsequent growth until measurements were recorded. The seed germination, shoot and root lengths, and dry mass were recorded.

Selected constituents, namely, kaempferol, sitosterol and pinoresinol from *C. campestris* were prepared with 1% of ethanol in deionized water (100μM) and the rest (1 and 10μM) were obtained by dilution. The same bioassay method was used to determine the allelopathic potential of these chemical constituents on lettuce seed germination and root and shoot lengths following exposures as such.

**Statistical Analysis**

The statistical analyses were performed on the data using analysis of variance (ANOVA) using the SPSS Program version 15.0. Any significant difference between treatment means was tested with Tukeys' test at $p<0.05$. The percentages of shoot and root growths were calculated according to the following formula:

$$\%\ growth\ inhibition = \frac{100(Pc - Pt)}{Pc}$$

Where *Pc* and *Pt* are the shoot or root lengths of the control and the treated samples, respectively.

## RESULTS AND DISCUSSION

### Identification of Chemical Constituents from *Cuscuta campestris*

Six different chemical constituents were isolated from *C. campestris*: three were flavonoids, one sterol, one lignan and one arbutin. The three flavonoids were kaempferol**4**, kaempferol-3-*O*-glycoside**6** and quercetin**5**. The concentration of kaempferol**4** was relatively high *vis-a-vis*



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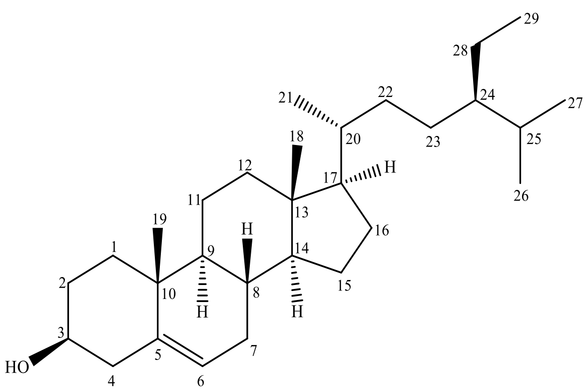
the six chemical constituents in the parasitic weed. All these constituents are putative allelochemicals which show allelopathic effects on the test plants (see section 2.2). The identifications of these constituents are based on the comparison of the spectral data with those reported. The chemical structures of sitosterol**1**, pinoresinol**2**, arbutin**3**, kaempferol**4**, quercetin**5**, and astragalin**6** are shown below.



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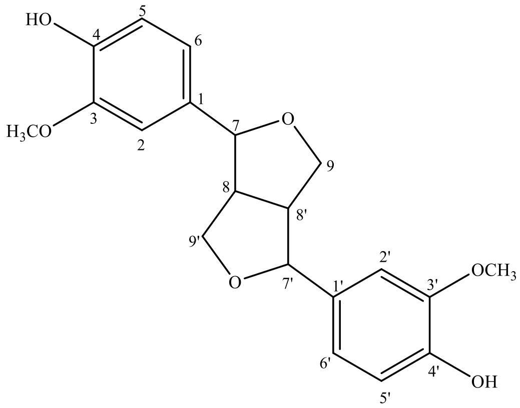

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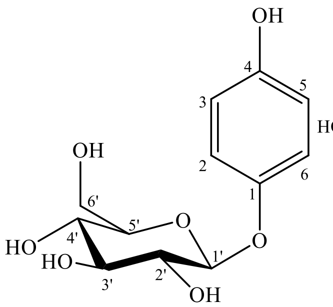

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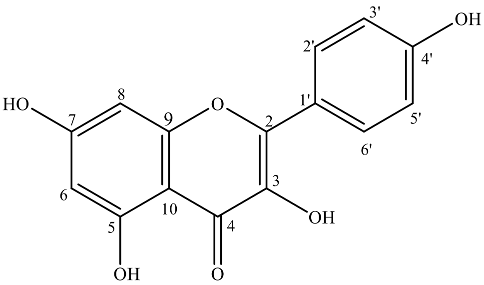

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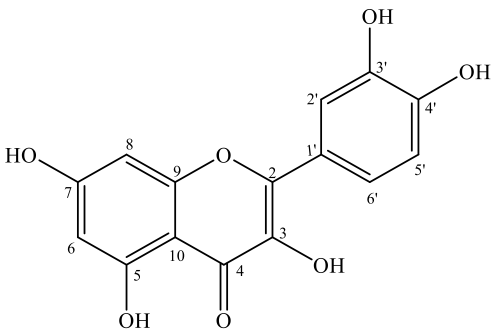

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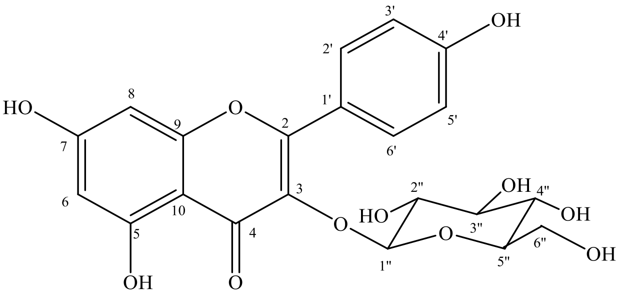

**6**

**Sitosterol (1)** (De-Eknamkul and Potduang, 2003; Rawat et al., 1998), $C_{29}H_{50}O$ : $^1$H NMR ($CDCl_3$) δ3.49 (1H, m, H-3), 5.32 (1H, m, H-6), 0.65 (3H, s, H-18), 0.98 (3H, s, H-19), 0.89 (3H,d, *J*=6.4Hz, H-21), 0.80 (3H, d, *J*=1.8Hz, H-26), 0.78 (3H, d, *J*=, H-27), 0.84 (3H, s, H-29). $^{13}$C NMR ($CDCl_3$) δ37.3 (C-1), 31.7 (C-2), 71.9 (C-3), 42.3 (C-4), 140.8 (C-5), 121.8 (C-6), 32.0 (C-7), 32.0 (C-8), 50.2 (C-9), 36.6 (C-10), 21.2 (C-11), 39.8 (C-12), 42.4 (C-13), 56.9 (C-14), 24.4 (C-15), 28.3 (C-16), 56.1 (C-17), 11.9 (C-18), 19.5 (C-19), 36.2 (C-20), 18.9 (C-21), 34.0 (C-22), 26.1 (C-23), 45.9 (C-24), 29.2 (C-25), 19.9 (C-26), 19.1 (C-27), 23.1 (C-28), 12.1 (C-29),.

**Pinoresinol (2)** (Do et al., 2009; Li Hui et al., 2004), $C_{20}H_{22}O_6$ : $^1$H NMR ($CDCl_3$) δ6.86 (1H, s, H-2 and H-2'), 6.87 (1H, d, *J*=2.28Hz, H-5 and H-5'), 6.81 (1H, dd, *J*=1.84, 6.64Hz, H-6 and



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H-6'), 4.71 (1H, d, *J*=4.12Hz, H-7 and H-7'), 3.07 (1H, m, H-8 and H-8'), 3.84 (2H, dd, J=4.12, 5.52Hz, H-9 and H-9'), 4.21 (2H, dd, J=6.88, 2.28Hz, H-9 and H-9'), 3.89 (3H, s, 3-$OCH_3$ and 3'-$OCH_3$). $^{13}C$ NMR ($CDCl_3$) δ132.95 (C-1 and C-1'), 108.67 (C-2 and C-2'), 146.78 (C-3 and C-3'), 145.31 (C-4 and C-4'), 114.35 (C-5 and C-5'), 119.06 (C-6 and C-6'), 85.97 (C-7 and C-7'), 54.23 (C-8 and C-8'), 71.75 (C-9 and C-9'), 56.05 (3-$OCH_3$ and 3'-$OCH_3$).

**Kaempferol (4)** (Hadizadeh et al., 2003; Rawat et al., 1998), $C_{15}H_{10}O_6$ : $^1H$ NMR ($CD_3OD$) δ6.15 (1H, d, J=1.84Hz, H-6), 6.37 (1H, d, J=2.28Hz, H-8), 8.05 (1H, d, J=9.16Hz, H-2' and H-6'), 6.87 (1H, d, J=9.16Hz, H-3' and H-5'). $^{13}C$ NMR ($CD_3OD$) δ146.7 (C-2), 135.8 (C-3), 176.0 (C-4), 161.2 (C-5), 97.9 (C-6), 164.2 (C-7), 93.1 (C-8), 156.9 (C-9), 103.2 (C-10), 122.4 (C-1'), 129.3 (C-2' and C-6'), 115.0 (C-3' and C-5'), 159.2 (C-4').

**Quercetin (5)** (Guvenalp and Demirezer, 2005; Rawat et al., 1998), $C_{15}H_{10}O_7$ : $^1H$ NMR ($CD_3OD$) δ6.17 (1H, d, *J*=2.0Hz, H-6), 6.38 (1H, d, *J*=2.0Hz, H-8), 7.72 (1H, d, *J*=2.2Hz, H-2'), 6.87 (1H, d, *J*=8.52Hz, H-5'), 7.61 (1H, dd, *J*=2.0, 6.6Hz, H-6'). $^{13}C$ NMR ($CD_3OD$) : δ146.4 (C-2), 136.0 (C-3), 176.1 (C-4), 161.2 (C-5), 97.9 (C-6), 164.2 (C-7), 93.1 (C-8), 156.9 (C-9), 103.0 (C-10), 120.3 (C-1'), 114.6 (C-2'), 144.9 (C-3'), 147.3 (C-4'), 114.9 (C-5'), 122.7 (C-6').

**Kaempferol-3-*O*-glucoside (6)**(Lee et al., 2004), $C_{21}H_{20}O_{11}$ : $^1H$ NMR ($CD_3OD$) δ6.20 (1H, d, J=1.96Hz, H-6), 6.39 (1H, d, J=1.96Hz, H-8), 8.05 (1H, d, J=8.8Hz, H-2' and H-6'), 6.88 (1H, d, J=8.8Hz, H-3' and H-5'), 5.25 (1H, d, J=7.1Hz, H-1''), 3.33-3.96 (5H, m, H-2'', H-3'', H-4'', H-5'', H-6''). $^{13}C$ NMR ($CD_3OD$) δ159.2 (C-2), 135.5 (C-3), 179.6 (C-4), 161.7 (C-5), 100.2 (C-6), 166.6 (C-7), 95.0 (C-8), 158.7 (C-9), 105.7 (C-10), 122.9 (C-1'), 132.4 (C-2' and C-6'), 116.2 (C-3' and C-5'), 161.7 (C-4'), 104.2 (C-1''), 75.8 (C-2''), 78.1 (C-3''), 71.5 (C-4''), 78.5 (C-5''), 72.7 (C-6'').

**Arbutin (3)**(Cepanec and Litvi, 2008), $C_{12}H_{16}O_7$ : $^1H$ NMR ($CD_3OD$) δ6.93 (1H, d, *J*=8.8Hz, H-2 and H-6), 6.66 (1H, d, *J*=8.8Hz, H-3 and H-5), 4.70 (1H, d, *J*=7.2Hz, H-1'), 3.25-3.88 (5H, m, H-2', H-3', H-4', H-5', H-6'). $^{13}C$ NMR ($CD_3OD$) : δ152.4 (C-1), 116.6 (C-2 and C-6), 119.4 (C-3 and C-5), 152.4 (C-4), 103.7 (C-1'), 75.0 (C-2'), 78.0 (C-3'), 71.4 (C-4'), 78.0 (C-5'), 62.6 (C-6').

**Bioassay of Ethanol Extract of *Cuscuta campestris***

Table 1 shows the results on the effect of different concentration of ethanol extract of *C. campestris* on the seeds of lettuce, radish and weedy rice. The effect of the extract of *C. campestris* on seed germination was not significant except lettuce. Interestingly, the growth of shoot and root of all three assayed species were severely affected. The efficacy of the extract was measurably higher on the roots than shoots of the lettuce and weedy rice seedlings. The responses of all assayed species were dose-dependent (Fig. 1 and Fig. 2).

At low concentration (100 -1000 ppm), there was negligible and non-significant reduction in germination of lettuce. At higher dose (5000 ppm), germination of lettuce seeds was markedly decrease (45% inhibition). The inhibitory effectsof the extract at 5000 ppm on the growth of lettuce shoots were highly significant at 89% compared with the control. Synergistic effects on shoot growth of lettuce seedlings were observed at other applied doses, explaining perhaps the hormonal role of the extracts at low concentrations. The roots of lettuce displayed higher sensitivity than the shoots to the ethanol extracts of *C. campestris.* A significant synergistic effect with 14-47% stimulation on root growth obtained when low concentrations of 100 -200 ppm. However, when treated with higher dosage, root growth decreased considerably at 43-95% (Table 1).

The ethanol extract of *C. campestris* did not show any significant effect on radish seed germination. However, the root growth appeared greatly affected. With parallel increase in concentration, root growth was inhibited with values ranging from 27% to 84%, although there was no obvious inhibition at low concentration (100 -200 ppm) (Fig. 2). The effect of *C. campestris* extract on radish shoot growth was not as great as the effect on roots. In fact, the



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growth of radish shoot was inhibited only when higher dosage (1000 -5000 ppm) applied (Table 1).

There were negligible effects of *C. campestris* extract on weedy rice germination. Yet, the growth of weedy rice seedlings is sensitive to lower concentration of *C. campestris* extract. The shoot and root growthsof weedy rice seedlings were inhibited by *C. campestris* ethanol extract at 200ppm, with differential sensitivity of 5-63% reductions of shoot growth and 15-93% reductions of growth compared with the respective controls (Table 1).

**Allelopathic Potential of Chemical Constituents of *Cuscuta campestris* on Lettuce**

Three bioactive compounds, kaempferol, pinoresinol and sitosterol were selected to determine their allelopathic potentials against the test plants. The results were reported as percentage differences in germination (Table 2), root growth and shoot growths and dry weight (Table 2) compared with the respective controls. Overall, exposures to each of the three chemical constituents failed to have any significant effect on the germination of lettuce seeds. Similar exposures to 1 – 100 µM showed synergistic effects on the growth of shoots and roots of lettuce seedlings *vis-à-vis* the control. The dose-mediated synergistic effects on the growth of lettuce seedlings were observed when the concentrations were increased. This shows that the responses of lettuce to kaempferol, pinoresinol and sitosterol were dose-dependent with the roots displaying greater sensitivity than the shoots.

Pinoresinol**2**showedthe greatest stimulatory effect on the growth of rootsand shoots of lettuce. Exposures to 1 – 100 µM of pinoresinol resulted in 60-76% increase in root growth of lettuce, and 21-64% in the shoot growth (Table 2). The parallel figures for exposures to kaempferol**4** were 20-60% increase in shoot growth and for the root growth these were 58-67% (Table 2). Sitosterol**1** showed the least growth synergistic effects on lettuce growth with only 13-49% in shoot growth the root growth increased up to 46-63% following exposures to 1 – 100 µM (Table 2). However, the beneficial effects of these three constituents on seedling growth of lettuce were reduced with parallel increase in doses in excess of 100 µM suggesting inhibitory effects at higher concentration.

## CONCLUSIONS

The biological and pharmacological activities of *C. campestris* are remarkable (Agha *et al.*, 1996; Istudor *et al.*, 1984). Nevertheless, not much research was done on the allelopathic potentials of the weed against other weed species and crops. The preliminary results in this research suggested that application of *C. campestris* extract at 500 ppm and above has allelopathic potential on other weeds (e.g. weedy rice) and crops (lettuce and radish). However, the pure compounds did not exhibit measurable allelopathic activity against the test plants at concentration of 100 µM and below. This observation on shoot and root growths of lettuce suggest that the allelopathic potential of these constituents may be synergistic at low doses, but inhibitory effects on growth may prevail at higher doses. Further investigation on a broader range of doses and application times of *C. campestris* in petri-dishes and soils, on different plants is commendable to be carried out to improve their efficacies for weed control.

**Table 1.** Effect of ethanol extract of *Cuscuta campestris* on the germination and growth of lettuce, radish, and weedy rice seedlings.

| Concentration (ppm) | Germination (%) | Shoot Length (mm) | Root Length (mm) | Dry Weight (g) |
|---|---|---|---|---|
| Lettuce | | | | |
| 0 | 100.0b (0.0) | 10.0b (0.0) | 39.3d (0.0) | 0.010a (0.0) |
| 100 | 100.0b (0.0) | 14.0c (-39.0) | 57.8f (-47.1) | 0.011a (-10.0) |
| 200 | 100.0b (0.0) | 13.0c (-30.0) | 44.7e (-13.8) | 0.012a (-20.0) |



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| | | | | |
|---|---|---|---|---|
| 500 | 98.3b (1.7) | 12.4bc (-23.1) | 22.4c (43.0) | 0.013 a (-30.0) |
| 1000 | 100.0 b (0.0) | 12.5bc (-24.1) | 11.2b (71.6) | 0.011a (-10.0) |
| 5000 | 55.0a (45.0) | 1.1a (88.9) | 1.9a (95.3) | 0.017b (-70.0) |
| | | | | |
| Radish | | | | |
| 0 | 100.0a (0.0) | 32.6b (0.0) | 71.9c (0.0) | 0.15a (0.0) |
| 100 | 98.3a (1.7) | 41.2c (-26.5) | 71.0c (1.2) | 0.17a (-13.3) |
| 200 | 98.3a (1.7) | 40.4c (-23.9) | 62.2bc (13.5) | 0.15a (0.0) |
| 500 | 100.0a (0.0) | 29.6b (9.2) | 52.2b (27.4) | 0.16a(-6.7) |
| 1000 | 100.0a (0.0) | 15.9a (51.1) | 16.1a (77.6) | 0.16a (-6.7) |
| 5000 | 95.0a (5.0) | 11.4a (65.1) | 11.6a (83.8) | 0.18a (-20.0) |
| | | | | |
| Weedy rice | | | | |
| 0 | 100.0a (0.0) | 52.4d (0.0) | 51.2c (0.0) | 0.34a (0.0) |
| 100 | 100.0a (0.0) | 59.3e (-13.1) | 50.3c (1.8) | 0.33a (2.9) |
| 200 | 100.0a (0.0) | 49.8cd (5.0) | 43.2b (15.6) | 0.35a (-2.9) |
| 500 | 98.3a (1.7) | 45.6bc (12.9) | 43.6b (14.8) | 0.36ab (-5.9) |
| 1000 | 98.3a (1.7) | 41.7b (20.4) | 40.6b (20.8) | 0.36ab (-5.9) |
| 5000 | 100.0a (0.0) | 19.4a (63.0) | 3.5a (93.1) | 0.39b (-14.7) |

Values in the column with the same letter are not significantly different at $p<0.05$.
Values in the parentheses are inhibition percentages over control.
Values in the parentheses with (-) are promotion percentages over control.

**Table 2.** Effect of three constituents from *Cuscuta campestris* on the germination and growth of lettuce (*Lactuca sativa*) seedlings.

| Concentration (µM) | Germination (%) | Shoot Length (mm) | Root Length (mm) | Dry Weight (g) |
|---|---|---|---|---|
| Kaempferol | | | | |
| 0 | 96.67a (0.0) | 8.52a (0.0) | 11.85a (0.0) | 0.032a (0.0) |
| 1 | 100.00a(-3.44) | 13.66b (-60.27) | 19.76b (-66.70) | 0.010a (69.29) |
| 10 | 98.33a(-1.72) | 12.56b (-47.43) | 18.94b (-59.83) | 0.010a (68.97) |
| 100 | 96.67a (0.0) | 10.25a (-20.31) | 18.71b (-57.89) | 0.009a (73.04) |
| | | | | |
| Pinoresinol | | | | |
| 0 | 96.67a (0.0) | 8.52a (0.0) | 11.85a (0.0) | 0.032a (0.0) |
| 1 | 100.00a (-3.44) | 13.99c (-64.18) | 20.91b (-76.48) | 0.011a (64.58) |
| 10 | 98.33a(-1.72) | 12.78c (-49.93) | 22.48b (-89.69) | 0.010a (67.40) |
| 100 | 100.00a (-3.44) | 10.33b (-21.18) | 18.97b (-60.06) | 0.010a (69.59) |
| | | | | |
| Sitosterol | | | | |
| 0 | 96.67a (0.0) | 8.52a (0.0) | 11.85a (0.0) | 0.032a (0.0) |
| 1 | 98.33a (-1.72) | 12.74b (-49.48) | 19.36b (-63.36) | 0.010a (68.97) |
| 10 | 100.00a (-3.44) | 12.34b (-44.85) | 20.67b (-74.40) | 0.010a (70.22) |
| 100 | 96.67a (0.0) | 9.62a (-12.83) | 17.30b (-45.98) | 0.009a (70.85) |

Values in the column with the same letter are not significantly different at $p<0.05$.
Values in the parentheses are inhibition percentages over control.
Values in the parentheses with (-) are promotion percentages over control



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## *Weed Management in Rice*

# EFFICACY AND RICE TOLERANCE TO PENOXSULAM+ CYHALOFOP HERBICIDE MIXTURES IN ASEAN COUNTRIES

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**ABSTRACT** Penoxsulam, a triazolopyrimidine sulfonamide rice herbicide, controls *Echinochloa* spp., annual sedges and many broadleaf weeds. Cyhalofop-butyl, an aryloxyphenoxypropionate rice herbicide, controls many grass weeds including *Echinochloa* spp. and *Leptochloa chinensis.* The pre-mix formulation of 10 g ai Penoxsulam + 50 g ai Cyhalofop-butyl/liter (Accept™60OD/TopShot™60OD) and the tank-mix of penoxsulam (Clipper™25OD/Rainbow™25OD)+Cyhalofop-butyl (Clincher ™100EC/Cranstan™100EC) are broad-spectrum herbicide products that are applied post-emergence and provide residual control of many grass, broadleaf and sedge weeds with excellent rice tolerance in ASEAN countries. Combination products containing penoxsulam + cyhalofop-butyl can increase rice productivity in direct-seeded, water-seeded and transplanted rice production systems in. Field research and on-farm demonstration trials were conducted from 1998 to 2011 at many locations across ASEAN countries. In on-farm demonstrations conducted from 2003 to 2011, tank mixes or premix formulations of penoxsulam + cyhalofop-butyl at 10 g ai+50 g ai/ha to12.5 g ai+62.5 g ai/ha, respectively, that were applied post-emergence at 7 to18 days after sowing or transplanting provided >90% control of common weeds in rice. This high level of weed control resulted in a 20 to 50 % yield increase when compared to rice produced in areas not treated with herbicides. Both active ingredients in the mixtures provided excellent *Echinochloa* spp. control. The herbicide mixtures were applied post-emergence at rates up to 5 times the labeled use rate ( 300 g/ha) at 7 to 18 days after sowing or transplanting and did not injure the rice crop or reduce yields. Each herbicide has a different mode of action, so their application is an effective means to manage *Echinochloa* spp. resistance. Penoxsulam + cyhalofop-butyl mixtures provided excellent weed control and with no injury to rice.

***Keywords:*** Penoxsulam, cyhalofop–butyl, efficacy, yield, direct-seed rice, transplanted rice, grasses, sedges, broadleaf weeds.

## INTRODUCTION

Rice is a major food staple for 600 million people in ASEAN countries with a planted area of around 29 million ha in 2009_(FA0) . Rice production must increase to meet demands of an increasing population. One of the most important methods to increase rice production is to minimize crop loss caused by weed competition. Weeds not only reduce rice production but also have an adverse effect on rice grain quality. Since the beginning of agriculture, growers have battled to control rice weeds by a variety of means. In recent times synthetic herbicides have become a common and reliable means farmers use and prefer a single application during the rice growing season to provide broad-spectrum weed control.

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A premix formulation of penoxsulam + cyhalofop-butyl has been developed by Dow AgroSciences and commercialized 2005 as TopShot™ 60 OD in Indonesia, Philippines, Vietnam and Accept™ 60 OD in Thailand. Penoxsulam is an ALS inhibitor in group B1 (triazolopyrimidine sulfonamides) and is a broad spectrum herbicide that controls *Echinochloa* spp, broadleaf and annual sedge weeds. Cyhalofop-butyl is an ACCase inhibitor in Group A (aryloxyphenoxypropionates) that controls grass weeds like *Echinochloa* spp and *Leptochloa* spp. Both herbicides are very safe to rice and have broad spectrum control efficacy. This report is a summary of 106 field trials conducted at locations in Vietnam, Philippines, and Thailand from 1998 to 2011.

## MATERIALS AND METHODS

Field experiments were conducted at Rice Research Institute field stations in Malaysia, Philippines, Indonesia and Vietnam and on farmer fields in Thailand. Experiments were designed as randomized complete blocks with 3 or 4 replications with plot area of 16 to 25 $m^2$. Target crop was direct-seeded or transplanted *Oryza sativa (Indica)* . In wet-seeded rice, water was partially drained from fields and post emergence foliar application made, and paddies were flooded within 48 hours after application. Rice production systems followed local common farming practice.

Tank mixtures o penoxsulam 25 OD (25 g/l) and cyhalofop-butyl 100 EC (100 g/l) were used in experiments during early stages of product development. The herbicide pre-mix, TopShot 60 OD (10 g/l penoxsulam + 50 g/l cyhalofop-butyl) was applied at 1, 1.25 and 1.5 L/ha in a total spray volume of 320 to 400 L/ha with a knapsack sprayer with a flat fan nozzle.

Weeds control (visual assessment of weed biomass reduction) was determined at 14 , 28 and 42 days after application (DAA). Rice injury was recorded at 1, 3, 5,7,14 and 28 DAA by visual assessment based on 1 to 9 injury scale where 1 was no injury and 9 was complete rice plant death. Rice grain was harvested in 5$m^2$ frame in each plot, weighed, and then rice yield was expressed on a per hectare basis. Weed biomass reduction and crop injury data were analyzed using one-way ANOVA.

## RESULTS AND DISCUSSION

**Rice Crop Injury:** Combinations of penoxsulam + cyhalofop at 10 + 50, 20 + 100, or 50 + 250 g ai applied 7 to 18 days after planting (DAP) did not injure rice at 1 to 28 DAA (Table 1).

**Table 1.** Rice injury 1 to 28 days after application (DAA) of penoxsulam + cyhalofop-butyl applied 3to18 days after planting at several locations across ASEAN countries.

| Days after planting | Herbicide | Rates (g ai/ha) | | | No. of obser-vations | Crop injury DAA | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 1 | 3 | 5 | 7 | 14 | 28 |
| **4 to 9** | Penoxsulam+cyhalofop | 10 | + | | 60 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | 20 | + | | 60 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | 50 | + | | 60 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Butachlor + propanil | 225 | + | | 52 | 1 | 3 | 2 | 1 | 1 | 1 |
| **10 to 14** | Penoxsulam+cyhalofop | 10 | + | | 54 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | 20 | + | | 54 | 1 | 1 | 1 | 1 | 1 | 1 |
| | | 50 | + | | 54 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Butachlor + propanil | 225 | + | | 48 | 3 | 4 | 5 | 3 | 1 | 1 |



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** Crop injury based on 1-9 scale with 1 = totally safe and 9 = complete kill.*

**Penoxsulam + cyhalofop tank-mix efficacy**

Penoxsulam at 10, 12.5 and 15 g ai/ha did not provide acceptable control of LEFCH. Application of a mixture of penoxsulam with cyhalofop-butyl provided excellent LEFCH control . Penoxsulam + cyhalofop-butyl at 10+50 or 12.5+62.5 g ai/ha applied 3 to16 days after planting provided more than 85% control of LEFCH (Table 2).

**Table 2.** *Leptochloa chinenses* (LEFCH) control (% biomass reduction) with penoxsulam + cyhalofop tank-mix 28 DAA

| Days after planting | Country | No. of observations | Penoxsulam + cyhalofop (g ai/ha) | | |
|---|---|---|---|---|---|
| | | | **10 + 50** | **12.5 + 62.5** | **15 + 75** |
| **3 to 5** | Vietnam | 12 | 91 | 94 | 100 |
| **6 to 9** | Thailand | 12 | 94 | 100 | 100 |
| | Vietnam | 12 | 90 | 94 | 98 |
| **10 to 12** | Philippines | 12 | 96 | 100 | 95 |
| | Thailand | n8 | 89 | 92 | 95 |
| | Vietnam | 12 | 91 | 94 | 98 |
| **13 to 16** | Philippines | 20 | 96 | 100 | 98 |
| | Thailand | 8 | 70 | 85 | 92 |
| | Vietnam | 16 | 95 | 95 | 99 |

Results from these experiments with tank mixes of penoxsulam + cyhalofop were used to develop premix formulation of penoxsulam + cyhalofop-butyl. Data presented in the following section are from experiments conducted with the premix formulation of penoxsulam + cyhalofop.

**Penoxsulam+Cyhalofop_butyl premix efficacy**

The premix of penoxsulam + cyhalofop at 1.0, 1.25, and 1.5 l/ha provided excellent control (>90%) of LEFCH (Table 3). In general, the 1.0 l/ha rate provided the same level of LEFCH control similar to that achieved with 1.25 and 1.5 l/ha in all experiments across ASEAN countries.

**Table 3**. *Leptochloa chinenses* (LEFCH) control (% biomass reduction) with penoxsulam + cyhalofop premix 28 DAA.

| Days after planting | Country | No. of observations | Rate of product (liter/ha) | | |
|---|---|---|---|---|---|
| | | | **1.0** | **1.25** | **1.5** |
| **4 to 9** | Thailand | 52 | 93 | 95 | 96 |
| | Vietnam | 24 | 92 | 99 | 96 |
| **10 to 14** | Philippines | 40 | 94 | 96 | 97 |
| | Thailand | 60 | 96 | 95 | 97 |
| | Vietnam | 64 | 95 | 99 | 98 |
| **15 to 18** | Philippines | 32 | 95 | 95 | 98 |
| | Vietnam | 12 | - | 88 | 100 |

Penoxsulam + cyhalofop-butyl premix at 1.0 l/ha provided >90% control *Echinochloa crus- galli* ECHCG of 0 to 18 DAP across ASEAN countries (Table 4). The penoxsulam + cyhalofop-butyl premix at 1.0 l/ha could be a good choice for ECHCG resistance management



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across ASEAN. It provides two different modes of action and appears to have a  a wide application window.

**Table 4.** *Echinochloa crus- galli* (ECHCG) control (% biomass reduction) with penoxsulam + cyhalofop premix 28 DAA.

| Days after planting | Country | No. of observations | Rate of product (liter/ha) | | |
|---|---|---|---|---|---|
| | | | **1.0** | **1.25** | **1.5** |
| **0 to 3** | Vietnam | 48 | 98 | 97 | 96 |
| **4 to 9** | Thailand | 52 | 91 | 94 | 97 |
| | Vietnam | 32 | 95 | 97 | 99 |
| **10 to 14** | Philippines | 40 | 94 | 93 | 96 |
| | Thailand | 40 | 98 | 97 | 99 |
| | Vietnam | 64 | 97 | 99 | 99 |
| **15 to 18** | Philippines | 24 | 93 | 98 | 91 |
| | Vietnam | 8 | - | 90 | 100 |

**Table 5.** *Echinochloa colona* (ECHCO) control (% biomass reduction) with penoxsulam + cyhalofop premix 28 DAA.

| Days after planting | Country | No. of observations | Rate of product (liter/ha) | | |
|---|---|---|---|---|---|
| | | | **1.0** | **1.25** | **1.5** |
| **10 to 14** | Philippines | 16 | 96 | 99 | 96 |
| | Thailand | 8 | 93 | 90 | 97 |
| **15 to 18** | Philippines | 8 | 91 | 95 | 97 |

The penoxsulam + cyhalofop-butyl premix at 1.0 l/ha applied 0 to 14 DAP provide very good control of *Cyperus difformis* CYPDI that was similar to efficacy observed with the premix at 1.25 or 1.5 l/ha (Table 6).

**Table 6.** *Cyperus difformis* CYPDI control (% biomass reduction) with penoxsulam + cyhalofop premix 28 DAA.

| Days after seeding | Country | No. of observation | Rate of product (liter/ha) | | |
|---|---|---|---|---|---|
| | | | **1** | **1.25** | **1.5** |
| **0-3** | Vietnam | N=40 | 98 | 96 | 100 |
| **4-9** | Thailand | N=30 | 91 | 92 | 98 |
| | Vietnam | N=8 | 100 | 99 | 99 |
| **10-14** | Thailand | N=70 | 94 | 95 | 95 |
| | Vietnam | N=32 | 97 | 98 | 99 |

The penoxsulam + cyhalofop-butyl premix at 1.0 l/ha provided >90% *Cyperus iria* CYPIR control when applied at 4 to14 DAP of in Thailand and 4 to18 DAP in Philippines.



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**Table 7.** *Cyperus iria* CYPIR control (% biomass reduction) with penoxsulam + cyhalofop premix 28 DAA.

| **Days after planting** | **Country** | **No. of observation** | **Rate of product (liter/ha)** | | |
|---|---|---|---|---|---|
| | | | **1** | **1.25** | **1.5** |
| **4 to 9** | Thailand | 9 | 97 | 98 | 98 |
| **10 to 14** | Philippines | 12 | 85 | - | 90 |
| | Thailand | 24 | 96 | 97 | 97 |
| **15 to 18** | Philippines | 48 | 93 | 95 | - |

The rate of 1.0 l/ha of penoxsulam + cyhalofop-butyl premix provided excellent *Fimbristylis miliacea* FIMMI control (94 to 99%) at 0 to18 DAP in Thailand, Philippines and Vietnam. Control provided by the premix at 1.0 l/ha was similar to level of control observed with 1.25 or 1.5 l/ha. In prior investigations in Vietnam, Thailand and Philippines,10 g a.i./ha of penoxsulam applied at 3 to 18 DAP provided 87 to 90 % FIMMI control (Lap *et al.* 2003).

**Table 8.** *Fimbristylis miliacea* (FIMMI) control (% biomass reduction) with penoxsulam + cyhalofop premix 28 DAA.

| **Days after planting** | **Country** | **No. of observations** | **Rate of product (liter/ha)** | | |
|---|---|---|---|---|---|
| | | | **1** | **1.25** | **1.5** |
| **0 to 3** | Vietnam | 48 | 99 | 97 | 97 |
| **4 to 9** | Thailand | 20 | 96 | 99 | 99 |
| | Vietnam | 40 | 97 | 99 | 98 |
| **10 to 14** | Philippines | 24 | 97 | 98 | 95 |
| | Thailand | 90 | 98 | 98 | 99 |
| | Vietnam | 72 | 95 | 97 | 98 |
| **15 to 18** | Philippines | 48 | 94 | 96 | 97 |

The penoxsulam + cyhalofop-butyl premix at 1.0 l/ha provided excellent *Sphenoclea zeylanica* (SPDZE) **control when applied** 0 to 14 DAP (Table 9).

**Table 9.** *Sphenoclea zeylanica* (SPDZE) control (% biomass reduction) with penoxsulam + cyhalofop premix 28 DAA.

| **Days after planting** | **Country** | **No. of observations** | **Rate of product (liter/ha)** | | |
|---|---|---|---|---|---|
| | | | **1** | **1.25** | **1.5** |
| **0 to 3** | Vietnam | 48 | 97 | 99 | 98 |
| **4 to 9** | Thailand | 52 | 96 | 91 | 96 |
| | Vietnam | 24 | 95 | 98 | 96 |
| **10 to 14** | Philippines | 24 | 100 | 100 | 100 |
| | Vietnam | 40 | 97 | 100 | 99 |



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| | | | | | |
|---|---|---|---|---|---|
| **15 to 18** | Philippines | 40 | 100 | 100 | 100 |

The penoxsulam + cyhalofop premix at 1.0 l/ha provided excellent control (95 to 100%) of MOOVA when applied 4 to 18 DAP in Philippines and Vietnam (Table 10).

**Table 10.** *Monochoria vaginalis* (MOOVA) control (% biomass reduction) with penoxsulam + cyhalofop premix 28 DAA.

| Days after planting | Country | No. of observations | Rate of product (liter/ha) | | |
|---|---|---|---|---|---|
| | | | **1** | **1.25** | **1.5** |
| **4 to 9** | Vietnam | 8 | 100 | 98 | 99 |
| **10 to 14** | Philippines | 24 | 100 | 100 | 100 |
| | Vietnam | 32 | 95 | 98 | 100 |
| **15 to 18** | Philippines | 24 | 100 | 100 | 100 |

**Penoxsulam + cyhalofop-butyl premix effect on rice yield**

Excellent weed control provided by the penoxsulam + cyhalofop premix applied at 1.0 l/ha increased rice grain yield by as much as 121% compared to yield from areas not treated with herbicide (Table 11). Premix rates 1.25 or 1.5 l/ha did not increase rice yields beyond that achieved with the premix at 1.0 l/ha. Rice yield appeared to be greater where the penoxsulam + cyhalofop premix (106 to 121% of yield from untreated areas) was applied compared to rice grain yield from areas treated with the commercial standards, butachlor + propanil or fenoxaprop_ethyl + ethoxysulfuron (96 to 85% of yield from untreated areas, respectively),

**Table 11.** Average rice yield from herbicide-treated and untreated plots.

| Days after planting | Herbicide | Rates (g ai/ha) | No. of observations | Average yield* (ton/ha) | Yield increased compared to untreated |
|---|---|---|---|---|---|
| **4 to 9** | Penoxsulam + cyhalofop | 10 + 50 | 60 | 7.6 | 121 % |
| | | 12.5 + 62.5 | 60 | 7.6 | 122 % |
| | | 15 + 75 | 60 | 7.7 | 124 % |
| | Butachlor + propanil | 225 + 225 | 52 | 6.7 | 96 % |
| | | Untreated | 60 | 3.4 | 0 % |
| **10 to 14** | | 10 + 50 | 54 | 7.3 | 106 % |
| | | 12.5 + 62.5 | 54 | 7.5 | 113 % |
| | | 15 + 75 | 54 | 7.6 | 114 % |
| | Fenoxaprop_ethyl + ethoxysulfuron | 34.5+ 10 | 48 | 6.5 | 85 % |
| | | Untreated | 54 | 3.6 | 0 % |

* *Calculated from harvested yield in 5m$^2$/ plot.*



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# CONTROL OF *ECHINOCHLOA COLONA*IN AEROBIC RICE: EFFECT OF DIFFERENT RATES OF SEED PADDY AND POST-PLANT HERBICIDES IN THE DRY ZONE OF SRI LANKA

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**ABSTRACT "**Aerobic rice" helps addressing water scarcity and environmental safety in a variable and changing climate. However, competition due to weeds is the major biotic constraint, and herbicides are the most commonly used weed control technique. A field experiment was conducted to identify a suitable weed management strategy to reduce the yield losses caused by *Echinochloacolona*, the dominant grass weed in aerobic rice (*Oryza sativa*) var. Bg300, under aerobic conditions. Three rates of seed paddy (100, 150 and 200 kg/ha) and post-plant herbicides (pretilachlor, 3-4 DPA and bispyribac-sodium at recommended rate) were evaluated with an un-weeded control in a factorial arrangement, in a randomized complete block design with three replicates. Three grams of *E. colona*seeds per plot was sown to provide a uniform soil seed bank. All other grass weedswere removed manually at weekly intervals, and sedges and broadleaves by application of MCPA at 21 days after sowing (DAS). Rainfall received during the experimental period was only 35.9 mm, and the crop was irrigated to field capacity at five-day intervals. There was no significant interaction (p>0.05) of the two factors on all parameters measured. Bispyribac-sodium showed the highest reduction of weed dry weight (94% at 40 DAS; p<0.05) compared to the un-weeded control. The post-plant herbicides 3,4 DPA, bispyribac-sodium and pretilachlor increased the rice yield by several fold (*i.e.*3.92, 7.84 and 6.36 fold, respectively; p<0.05) compared to the un-weeded control (0.25 t/ha). The higher rates of seed paddy (150 and 200 kg/ha)showed a decrease in grain yield (0.49 and 0.33 t/ha, respectively) compared to the recommended seed rate (100 kg/ha).The results showed that *E. colona* significantly reduced the yield of aerobic rice. Application of bispyribac-sodium or3,4 DPA at recommended rates with seed paddy used at 100 kg/ha is effective in control of *E. colona*in aerobicrice.

***Keywords*:** Aerobic rice, *Echinochloa colona*, rates of seed paddy, post-plant herbicides

## INTRODUCTION

Rice (*Oryza sativa*) requires moist growing conditions making it unsuitable to grow in areas with low rainfall unless the rice fields are provided with supplementary irrigation. The major advantage of growing rice under submerged conditions is the weed control. Flooded fields have less available oxygen for plant roots and thus, prevent germination and growth of weeds that would compete with the rice plant for basic requirements of growth and development. Standing water is also known as a deterrent to insects such as the army worm and chinch bug that threaten rice, and helps to prevent excessive salt accumulation and soil depletion. Standing water also offers a temperature control for the crop. However, conditions of the monsoons have totally changed due to climate change, resulting in change in the onset and high variability resulting drier dry areas and wetter wet areas (Marambe *et al*., 2012). All areas of the dry zone are highly vulnerable to droughts where the decreasing water availability would lead to reductions in both cultivated and harvested rice extents. Less water availability of minor and major irrigation tanks would also limit the cultivable land area (Gamage, 2002).

With the increase in frequency and severity of drought events during the recent past, a cropping system which can produce adequate amount of rice under low water availability is essential to sustain feeding the increasing population. In this context, aerobic rice has been



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introduced as a fundamentally different concept of growing rice with referred to as growing high yielding rice grown in non-puddled and non-flooded fields under low irrigation and external inputs (Wang *et al*., 2002;Bouman *et al*., 2005). In aerobic rice, soil water is maintained around field capacity in the root zone either by rainfall or irrigation, where as the traditional irrigated lowland rice production requires continuous flooding and relatively high water inputs. At present, rice is also grown under rainfed systems in Sri Lanka with alternate wetting and drying cycles. Aerobic rice consumes less than 50% of the water compared to lowland rice, and increases water productivity up to 0.6 kg/ $m^3$ from 04 kg/$m^3$ (Bouman *et al*., 2005; Wang *et al*., 2002).

Aerobic rice experiences higher weed pressure (Balasubramanian and Hill, 2002) when compared to the flood irrigated rice mostly due to lack of 'head start' over weeds and absence of a standing water layer to suppress weeds (Moody, 1983; Zhao *et al*., 2006). Weed infestation has been reported as a major threat to the success of aerobic rice cultivation and can reduce yield by 38-92%.The composition of weed flora and their abundance in aerobic rice is different to that of puddled flooded rice system (Mahajan *et al*., 2009).Developing a sustainable weed management strategy has been a challenge for widespread adoption of aerobic rice. Phuong *et al*.(2005) have shown that increasing the seed rate can strengthen the ability of the crop to suppress weeds and increase crop yields by reducing weed densities in low land rice. The efficacy of herbicides under aerobic soil conditions still requires screening done through scientific protocols in order to achieve a successful weed management in this system. However, the information on weed control under aerobic rice under Sri Lankan condition is scarce. Knowing the significance of the production system in the dry zone of Sri Lanka under variable and changing climate, the present study attempted to determine a suitable weed management strategy by changing seed rates and applying different post-plant herbicides to reduce the yield loss due to competition from *Echinochloa colona*, a dominant grass weed in aerobic rice.

## MATERIALS AND METHODS

The field experiment was carried out during the *Yala* season at the Mahailluppallama sub campus of the Faculty of Agriculture, University of Peradeniya, Sri Lanka. The study site was located in the Anuradhapura district in the low country Dry zone ($DL_{1b}$) of Sri Lanka (8° 05' 53 N 80° 26' E, Elevation 53 m amsl). The soil was the Reddish Brown Earth (RBE) with the pH of 6.9. The experiment was conducted as a two factor factorial with (a) three rates of seed paddy (*i.e.* 100, 150 and 200 kg/ha) and (b) three post-plant herbicides (*i.e.* pretilachlor at 0.48 kg a.i./ha; 3,4 DPA at 2.7 kg a.i./ha; bispyribac sodium at 0.03 kg a.i./ha) as the main factors. The herbicides were applied at the rates recommended by the Department of Agriculture of Sri Lanka when the grass weed were at 2-3 leaf stage, in a randomized complete block design with three replicates. An un-weeded control was maintained for comparison of data. The experimental plots were at the size of 6 m (length) × 3 m (width), and were separated by using bunds of 0.4 m width and 0.3 m height.

Pre-germinated seeds of the rice variety Bg300 were broadcasted evenly to the plots. Three grams of *Echinochloa colona* seeds (containing around 1500 seeds) was sown to each plot to facilitate a uniform soil seed bank. Previous observations confirmed that the experimental site selected was not infested with *E. colona* but dominant with sedges and broadleaf weeds. Irrigation was done up to the field capacity by using a water pump at five day intervals while maintaining the aerobic conditions. Soil moisture was measured gravimetrically before each irrigation. The rainfall received during the 3-months long experimental period was only 35.9 mm. Fertilizer management and pest and disease control were carried out as recommended by the Department of Agriculture. When emerged, grass weeds except *E. colona* were manually removed in weekly intervals, and the herbicide M.C.P.A was used to control broadleaf weeds at 21 days after sowing (WAS).

Weed count and dry weight of weeds were taken by using a 50 cm ×50 cm quadrate. The total leaf area of rice plants in a 20 cm × 20 cm quadrate was measured using a portable



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leaf area meter and leaf dry weight was taken after oven drying. The plant dry weight of rice was taken after oven drying of destructive samples within the same quadrate. The weed and crop growth measurements were taken at 20, 30, and 40 DAS. Dry weights were obtained by oven drying samples at 70 °C to a constant weight and weighed using top loading balance. The yield components of rice namely, the number of panicles and number of grains per panicle were counted in an area of 50 cm ×50 cm quadrate. The filled grain percentage, 1000 grain weight and total harvest were also measured. Data collected were statistically analyzed by using the SAS computer software package.

## RESULTS AND DISCUSSION

### Weed dry weight

There was no significant interaction effect ($p>0.05$) between the two factors tested on the total weed dry weight. The un-weeded control had the highest weed dry weight even at 20 DAS and increased with time (Fig. 1). At 40 DAS, the post-plant herbicides tested was effective ($p<0.001$) in weed control in aerobic rice plot recording the highest weed dry weight however, there were no significant difference among the three herbicides ($p>0.05$). The results indicated that the post-plant herbicides evaluated in this study, which were recommended by the Department of Agriculture for conventional lowland rice cultivation, can also be effectively used for weed control in aerobic rice.

Changing the seed rate did show a significant impact on the total weed dry weight at all sampling dates ($p>0.05$; Fig. 2).The rate of seed paddy also exhibited a significant influence on weed density (data not presented). The results are in agreement with Nice *et al*. (2001) and Phuong *et al*. (2005) who confirmed that reduced crop densities may provide a suitable environment for weed growth and may enhance the survival and pressure of weed.

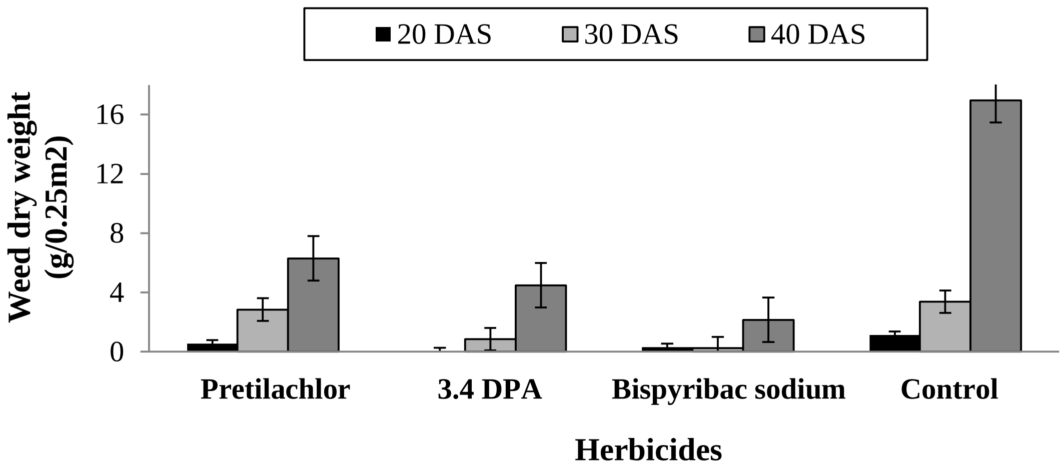


**Fig 1**. The change in total weed dry weight over time under herbicide treatments. Vertical bars represent the standard error of the mean.



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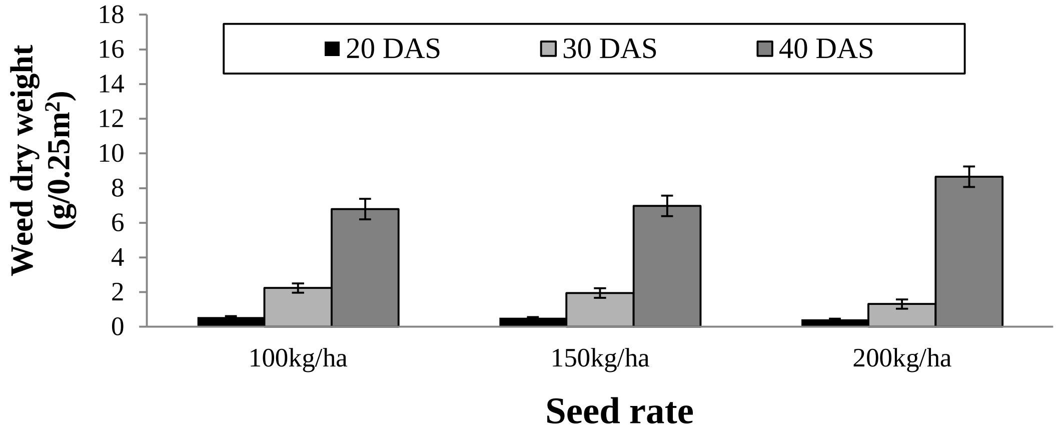


**Fig 2.** The change in total weed dry weight over time under different seed rates. Vertical bars represent the standard error of the mean

**Leaf area index and total dry weight of rice**

There was no significant interaction effect between the herbicides and the rate of seed paddy on the leaf area index and total dry weight of rice ($p>0.05$; Table 1). The LAI of the rice increased with the date of sampling in all treatments, and was significantly different among the herbicide treatments ($p<0.05$), among different seed rates ($p<0.001$) and were higher compared to that of the control at all sampling dates. The LAI was the lowest on pretilachlor-treated plots ($p<0.05$). The higher plant dry weight observed in plots treated with 3.4. DPA and bispyribac sodium was under lower weed density and weed dry weight. The total plant dry weight followed a similar pattern to that of the LAI in response to different herbicide treatments.

Use of a seed paddy rate of 200 kg/ha, which is twice as the recommended rate, showed the highest LAI and 100 kg/ha showed the lowest among the seed rates. Despite this, the rate of seed paddy did not have a significant impact ($p>0.05$) on the total plant dry weight suggesting intra-specific competition by rice plants for limited resources.

**Table 1.** Leaf area index and dry weight of rice under different treatment combinations at 40 DAS.

| Treatment | Leaf Area Index | Plant Dry Weight(g/0.04m$^2$) |
|---|---|---|
| Un-weeded control | 3.07 ± 0.18 | 6.62 ± 1.04 |
| Post-plant herbicides | | |
| Pretilachlor | 3.17 ± 0.31 | 7.62 ± 0.82 |
| 3.4 DPA | 3.53 ± 0.11 | 8.73 ± 0.54 |
| Bispyribac sodium | 3.70 ± 0.26 | 9.65 ± 0.32 |
| Seed rates | | |
| 100 kg/ha | 2.58 ± 0.11 | 7.15 ± 0.96 |
| 150 kg/ha | 3.82 ± 0.20 | 7.83 ± 0.77 |
| 200 kg/ha | 3.69 ± 0.24 | 8.59 ± 0.69 |

**Values are presented as mean ± standard error**

**Yield components of rice**

The interaction effect between the herbicides and seed rates were not statistically significant on the number of panicles per sq. meter ($p>0.05$). A significant impact of herbicides on the panicle number was observed ($p<0.05$) where 3.4 DPA-treated plots had the highest panicle number with the un-weeded control plots having the lowest (Table 2).



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**Table 2**. Selected yield components of rice and the final yield.

| Treatment | No. of seeds per panicle | Filled grain % | Grain yield (kg/ha) |
|---|---|---|---|
| Un-weeded control | 30 ± 6 | 74 ± 2 | 250 ± 26 |
| Post-plant herbicides | | | |
| Pretilachlor | 52 ± 9 | 82 ± 4 | 980 ± 43 |
| 3.4 DPA | 75 ± 8 | 87 ± 5 | 1960 ± 82 |
| Bispyribac sodium | 72 ± 8 | 84 ± 3 | 1590 ± 58 |
| Seed rates | | | |
| 100 kg/ha | 62 ± 4 | 83 ± 6 | 1390 ± 81 |
| 150 kg/ha | 58 ± 6 | 72 ± 4 | 900 ± 36 |
| 200 kg/ha | 56 ± 6 | 87 ± 4 | 1060 ± 34 |

*Values presented as mean±standard error

There was no significant interaction effect between the two factors evaluates on the yield and yield components of rice ($p>0.05$; Table 2). The un-weeded control showed the lowest number of seeds per panicle, filled grain % and the final grain yield.

The number of seeds per panicle was significantly increased ($p<0.001$) with the use of different herbicides and different seed rates compared to that recorded in the un-weeded control. Among the herbicides tested, pretilachlor showed a significantly lower number of seeds per panicle ($p<0.05$) but there was no significant difference between3.4 DPA and bispyribac sodium (Table 2). The number of seeds per panicle of rice plants was not significantly affected by the rate of seed paddy used ($p>0.05$).The filled grain % of rice was significantly increased by the herbicide treatments ($p<0.05$)when compared to the un-weeded control. Plots sown with 100 kg/ha and 200 kg/ha showed statistically similar results where as those with 150 kg/ha showed a significantly lower filled grain % ($p<0.05$) compared to the rest of the seed rates. The 1000 grain weight did not show any significant difference among the treatments (data not shown).

The grain yield was increased significantly by the use of herbicides ($p<0.001$) compared to the un-weeded control, while3.4 DPA-treated plots recording the highest (Table 2). Plots treated with 3.4 DPA and bispyribac sodium recorded statistically similar grain yield ($p>0.05$) but significantly higher yield when compared to those treated with pretilachlor ($p<0.05$). The rate of seed paddy showed a significant impact on grain yield, too ($p<0.05$). Use of a seed rate of 100 kg/ha resulted in the highest and 150kg/ha the lowest grain yield ($p<0.05$) (Figure22). No significant difference was observed between the seed rates of 100 kg/ha and 200 kg/ha ($p>0.05$).

Increasing in rice grain yield observed in this experiment with effective weed control may be attributed to better crop growth and reduced weed-crop competition (Akbari *et al*., 2011). Improvements in yield components including the number of panicle bearing tillers (data not shown) and seeds per panicle in the treated plots compared to the control would have resulted in an improved grain yield (Jabran *et al*., 2008; Jabran *et al*., 2010). The grain yield was significantly higher in all the plots with herbicide applications. The post-plant herbicides 3.4 DPA, bispyribac sodium and pretilachlor increased the grain yield of rice by several fold (*i.e.* 3.92, 7.84 and 6.36 fold, respectively) over the control (0.25 t/ha), in line with the weed control efficacy shown by the herbicides. However, 3.4 DPA showed a higher number of panicles per plant as well as a lower 1000 seed weight and pretilachlor showed the lower number of seeds per panicle as well as relatively higher 1000 seed weight (data not shown; results are not statistically significant).

Anwar *et al*. (2011) reported that the highest number of panicles/$m^2$ was accompanied by the lowest filled grains/panicle and 1000 grain weight resulting in the low grain yield. In the present study, the increase in the rate of seed paddy has resulted in no significant increase of grain yield, but a reduction in yield by 0.47 and 0.33 t/ha at 150 and 200 kg seed paddy/ha.



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Findings of Zhao *et al*. (2006), Mahajan *et al*. (2009) and Anwar *et al*. (2011) are in contrast with the results of the present study. However, Baloch *et al*. (2002) reported that under increased plant density, intra-specific competition for light and nutrient would lead to reduction in grain yield.

The results revealed that *Echinochloa colona* significantly reduce rice growth and development and the final yield under aerobic condition highlighting the importance of its management to reap richer harvests. Among the treatments used, application of 3.4 DPA or bispyribac sodium at the recommended dosage at 2/3 leaf stage of the weed combined with the recommended rate of seed paddy at 100 kg/ha is an effective integrated weed management strategy for the control of *E. colona* in aerobic rice.

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# EFFECT OF PROPYRISULFURON ON GROWTH AND ACETOLACTATE SYNTHASE ACTIVITY OF FIVE WEED SPECIES AND THREE RICE (*ORYZA SATIVA* L.) CULTIVARS

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**ABSTRACT** Propyrisulfuron, a recently developedsulfonylurea, has shown high activity against broadleaf weeds, sedges, and grasses, unlike older sulfonylureas which are activeonly against broadleaf weedsand sedges. Greenhouse and laboratory studies wereconducted to determine if the differential response of three rice (*Oryza sativa*) cultivars and five weed species to propyrisulfuron is a function of its acetolactate synthase (ALS) activity. Propyrisulfuronat 50 to 200 g ai $ha^{-1}$inhibited *in vitro* ALS activity of both rice (tolerant) and weeds (susceptible) indicating that their differential response to the herbicide is not due to differences at the target site.Inhibition of*in vivo* ALS activitywas significantly greater in weeds than in rice, which increased progressively over a 6-day period and was greater in two-leaf than in five-leaf seedlings. At 6 DAT, shoot fresh weights of weed seedlings were reduced by 95 to 99%in *Ludwigiahyssopifolia*, *Cyperusiria*, *Echinochloacrusgalli*, and *Echinochloacolona* and by 80% in*Leptochloachinensis* resulting in plant death. Lower inhibition of ALS activity in rice caused slight to moderate reduction in shoot fresh weight from which rice recovered, with*aus*cultivar more tolerant than*indica* or *japonica* cultivars. Addition of valine, leucine, and isoleucine alleviated the inhibitory effects on *in vivo*ALS activity and seedling growth of the five weeds. Our studies confirm the susceptibility of grasses, broadleaf weeds and sedges to propyrisulfuron, with high selectivity to rice. Our results also show that propyrisulfuron can be used for control of weedsinfesting rice as additional option for rice farmers using herbicide rotation, a practice recommended to avoid or delay evolution of herbicide-resistant weeds.

***Keywords*:** mode of action,sulfonylureas,selectivity, tolerance, susceptibility, ALS inhibitor

## INTRODUCTION

Weeds reduce yields by 34% in transplanted rice (*Oryza sativa* L.), 50% in direct-seeded rice and 70% in upland rice. Among the more than 100 weed species growing withrice, the most troublesome are *Echinochloacrusgalli*, *Echinochloacolona*, *Leptochloachinensis*, *Ludwigiahyssopifolia* and *Cyperusiria* (Caton*et al*, 2004). About US$28 billion in global herbicide sales, constituting 47% of the worldwide total sale of agrochemicals, indicates the significant economic impact of weeds on agriculture (Singh *et al*., 2006).

The sulfonylureas were developed in the 1970s and are widely used to control broadleaf weeds and sedges infesting rice, starting withbensulfuron in the 1980s (Beyer et al, 1988; Takeda et al, 1986). Sulfonylureas inhibitacetolactate synthase (ALS), the key enzyme catalyzing the first common step in the biosynthesis of branched-chain amino acids, valine, leucine, and isoleucine(McCourt *et al*, 2006; Ikeda *et al*, 2011a). ALS inhibition causes rapid cessation of cell divisionresulting in growth inhibition and plant death (Yoon *et al*., 2003).However, continuous use of bensulfuron and other sulfonylureas for weed control in rice for the past 20 years has resulted in the evolution of sulfonylurea-resistant weeds in California, Australia, Japan, Korea and other rice-growing countries (Itoh et al, 2005; Valverde and Itoh, 2001; Uchino, 2011; Heap 2012).

Propyrisulfuron is a new pyrimidinylsulfonylureadeveloped in 2008 for weed control in ricewhich has high activity not only against broadleaf weeds and sedges but also against grasses



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(Ikeda *et al*, 2011a). It has a fused heterocyclic moiety bonded to a sulfonyl group, a property which was shown to have high activity against sulfonylurea-resistant weeds (Tanaka et al, 2006; Ikeda et al 2011a).Due to the increasing rate of evolution of sulfonylurea-resistant weeds, use of herbicide rotation is now a recommended practice in managing herbicide resistance. Use of propyrisulfuron will broaden the weed control spectrum to include grasses as well as broadleaf weeds and sedges. It will also serve as additional herbicide option to enable rice farmers to practice herbicide rotation in the management of sulfonylurea-resistant weeds.

Thisstudy was conducted to determine if the differential responsesin rice and weeds to propyrisulfuronis a function of itsALS activity.The specific objectives were to determine the effect of different rates and times of application of propyrisulfuronon: 1) *in vitro* and *in vivo* ALS activities of rice and weeds, 2) *in vivo*ALS activity over time in rice and weeds; 3) early seedling growth of rice and weeds;and 4) the effect of exogenous addition of branched chain amino acids valine, leucine, isoleucine on *in vivo*ALS activity and seedling growth of rice and weeds treated with propyrisulfuron.

## MATERIALS AND METHODS

### Seed collection and site of study

Seeds of three rice cultivars;IR-64 (*indica*), Azucena (*japonica*) and N22 (*aus*) were obtained from the International Rice Genebank Collection at the International Rice Research Institute (IRRI), Los Banos, Laguna, Philippines. Seeds of weeds (*E. colona*, *E. crusgalli*, *Lchinensis*, *L.hyssopifolia*, *C.iria*)were obtained from the weed seed collection of the Weed Science laboratory at IRRI.All studies were conducted at the greenhouse and laboratory facilities of the IRRI Weed Science and Plant Physiology Division from December 2011 to December 2012.

### Study 1. Effect of propyrisulfuron on seedling growth of rice and weeds

Rice and weed seeds were sown 1 cm deep in plastic containers (100 mm x 100 mm x 70 mm) filled with sterilized soil. Plants were grown in the greenhouse under natural conditions (27 C/30C, night/day temperatures) and watered daily. At 5-6 days after emergence, the seedlings were thinned to one plant per pot.At the two-leaf stage, propyrisulfuron 10 SCwas sprayedon rice and weed seedlings at 0, 12.5, 25, 50, 100 and 200 g ai $ha^{-1}$using a stationary belt sprayercalibrated to deliver 214 L $ha^{-1}$. At 2, 4 and 6 days after treatment (DAT), seedling heights were measured. At 6 DAT, seedlings were harvested and separated into roots and shoots and fresh weights of shoots were recorded.

### Study 2. Effect of propyrisulfuron on *in vitro* and *in vivo* ALS activitiesof rice and weeds

*Preparation of plant samples*

Rice and weeds were grown in the greenhouse as described in Study 1. For the *in vitro* assay, seedlings were grown to two-leaf stage, then shoots were harvested and leaf tissues cut into small pieces and stored at -20 $^{o}$C prior to ALS extraction and assay. For the *in vivo* assay, seedlings were treated with 0, 12.5, 50, 100 and 200 g aiha$^{-1}$propyrisulfuron at the two-leaf and five-leaf stages. At 21 hrs after treatment, seedlings were sprayed with 766 g $ha^{-1}$ of 1,1-cyclopropanedicarboxylic acid (CPCA) containing 0.25% v/v non-ionic surfactant Tween 20 (sorbitanmonolaureate). After 3 hrs, seedlings were harvested. Leaf tissues were cut into small pieces and frozen at -20 $^{o}$C for 24 hrs prior to ALS extraction and assay.

*ALS extraction and determination of ALS concentration*

Extraction of ALS was done following the method of Yoon *et al*. (2003). All operations were carried out at 0-4$^{o}$C with minimum exposure to sunlight. The frozen leaf tissues were suspended in 5 mL $g^{-1}$ of ice-cold standard buffer (20 mM potassium phosphate (pH 7.5) containing 20% by volume glycerol, 1 mM magnesium chloride, 0.25 mMdithiothreitol, 0.1 mM TPP and 0.01 mM FAD). The mixture was homogenized in a Teflon-in-glass potter homogenizer and 10 mg $mL^{-1}$ polyvinyl polypyrrolidone was added. The mixture was centrifuged at 20,000 *g* for 15 min



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and the supernatant containing ALS was collected.ALS concentration was determined following the method of Bradford (1976) using bovine serum albumin (BSA) as standard. Protein solution (50 µL) was pipetted into microfuge tubes, added with 850 µL distilled water and 100 µL Bradford reagent. After 5 min,absorbance was read at 595 nm using a UV-Vis Shimadzu 1800 spectrophotometer.

**Study 2a. *In vitro*ALS assay**

The assay was conducted following the method ofHwang *et al*. (2000). All operations were carried out at 0-4 $^oC$. One mL crude enzyme extract in 0.9 mL of 50 mM potassium phosphate buffer (pH 7.5), (10 mM magnesium chloride, 0.1 mM TPP, 10 µM FAD and 10 mM sodium pyruvate) was added with 0.1 mL of 5, 10, 15, 20 and 25 nMpropyrisulfuron. To terminate the reaction, 0.05 mL of 6 N $H_2SO_4$was added. For decarboxylation ofALS, the reaction mixture was heated at30 $^oC$ for 15 min, added with 0.5 mL of 0.5% (w/v) creatine and 5% (w/v) 1-napthol dissolved in 10% NaOH, and heated at 60 $^oC$ for 15 min, thencentrifuged at 3000*g*for3 min. Absorbance of the supernatant was measured at 530 nm using a UV-Vis Shimadzu 1800 spectrophotometer. A standard curve using acetoin was plotted to quantify enzyme reaction products. Specific enzyme activity was determined from the standard curve. One unit of enzymatic activity is defined as the amount of acetoinrequired to form 1 nmol/hr of acetolactate. The $I_{50}$ values were obtained from the dose response curve.

**Study 2b. *In vivo* ALS assay**

The assay was conducted using the procedure ofUchino et al. (2007).Frozen leaf tissues (0.1 g) were added with 3 mL distilled water and homogenized in a vortex mixer at 25°C for 45 min.Leaf tissue residue was discarded and the 3 mL homogenate aliquot was treated with 50 µL 6N $H_2SO_4$, mixed for 20 sec and incubated at 60°C for 30 min to allow conversion of acetolactate to acetoin. To stop the reaction, 1 mL aliquot of creatine and α-naphthol solution (0.09 and 0.9 w/v, respectively) in 2 N NaOH was added and stirred in a vortexmixer for 10 seconds. The solution was placed in a 60°C water bath for 30 min to allow color change from white to pink/red, with higher color intensity indicating higher amount of acetoin, hence, higher ALS activity. Samples were cooled down to ambient room temperature and centrifuged at 10,000 *g* for 5 min. Absorbance of the supernatant wasread at 530 nm with a UV-Vis Shimadzu 1800 spectrophotometer, using leaf extracts from untreated control plants(without herbicide and CPCA) as background. The absorption values were converted into µg acetoin/hr-g foliage fresh weight using a standard curve.

**Study 2c. *In vivo*assay ofALS activity over time**

Plants were grown in the greenhouse in the same manner as described in Study 1. At the two-leaf stage, seedlings were treated with 50 g aiha$^{-1}$propyrisulfuron. At 2, 4 and 6 DAT, seedlings were harvested and shoots were separated from the roots. Leaf tissues were cut and stored at -20 $^oC$ prior to ALS extraction and assay. At each sampling time (2,4 and 6 DAT), the *in vivo* ALS activity was assayed as described in Study 2b.

**Study 3. Effect of adding exogenous valine, leucine and isoleucine on seedling growth and ALS activity of propyrisufuron-treated rice and weeds**

Rice and weeds were grown in the greenhouse similar to those described in Study 1. At the two-leaf stage, the seedlings were sprayed with 50 and 100 g aiha$^{-1}$propyrisulfuron and added with 10, 50 or 100 ppm each of valine, leucine and isoleucine or 100 ppm each of valine and isoleucine. Control plants (with propyrisulfuron but without valine, leucine and isoleucine) were grown for comparison. At 2,4, and 6 DAT, visual phytotoxicity ratings and plant heights were measured. At 6 DAT, the seedlings were harvested and shoot fresh weights were recorded. A separate set of plants, grown in the same manner as those described above, were treated with 50 g aiha$^{-1}$propyrisulfuron and added with10, 50 or 100 ppm each of valine, leucine, and



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isoleucine, or 100 ppm each of valine and isoleucine. At 6 DAT, seedlings were harvested and leaf tissues were cut and stored at -20°C prior to*in vivo*assay of ALS activity.

**Experimental design and data analysis**

Pots in all studies were arranged in a completely randomized design with three replications per treatment. Each replication was an average of three repeat measurements. ANOVA was done using SAS version 9.1.3 PROC MIXED and PROG REG (SAS Institute Inc., Cary, North Carolina). Treatment means were compared using LSD at $P \leq 0.05$. Herbicide concentration causing 50% inhibition ($I_{50}$) of ALS activity, 50% growth reduction ($GR_{50}$) of plant height and shoot fresh weight, and 50% protein concentration reduction ($ED_{50}$) were estimated using Sigma Graph 2005-2010 SIDI.CC data graphing and analysis software employing non-linear computer analysis based on the log-logistic model(Seefeldt*et al.*, 1995). To obtain dose response curves, data for each replication were regressed using the following model: $y = C + (D-C)/(1 + \exp((\log ED_{50} - \log X) b)$. Quadratic equation provided the best fit for regression analysis, as reflected by good coefficient of determination ($R^2$).

## RESULTSAND DISCUSSION

**Study 1. Effect of propyrisulfuronon seedling growth of rice and weeds**

The rice seedlings showed slight to moderate reduction in plant height (data not shown) and shoot fresh weight (Table 1). Shoot fresh weightof N22 was reduced by1 to 35% whileIR-64 had about 50% reduction at the highest rate (Table 1). Azucena was the least resistant with 65%to 70% reduction at the two highest rates (Table 1). Of the 3 cultivars, N22 was more tolerant than either Azucena or IR-64. All rice the seedlings recovered and had normal growth at 14 DAT (data not shown).All the weed seedlings had greater reduction in plant height (data not shown) and shoot fresh weight compared to those in the rice seedlings (Table 1). At 6 DAT,*E. crusgalli*, *E, colona*, *L. hyssopifolia* and *C. iria* had 95 to 99% reduction at 50 g ai $ha^{-1}$, while it took about 100 to 200 g ai$ha^{-1}$ to reduce the shoot fresh weight of *L. chinensis* by 80% (Table 1).At 6 DAT, seedlings of all five weed species were killed. Ikeda *et al.* (2011b) also reported that 90 g ai/ha propyrisulfuron showed high activity against grasses such as *Echinochloaoryzicola*, *E. crusgalli*, *E. crusgalli* var. *formosensis*, sedges such as *C. difformis*, *C. serotinus*, and broadleaf weeds such as *Monochoriavaginalis* at one-leaf to six-leaf stages.

**Table 1.** Shoot fresh weights of rice and weed seedlings treated with increasing concentrations of propyrisulfuronandgrown in the greenhouse[1].

| Propyrisulfuron (g ai$ha^{-1}$) | Shoot fresh weight (g $plant^{-1}$) at 6 DAT and percent reduction (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *E. colona* | *E.crus-galli* | *L. chinensis* | *C. iria* | *L. hyssopifolia* | IR-64 | Azucena | N22 |
| 0 | 4.0 (0) | 2.8 (0) | 1.46 (0) | 2.27 (0) | 1.57 (0) | 2.98 (0) | 2.21 (0) | 2.04 (0) |
| 12.5 | 2.2 (45) | 1.7 (38) | 1.09 (25) | 1.39 (38) | 0.92 (41) | 2.84 (4) | 1.89 (10) | 2.01 (1) |
| 25 | 1.8 (55) | 1.3 (55) | 0.96 (34) | 1.02 (55) | 0.62 (60) | 1.73 (42) | 1.60 (24) | 1.92 (5) |
| 50 | 0.13 (97) | 0.11 (95) | 0.51 (65) | 0.08 (96) | 0.08 (94) | 1.54 (8) | 0.73 65() | 1.62 (20) |
| 100 | 0.05 (98) | 0.08 (97) | 0.32 (78) | 0.04 (98) | 0.07 (95) | 1.34 (55) | 0.64 (69) | 1.37 (32) |
| 200 | 0.03 (99) | 0.06 (98) | 0.28 (81) | 0.04 (98) | 0.04 (97) | 1.31 (56) | 0.63 (70) | 1.32 (35) |

[1]Numbers in parentheses indicate percent reduction in shoot fresh weight based on non-treated control

**Study 2. Effect propyrisulfurononin *in vitro* and *in vivo*ALS activity of rice and weeds**

The *in vitro* ALS activity of both rice and weeds were inhibited by propyrisulfuron at all rates, with similar $I_{50}$ values, ranging from 19 to 22 nM (Table 2). This indicates that propyrisulfuron inhibits ALS activity *in vitro* regardless of species, growth stage, and plant tolerance or



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susceptibility. In other studies, similar $I_{50}$ values in *in vitro*ALS activity assay of rice and the weeds*C. serotinus*, *E. crusgalli* and *E oryzicola* treated with cinosulfuron, imazosulfuron, and flucetosulfuron were reported, regardless of tolerance or susceptibility to these herbicides(Park *et al*, 1993; Tanaka and Yoshikawa 1998; Hwang *et al*, 2000).

**Table 2**. *In vivo* and *in vitro*ALS activity ($I_{50}$) of rice and weeds treated with increasing rates of propyrisulfuron at two-leaf and five-leaf stages.

| PLANTS | $I_{50}$ values[1] *In vitro*, nM (two-leaf) | *In vivo*, g a.i./ha (two-leaf) | *In vivo*, g a.i./ha (five-leaf) |
|---|---|---|---|
| *E.colona* | 22.40 ± 0.27 | 67.93 ± 2.32 | 75.32 ± 1.83 |
| *E.crus-galli* | 22.22 ± 0.17 | 66.88 ± 2.53 | 74.04 ± 0.77 |
| *L.chinensis* | 22.10 ± 0.12 | 73.01 ± 2.52 | 76.58 ± 2.67 |
| *C.iria* | 22.16 ± 0.27 | 42.02 ± 4.42 | 67.87 ± 4.51 |
| *L.hyssopifolia* | 19.03 ± 0.47 | 31.68 ± 1.39 | 65.34 ± 4.57 |
| IR-64 (*indica*) | 22.05 ± 0.23 | 80.21 ± 2.30 | 86.94 ± 2.32 |
| Azucena (*japonica*) | 21.81 ± 0.71 | 72.74 ± 1.58 | 81.04 ± 4.84 |
| N22 (*aus*) | 21.93 ± 0.68 | 87.15 ± 3.43 | 92.63 ± 3.16 |

[1]$I_{50}$ indicates the effective herbicide dose that inhibited ALS activity by 50% based on non-treated control

In contrast, inhibition of *in vivo* ALS activity was much greater in all the weed species and only slight inhibition of *in vivo* ALS activity occurred in the rice seedlings (Table 2). Greatest inhibition occurred in *L. hyssopifolia* and *C. iria*, followed by*E. crusgalli* and *E. colona*,while*L. chinensis* showed the least inhibition (Table 2). Among the rice cultivars, N22 showed the least inhibition, IR64 had moderate inhibition whileAzucena showed the highest inhibition (Table 2). In both rice and weeds, there was greater reduction in *in vivo* ALS activity in plants treated with propyrisulfuron at the two-leaf stage than in plants treated at the five-leaf stage (Table 2).Tanaka and Yoshikawa (1998) also observed higher *in vivo* ALS activity in older rice than in younger rice seedlings treated with imazosulfuron.

**Study 2c. Effect of propyrisulfuron on *in vivo* ALS activity over time**

Inhibition of in vivo ALS activity in both rice and weeds increased progressively over a 6-day period, as reflected in decreasing amounts of acetoin from 0 to 6 DAT (Fig 1). Fastest inhibition occurred in *L. hyssopifolia* and *C. iria*, followed by *E. crusgalli*, *E. colona* and slowest inhibition in *L. chinensis* (Fig 1). *In vivo* ALS activity in weeds continued to decrease and at 6 DAT, all the weed seedlings were killed. There was minimal inhibition of *in vivo* ALS activity in rice, with least inhibition in N22 (Fig 1). After 6 DAT, rice seedlings recovered and all three cultivars had normal growth by 14 DAT (data not shown).



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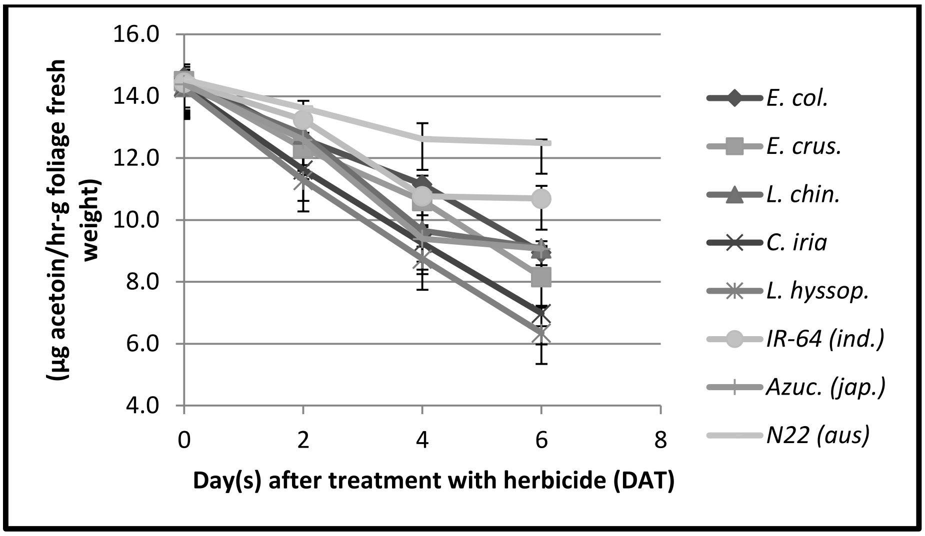


**Fig. 1.** *In vivo* ALS activity at 0, 2, 4, and 6 daysafter treatment with 50 g aiha$^{-1}$propyrisulfuronin rice and weeds at two-leaf stage.

**Study 3a. Effect of exogenousaddition of amino acids on seedling growth of propyrisulfuron-treatedrice and weeds**

Exogenous addition of 50 to 100 ppm each of valine, leucine and isoleucine increased the shoot fresh weights of rice and weeds treated with 50 to 100 g aiha$^{-1}$propyrisulfuron compared with propyrisulfuron-treated rice and weeds not added with amino acids (Table 3). Shoot fresh weightsin rice increased by 87 to 104% while those in weeds increased by 61 to 76%. Increase was highest in N22 for rice and in *L. chinensis* for weeds. Increasing propyrisulfuronto 100 g aiha$^{-1}$and increasing amino acids to 100 ppm caused no further increase in shoot weights in rice and weeds (Table 3).Recovery in terms of increased weight was less prominent in the rice cultivars than in the weeds possibly because the rice seedlings exhibited less injury to propyrisulfuron compared with injury in the weeds. Other studies have reported recovery of rice treated with cinosulfuron or bensulfuron then added with 1mMvaline, leucine and isoleucine compared with sulfonylurea-treated rice not added with amino acids (Sengnil*et al*., 1992; Park *et al* 1993). Plantdeath occurs once the amino acid pools have decreased below a critical concentration (Scheel and Casida, 1985).

**Table 3.** Shoot fresh weights of rice and weeds added exogenously with amino acids after treatment with propyrisulfuron.

| Weed Species or Rice cultivar | Increase in shoot fresh weight (%) | | | | |
|---|---|---|---|---|---|
| | With 100 ppm V, I, L and no herbicide | 50 g a.i./ha propyrisulfuron and no V,I,L | 50 g a.i./ha propyrisulfuron + 50 ppm V,I,L | 100 g a.i./ha of propyrisulfuron and no V, I, L | 100 g a.i./ha of propyrisulfuron + 100 ppm V,I,L |
| *E.colona* | 106 | 4 | 60 | 2 | 8 |
| *E.crus-galli* | 108 | 6 | 65 | 2 | 8 |
| *L.chinensis* | 106. | 38 | 76 | 25 | 29 |
| *C.iria* | 101 | 5 | 64 | 2 | 9 |
| *L.hyssopifolia* | 105 | 5 | 61 | 3 | 8 |
| IR-64 | 104 | 58 | 96 | 49 | 71 |
| Azucena | 103 | 37 | 87 | 26 | 44 |
| N22 | 103 | 78 | 104 | 71 | 91 |



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**Study 3b. Effect of exogenous addition of amino acids on *in vivo*ALS activity of propyrisulfuron-treated rice and weeds.**

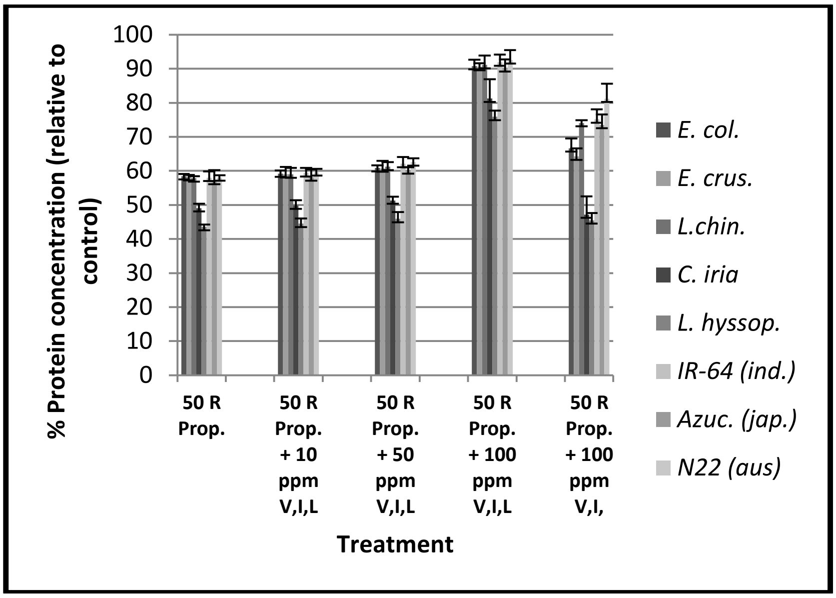


**Fig. 2.** *In vivo*ALS activity of three rice cultivars and five weed species added

The *in vivo* ALS activity of propyrisulfuron-treated plants added with 10, 50 or 100 ppm valine, leucine and isoleucine also increased over those of propyrisulfuron-treated plants not added with amino acids, with highest increase in those treated with 100 ppm amino acids (Fig 2). Increase in *in vivo* ALS activity was higher in the three rice cultivars than in the five weed species. There was a lower increase in ALS activity when only valine and isoleucine were added compared to those added with all three amino acids (Fig 2). Our results showed that exogenous addition of branched chain amino acids valine, leucine and isoleucine alleviated ALS inhibition, resulting in increased shoot fresh weight and increased *in vivo* ALS activity of propyrisulfuron-treated plants. Shimizu *et al.* (1996) also observed alleviation effect on *in vivo* ALS activity and growth of sulfonylurea-treated pea roots resulting from the addition of valine, leucine and isoleucine.

Our results agree with those of previous studies (Tanaka *et al*, 2006; Ikeda *et al*, 2011a) showing ALS inhibition as the mode of action of propyrisulfuron. Inhibition of ALS activity resulted in stunted growth and reduced shoot biomass of *L. hyssopifolia* and *C. iria*, broadleaf and sedge weeds which are known to be susceptible to sulfonylureas. Propyrisulfuron also inhibited ALS activity with reduced shoot biomass and subsequent growth inhibition in *L. chinensis*, *E. colona* and *E. crus-galli*, confirming its high activity against grasses. Slight inhibition in *in vivo* ALS activity and minimal growth reduction in rice confirm that propyrisulfuron is selective to rice, with N22 (*aus*) as the most tolerant, followed by the *indica*cultivar IR-64, and the *japonica*cultivar, Azucena, as the least tolerant. Other studies also reported greater tolerance of *indica* than *japonica* cultivars to sulfonylureas (Ohno*et al*., 1991; Park *et al*, 1993).

Similar inhibition of *in vitro* ALS activity in rice and weeds but significant inhibition of *in vivo* ALS activity of weeds with minimal inhibition in rice indicate that selectivity of propyrisulfuron is not due to differential response at the target site but due to other selectivity mechanisms. Tolerance of rice to bensulfuron, cinosulfuron, imazosulfuron and flucetosulfuron is due to faster herbicide degradation in rice than in weeds (Hwang *et al*, 2000; Takeda *et al*, 1986; Ohno*et al*,1991). Sulfonylureas are metabolized by cytochrome P450 monooxygenase systems or glutathione-s-transferase (GST) systems in tolerant crops (Naylor, 2002). Ikeda *et al*. (2011b) reported that rice degraded propyrisulfuron through demethylation and cleavage of



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the sulfonylurea linkage followed by conjugation with glucose and other natural products in rice.

Our study showed that propyrisulfuron can be used for control of broadleaf weeds and sedges as well asgrasses, with high selectivity to rice.In addition to grass, broadleaf and sedge control, propyrisulfuroncan also be used for control of sulfonylurea-resistant weeds such as *Schoenoplectusjuncoides*, *M.vaginalis*, *Linderniaprocumbens*, *Linderniadubia*, *Rotalaindica* and *Sagittariatrifolia*as reported in previous studies (Tanaka *et al,* 2006; Ikeda *et al*., 2011b). Thus, propyrisulfuronas additional control option will widen farmers' choices in herbicide rotation, arecommended practiceto delay or prevent evolution of sulfonylurea-resistant weeds in rice.

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# EVALUATION OF BROAD SPECTRUM HERBICIDE- BISPYRIBACSODIUM + METAMIFOP ON WEED CONTROL AND PRODUCTIVITY OF DIRECT-SEEDED RICE IN KUTTANAD

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**ABSTRACT** In wet-sown direct seeded rice (*Oryza sativa* L.) the weed infestation and competition is severe, as the crop and weed seeds germinate simultaneously and compete for the same pool of resources. A field experiment was conducted during the *kharif* and *rabi* seasons 2012-13 at the Rice Research Station Moncompu, India to evaluate bispyribac-sodium + metamifop 14 % SE for its weed control efficacy and productivity of direct seeded rice. The experiment was laid out in a randomized complete block design in three replicates with eight treatments *viz*., bispyribac-sodium + metamifop 14 % SE at 70 g a.i./ha + PIW-111 wetter on 10-15 DAS, bispyribac-sodium + metamifop 14 % SE at 140 g a.i./ha + PIW-111 wetter on 10-15 DAS, chlorimuron ethyl + metsulfuron methyl 20 % WP at 4 g a.i./ha on 10-15 DAS, bispyribac-sodium 10 % SC at 20 g a.i. /ha + PIW-111 wetter on 10-15 DAS, metamifop10 % SE at 50 g a.i./ha + PIW - 111 wetter on 10-15 DAS, bispyribac-sodium + metamifop 14 % SE at 70 g a.i./ha alone, weed free situation and un-weeded control. Application of bispyribac-sodium + metamifop 14 % SE at 70 g a.i./ha + PIW-111 wetter on 10-15 DAS gave better control of grassy weeds, broad leaf weeds and sedges with better weed control efficiency and lower weed index. The yield recorded was comparable with its higher concentration at 140 g a.i./ha + PIW-111 wetter on 10-15 DAS. The combined application of bispyribac-sodium and metamifop was better than their individual application in reducing the weed density, weed dry matter and enhancing the rice yield. It could be concluded that bispyribac-sodium + metamifop 14 % SE at 70 g a.i./ha + PIW-111 wetter on 10-15 DAS can be recommended for the broad spectrum control of weeds with higher yield in direct seeded rice.

***Keywords:*** Bispyribac-sodium, direct seeded rice, metamifop, weed control efficiency, weed index

## INTRODUCTION

Broadcasting of pre-germinated seeds is the common practice of crop establishment in Kuttanad of India; a unique wetland ecosystem, which lies 0.5 to 2.0 m below the mean level. Weeds are the prime biological constraint in wet-sown direct seeded rice, as both weed and crop seeds emerge at the same time and compete with each other for the same pool of resources. Uncontrolled weeds reduce the grain yield by 96 % in dry direct seeded rice and 61 % in wet direct-seeded rice (Maity and Mukerjee, 2008). The yield decrease in direct-seeded rice increases with the increase in competition duration during the initial period. Weed control during the critical period of crop- weed competition is essential to reduce the weed competition and effective utilization of available resources for enhanced productivity of rice crop. Direct-seeded rice could yield equal to those of transplanted rice, when weed control was optimum and crop establishment was good.

Manual weeding, though effective, is becoming increasingly difficult due to labour scarcity, rising wages and its dependence on weather conditions. Moreover, it is incomplete and impractical due to escape or regeneration of perennial weeds having many flushes. Delayed weeding results in crop losses and increased cost of production. Thus, herbicide usage seems indispensable for weed management in direct-seeded rice. It offers selective and economic control of weeds right from beginning, giving the crop an advantage of good start and competitive superiority (Saha, 2005). However, the continuous use of single herbicide or herbicides having the same mode of action may lead to herbicide resistance in weeds.



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Herbicide rotation and use of herbicide mixtures are the two major strategies to prevent development of herbicide resistance in weeds. Herbicides with different mode of action when mixed together, bind to different target site in weeds and prevent the probability of target site resistance in susceptible species (Paswan *et al*., 2012). In view of the above facts, the present study was undertaken to evaluate the performance of the new broad spectrum combination herbicide bispyribac-sodium + metamifop 14 % SE for weed control in direct seeded rice under puddle condition.

## MATERIALS AND METHODS

Field experiment was conducted during *kharif* 2012 (June-October) and *rabi* 2012-13 (December 2012-April 2013) at the experimental farm of Rice Research Station, Moncompu, Kerala (geographically situated at 9º 5´ N latitude and 76º 5´E longitude and at an altitude 1 m below MSL) in India. The soil of the experimental site was silty clay in texture with pH 6.2 (wet), O.C 2.55 %, available P 26.88 kg/ha and available K 239.11 kg/ha. The experiment was laid out in a randomized complete block design replicated thrice with eight treatments *viz*., bispyribac-sodium + metamifop 14 % SE at 70 g a.i./ha + PIW-111 wetter on 10-15 DAS (days after sowing), bispyribac-sodium + metamifop 14 % SE at 140 g a.i./ha + PIW-111 wetter on 10-15 DAS, chlorimuron ethyl + metsulfuron methyl 20 % WP at 4 g a.i./ha on 10-15 DAS, bispyribac-sodium 10 % SC at 20 g a.i./ha +PIW-111 wetter on 10-15 DAS, metamifop10 % SE at 50 g a.i./ha + PIW -111 wetter on 10-15 DAS, bispyribac-sodium + metamifop 14 % SE at 70 g a.i./ha alone on 10-15 DAS, weed-free situation and un-weeded control.

The total rainfall received during the cropping season was 1150.6 mm and 217.1 mm, respectively, during *kharif* 2012 and *rabi* 2012-13. The pre-germinated seeds of medium duration rice variety 'Uma' were sown on 6 June 2012 and 13 December 2012. The seed rate adopted for sowing was 100 kg/ha. The crop was fertilized with 90-45-45 of N-$P_2O_5$-$K_2O$ kg/ha. One third dose of N and K and half P was applied at 15 DAS, one third dose of N and K and half P at 35 DAS and remaining one third N and K at 55 DAS. Herbicides were applied with the help of a hand operated knapsack sprayer fitted with flat fan nozzle at a spray volume of 300 L/ha. Observations on weed density were recorded with the help of a quadrate (0.25 x 0.25 m) placed randomly at two representative sites in each plot at 30 and 60 DAS. Weed samples were collected from the same area at 60 DAS for recording the total weed dry weight. Weed samples were sun dried before oven drying at 65 ºC until constant weight was attained. The data on weed density and weed dry weight were subjected to square root transformation ($\sqrt{x+0.5}$) to normalize their distribution. Weed control efficiency (WCE) was computed using the dry weight of weeds and weed index (WI) was computed using the grain yield of weed free check and yield of treated plot. The yield attributing characters like panicles per square meter, panicle weight, fertile grains per panicle and 1000 grain weight were recorded from 10 randomly selected hills at harvest. Number of panicles was recorded by placing a quadrate (0.25 m x 0.25 m) at two spots in each plot. The grain yield was recorded at 13 % moisture. The data were analyzed separately for *kharif* 2012 and *rabi* 2012-13 using the ANOVA

## RESULTS AND DISCUSSION

### Weed Flora

During *kharif* season, the experimental field was dominated by broad leaf weeds followed by grassy weeds and sedges while during *rabi* season the field was dominated by sedges followed by broad leaf weeds and grasses. *Echinochloa stagnina* (Retz.) P. Beauv. and *Echinochloa glabrescens* Kossenko. were the major grassy weeds and *Fimbristylis miliacea* (L.) Vahl., *Cyperus difformis* L., *Cyperus iria* L., *Schenoplectus pungens* (Vahl) Palla were the major sedges in the experimental field. *Monochoria vaginalis* (Burm. f.) C. Presl *ex* Kunth, *Ludwigia perennis L.* and *Sphenoclea zeylanica* Gaertn. were the dominant broad leaf weed species present in the field during both the seasons.



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On an average total weed population at 30 DAS (days after sowing) comprised of grasses (21.8 %), broad leaf weeds (39.3 %) and sedges 38.9 % respectively, during *kharif* season and grassy weeds (0.5 %), broad leaf weeds (2.7 %) and sedges (96.8 %) respectively, during *rabi* season. At 60 DAS, total weed population comprised of grasses (22.8 %), broad leaf weeds (62.0 %) and sedges (15.2 %) respectively, during *kharif* season and grassy weeds (3.7 %), broad leaf weeds (1.9 %) and sedges (94.4 %) respectively, during *rabi* season.

**Weed density**

The density of different species recorded under herbicidal treatments was significantly less than the un-weeded control (Tables 1 and 2). Weed density at 30 DAS during *kharif* season indicated that the lowest population of sedges and broad leaf weeds were observed in chlorimuron ethyl + metsulfuron methyl 20 % WP treated plots and was found to be comparable with bispyribac-sodium + metamifop 14 % SE at 70 g a.i./ha with wetter. The lowest population of grassy weeds were observed in bispyribac-sodium + metamifop 14 % SE at 70 g a.i./ha and was found to be on par with its higher concentration and bispyribac-sodium 10 % SC at 20 g a.i./ha with wetter. During *rabi* season, lower population of broad leaf weeds was recorded in chlorimuron ethyl + metsulfuron methyl 20 % WP at 4 g a.i./ha, but sedges population was found to be low in bispyribac-sodiuim + metamifop at 140 g a.i./ha.

As the population of grassy weeds was found to be low in *rabi* season, bispyribac-sodium + metamifop applied at low and high concentration, metamifop 10 % SE applied alone with wetter and bispyribac sodium 10 % SC applied at 20 g a.i./ha registered 100 % control.

**Table 1.** Effect of treatments on weed density, total weed dry matter and weed control efficiency during *kharif* 2012

| Treatment | Weed density at 30 DAS (No./m$^2$) | | | Weed density at 60 DAS (No./m$^2$) | | | Total weed dry matter (g) at 60 DAS | WCE % |
|---|---|---|---|---|---|---|---|---|
| | BL | G | S | BL | G | S | | |
| $T_1$ | 1.6 (1.5) | 1.24 (1.3) | 0.5 (1.0) | 3.7 (2.1) | 1.6 (1.5) | 2.1 (1.6) | 3.46 (1.99) | 94.9 |
| $T_2$ | 9.4 (3.2) | 5.2 (2.4) | 9.6 (3.2) | 12.8 (3.7) | 2.5 (1.7) | 2.3 (4.7) | 17.65 (4.26) | 74.4 |
| $T_3$ | 0 (0.7) | 8.3 (2.9) | 0.5 (1.0) | 0 (0.7) | 17.4 (4.2) | 2.2 (1.7) | 12.25 (3.57) | 82.2 |
| $T_4$ | 3.4 (1.9) | 4.3 (2.2) | 2.1 (1.6) | 0 (0.7) | 8.4 (2.9) | 0 (0.7) | 5.45 (2.44) | 92.1 |
| $T_5$ | 45.9 (6.8) | 10.8 (3.4) | 36.8 (6.1) | 41.2 (6.5) | 13.0 (3.7) | 31.1 (5.6) | 62.38 (7.93) | 9.5 |
| $T_6$ | 7.9 (2.9) | 5.9 (2.5) | 3.99 (2.1) | 3.9 (2.1), | 9.9 (3.2) | 3.2 (1.9) | 24.80 (5.03) | 64.0 |
| $T_7$ | 43.4 (6.6) | 5.6 (2.5) | 43.4 (6.6) | 45.3 (6.8) | 1.7 (2.5) | 7.3 (2.8) | 3.91 (2.1) | 94.3 |
| ($T_8$) | 52.1 (7.3) | 28.9 (5.4) | 51.6 (7.2) | 62.2 (7.9) | 22.9 (4.8) | 15.2 (3.9) | 68.89 (8.3) | |
| CD (P=0.05) | 2.7 | 1.9 | 3.2 | 3.2 | NS | 2.4 | 3.7 | |

$T_1$ - Bispyribac-sodium + metamifop 14 % SE @ 70 g a.i./ha+ PIW-111 wetter ; $T_2$ - Bispyribac-sodium + metamifop 14 % SE @ 140 g a.i./ha + PIW-111 wetter; $T_3$ - Chlorimuron ethyl +metsulfuron methyl 20 % WP @ 4 g a.i./ha; $T_4$ - Bispyribac-sodium 10 % SC @ 20 g a.i/ha+ PIW -111 wetter; $T_5$ - Metamifop 10 % SE @ 50 g a.i./ha+ PIW -111 wetter; $T_6$ - Bispyribac-sodium + Metamifop 14 % SE @ 70 g a.i./ha (alone); $T_7$ - Weed free situation ; $T_8$ - Un-weeded control ; BL - Broad leaf weeds, G - Grasses, S - sedges, WCE - Weed control efficiency, CD - Critical difference. Values in parenthesis are transformed values



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At 60 DAS, during *kharif* season the lowest population of grassy weeds was recorded in bispyribac-sodium + metamifop at 70 g a.i./ha with wetter followed by weed free situation, bispyribac-sodium + metamifop at 140 g a.i./ha and bispyribac-sodium 10 % SC at 20 g a.i./ha along with wetter, while during *rabi* season bispyribac-sodium 10 % SC at 20 g a.i./ha with wetter registered the lowest population followed by bispyribac- sodium + metamifop at 140g a.i./ha with wetter and its lower concentration ($T_1$). During both seasons, chlorimuron ethyl + metsulfuron methyl 20 % WP at 4 g a.i./ha registered lower population of broad leaf weeds and was found to be comparable with bispyribac- sodium 10 % SC at 20 g a.i./ha and in case of sedges during *kharif* season bispyribac- sodium 10 % SC at 20 g a.i./ha registered lower density and was found to be comparable with chlorimuron ethyl + metsulfuron methyl 20 % WP and bispyribac- sodium + metamifop at 70 and 140 g a.i./ha.

Results of both season revealed that hand weeding failed to control the sedges effectively (Tables 1 and 2). This was mainly because of the escape or regeneration of weeds in manual weeding. Irrespective of the stages, un-weeded control registered the highest density of broad leaf weeds, sedges and grassy weeds.

**Table 2.** Effect of treatments on weed density, total weed dry matter and weed control efficiency during *rabi* 2012-13

| Treatment* | Weed density at 30 DAS *rabi* 2012 (No./m$^2$) | | | Weed density at 60 DAS *rabi* 2012 (No./m$^2$) | | | Total weed dry matter (g) at 60 DAS | WCE % |
|---|---|---|---|---|---|---|---|---|
| | BL | G | S | BL | G | S | | |
| $T_1$ | 2.2 (1.7) | 0 (0.7) | 13.4 (3.7) | 7.6 (2.8) | 4.6 (2.3) | 13.2 (3.7) | 7.28 (2.79) | 98.1 |
| $T_2$ | 2.2 (1.7) | 0 (0.7) | 6.9 (2.7) | 0.9 (1.2) | 0.5 (1.0) | 11.8 (3.5) | 3.91 (2.10) | 98.9 |
| $T_3$ | 0 (0.7) | 5.1 (2.4) | 25.3 (5.1) | 0.9 (1.2) | 27.1 (5.3) | 27.9 (5.3) | 20.94 (4.63) | 94.5 |
| $T_4$ | 6.2 (2.6) | 0 (0.7) | 14.7 (3.9) | 1.6 (1.5) | 0 (0.7) | 10.6 (3.3) | 7.91 (2.90) | 97.9 |
| $T_5$ | 7.7 (2.9) | 0 (0.7) | 156 (12.5) | 37.8 (6.2) | 18.3 (4.3) | 216.5 (14.7) | 138.7 (11.80) | 64.1 |
| $T_6$ | 7.7 (2.9) | 5.5 (2.4) | 42.8 (6.6) | 11.5 (3.5) | 11.3 (3.4) | 51.8 (7.2) | 18.42 (4.35) | 95.2 |
| $T_7$ | 7.2 (2.8) | 0.9 (1.2) | 41.1 (6.5) | 22.3 (4.8) | 12.3 (3.6) | 56.2 (7.5) | 12.07 (3.55) | 96.9 |
| $T_8$ | 20.4 (4.6) | 3.9 (2.1) | 728.5 (27.0) | 15.2 (3.9) | 29.3 (5.5) | 754.7 (27.5) | 386.80 (19.67) | |
| CD (P=0.05) | 1.8 | NS | 6.3 | 3.0 | 3.2 | 4.2 | 4.9 | |

*refer to the footnote of the Table 1 for the description of treatments. BL- Broad leaf weeds, G - Grasses, S - Sedges, WCE - Weed control efficiency, CD - Critical difference. Values in parenthesis are transformed values

**Total weed dry matter and weed control efficiency**

The dry matter accumulation by weeds in different treatments varied in accordance with weed population recorded under these treatments. Significant reduction in weed dry weight was observed due to decrease in their population under herbicide treatments. The maximum reduction in weed dry weight was observed under bispyribac-sodium + metamifop 14 % SE at 70 g a.i./ha with wetter with a weed control efficiency of 94.9 % which was on par with weed free situation, bispyribac-sodium 10 % SC at 20 g a.i./ha with wetter, chlorimuron ethyl + metsulfuron methyl 20 % WP at 4 g a.i./ha and bispyribac-sodium + metamifop 14 % SE at 140



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g a.i./ha with wetter during *khari*f season. During *rab*i season, bispyribac-sodium + metamifop 14 % SE applied at 140 g a.i./ha with wetter registered the lowest total weed dry weight with weed control efficiency of 98.9 % which was on par with bispyribac sodium + metamifop 14 % SE at 70 g a.i./ha with wetter (98.1 %), bispyribac sodium 10 % SC at 20 g a.i./ha with wetter and weed free situation (Tables 1 and 2). The higher weed control efficiencies in these treatments might be due to lower weed dry matter accumulation as compared to other treatments. Among the herbicides tested, the lowest weed control efficiency was recorded in metamifop 10 % SE with wetter during both the seasons. This was due to its inefficacy in controlling broad leaf weeds and sedges as evident in Tables 1 and 2, which contributed to higher weed dry matter accumulation.

**Yield attributes**

Weed control treatments brought out a significant effect on yield attributes as compared to the weedy check (Table 3).

**Table 3.** Effect of weed control treatments on yield attributes of direct seeded rice during *kharif* 2012 and *rabi* 2012-13.

| Treatment* | Panicles per square meter | | Panicle weight ( g) | | Fertile grains per panicle | | 1000 grain weight (g) | |
|---|---|---|---|---|---|---|---|---|
| | *kharif* 2012 | *rabi* 2012 | *kharif* 2012 | *rabi* 2012 | *kharif* 2012 | *rabi* 2012 | *kharif* 2012 | *Rabi* 2012 |
| $T_1$ | 352 | 237 | 2.71 | 2.34 | 104.6 | 114.2 | 26.1 | 22.2 |
| $T_2$ | 317 | 227 | 2.75 | 2.52 | 100.1 | 101.6 | 25.3 | 23.2 |
| $T_3$ | 304 | 215 | 2.36 | 1.94 | 89.1 | 89.2 | 25.2 | 23.0 |
| $T_4$ | 308 | 243 | 2.65 | 2.32 | 101.3 | 96.0 | 26.1 | 21.9 |
| $T_5$ | 363 | 203 | 2.49 | 2.09 | 91.6 | 91.9 | 25.1 | 21.5 |
| $T_6$ | 336 | 217 | 2.63 | 2.14 | 100.1 | 91.3 | 25.1 | 22.3 |
| $T_7$ | 387 | 343 | 3.05 | 2.53 | 115.5 | 120.7 | 25.4 | 23.5 |
| $T_8$ | 242 | 80 | 2.10 | 1.15 | 79 | 30.9 | 24.4 | 21.4 |
| CD (p=0.05) | 68 | 40 | 0.42 | 0.32 | 15.6 | 23.9 | 0.87 | NS |

*refer to the footnote of the Table 1 for the description of treatments. CD - Critical difference.

The highest number of panicles per square meter was observed in weed free situation during both the seasons (Table 3). Among the tested herbicides, during *kharif* season the highest number of panicles was observed in metamifop 10 % SE with wetter treated plots and it was on par with bispyribac-sodium + metamifop 14 % SE at 70 g a.i./ha with wetter, bispyribac-sodium + metamifop 14 % SE (alone) at 70 g a.i./ha and bispyribac-sodium + metamifop 14 % SE at 140 g a.i./ha with wetter. In *rabi* season, the highest number of panicles was observed in bispyribac-sodium 10 % SC at 20 g a.i./ha with wetter followed by bispyribac-sodium + metamifop 14 % SE at 70 g a.i./ha and its higher concentration with wetter. In case of panicle weight, during *khari*f season panicles with higher weight was recorded in weed free situation (3.05 g) followed by bispyribac sodium + metamifop 14 % SE at 140 g a.i./ha (2.75) and its lower concentration (2.71). Similarly during *rabi* season, weed free situation recorded panicles with higher weight (2.53) followed by bispyribac-sodium + metamifop 14 % SE at 140 g a.i./ha (2.52 g) and bispyribac-sodium + metamifop at 70 g a.i./ha with wetter (2.34 g). During both the seasons weed free situation recorded higher number of fertile grains per panicle, while un-weeded control recorded the least number of fertile grains per panicle during both seasons.

The highest test weight was recorded in the treatment bispyribac-sodium + metamifop at 70 g a.i./ha with wetter during *kharif* season and weed free situation during *rabi* season, respectively. The higher values of yield attributes in bispyribac-sodium + metamifop at 70 g a.i./ha during both the seasons might be due to better control of weeds, which resulted in lesser



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competition for space, light, moisture and nutrients and ultimately led to better growth and development of the crop.

**Grain yield and weed index**

Weed control treatments significantly influenced the grain yield of direct seeded rice. Adoption of different weed management measures enhanced the grain yield from 1387 kg/ha to 4133 kg/ha in *kharif* season and from 997 kg/ha to 3983 kg/ha in *rabi* season.

**Table 4.** Effect weed control treatments on grain and weed index of direct seeded rice

| Treatment* | Grain yield kg/ha | | Weed index | |
|---|---|---|---|---|
| | *kharif* 2012 | *rabi* 2012 | *kharif* 2012 | *rab*i 2012 |
| $T_1$ | 3960 | 3683 | 4.2 | 7.5 |
| $T_2$ | 3893 | 3717 | 5.8 | 6.7 |
| $T_3$ | 2667 | 3133 | 35.5 | 21.3 |
| $T_4$ | 3213 | 3537 | 22.3 | 11.2 |
| $T_5$ | 3000 | 2982 | 27.4 | 25.1 |
| $T_6$ | 3027 | 3330 | 26.8 | 16.4 |
| $T_7$ | 4133 | 3983 | - | - |
| $T_8$ | 1387 | 997 | 66.4 | 75.0 |
| CD (p=0.05) | 531 | 310 | | |

*refer to the footnote of the Table 1 for the description of treatments. CD - Critical difference

The weed-free situation registered the highest grain yield in both the seasons (Table 4). Among the herbicides, bispyribac-sodium+ metamifop 14 % SE at 70 g a.i./ha recorded the highest grain yield (3960 kg/ha), which was on par with weed free situation and bispyribac-sodium+ metamifop 14 % SE at 140 g a.i./ha in *kharif* season. During *rabi* season bispyribac-sodium+ metamifop 14 % SE at 140 g a.i./ha (3717 kg/ha) recorded the highest grain yield which was on par with its lower concentration and bispyribac-sodium 10 % SC at 20 g a.i./ha with wetter. The yield improvement observed in these herbicide treatments might be due to better control of weeds and weed control efficiency (Table 1 and 2) and higher values of yield attributing characters (Table.3). During *kharif* season the lowest weed index was recorded in bispyribac-sodium+ metamifop 14 % SE at 70 g a.i./ha (4.2) followed by its high concentration (5.8) and bispyribac-sodium 10 % SC at 20 g a.i./ha with wetter. During *rabi* season, the lowest weed index was recorded in bispyribac-sodium+ metamifop 14 % SE at 140 g a.i./ha (6.7) followed by its lower concentration (7.5) and bispyribac sodium at 20 g a.i./ha with wetter (11.2). During *kharif* season chlorimuron ethyl + metsulfuron methyl 10 % WP at 4 g a.i./ha and during *rabi* season metamifop 10 % SE applied at 50 g a.i./ha with wetter recorded the highest weed index with lowest grain yield. This might be due to the production of panicles with lesser weight and less number of panicles per square meter (Table 3). Un-weeded control recorded 66.4 % lesser yield in *kharif* season and 75.0% lesser yield in *rabi* season. This was due to very severe weed competition and improper utilization of nutrient, space and light. The result is in conformity with the findings of Mohan *et al*. (2010) and Kachroo and Bayaza (2011).

## CONCLUSIONS

The predominance of weed flora is influenced by the season of cultivation. Post emergence application of bispyribac-sodium + metamifop 14 % SE at 70 g a.i./ha with wetter applied on 10 to 15 DAS was found effective in controlling grassy weeds, sedges and broad leaf weeds. Based on the overall rice yield across herbicide treatments, bispyribac-sodium + metamifop 14 % SE applied at 70 g a.i./ha and 140 g a.i/ha were comparable during both the seasons. Hence



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bispyribac-sodium + metamifop 14 % SE applied at 70 g a.i./ha can be recommended for weed control and higher productivity in direct seeded rice under puddle condition.

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# BIOLOGY AND MANAGEMENT OF WEEDY RICE IN DIRECT-SEEDED PUDDLED RICE

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**ABSTRACT** Studies were conducted on the biology and management of weedy rice during 2009-13 at Rice Research Station, Moncompu and farmer's fields of Kuttanad with severe weedy rice infestation (more than 15 weedy rice plants per square meter). Invasion and spread of weedy rice in the cultivated rice fields have forced farmers to abandon rice farming leading to socio economic and ecological impacts in this rice bowl of Kerala. Survey in the infested area has revealed the presence of weedy rice with variation in morphological characters like ligule length (0.2-2 cm), panicle type (closed to open), spikelet colour (straw to black),length of grains (0.7-0.9 cm), colour of awns (straw to red) and length of awns (0.5-3.5 cm). Investigations on dormancy revealed that hull induced dormancy in weedy rice could be broken by exposing seeds to 22ºC for 48 hours, treating seeds with 0.6% nitric acid for six hours, rupturing of seed coat or maintaining electrical conductivity of 5 dS $m^{-1}$ for six hours in the growing media. Scanning electron microscope studies confirmed that the differences in the tightness of packing of lemma and palea contributed to quick and efficient germination of half matured straw coloured weedy rice grains compared to black coloured fully matured dried ones. Presence of silica deposits and trichomes on the leaf surface contributed to the growth vigour of weedy rice. Management options found effective for the control of weedy rice in direct seeded puddled rice include pre sowing surface application of oxyfluorfen @ 0.3 kg ai $ha^{-1}$, three DBS in thin film of water and selective drying of weedy rice panicles by direct contact application of glufosinate ammonium or glyphosate or paraquat @ 15- 20% concentration at 60-65 DAS using specially designed wick applicator. Stale seed bed technique with dry and wet ploughing followed by the application of a broad spectrum herbicide and flooding proved to be effective in exhausting soil seed bank.

***Keywords:*** Dormancy, oxyfluorfen, pre sowing application, stale seed bed, weedy rice, wick applicator.

## INTRODUCTION

Weedy rice is a complex of *Oryza* morphotypes widely distributed in commercial rice fields, especially in areas where farmers have switched to direct seeding. India has the earliest history of rice cultivation and hence highly variable population of weedy/wild rice types due to introgression are seen in the rice growing areas of the country. Indian weedy rice has been reported to be *Oryza sativa* f.sp. *spontanea*, belonging to *indica* group (Vaughan, 1994).Heavy infestation of weedy rice and subsequent reduction in crop yield (60-80%) in rice fields of Kuttanad and other locations of Kerala state during recent years have forced farmers to abandon rice cultivation. The recognition and recent declaration of the below sea level farming system in this rice bowl of Kerala as a GIHAS (Globally Important Agricultural Heritage System)by FAO (International Forum at Ishikawa Prefecture, Japan, during June 2013), also calls for sustaining the rice cultivation of the area.

Morphological similarity with cultivated rice, variable seed dormancy, early seed shattering nature, staggered germination and high competitiveness of weedy rice make hand weeding incomplete and ineffective.The spread of weedy rice as contaminants with seed material, its distribution through irrigation water, machinery and animals, and efficient replenishment to the soil seed bank also add to the severity of infestation and invasion to newer



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areas. As the present recommendations of chemical weed control in rice are not effective for selective control of weedy rice, modifications in the time and method of herbicide application were evaluated for the management of weedy rice. Biology of weedy rice was also studied as it can help in formulating suitable package for integrated management of weedy rice.

## MATERIALS AND METHODS

Studies on the biology and management weedy rice were conducted during 2009-13, at Rice research Station, Moncompu, Kerala and in the farmer's fields of Kuttanad (9º 5' N latitude and 76º25' E longitude lying 0.5 m to 2.0 m below MSL) with severe weedy rice infestation (more than 15 weedy rice plants per square meter).

### Survey of the area

Roving survey was conducted in the major rice growing tracts of Kerala during the two rice growing seasons in 2010-11 and 2011-12 to understand the extent of occurrence of weedy rice, morphology and habit of variants of weedy rice and traditional knowledge of the farmers on the occurrence, nature, spread, competition and management of weedy rice.

### Morphology and seed dormancy studies

The weedy rice variants, both plants and seeds, were collected from different locations to study the morphological characters. Scanning electron microscopic studies were done to identify the topographical and anatomical differences between rice and weedy rice grains. Seed dormancy studies were conducted with acid scarification by treating with 0.6% nitric acid (pH-3) for six hours, exposing seeds to 22ºC for 48 hours, 40ºC for six hours, soaking in salt water with electrical conductivity of 5 and 15 dS $m^{-1}$, mechanical scarification by scraping the hull with a razor blade, and germinating weedy rice without seed treatment (control), with four replications. The pre treated seeds were washed with distilled water and kept at room temperature for germination.

### Management options for the control of weedy rice

The experiment on management of weedy rice aimed at tackling the problem at different phases of the crop viz., adopting Stale Seed Bed (SSB) technique during land preparation, pre sowing application of herbicides and direct contact application (DCA) of herbicides to ear heads using specially designed wick applicator at around 60-65 days after sowing (DAS).

In Stale Seed Bed technique, infested fields were subjected to a sequence of operations including burning of stubbles followed by germination of weed seeds from the moist soil by providing conditions for seed germination for 12-15 days. Depending on the availability of time and intensity of infestation, the seedlings emerged from the soil seed bank were destroyed by dry ploughing followed by wet ploughing (to expose soil seed bank), or by applying broad spectrum herbicide (glyphosate @ 0.8 kg ai $ha^{-1}$) after germination. Ten days after glyphosate application the plots were flooded for 10-15 days.

In chemical control, infested fields were ploughed, puddled, levelled and different herbicides viz., oxyfluorfen, pretilachlor and butachlor, were applied on the surface maintaining a thin film of water of about 2 cm to improve the weed control efficiency. Pre germinated seeds were broadcasted in the field three days after herbicide application. The broadspectrum herbicide bispyribac sodium was applied @ 30 g ai/ha at 15 DAS in all the experimental plots to avoid competition from other weeds on the growth of rice and weedy rice.

Post emergence management of weedy rice by direct contact application of herbicides (DCA) was experimented by utilising a specially designed wick applicator for selective drying of ear heads of weedy rice which flowers earlier than rice and emerge above the canopy of rice at 60 to 75DAS (Stroud and Kempen, 1989) . The broad spectrum herbicides used in the study were paraquat dichloride, glyphosate and glufosinate ammonium at different concentrations.



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The above experiments were laid out in Randomised Block Design with five replications in 8 x 5 $m^2$or bigger plots of 200 $m^2$ as in SSB studies.

**Participatory technology demonstration on integrated weedy rice management**
Integrated management of weedy rice was demonstrated in severely infested rice polders of Kuttanad using appropriate combination of the three management options viz., SSB technique, pre sowing herbicidal control and post emergence control by DCA of herbicides.

## RESULTS AND DISCUSSION

**Occurrence and biology of weedy rice**
Extensive survey of the rice fields could categorise 20% of the cultivated area under low incidence (1-5 weedy rice plants per square meter), 35 % with medium (6-10 weedy rice plants per square meter) and 20% with heavy incidence (> 10 weedy rice plants per square meter). Though more than 75% of the farmers were aware of the aggressiveness of weedy rice, hand weeding was practically incomplete and ineffective. Indigenous traditional knowledge (ITKs) of the farmers on the management practices include reduced tillage to prevent enrichment of soil seed bank, deep ploughing to prevent the germination, higher seeding rate, early flooding and cutting the panicles at harvest etc., to reduce weedy rice infestation.

The wild nature of weedy rice was clearly evident from its plant height, high biomass production, awned grains and shattering of both fully matured and half matured grains. Though most of the variants were tall with 130-145 cm height, there were also dwarf plants of 110 cm similar to cultivated rice. Weedy rice had a few tillers (3-5 per plant) with three to four leaves per tiller and ligule length varied from 0.2 -2 cm. The panicles were either open or closed type. The L/B ratio of grains varied from 2.2 to 3.0. All the variants possessed red or straw coloured awns (2.5-8 cm long). Ninety percentage or more of the seeds in a panicle turned deep brown to black colour on maturity with 10% remaining yellow; either fully matured or half matured. Number of filled grains per panicle was highly variable, ranging from 30-80. Chaffy grains per panicle accounted to 30-60%. The 1000 grain weight of weedy rice was 18-19.5 g.

**Dormancy studies**
The seeds in a panicle had variable dormancy ranging from two months to one year or more when exposed to germination in petridish. Pot culture studies also revealed that weedy rice seeds remained dormant under flooded condition and germinated under moist condition even from 4 cm of soil depth. Among the various treatments evaluated for breaking hull induced seed dormancy of weedy rice (Fig. 1), soaking seeds in salt water for six hours (EC–5 dS $m^{-1}$and 15 dS $m^{-1}$) and scraping of hull gave quick germination (80%) as reported by Naredo et al. (1998). But, seeds exposed to low temperature of $22^0$C for 48 hours and soaking in 0.6% nitric acid for six hours had 80% germination only on the sixth day. The scraped and salt water treated seeds had lesser root and shoot length and were found to decay as days proceeded, probably due to the decay of endosperm and death of young tissues. It was observed that after 12 days of soaking all the treatments had 90 – 100% germination (15-25% over and above the control) except on exposing seeds to high temperature of $40^0$C for six hours. At high temperature, the seeds were found to lose viability. The dormancy breaking treatments in the descending order of efficiency were: (i) subjecting seeds to low temperature of 22ºC for 48 hours, (ii) followed by scraping of seed hull, (iii) salt water treatment and (iv) 0.6% nitric acid soaking. These treatments can be effectively opted alone or in combination during different seasons in stale seed bed preparation for weedy rice management under different field situations.



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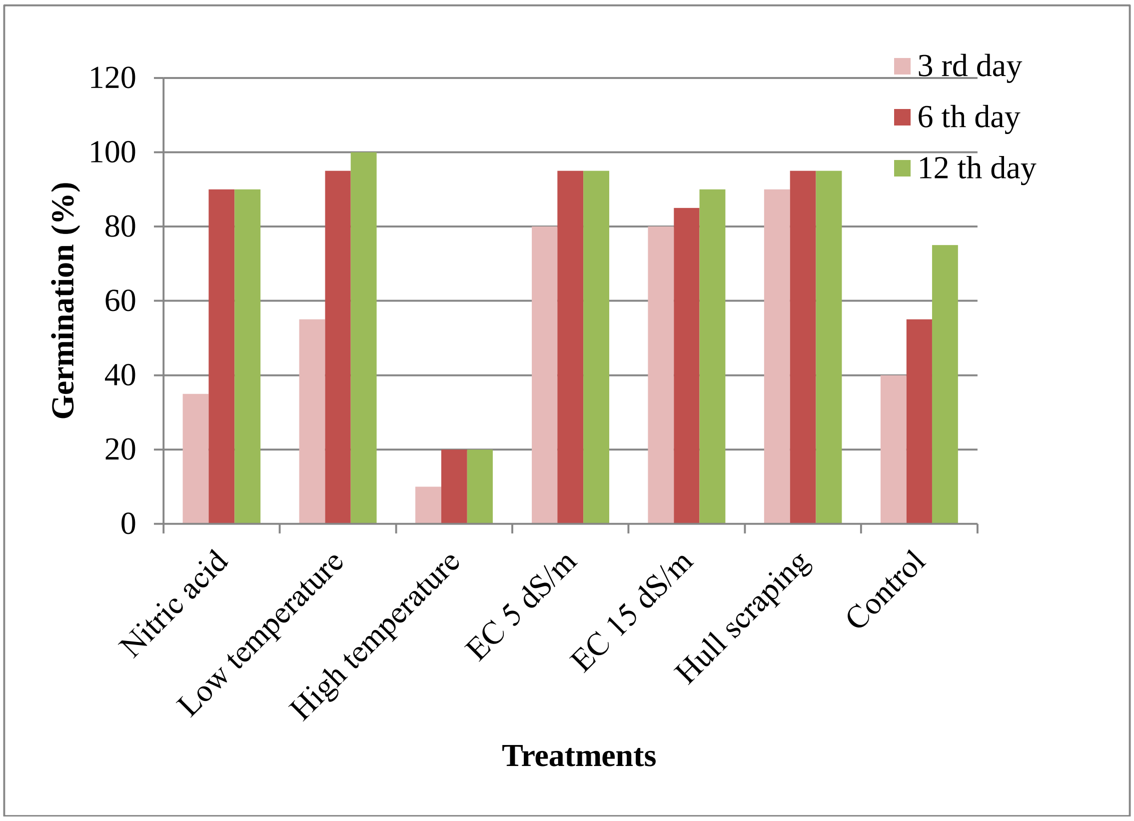


**Fig 1**. Effect of treatments on breaking the dormancy of weedy rice seeds

Scanning electron microscope studies of weedy rice seeds revealed the presence of indentations on the exterior surface with silica in the mid region. The seed surface had parallel rows of trichomes which help in dispersal of seeds, give better grip for seeds in soil facilitating germination and prevent wash out during heavy rains. Mature weedy rice seeds had tight packing of the glumes and the overlapping region of the glumes extended up to 319.8 µm, whereas, in immature seeds it was only 210.0 µm. The thickness of the lemma and palea was 87.4 µm and 73.6 µm in mature grains and that for immature grains was 63.24 µm and 55.73 µm, respectively. The maximum angular distance between the rudimentary glumes were higher in immature grains (2.675 mm) compared to the mature ones (2.140 mm). These observations confirmed the tightness of packing of the glumes which delayed the germinability of matured seeds compared to immature seeds. The weedy rice leaves had more micro hairs and epicuticular wax on the adaxial surface compared to the abaxial surface, which can reduce transpiration, reflect away excessive heat and enhance water use efficiency. These features enhance the competitive efficiency of weedy rice plants compared to the cultivated rice (Hamid et al., 2007).

**Effect of SSB in reducing weedy rice infestation in the succeeding crop**

In moderately infested fields, two SSB by ploughing the field in between two germinations at 25-30 days interval prior to sowing provided conditions for germination of majority of weed seeds in the soil seed bank. Ploughing in between two SSB operations took the buried seeds to the top soil for promoting germination, as weedy rice seeds do not germinate under continuous submergence and emerged only from top 4cm of soil (Chin et al., 2000). In double cropped fields, where farmers do not get ample time for doing the SSB operations twice, burning of straw before single SSB preparation broke seed dormancy and ensured uniform germination of weeds from the soil surface. It was found that in severely infested areas it is better to skip one crop and repeat SSB operations twice to prepare a weed free field, giving maximum time for exhausting soil seed bank. Weed control efficiency (WCE) and cost benefit ratio on partial budgeting was also higher in skipped crop with intensive ploughing and repeated germination



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than in single SSB (Table 1). All the different methods of SSB significantly decreased the weedy rice population and increased yield in the succeeding crop, depending on the duration and frequency of various operations.

**Effect of pre sowing application of herbicides in reducing soil seed bank**

Experiments conducted at different locations during the study revealed that pre sowing surface application of herbicides was better than its soil incorporation or pre emergence application after sowing. Sadohara et al. (2000) suggested that surface application of herbicides three to four DBS was effective for reducing soil seed bank and early weed growth. Among the different herbicides tried (Table 2), surface application of oxyfluorfen @ 0.3 to 0.4 kg ha$^{-1}$ in 2cm standing water three DBS effectively controlled weeds (84% reduction in weedy rice dry weight) during the initial critical period. Pre sowing surface application of oxyfluorfen at 0.1 kg ha$^{-1}$ gave broad spectrum control of all kind of weeds emerging from the soil (density of weedy rice reduced to about 75 %), at the same time the emerging shoots of pre germinated crop seeds sown later were protected from the herbicide by spatial and temporal difference. Contact and residual action of oxyfluorfen destroyed weed seeds in the top layer of soil, reduced initial crop weed competition and standing water inhibited further seed germination. Yield obtained in oxyfluorfen (@ 0.2, 0.3, 0.4 kg ha$^{-1}$) applied plots (3690 kg ha$^{-1}$) was statistically on par with that in hand weeding. Unweeded control plots gave significantly lower grain yield of 1715 kg ha$^{-1}$.

**Table 1**. Effect of stale seed bed technique in managing weedy rice

| Treatment | Plant density at 45 DAS (No. m $^{-2}$) | | Tiller count at 45 DAS (No. m $^{-2}$) | | Grain yield (kg ha$^{-1}$) | WCE (%) |
|---|---|---|---|---|---|---|
| | Weedy rice | Rice | Weedy rice | Rice | | |
| One SSB | 6 | 75 | 16 | 201 | 5688 | 49.8 |
| Two SSB | 3 | 75 | 8 | 241 | 6562 | 75.2 |
| SSB with skip crop | 1 | 74 | 4 | 323 | 7500 | 89.6 |
| Control | 12 | 73 | 29 | 195 | 2420 | 0 |
| CD (0.05) | 2 | NS | 3.2 | 48 | 587 | - |

**Table 2**. Effect of pre sowing surface application of herbicides on weedy rice management

| Treatments | Dose | Countat 25 DAS (No. m$^{-2}$) | Plant Height 25 DAS (cm) | Plant Height 70 DAS (cm) | Dry weight 70 DAS (g m$^{-2}$) | Rice grain yield |
|---|---|---|---|---|---|---|
| | (kg ai ha$^{-1}$) | Weedy rice | Weedy rice | Weedy rice | Weedy rice | (kg ha$^{-1}$) |
| Butachlor 50 EC | 2.5 | 14 | 33.6 | 65.7 | 52.8 | 2975 |
| Butachlor 50 EC | 2 | 12 | 34.0 | 64.4 | 56.0 | 3453 |
| Pretilachlor 50 EC | 2 | 8 | 33.7 | 70.2 | 50.8 | 3227 |
| Pretilachlor 50 EC | 1.5 | 12 | 33.6 | 64.6 | 55.2 | 3565 |
| Oxyfluorfen 23.5 EC | 0.4 | 11 | 32.8 | 72.2 | 45.3 | 3759 |
| Oxyfluorfen 23.5 EC | 0.3 | 9 | 34.9 | 74.1 | 45.3 | 3686 |



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| | | | | | | |
|---|---|---|---|---|---|---|
| Oxyfluorfen 23.5 EC | 0.2 | 8 | 33.4 | 74.8 | 42.2 | 3640 |
| Oxyfluorfen 23.5 EC | 0.1 | 11 | 33.4 | 69.5 | 43.8 | 2940 |
| HW | | 4 | 35.6 | 77.8 | 30.6 | 3717 |
| UWC | | 15 | 34.1 | 59.1 | 65.5 | 1715 |
| CD (0.05) | | 4 | NS | 4.5 | 8.3 | 390 |

HW- hand weeding, UWC- unweeded control

**Use of wick applicator for selective killing of panicles of weedy rice**

It was noticed that mature to near mature seeds of weedy rice normally got shattered by around 80-100 DAS and replenished the soil seed bank. Lab studies conducted in petriplates revealed that fully mature seeds of weedy rice remained dormant for more than one year while those in the late dough to near mature stage germinated within a week or two. The result confirmed the induction of pericarp induced dormancy on seed maturation as reported by Gu et al. (2003), which emphasises the need for destruction of weedy panicles before dough stage of grains to reduce the replenishment of soil seed bank.

The experiment proved that better WCE can be obtained by selective killing of weed panicles by DCA using wick applicator at 60-65 DAS, with broad spectrum herbicides glufosinate ammonium, paraquat or glyphosate @ 15-20% (Table 3), taking advantage of 15-20 cm height difference between rice and weedy rice plants. Quick drying of weedy rice panicles was observed on using paraquat for sweeping followed by ammonium glufosinate. This method of weed management has the advantage of being eco friendly as the herbicide is not coming in contact with soil or the crop.



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**Table 3**. Effect of direct contact application on the control of weedy rice panicles

| Herbicides | Dose (%) | Plant height at 65 DAS (cm) | | Panicles before drying (No. $m^{-2}$) | Skipped panicles (%) | Dried panicles (%) |
|---|---|---|---|---|---|---|
| | | Weedy rice | Rice | | | |
| Paraquat 24 SL | 10 | 93.3 | 73.3 | 38 | 25 | 75 |
| Paraquat 24 SL | 15 | 89 | 74.3 | 29 | 33 | 67 |
| Glyphosate 41 SL | 10 | 93.3 | 77 | 36 | 30 | 70 |
| Glyphosate 41 SL | 15 | 89 | 71 | 40 | 28 | 72 |
| Glufosinate Ammonium 14 SL | 5 | 94.3 | 81.7 | 33 | 52 | 48 |
| Glufosinate Ammonium 14 SL | 10 | 91.7 | 75 | 36 | 37 | 63 |
| Glufosinate Ammonium 14 SL | 15 | 91.3 | 79 | 27 | 37 | 63 |
| Glufosinate Ammonium 14 SL | 20 | 87.7 | 72.7 | 45 | 38 | 62 |
| Glufosinate Ammonium 14 SL | 25 | 96.3 | 79.7 | 44 | 23 | 77 |
| Control | | 89 | 75.3 | 57 | 100 | 0 |
| CD (0.05) | | NS | NS | NS | 18.7 | 18.7 |

**Participatory technology demonstration on integrated weedy rice management**

Integrated management of weedy rice was successfully demonstrated in rice polders by exhausting soil seed bank with stale seed bed technique and pre sowing herbicide application, and its further addition to soil by direct contact application. Other management options like higher seed rate (Bakar et al., 2000), scientific water management, straw burning, appropriate tillage practices, adoption of mechanized transplanting or dibbling and hand weeding also proved to be successful in the integrated approach. The weedy rice infested rice fields which yielded less than 1250-1500 kg $ha^{-1}$ before management could produce nearly 6250-6500 kg $ha^{-1}$ on adoption of the integrated management technology. The technology is getting wider adoption as it is effective in eradicating weedy rice infestation and increase the yield to sustain rice farming in Kuttanad.

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# EFFECT OF APPLICATION TIMINGS OF SOIL APPLIED HERBICIDES ON WEED GROWTH AND CROP YIELD IN DRY-SEEDED RICE IN BANGLADESH

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**ABSTRACT** Dry-seeded rice (DSR) is an emerging production system in many Asian countries, including Bangladesh. Weeds, however, are a major constraint to the success of DSR. The use of a pre-emergence herbicide is must to manage weeds effectively in DSR. However, the timing of herbicide application is very crucial. A field study was conducted during the period of February to June, 2013, in the south-west part of Bangladesh to evaluate the effect of application time of soil applied herbicide (3 times - before crop sowing, after crop sowing but before the first irrigation, and after sowing and first irrigation) and 4 weed control method (weed free, weedy check, oxadiargyl 80 g ai ha$^{-1}$, and pendimethalin 1000 g ai ha$^{-1}$) on weed growth and crop performance of DSR. Rice plant stand was highly influenced by application time and weed control method. Pendimethalin significantly reduced rice plant density, more so as sowing was advanced. Compared with the non-treated (weedy) treatment (195 rice plants m$^{-2}$), pendimethalin application before sowing, after sowing but before irrigation, and after sowing and irrigation reduced rice plant density by 43, 19 and 12%, respectively. There was no effect of oxadiargyl on plant density, regardless of application time. However, weed density and biomass were not reduced when this herbicide was applied before sowing. Grain yield was significantly increased as the time of herbicide was delayed from before sowing (2.2-2.4 t ha$^{-1}$), after sowing before irrigation (2.5-2.6 t ha$^{-1}$), and after sowing after irrigation (4.0-4.1 t ha$^{-1}$). The results suggest that to manage weeds effectively and reduce crop toxicity in dry-seeded rice, soil applied herbicides should be applied after sowing and irrigation.

***Keywords:*** oxadiargyl, pendimethalin, phytotoxicity

## INTRODUCTION

Due to the decreasing availability of labor and water, farmers in many Asian countries are shifting their traditional rice cultivation method of puddled transplanted rice to dry seeded rice (DSR) (Pandey and Velasco 2005; Chauhan 2012; Mahajan et al., 2012). DSR can be established directly in the field with no prior tillage (zero tillage) or following dry tillage. Dry seeding reduces labor requirement by eliminating seedling rearing in nursery beds, seedling uprooting, repeated tillage under wet condition to puddle the soil and manual transplanting (Farooq et al., 2011; Chauhan et al., 2012). Dry-seeding readily enables mechanization of rice crop establishment using a 'power tiller operated seeder' (PTOS) powered by a 2-wheel tractor, or using 4-wheel tractor mounted seed drills, with low labor requirement (Riches et al., 2008). Dry seeding also reduces irrigation water requirement through elimination of puddling. While dry-seeding has many benefits over puddled transplanted rice, weeds are a major constraints to its success when grown under non-ponded conditions (Singh et al., 2007; Mahajan and Chauhan, 2011; Chauhan and Opeña, 2012). In the DSR system, weeds and rice germinate simultaneously and most often weeds show more vigorous growth than rice, so the rice seedlings are suppressed by weeds at an early growth stage (Chauhan and Johnson, 2010b; Chauhan, 2012). In Asian countries, manual weeding is very common but because of increasing labor scarcity and its high price, manual weeding alone is not an option. In addition, at the early growth stage, the morphology of rice seedlings and some weeds (especially grasses) are similar, so manual weeding is difficult (De Datta, 1981; Rahman et al., 2012). Herbicides are considered to be the best tool to manage weeds in DSR. Herbicides can be applied as a pre-emergence



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(control weeds before emergence) spray and/or a post emergence (control weed after emergence) spray. However, pre-emergence herbicide is essential to manage weeds effectively in DSR (Singh et al., 2007; Khaliq et al., 2011). Several pre-emergence herbicides are now available in the market, but selection of right herbicide is very important to suppress weeds from the existing weed seed bank, in an economical way. In addition, environmental conditions, such as soil water content at the time of application, can influence both herbicide efficacy and crop phytotoxicity by altering herbicide absorption, translocation, or metabolism (Levene and Owen 1995; Chauhan and Johnson, 2011). For example, Chauhan and Johnson (2011) reported that the pre-emergence herbicide oxadiazon at 1.0 kg $ha^{-2}$ reduced rice shoot biomass by 22 to 36% in dry soil conditions, and by 43 to 56% in saturated conditions. So, herbicide application timing and soil moisture condition are very important to reduce both the crop phototoxicity and to increase the herbicide efficacy on weeds. Therefore, the aim of our study was to determine the effect of time of application of soil applied herbicide on rice plant establishment, weed growth and crop yield in dry-seeded rice.

## MATERIALS AND METHODS

### Experimental site

The field experiment was conducted at the research farm of the Regional Agricultural Research Station (RARS) of Bangladesh Agricultural Research Institute (BARI), Jessore (23°11' N, 89°14' E and 6.71 m above mean sea level) in the boro (dry) season of 2013. The area belongs to the agro ecological zone number 11, known as the High Ganges River Floodplain. The climate of the area is subtropical, with an average annual rainfall of 1590 mm (90% of which is received during May to September), minimum temperature of 6–9 $^0$C in January, and maximum temperature of 36–44$^0$ C in April and May (Fig. 1 shows the minimum and maximum temperature and rainfall during the experimental period). The soil at 0–15-cm depth was a clay loam, with a bulk density of 1.58 Mg $m^3$, a pH of 7.8, organic carbon of 1%, sand of 31.5 %, silt of 30.7 %, and clay of 37.8%.

### Experimental design

There were three times of herbicide application and four methods of weed control, in a split plot design with 3 replicates. Sub-plot size was 13.5 (4.5 x 3) $m^2$.

Main plots – time of herbicide application

- (i) before sowing (BS) - herbicide was applied after land leveling and one day before sowing
- (ii) after sowing but before irrigation (ASBI) (irrigation was applied shortly after sowing, on the same day)
- (iii) after sowing and irrigation (ASI) - herbicide was applied two days after irrigation

Sub-plots – weed control method

- (i) weed-free
- (ii) weedy(control)
- (iii) oxadiargyl 80 g active ingredient (ai) $ha^{-1}$
- (iv) pendimethalin 1000 g ai $ha^{-1}$

Herbicides were applied using a knapsack-sprayer with a 1.2 m boom with three flat fan nozzles and spray volume was 450 L $ha^{-1}$. To prevent the movement of herbicide after irrigation, the plots were separated by bunds and irrigated one plot at a time. In the weed-free treatment, weeds were removed by manual hand weeding (HW) (5 times). In the 'weedy' control, one hand weeding was performed at 40 DAS and weeds were allowed to grow before and after the hand weeding. One weeding was applied as in the absence of any weed control; there may be no or very little yield of DSR (Chauhan and Johnson, 2011b). In addition, it is rare that farmers leave their rice fields infested with weeds throughout the season in irrigated areas. In the oxadiargyl and pendimethalin treated plots there was also one hand weeding at 40DAS.



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**Crop management**

The experimental site was cultivated twice and leveled before sowing using a four wheel tractor with metal leveler. Dry seed of the rice variety BRRI dhan28 (140 d duration) was sown on 1 February 2013 at 40 kg $ha^{-1}$ and a row spacing of 20 cm. The crop was sown using a seed-drill with a fluted roller seed-metering device and a power tiller linked to a 2-wheel tractor. Fertilizer were applied at 160-20-60-12-2.2 kg $ha^{-1}$ of N, P, K, S and Zn, respectively, in the forms of urea, triple superphosphate (TSP), MoP, gypsum, and zinc sulphate, respectively. All of the TSP, MoP, gypsum, and zinc sulphate were broadcast after land preparation and prior to sowing. The urea was applied in four equal splits at 14 DAS, start of tillering (35DAS), maximum tillering (55 DAS), and panicle initiation (65 DAS).A light irrigation was given just after sowing and the field was kept saturated up to 20 DAS and then irrigations were given based on soil water tension using a threshold value of 15 kPa at 15 cm soil depth (Sudhir-Yadav et al., 2011). At each irrigation, water was added until the depth of water on the soil surface reached 5cm.

**Measurements and analyses:**

Rice plant density (no. $m^{-2}$) was determined at 14 DAS by counting the number of plants in 1 m of row length in five randomly selected locations in each plot. Weed density was determined at 20 DAS, and weed density and weed biomass were determined at 40 DAS and at anthesis, separated into grasses, broad leaves and sedges. Total weed biomass was also determined at the time of rice harvest. At each sampling time, all weeds were collected from two randomly located quadrats of 40 cm × 40 cm. The biomass was measured after oven drying the samples at 70 $^0$C for 72 h.

At harvest, panicle density (no. $m^{-2}$) was determined by counting the number of panicles in 1 m row lengths at 4 randomly selected locations in each plot. The number of florets per panicle (filled and unfilled) was counted on 20 randomly selected panicles per plot. Rice grain yield was determined from an area of 6.6 (3.0 x 2.2) $m^2$. Grain yield was converted to t $ha^{-1}$ at 14% moisture content.

Data were analyzed using ANOVA to evaluate differences between treatments and the means were separated using least significant differences (LSD) at the 5% level of significance using Crop Stat 7.2.

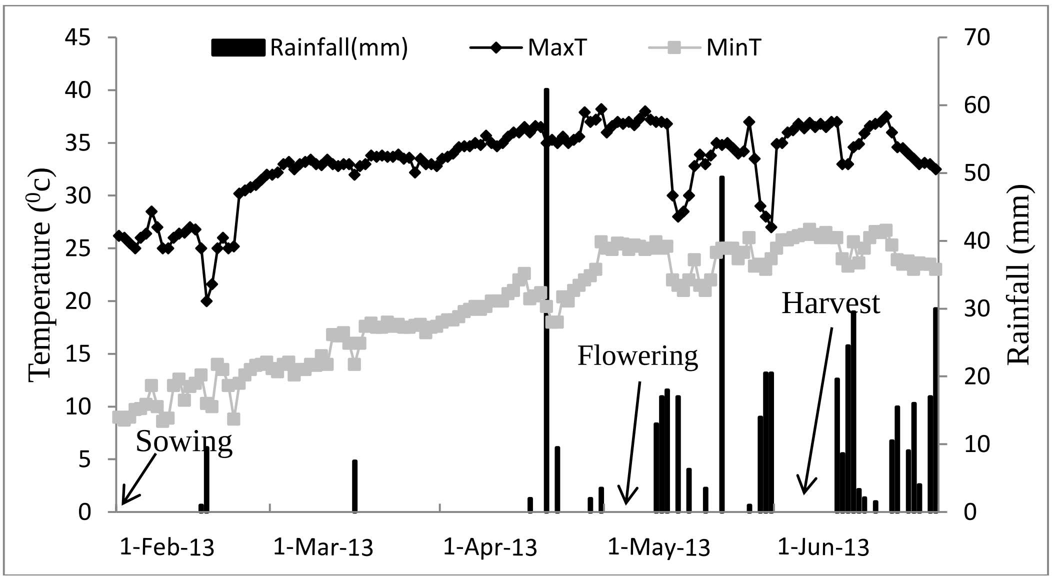


**Fig.1**. Maximum and minimum temperature, and total rainfall (mm) at the experimental site during the cropping period



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## RESULTS AND DISCUSSION

### Effect of application time and weed control method on rice plant stand

Rice plant stand was highly affected by application time and weed control method (Table 1). Plant density ranged from 111 to 195 plant $m^{-2}$. There was a significant interaction between application time and weed control treatments. Rice plant density was least with pendimethalin at all application times, by 43 (BS), 19 (ASBI) and 12% (ASI) compared with the non treated plots (188-195 rice plants $m^{-2}$). In contrast, oxadiargyl did not affect plant density, regardless of application time. Adequate and uniform plant density is pre-requisites to achieving optimum yield in dry-seeded rice (Chauhan et al., 2011). In our study, application of pendimethalin to dry soil before irrigation had more phyto-toxicity than when applied after irrigation. Chauhan and Johnson (2011) found that phytotoxicity on rice seedling emergence was more when oxadiazon was applied to saturated soil than to dry soil. Thus, soil moisture at the time of application has an important influence on herbicide phytotoxicity in rice.

### Effect of application timing and weed control method on weed density and biomass

The dominant weed species in the experiment were: *Celosia argentea* L., *Cyperus rotundus* L., *Dactyloctenium aegyptium* (L.) Willd., *Digitaria ciliaris* (Retz.)Koel., *Echinochloa colona* (L.) Link., *Eleusine indica* (L.) Gaertn, *Leptochloa chinensis* L., *Phyllanthus niruri* L., *and Spilanthes paniculata* Wall. Ex Dc. At 20 DAS, there was a significant interaction between herbicide timing and weed control method on density of grass and broad leaf weeds, but not sedges (Table 2). Pendimethalin was very effective against grasses regardless of application time, reducing the grass population to zero when applied before sowing or after sowing but before irrigation, and reducing it to 8% of the grass weed density in the weedy plots when applied after sowing

**Table 1.** Effect of application time and weed control method on rice plant density at 14 DAS

| Application time | Plant density (number $m^{-2}$) | | | |
|---|---|---|---|---|
| | Weed free | Weedy | Oxadiargyl | Pendimethalin |
| BS | 192 | 189 | 185 | 111 |
| ASBI | 190 | 188 | 186 | 157 |
| ASI | 195 | 193 | 189 | 171 |
| Lsd $_{0.05}$ $^{a}$ | | | 5.36 | |
| Lsd $_{0.05}$ $^{b}$ | | | 6.15 | |

BS= before sowing, ASBI=after sowing but before irrigation, and ASI= after sowing and irrigation

a=same level of application time

b=same level of weed control method

**Table 2.** Effect of application time and weed control method on weed density (number $m^{-2}$) of grasses, broad leaf weeds and sedges at 20 DAS.

| Weed control methods | Weed density (number $m^{-2}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Grass | | | Broad leaf | | | Sedges | | |
| | BS | ASBI | ASI | BS | ASBI | ASI | BS | ASBI | ASI |
| Weedy | 266.7 | 230.2 | 290.6 | 331.3 | 356.3 | 334.4 | 137.1 | 153.1 | 163.5 |
| Oxadiargyl | 166.7 | 53.1 | 34.4 | 304.2 | 236.5 | 80.2 | 110.4 | 99.3 | 114.5 |
| Pendimethalin | 0.0 | 0.0 | 24.0 | 243.8 | 153.1 | 126.0 | 168.8 | 146.9 | 154.2 |
| Lsd $_{0.05}$ $^{a}$ | | 39.04 | | | 43.67 | | | ns | |
| Lsd $_{0.05}$ $^{b}$ | | 76.31 | | | 60.60 | | | ns | |



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**Table 3**. Effect of application time and weed control method on weed density (number $m^{-2}$) and biomass ($g\ m^{-2}$) at 40 DAS (before hand weeding).

| | Weed density (number $m^{-2}$) | | | Weed biomass ($g\ m^{-2}$) | | |
|---|---|---|---|---|---|---|
| | BS | ASBI | ASI | BS | ASBI | ASI |
| Weedy | 511.04 | 498.13 | 557.29 | 44.53 | 45.96 | 47.17 |
| Oxadiargyl | 495.83 | 314.54 | 223.96 | 41.15 | 37.03 | 26.94 |
| Pendimethalin | 354.17 | 288.58 | 285.83 | 33.14 | 32.32 | 29.15 |
| $Lsd_{0.05}$ [a] | | 56.48 | | | 4.64 | |
| $Lsd_{0.05}$ [b] | | 82.40 | | | 5.35 | |

Oxadiargyl was also effective against grasses when applied after sowing before and after irrigation (grass density was reduced 77 and 23 88%, respectively, in comparison with the weedy treatment). But the performance of oxadiargyl was very poor when applied before sowing and reduced the grass density by only 38 %. Oxadiargyl was more effective then pendimethalin in controlling broad leaf weeds, however, neither herbicide gave good control. Their effectiveness was greatest when applied after sowing and irrigation. Neither herbicide was able to control sedge (*Cyperus rotundus*).

**Table 4.** Effect of application time and weed control method on weed density (number $m^{-2}$) and biomass ($g\ m^{-2}$) at anthesis.

| | Weed density (number $m^{-2}$) | | | Weed biomass ($gm^{-2}$) | | |
|---|---|---|---|---|---|---|
| | BS | ASBI | ASI | BS | ASBI | ASI |
| Weedy | 529.17 | 560.42 | 583.33 | 182.33 | 186.90 | 192.54 |
| Oxadiargyl | 520.83 | 365.63 | 314.58 | 166.34 | 130.03 | 105.13 |
| Pendimethalin | 420.83 | 345.00 | 336.46 | 143.89 | 123.96 | 113.69 |
| $Lsd_{0.05}$ [a] | | 59.91 | | | 10.91 | |
| $Lsd_{0.05}$ [b] | | 72.23 | | | 18.24 | |

At both 40 DAS (before hand weeding) and anthesis, the interactions between application time and weed control method on total weed density and total weed biomass were significant (Tables 3 and 4). This was largely due to the fact that application of pendimethalin before sowing had a significant effect on weed density and weed biomass, while oxadiargyl did not. None the less, weed control with pendimethalin applied before sowing was poor. There was a consistent trend for better herbicide performance with application after sowing and irrigation than before irrigation, but only in the case of oxadiargyl were the differences significant. The net result was that weed density and biomass of oxadiargyl and pendimethalin applied after sowing and irrigation were similar (except for higher weed density with pendimethalin 40 DAS). At 40 DAS and anthesis, both herbicides reduced total weed biomass by around 40%.

**Effect of application time and weed control method on rice panicle density**

Panicle density was strongly influenced by both application time and weed control method (Table 5). Application of herbicide significantly reduced panicle density compared with the weed free plots, more so as application time was advanced. The effect of oxidargyl and pendimethalin on panicle density was similar within each application time. The mean reduction in panicle density was 50, 40 and 12% with application before sowing, after sowing, and after sowing and irrigation, respectively. Panicle density was significantly higher in all herbicide treatment combinations than in the weedy control (161 panicles $m^{-2}$). Application of herbicide after sowing and irrigation increased panicle density to around 294 panicles $m^{-2}$, which was still significantly lower than panicle density in the weed free treatment (333 plants $m^{-2}$).



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**Effect of application time and weed control method on rice yield**

Trends in the effects of the treatments on grain yield were similar to the effects on panicle density, with higher yield as the time of herbicide application was delayed (Table 6). Yields with oxidiargyl and pendimethalin were similar, with maximum yield (4.0 t $ha^{-1}$) with application after sowing and irrigation, 1 t $ha^{-1}$ lower than yield of the weed free treatment. Herbicide application after sowing and irrigation increased yield by 2.1 t $ha^{-1}$compared with the weedy treatment (1.9 t $ha^{-1}$) Rice grain yield was negatively correlated with weed biomass at harvest and 73% ($R^2$ = 0.73) of the variation was explained by this relationship (Fig. 2).

**Table 5**. Effect of application time and weed control method on panicle density (number $m^{-2}$).

| Application timings | Panicle(number $m^{-2}$) | | | |
|---|---|---|---|---|
| | Weed free | weedy | Oxadiargyl | Pendimethalin |
| BS | 330 | 160 | 174 | 161 |
| ASBI | 335 | 171 | 196 | 210 |
| ASI | 335 | 165 | 298 | 289 |
| Lsd $_{0.05}$ $^{a}$ | 10.80 | | | |
| Lsd $_{0.05}$ $^{b}$ | 15.10 | | | |

**Table 6.** Effect of application time and weed control method on grain yield (t $ha^{-1}$)

| Application timings | Yield (t $ha^{-1}$) | | | |
|---|---|---|---|---|
| | Weed free | Weedy | Oxadiargyl | Pendimethalin |
| BS | 4.99 | 1.98 | 2.20 | 2.33 |
| ASBI | 5.03 | 2.07 | 2.47 | 2.55 |
| ASI | 4.96 | 1.90 | 4.09 | 3.97 |
| Lsd $_{0.05}$ $^{a}$ | 0.15 | | | |
| Lsd $_{0.05}$ $^{b}$ | 0.22 | | | |

BS= before sowing, ASBI=after sowing but before irrigation, and ASI= after sowing and irrigation

a=same level of application timing

b=same level of weed control methods

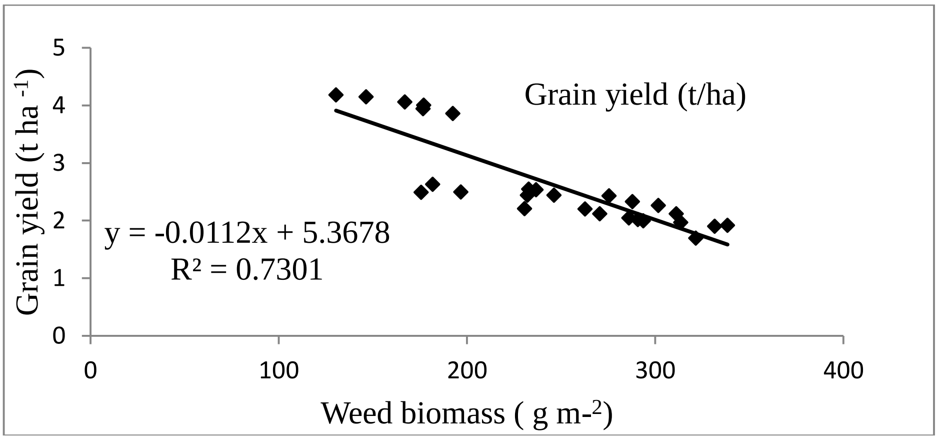


**Fig. 2.** The relationship between grain yield and weed biomass at harvest

## CONCLUSIONS

The study suggests that to increase herbicide efficacy on weeds and reduce phytotxicity to dry seeded rice, both oxadiargyl and pendimethalin should be applied after sowing and irrigation. These results are consistent with the findings of Jordan *et al*. (1998) who suggested that pendimethalin and benthiocarb should be applied once the rice seed has imbibed water but prior



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to the emergence of both rice and weeds. In addition, our study also suggests, preplant application of pendimethalin may not be feasible, and this may reduce some mechanization options (e.g., spray with a tractor before irrigation/wet conditions). The results also indicate that there is a need to further strengthen weed management strategies as the gap was around 1 t ha$^{-1}$ between the weed free treatment and treatment with pre-emergence herbicide followed by one hand weeding."

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## *Weed Management Field Crops*

# EFFICACY AND CROP SELECTIVITY OF TOPRAMEZONE FOR POST-EMERGENCE WEED CONTROL IN MAIZE

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**ABSTRACT** Pre-emergence herbicides with residual action are currently a key tool for effective weed control in New Zealand maize crops. However, the long term sustainability of some of these is under threat due to problems associated with resistance and accelerated breakdown by soil microbes. Thus there is a need to develop weed control programmes that rely more on post-emergence herbicides. Topramezone is a recent, 4-HPPD inhibitor post-emergence herbicide with good efficacy against the major broadleaf and the warm season annual grass weeds. It was evaluated in four field trials between 2010 and 2012, with the adjuvant Hasten (0.5% v/v), for weed control in maize crops. At sites with a mixed spectrum of broadleaf and annual grass weeds, topramezone applied early post-emergence (4-5 leaf stage of maize), provided effective season-long control at rates ≥67 g ai/ha. Addition of atrazine (500 g ai/ha) improved the speed of initial knockdown and was required for effective control of certain broadleaf species such as *Lepidium didymum*, *Oxalis vallicola*, *Polygonum aviculare* and *Solanum nigrum*. Topramezone was also effective for control of large broadleaf weeds when used as a late post-emergence treatment (12-13 leaf stage of maize) following pre-emergence metolachlor at 1.92 kg ai/ha. Addition of atrazine at this stage significantly improved its rate of knockdown and efficacy. Some large (>50 cm) *Chenopodium album* and multi-crowned *Rumex obtusifolius* plants survived the rates and combinations evaluated. Topramezone showed better efficacy than mesotrione, especially at sites with greater proportion of annual grass weeds but 100 g ai/ha was required for control of tillering *Digitaria sanguinalis* plants. Some minor phytotoxicity to uppermost maize leaves at application time was observed in the second year with cooler early season conditions, but the symptoms disappeared quickly. All topramezone treatments resulted in considerably higher maize silage and grain yields compared to the untreated control.

***Keywords:*** herbicide, maize, post-emergence, pyrazolone, topramezone, *Zea mays*.

## INTRODUCTION

Pre-emergence herbicides, often in combination with atrazine or terbuthylazine, are the main chemical weed control options currently available to maize growers in New Zealand (James et al. 2010). This spray programme usually provides satisfactory control of most annual weeds in maize crops. However, in recent years growers have faced problems with herbicide resistance (James and Rahman 2005; Rahman et al. 2001; 2008), herbicide persistence (Rahman and James 2002), enhanced herbicide degradation by soil microbes (James et al. 2010) and changes in weed spectrum towards more difficult to control weeds (James et al. 2007; Rahman 1988). Current research efforts are directed therefore to develop weed control programmes that rely more on post-emergence herbicides and can avoid some of the above mentioned problems.

Topramezone is a selective post-emergence herbicide that has been recently introduced for use in maize crops (Bollman et al. 2008; Gitsopoulos et al. 2010; Soltani et al. 2007). It belongs to the chemical class called pyrazolones. Its mode of action is to inhibit the enzyme 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) and the biosynthesis of plastoquinone, with subsequent carotenoid pigment formation, membrane structure and chlorophyll disruption (Grossmann and Ehrhardt 2007; Schonhammer et al. 2006). Plant damage is expressed in pronounced bleaching symptoms of the growing shoot tissue and subsequent necrosis of the



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above ground foliage. Its moderate water solubility and persistence in soil allows root as well as foliar uptake by plants. This herbicide has been introduced to New Zealand as a 336 g/L SC formulation and is pending registration for control of weeds in maize. This paper reports on a series of field trials carried out to determine its crop selectivity, optimum use rates, and weed control efficacy under New Zealand conditions where the weed seed bank in maize fields is frequently very large (Rahman et al. 2006).

## MATERIALS AND METHODS

Four field trials were conducted between 2010 and 2012 on commercial maize properties in Waikato and Bay of Plenty regions, with two trials laid down in each growing season. Location of these trials is listed in Table 1, along with some soil details, planting dates and herbicide spraying dates for each trial.

**Table 1** Some details of the four field trials.

| Trial No. | Location | Soil texture | Soil pH | Organic C (%) | Planting date | Pioneer® cultivar | Spraying date Post-em |
|---|---|---|---|---|---|---|---|
| 1 | Matamata | Silt loam | 6.7 | 4.0 | 30.10.10 | 35Y33 | 23.11.10 |
| 2 | Parawera | Silt loam | 5.5 | 4.1 | 5.10.10 | 34P88 | 7.12.10[b] |
| 3 | Gordonton | Peaty loam | 5.6 | 19.3 | 26.10.11 | 34P88 | 23.11.11 |
| 4 | Matata[a] | Loamy sand | 6.2 | 3.3 | 10.10.11 | 34P88 | 8.11.11 + 15.11.11 |

[a] Bay of Plenty region, the other three sites all in Waikato region.
[b] Trial 2 also received a pre-emergence treatment of S-metolachlor on 7.10.10.

All trials were of a randomized block design and the treatments were replicated four times. Each plot was 10 m by 3 m with five or six 50 cm wide maize rows in Trial 3, and four 75 cm wide maize rows at the other sites. All herbicide treatments were applied with a precision $CO_2$ pressurized back pack sprayer fitted with four TeeJet 11003 nozzles at 75 cm spacing and applying 200 litres/ha spray mix at 190 kPa. Starter fertiliser and a side dressing of urea were applied as per commercial practice.

The herbicide treatments included an untreated control and various rates of topramezone (BAS 670 01H, 336 g/L) with or without atrazine. Mesotrione alone or in combination with atrazine was included for comparison. All topramezone treatments were applied with the adjuvant Hasten (0.5% v/v) while mesotrione treatments included Synoil (1% v/v). In Trials 1 and 3 all treatments were applied at an early post-emergence stage, while in Trial 2 these were applied late post-emergence following a pre-emergence application of S-metolachlor at 1.92 kg/ha. Trial 4 included both early and late applications of treatments with no pre-emergence herbicide. Growth stages of the weeds and the maize crop at each application time are summarized in Table 2.

Weed species present at the time of post-emergence applications were recorded along with their relative abundance (%) and growth stage (size and leaf/tiller number). Herbicide efficacy was assessed visually on three occasions using relative weed ground cover scores (%) and a percent damage score rating along with observational notes. Individual weed species were identified and counted on two occasions in each plot using two representative 0.1 $m^2$ quadrats/plot.

**Table 2.** Growth stages of weeds and maize crop at the time of post-emergence spraying in the four trials.

| | | Maize (average) | | Grass weeds | | Broadleaf weeds | |
|---|---|---|---|---|---|---|---|
| Trial No. | Time of spraying | Leaf No. | Height (cm) | Tiller No. | Diameter (cm) | Leaf No. | Height (cm) |



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|---|---|---|---|---|---|---|---|
| 1. | Early – 2 WPE [a] | 5 | 23.4 | 1 – 3 | 1 – 2 | 2 – 8 | 1 – 5 |
| 2. | Late – 8 WPE | 13 | 136.2 | –[b] | – | 6 – 50 | 4 – 80 |
| 3. | Early – 3 WPE | 4 | 13.5 | 1 – 2 | 2 – 4 | 2 – 7 | 2 – 6 |
| 4. | Early – 2 WPE | 4 | 13.1 | 0 – 1 | 2 – 3 | 5 – 7 | 2 – 3 |
| | Late – 3.5 WPE | 6 | 21.2 | 2 – 3 | 5 – 6 | 8 – 12 | 4 – 5 |

[a] Weeks post-crop emergence.
[b] Trial sprayed with metolachlor 1.92 kg ai/ha, so very few grass weeds were present.

Maize silage yields were measured in March at the time of commercial silage harvest. Ten representative maize plants per plot were cut at 10 cm above ground level and dried in the oven at 80°C to determine dry matter (DM) weights and calculate silage yield (t/ha). Grain yield was measured in April/May by collecting all cobs along two 4 m representative strips from two central rows of maize in each plot. The number of maize plants was counted in these two strips to determine plant populations. The cobs were shelled, the grain weight recorded and a sub-sample was dried at 105°C for 40 h to determine the moisture content of the grain. Grain yields per hectare were calculated using the average final plant population and adjusted to 14% moisture content.

All data were subjected to analysis of variance (Genstat, version 11) to separate the means. The treatment arithmetic means and least significant difference (LSD) are presented for the weed scores and silage and grain yields for each trial site.

## RESULTS AND DISCUSSION

### Herbicide phytotoxicity

No visual phytotoxicity due to topramezone treatments was evident in the two trials conducted during the 2010/11 growing season, even after the late post-emergence application where direct spray contact occurred due to the height of maize plants (13th leaf stage). However, some minor damage was observed in the first or second week after spraying during the 2011/12 growing season in both trials (damage scores between 0.5 and 8.3%). The phytotoxicity symptoms were in the form of bleaching and included slight whitening or yellowing of the leaves which were uppermost at the time of spraying and were more concentrated on the leaf shoulders and tips, i.e. the parts of leaf most susceptible to spray contact. The damage was slightly greater in combinations with atrazine and in the late application in Trial 4 compared to the early application. The phytotoxicity was similar at all rates of topramezone and disappeared within two weeks, with no measurable effect on plant height or growth. Similar slight levels of damage to maize plants were reported by Gitsipoulos et al. (2010) which disappeared by 20 DAT. Growing conditions early in the season in our 2011/12 trials were typically cooler than the long term average and the resulting slower growth of maize plants could have made them more susceptible to herbicide damage (Peterson et al. 2010).

**Table 3.** Percent weed control scores for different weeds and maize yields for *early* post-emergence treatments in Trial 1.

| Treatment | Rate (g ai/ha) | Oxalis 2 WAT[b] | Oxalis 6 WAT | BL[a] weeds 6 WAT | Grass weeds 2 WAT | Grass weeds 6 WAT | Maize Grain (t/ha) |
|---|---|---|---|---|---|---|---|
| Untreated | – | 0 | 0 | 0 | 0 | 0 | 8.84 |
| Mesotrione | 72 | 39 | 0 | 96 | 30 | 41 | 14.42 |



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|---|---|---|---|---|---|---|---|
| Mesotrione + atrazine | 72 + 500 | 50 | 11 | 100 | 39 | 68 | 14.01 |
| Topramezone | 50 | 80 | 9 | 99 | 88 | 86 | 13.67 |
| Topramezone | 67 | 74 | 18 | 97 | 89 | 93 | 13.06 |
| Topramezone + atrazine | 67 + 500 | 83 | 29 | 100 | 94 | 94 | 16.07 |
| Topramezone | 100 | 86 | 41 | 100 | 96 | 98 | 14.35 |
| Topramezone | 200 | 84 | 53 | 100 | 100 | 100 | 14.13 |
| LSD (P <0.05) | | 11.5 | 15.1 | 3.5 | 11.0 | 12.3 | 2.94 |

[a] Broadleaf weeds excluding oxalis.
[b] Weeks after post-emergence treatment.

**Herbicide efficacy: *early* post-emergence applications**

The efficacy of herbicides applied at early post-emergence (2-3 weeks post-crop emergence) was evaluated in 3 of the 4 trials. The weed spectrum in Trial 1 comprised approximately 68% broadleaf weeds (45% *Oxalis vallicola,* 15% *Chenopodium album,* 5% *Solanum nigrum* , 2% others) and 32% grass weeds (20% *Digitaria sanguinalis,* and 5% each of *Panicum dichotomiflorum* and *Setaria pumila* plus *2% Echinochloa crus-galli*). In this trial all treatments provided excellent control of broadleaf weeds (with the exception of *Oxalis*) for the entire growing season as evidenced by the weed control scores in Table 3. However, it was the control of *Oxalis* and the grass weeds where differences between treatments were evident. All topramezone treatments provided close to 80% control of *Oxalis* at 2 WAT, significantly greater than mesotrione and these differences continued at the 6 WAT assessment for the two high rates of topramezone and its combination with atrazine (Table 3). Topramezone showed good activity also against the three grass weeds present, although by 4 WAT some new seedlings were emerging, with fewer and smaller plants in the plots treated with higher rates. Based on the recovery at the low rates and appearance of new seedlings, *E. crus-galli* appeared most susceptible to topramezone followed by *P. dichotomiflorum*, *S. pumila* and *D. sanguinalis*. The weed cover scores and the weed counts (data not presented) tended to confirm the weed control data in Table 3, particularly the decline in total grass numbers with increasing rates of topramezone.

The weed spectrum in Trial 3 consisted of a mix of broadleaf and grass weeds in proportions similar to Trial 1, with *C. album* (27%), *Persicaria maculosa*. (16%), *D. sanguinalis* (27%) and *P. dichotomiflorum* (8%) being the main species. All topramezone treatments showed good efficacy with a significant reduction in weed cover and had weed control scores of >93% at 2 WAT (Table 4). At the early stage, topramezone combinations with atrazine had significantly less weed cover compared to when used alone (data not presented), indicating a faster knockdown with the addition of atrazine. At 6 WAT, all topramezone treatments were still showing excellent weed control (in the high 90's) with less than 2% weed cover. *Lepidium didymum* was less susceptible to topramezone alone as higher plant numbers (3-9/$m^2$) were recorded in these treatments compared to its combinations with atrazine (0-3/$m^2$). Mesotrione showed much lower efficacy on *P. dichotomiflorum* than topramezone treatments.

**Table 4.** Average percent control scores for all weeds and maize yields for the *early* post-emergence treatments in Trials 3 and 4.

| Treatment | Rate (g ai/ha) | Trial 3 | | Trial 4 | | Trial 3 | Trial 4 |
|---|---|---|---|---|---|---|---|
| | | 2 WAT[a] | 6 WAT | 2 WAT | 6 WAT | Grain (t/ha) | Grain (t/ha) |
| Untreated | – | 0 | 0 | 0 | 0 | 10.5 | 2.2 |
| Mesotrione + atrazine | 72 + 500 | 91 | 83 | –[b] | – | 15.6 | – |



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|---|---|---|---|---|---|---|---|
| Mesotrione + atrazine | 96 + 500 | 89 | 80 | 90 | 56 | 15.2 | 10.1 |
| Topramezone | 67 | 93 | 95 | 96 | 78 | 15.3 | 9.9 |
| Topramezone | 84 | 96 | 96 | – | – | 14.7 | – |
| Topramezone | 100 | 96 | 97 | 97 | 91 | 15.3 | 10.0 |
| Topramezone + atrazine | 67 + 500 | 97 | 97 | 97 | 77 | 15.2 | 9.3 |
| Topramezone + atrazine | 84 + 500 | 98 | 98 | – | – | 15.1 | – |
| Topramezone + atrazine | 100 + 500 | 99 | 99 | 97 | 79 | 14.8 | 10.7 |
| LSD (P<0.05) | | 2.9 | 5.2 | 1.5 | 8.8 | 1.83 | 1.64 |

[a] Percent weed control scores, weeks after post-emergence treatment.
[b] Treatment not included in this trial.

Trial 4 had a very high weed pressure, with the weed density of around 1000 plants/m$^2$, consisting of 98% grass weeds (almost entirely *D. sanguinalis* with a few *P. dichotomiflorum* plants) and 2% broadleaf species, mainly *C. album*. At this high weed infestation site, the early post-emergence treatments of topramezone provided good knockdown of weeds 2 WAT as evidenced by the high weed control scores (Table 4) and very low weed cover in these plots (4-7%). The mesotrione treatment had significantly lower control scores due to its lower efficacy on grass weeds compared to topramezone. However, as no pre-emergence herbicide was used in this trial, new grass weeds were emerging in most treatments by 3 WAT and efficacy scores had declined considerably by the 6 WAT assessment (Table 4).

**Herbicide efficacy: *late* post-emergence applications**

Late post-emergence treatments of topramezone were evaluated in Trials 2 and 4. In Trial 2, S-metolachlor was applied pre-emergence at 1.92 kg/ha to the entire trial area which provided good control of grass weeds. As a result the weed spectrum at the time of application (8 weeks post-crop emergence) consisted mostly of broadleaf weeds ranging 4-80 cm tall. In this situation, both topramezone and mesotrione required longer time to show maximum efficacy and their combinations with atrazine generally provided a better knockdown (Table 5).

**Table 5.** Average percent control scores for all weeds and maize yields for the *late* post-emergence treatments in Trials 2 and 4[a].

| Treatment | Rate (g ai/ha) | Trial 2 | | Trial 4[a] | | Trial 2 | Trial 4 |
|---|---|---|---|---|---|---|---|
| | | 2 WAT | 6 WAT | 1 WAT | 5 WAT | Silage (t/ha) | Grain (t/ha) |
| Untreated | – | 0 | 0 | 0 | 0 | 30.6 | 2.2 |
| Mesotrione | 96 | 45 | 93 | –[b] | – | 30.0 | – |
| Mesotrione + atrazine | 96 + 500 | 71 | 98 | 77 | 51 | 28.4 | 9.4 |
| Topramezone | 50 | 68 | 92 | – | – | 29.3 | – |
| Topramezone | 67 | 58 | 91 | 71 | 87 | 29.0 | 9.6 |
| Topramezone | 100 | 65 | 94 | 73 | 95 | 29.4 | 10.1 |
| Topramezone | 200 | 68 | 94 | – | – | 29.2 | – |
| Topramezone + atrazine | 50 + 500 | 77 | 99 | – | – | 30.6 | – |
| Topramezone + atrazine | 67 + 500 | 79 | 100 | 75 | 82 | 29.3 | 10.9 |
| Topramezone | 100 | 81 | 98 | 76 | 95 | 29.8 | 10.7 |



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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| + atrazine | + 500 | | | | | | |
| LSD (P<0.05) | | 15.0 | 5.0 | 1.5 | 8.8 | 4.72 | 1.64 |

[a]Trial 4 included both early (Table 4) as well as late post-emergence treatments.
[b]Treatment not included in this trial.

At 2 WAT in Trial 2, the efficacy of topramezone on *C. album*, *Nicandra physalodes* and *Rumex obtusifolius* was greater on smaller plants (4-10 cm tall) compared to larger plants (50 cm tall), but on *P. maculosa* and *S. nigrum* the efficacy was similar regardless of their size. *Polygonum aviculare* was also well controlled by topramezone, in combination with atrazine. At the final assessment 6 WAT, excellent weed control was recorded in most plots (Table 5), with only some large *C. album* or multi-crown *R. obtusifolius* plants surviving the treatment.
In Trial 4 no pre-emergence herbicide was used for grass weed control. The late treatments were applied about 3.5 weeks post-crop emergence. At this high weed infestation site dominated by grass weeds, there was a reasonable initial knockdown of all weeds at 1 WAT (Table 5). Later assessments showed that new grass weeds had started emerging, but the level of weed control was still quite good, especially at the 100 g/ha rate of topramezone which provided the greatest suppression of both recovering and seedling *D. sanguinalis* plants. In general, the addition of atrazine to topramezone did not improve the level of weed control at this site infested mostly with grass weeds.

**Maize silage and grain yields**

Conditions for maize growth were favourable during the 2010/11 growing season (Trials 1 and 2) with warm soil temperatures, long sunshine hours and adequate moisture. In the early post-emergence trial (Trial 1) all herbicide treatments provided significantly higher grain yields than the untreated control, but differences between herbicides were not significant (Table 3). Results were very similar with the maize silage yields at this site (data not presented). In Trial 2, with a low weed pressure due to pre-emergence herbicide use, no significant differences were recorded in the silage yield between different treatments (Table 5). Maize grain yield could not be determined at this site as the field was harvested for silage.

In general the silage and grain yields were better than average also in the 2011/12 growing season. With the high level of efficacy that the early applied herbicide treatments provided in Trial 3, they all resulted in significantly higher grain yields than the untreated control, but once again no significant differences were recorded between them (Table 4).

In Trial 4 both silage and grain yields were lower than in other trials due to the high weed pressure and additional weed flushes at this site. The very low yields in the untreated plots (Tables 4 and 5) were significantly different from the herbicide treatments and clearly highlight the importance of good weed management. However, again there were no significant differences in the yields between herbicide treatments.

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# BIO-EFFICACY OF ETHOXYSULFURON 15 WG (SUNRICE 15 WG) FOR MANAGEMENT OF SEDGE AND BROADLEAVED WEEDS IN SUGARCANE

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**ABSTRACT** A field study was conducted for consecutive two times on sandy clay loam soil of Agricultural Research Station, Kathalagere, Davanagere, University of Agricultural Sciences, Bangalore. The study was conducted to know the bio-efficacy of ethoxysulfuron 15 WG (Sunrice 15 WG, formulated by M/S Bayer Crop Science Limited, Mumbai) in relation to other pre-emergence herbicides – atrazine and metribuzin, post-emergence herbicide 2,4-D sodium salt and hand weeding at 20 DAP (days after planting) followed by earthing up at 45, 70 and 100 DAP as farmers' practice in sugarcane crop during 2009 – 10 and 2011 - 12. These treatments were replicated three times in a RCBD experiment using sugarcane variety Co 86032**.** The soil was sandy clay loam with fertility status of 0.65% OC, available $P_2O_5$ of 35.0 kg/ha and $K_2O$ of 225.0 kg/ha. The major weeds observed in the experimental fields were *Cyperus rotundus, Cynodon dactylon, Digitaria marginata, Amaranthus viridis, Cyanotis axillaris* and *Phyllanthus niruri.* The new molecule, ethoxysulfuron 15 WG at 56.25 to 60.0 g ai/ha – 12 DAP gave cane yield of 99.9 to 101.2 t/ha during 2009-10 and 110.2 to 115.8 t/ha during 2011-12, similar to pre-emergence herbicide, atrazine 50 WP at 1.0 kg ai/ha (94.7 t/ha and 102.8 t/ha), metribuzin 70 WP at 0.5 kg ai/ha – 3 DAP (99.6 t/ha and 110.1 t/ha). In addition, these treatments gave cane yield slightly lower than hand weeding (124.1 t/ha and 132.9 t/ha) in addition to saving in weeding cost by Rs.4000 /ha under present labour constraint conditions. The stiff competition offered in unweeded control lowered the growth aspects like cane height and number of millable canes/ha considerably. All these lowered traits caused lower cane yield by 63.9 % (44.8 t/ha) and 65.0 % (44.8 t/ha) in unweeded control mainly due to competition offered by sedge and broad leaf weeds.

***Keywords:*** Bio-efficacy, competition, ethoxysulfuron, cane yield.

## INTRODUCTION

Weeds are regarded as one of the major negative factors of crop produce loss due to competition for nutrients, moisture, light and space which have been reported as high as 30 – 70 per cent. Weeds are one of the major constraints in achieving potential yield of sugarcane. Various studies have shown that weed competition has resulted in an estimated sugarcane yield loss of 12 to 83% (Sathyavelu *et al.*2002, Kanwar *et al.*1992). Sugarcane, being a long duration crop with slow initial growth habit is severely affected by weed infestation. A weed free environment during the germination and tillering phase is important for getting higher cane yield. Now a days, constraints in timely availability of labour, higher economic cost of labour for manual weeding and its lower efficacy, farmers are relying heavily on herbicides for effective weed control in different crops including sugarcane. Selection of appropriate herbicide along with accurate dose and time of application is the key to success for controlling weeds. Sometimes farmers will not be in a position to apply pre-emergence herbicides due to unfavourable weather conditions and they demand effective post-emergence herbicides for management of weeds in sugarcane. So the present investigation was undertaken to study the bio-efficacy of early post-



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emergence herbicide ethoxysulfuron 15 WG (Sunrice 15 WG) formulated by M/S Bayer Crop Science Limited, Mumbai for management of sedge and broad leaf weeds in sugarcane.

## MATERIALS AND METHODS

A field study was conducted for consecutive two times during summer 2009 to summer 2010 and summer 2011 to summer 2012 on sandy clay loam soil of Agricultural Research Station, Kathalagere, Davanagere District, University of Agricultural Sciences, Bangalore. The study was conducted to know the bio-efficacy of ethoxysulfuron 15 WG (Sunrice 15 WG, formulated by M/S Bayer Crop Science Limited, Mumbai) in relation to other pre-emergence herbicides – atrazine and metribuzin, post-emergence herbicide, 2,4-D sodium salt and hand weeding followed by earthing up as farmers' practice in sugarcane crop during 2009 – 10 and 2011-12. The soil type was sandy clay loam with average fertility status of 0.65% OC, available $P_2O_5$ of 35.0 kg/ha and $K_2O$ of 225.0 kg/ha. Eight treatments tried were as follows: $T_1$ – ethoxysulfuron 15 WG (Sunrice 15 WG) at 46.9 g ai/ha – 12 days after planting (3-4 leaf stage of *Cyperus rotundus*) (DAP), $T_2$ – ethoxysulfuron 15 WG at 56.25 g ai/ha – 12 DAP, $T_3$ – ethoxysulfuron 15 WG at 60.0 g ai/ha – 12 DAP, $T_4$ – atrazine 50 WP at 1.0 kg ai/ha – 3 DAP, $T_5$ – metribuzin 70 WP at 0.5 kg ai/ha - 3 DAP, $T_6$ – 2,4-D sodim salt 80 WP at 1.0 kg ai/ha - 12 DAP, $T_7$ – hand weeding (20 DAP fb earthing up at 45 DAP, 70 and 100 DAP) and $T_8$ – unweeded control. These treatments were replicated three times in a RCBD experiment using Cv. Co 86032. The pre-emergence herbicides – atrazine 50 WP and metribuzin 70 WP were sprayed using spray volume of 750 litres/ha on 3 DAP with flood jet nozzle (WFN 72) attached to the Knapsack sprayer, while post-emergence herbicides – ethoxysulfuron and 2,4-D sodium salt were sprayed using a spray volume of 500 litres/ha using flood jet nozzle (WFN 60). The crop was planted at a common row spacing of 90 cm and 30 cm between sets with an uniform fertilizer dose of 250 kg N, 75 kg $P_2O_5$, 75 kg $K_2O$ per ha. At planting, 10% of N, 100% $P_2O_5$ and 100% $K_2O$ were applied in the form of urea, super phosphate and muriate of potash as basal dosage. The remaining N was applied as 20% at 6 weeks after planting, 30% at 10 weeks and 40% at 14th week after planting. The gross and net plot sizes were 9.0 m X 4.5 m and 6.0 m X 3.9 m, respectively.

The data on species wise weed count specially in sedge and broad leaf weeds in a quadrant of 50 cm X 50 cm were collected on 12 days after planting (DAP), 42, 72 DAP and at harvest. Weeds' density and dry weight category wise - sedge, grasses and broad leaf weeds at 12 days after planting (DAP), 42, 72 DAP and at harvest are provided in Table 2 and 3. The data on weeds' density and dry weight were analysed using suitable transformations like square root transformation {square root of (X + 1)} and logarithmic transformation {$\log_{10}$ (X + 2)}, depending on the extent of variability. The plant observations like cane height, number of millable canes/ha and cane yield (t/ha) were collected at harvest and the data on these are presented in Table 3. The grassy weeds were removed manually in ethoxysulfuron sprayed plots around 45-50 DAP to avoid the competition from grasses.

## RESULTS AND DISCUSSION

**a) Weed Flora.** The major weeds observed in the experimental fields were *Cyperus rotundus* (from initial stage) (among sedges), *Euphorbia geniculata, Desmodium triflorum*, *Cyanotis axillaris, Ocimum canum, Portulaca oleracea* (from initial stages), *Phyllanthus niruri* (from 12 DAP), *Mimosa pudica, Commelina benghalensis* (from 42 DAP onwards), *Spilanthes acmella* (from 72 DAP onwards) (among broad leaf weeds). The other weeds observed in lesser number were *Amaranthus viridis, Emilia sonchifolia, Ageratum conyzoides* and *Cleome monophylla* (among broad leaf weeds).

**b) Weed Density.** At 12 days after planting (DAP), pre-emergence application of metribuzin at 0.5 kg/ha lowered density of sedge and broad leaf weeds as compared to other herbicides –



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pre-emergence application of atrazine at 1.0 kg ai/ha – 3 DAP, post-emergence application of ethoxysulfuron 15 WG at 46.9 to 60.0 g ai/ha – 12 DAP and hand weeding, which were yet to be imposed (Table 1). However, hand weeding had higher weeds' density and was similar to unweeded control, as the first weeding was yet to be imposed on 20 days after planting. At this stage, the density of sedge was more, followed by broad leaf weeds (particularly *D. triflorum, C. axillaris* and *P. oleracea*) (Table 1). At 42 DAP, application of post-emergence herbicide – ethoxysulfuron 15 WG at 56.25 to 60.0 g ai/ha gave density of sedge and broad leaf weeds slightly lower or similar to plots treated with pre-emergence herbicides – metribuzin and atrazine, post-emergence 2,4-D sodium salt. Further, all these treatments showed higher weeds' density as compared to hand weeding, but eventually lower than unweeded control (Table 1). Increasing the dose of ethoxysulfuron 15 WG from 46.9 to 60.0 g ai/ha lowered the density of sedge and broad leaf weeds considerably. At this stage, the density of broad leaf weeds (particularly *A. indica, M. pudica, O. canum, D. triflorum*) was higher, followed by sedge.

At 72 DAP, use of post - emergence herbicide – ethoxysulfuron 15 WG at 56.25 to 60.0 g ai/ha resulted in lower weeds' density or similar to plots treated with pre-emergence herbicides – metribuzin, atrazine and post-emergence 2,4-D sodium salt. Further, all these treatments showed higher weeds' density as compared to hand weeding, but eventually lower than unweeded control. Similar trend in reduction in weed density due to use of herbicides was noticed by Srivastava (2001). Increasing the dose of ethoxysulfuron 15 WG from 46.9 to 60.0 g ai/ha lowered the density of broad leaf weeds and sedge considerably. At this stage, the density of broad leaf weeds (particularly *M. pudica, P. niruri* and *O. herbacea*) was higher, followed by sedge. Compared to previous stage, the density of sedge showed increase in all herbicide treatments. The same trend continued in most of the treatments even at harvest.

**c) Weed dry weight** Regarding the dry weight of weeds under unweeded control, the sedge dominated at 12 DAP, while broad leaf weeds dominated at other stages. (Table 2). At 12 DAP, dry weight of weeds – sedge and broad leaf weeds was higher in all treatments except pre-emergence metribuzin and 2,4-D, yet to be applied. Further, hand weeding showed higher dry weight of all weeds, as first hand weeding yet to be imposed on 20 DAP and compared similar to unweeded control. At 42 DAP the dry weight of weeds – sedge and broad leaf weeds was pretty lower in plots treated with ethoxysulfuron 15 WG at 56.25 to 60.0 g ai/ha and pre-emergence herbicides – atrazine and metribuzin, post-emergence 2,4-D sodium salt. Further, ethoxysulfuron treatment at 46.9 g ai/ha showed slightly higher weeds' dry weight, perhaps due to lower dosage. Nevertheless, unweeded control had considerably higher weeds' dry weight as compared to herbicide treated plots, while hand weeding had the lowest dry weights of all weeds as compared to herbicide treatments (Table 2).

At 72 DAP, the trend in weeds' dry weight continued similar to previous stage in most of the treatments and there was increase in the dry weight of sedge and broad leaf weeds as compared to previous stage. The dry weight of weeds – sedge and broad leaf weeds were comparable in treatments receiving herbicide - ethoxysulfuron 15 WG at 56.25 to 60.0 g ai/ha and pre-emergence herbicides – atrazine and metribuzin, post-emergence 2,4-D sodium salt. Nevertheless, unweeded control had considerably higher dry weight of sedge and broad leaf weeds as compared to herbicide treated plots. Compared to 42 DAP, weeds' dry weight showed an increase in all treatments due to increase in weed's density and or increase in growth of weeds with advance in age. The same trend continued at harvest also in most of the treatments.

**d) Growth and Cane Yield:**

The stiff competition offered in unweeded control lowered the growth aspects like cane height and number of millable canes/ha considerably. All these lowered traits caused lower cane yield by 64 and 65% in unweeded control during 2009-10 and 2011-12, respectively(Table 3), mainly due to competition offered by sedge and broad leaf weeds.



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The new molecule, ethoxysulfuron 15 WG at 56.25 to 60.0 g ai/ha – 12 DAP gave cane yield (99.9 to 101.2 t/ha during 2009-10) and (110.2 to 115.8 t/ha during 2011-12) similar to pre-emergence herbicide, atrazine 50 WP at 1.0 kg ai/ha (94.7 and 102.8 t/ha), metribuzin 70 WP at 0.5 kg ai/ha – 3 DAP (99.6 and 110.1t/ha). In addition, these treatments gave cane yield slightly lower than hand weeding (124.1 and 132.9 t/ha). These results corroborate with the findings of Srivastava (2001). Lower dose of ethoxysulfuron 15 WG at 46.9 g ai/ha gave slightly lower yield (92.0 and105.9 t/ha) owing to less control of sedge and broad leaf weeds. However the yield at this dosage was comparable to atrazine and metribuzin treated plots (Table 3, Fig. 1 and 2).

The weed index, an indicative of weeds' competition effect on cane yield, was 64% and 65% in unweeded control, mainly due to severe competition offered by sedge and broad leaf weeds right from initial stages. This is in conformity with the findings of Rana and Singh (2004). The use of ethoxysulfuron 15 WG at 56.25 to 60.0 – 12 DAP gave a weed index of 18.5 to 21.9% and 15.6 to 23.0% during both the years, similar to the plot treated with atrazine at 1.0 kg ai/ha – 3 DAP (23.7 and 23.1%) and metribuzin at 0.5 kg/ha – 3 DAP (19.7 and 18.4%) (Table 3 & Fig. 1 and 2).

## CONCLUSIONS

New post-emergence herbicide, ethoxysulfuron 15 WG (Sunrice 15 WG) formulated by M/S Bayer Crop Science Limited, Mumbai at 56.25 to 60.0 g ai/ha - 12 DAP, can be used safely for control of sedge and broad leaf weeds in irrigated sugarcane, as it gave good control of major weeds and resulted in cane yields similar to pre-emergence metribuzin at 0.50 kg ai/ha – 3 DAP, atrazine at 1.0 kg ai/ha – 3 DAP, 2,4-D sodium salt at 1.0 kg ai/ha – 12 DAP and hand weeding (20 DAP fb earthing up at 45, 70 and 100 DAP). Use of ethoxysulfuron 15 WG at 56.25 to 60.0 g ai/ha restricted the emergence and growth of sedge and broad leaf weeds up to 72 DAP. This suggests that usage of ethoxysulfuron 15 WG (Sunrice 15 WG) at 56.25 to 60.0 g ai/ha as post-emergence herbicide at 12 DAP appeared ideal for management of sedge and broad leaf weeds, safety to sugarcane without causing any phytotoxicity and obtaining higher cane yield under irrigated conditions.

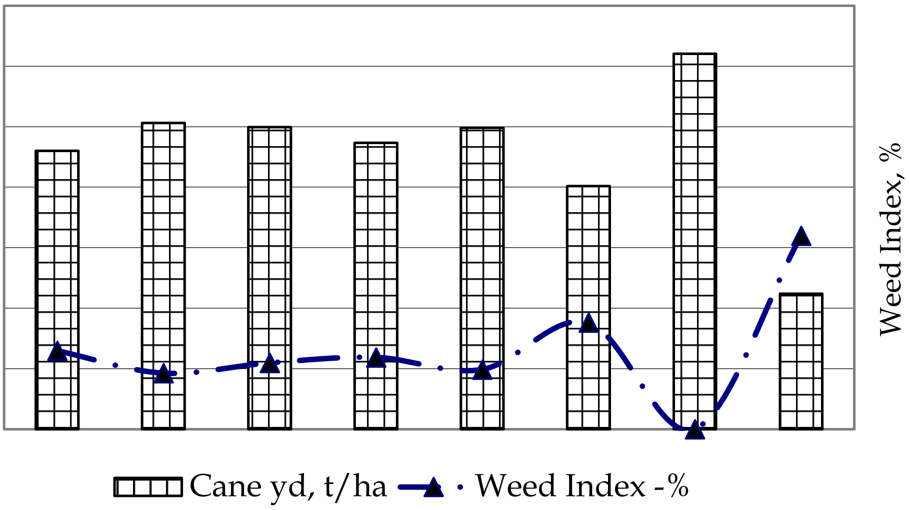


**Fig. 1.** Cane yield and weed index in doses of ethoxysulfuron 15 WG in relation to other weed control treatments in sugarcane during 2009-10 at UAS, Bangalore (For treatment details, refer Table 3)



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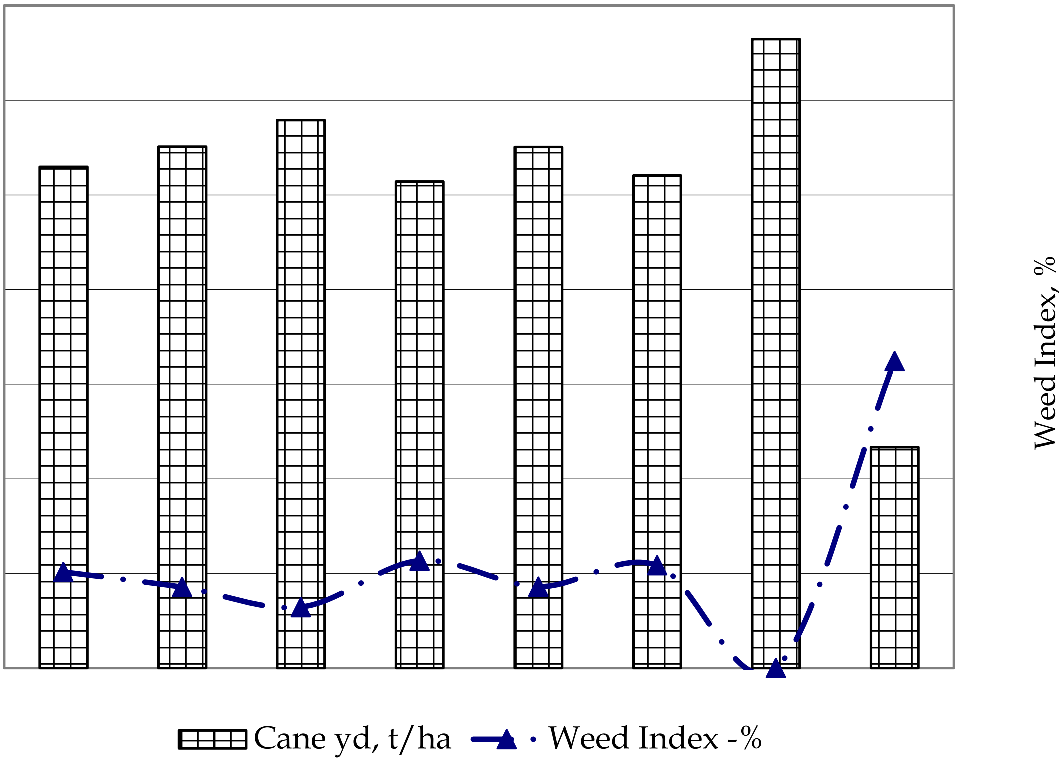


**Fig 2**. Cane yield and weed index during 2011-12 at UAS, Bangalore.

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# EFFECTS OF OIL PALM RACHIS RESIDUE MULCH IN COMBINATION WITH PRETILACHOR ON CONTROL OF GOOSEGRASS (*Eleusine indica*)

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**ABSTRACT** Pre-emergent herbicides play important role in early elimination of goosegrass and protect rice seedlings during the critical period. However, excessive use of the herbicides may cause environmental pollution and development of herbicide-resistant weed. This study was conducted to determine effects of oil palm rachis residues (OPR) in combination with or without pretilachlor on goosegrass grown with Chinese spinach under glasshouse conditions. It was found that rates which gave 75% inhibition of emergence ($ED_{75}$) and 90% inhibition of shoot fresh weight ($ED_{90}$) of goosegrass were 16.7 and 37.5 g/ha, respectively, when treated with pretilachor alone. Interestingly, the respective $ED_{75}$ and $ED_{90}$ values were reduced by 70 and 87% when being mixed with OPR at 2t/ha. These results suggest that OPR has potential to reduce rate of pretilachlor for goosegrass suppression.

***Keywords:*** goosegrass, oil palm rachis, pretilachlor

## INTRODUCTION

Herbicide has been the main strategy for effective weed control in Malaysia agriculture. However, the intensive use of single herbicide for weed control has led to development of herbicide-resistant weed to various groups of herbicides. For instance, grassy weed of goosegrass has developed resistance to fluazifop (Marshall *et al.*, 1994), glyphosate (Lee and Ngim, 2000), paraquat (Itoh *et al.*, 1990), dinitroaniline (Mudge *et al.*, 1984), glufosinate (Chuah *et al.*, 2010). This has resulted in a serious trouble for the growers because of less herbicide options are available in controlling weed infestation. Furthermore, excessive use of herbicide may have adverse impacts on our environment as well as human health (Qasem, 2011). Hence, there is a need to diversify weed management strategy by reducing heavy reliance upon herbicide and one of the strategies is through organic mulching.

In the year of 1998, Malaysia generates about 27.2 mil tons of pruned fronds and 1.38 mil tons of trunks and frond during replanting (Khalid *et al.*, 1999). One of the common practices to utilize oil palm frond residues is disposal as mulches which can suppress weed growth while increasing the overall profitability and sustainability when nutrients are recycled efficiently to the fields. Alternatively, oil palm frond residues can be also processed as dietary supplement, animal feed or raw materials for furniture, pulp and paper industry (Elbersen *et al.*, 2005). Currently, however, the use of mulches alone for weed control is still below the commercially acceptable levels (Somireddy, 2012). It has been suggested that the organic mulches should be combined with pre-emergent herbicide to offer extra benefits for weed control by decreasing the amount of herbicide needed and extending duration of weed control (Azhar, 2010). Hence, this study aimed to determine whether incorporation of oil palm rachis residue mulch with pre-emergence herbicide of pretilachlor could reduce the application rate of pretilachlor on control of goosegrass.



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## MATERIALS AND METHODS

Pretilachlor was supplied from Halex (m) Sdn. Bhd. Goosegrass seeds and oil palm frond residues were harvested from an oil palm plantation around Terengganu area. Goosegrass seeds were scarified using sandpaper. Oil palm fronds were separated into rachis and leaflets, then dried under full sunlight, respectively. The rachis was ground in mill grinder to pass 5 mm screen. Sandy loam soil was obtained from Agticultural Department of Rhu Tapai, Setiu, dried at 40°C and sieved to pass 2 mm screen.

### Experiement 1: Phytotoxic test of single application of pretilachor

100 g soil was placed in each 6-diameter paper cup. 20 goosegrass seeds were sown at 1 cm deep of soil in each cup. A series of pretilachlor rates at 0, 1.2, 4.7, 18.8, and 75.0 g ai/ha was applied to the soil surface, respectively. The goosegrass emergence and shoot fresh weight were measured four weeks after treatment.

### Experiment 2: Phytotoxic test of oil palm rachis residues mulch in combination with pretilachlor or without pretilachlor at a reduced rate

20 goosegrass seeds were sown at 1 cm deep of soil. The rachis residues at 0, 0.5, 1.0 and 2.0 t/ha were incorporated to soil at the surface, respectively. Then, a reduced rate of pretilachlor, $ED_{50}$ (rate that causes 50% inhibition on goosegrass emergence) was applied to the surface of soil and/or the mulch. 9 gram chicken dung was applied at week 2 and 3 after transplanting, respectively. All treatments were arranged in completely randomized design with eight replicates. Four weeks after treatment, goosegrass emergence and shoot fresh weight were recorded.

### Statistical analysis

The data of goosegrass were expressed as percentage of control and fitted to a logistic regression model (Kuk *et al.* 2002)..

## RESULTS AND DISCUSSION

It is noted that oil palm rachis residue (OPR) mulches alone can reduce the emergence and shoot fresh weight of goosegrass. The inhibitory effect was enhanced when increasing the rate of the residues (Fig 1). Similarly, it has been demonstrated that croton leaf residue with different rates from 0.5 to 3t/ha caused varying degrees of inhibition of broadleaves and grassy weeds where higher rates provided more inhibitory effect; this may be attributed to the relative different amounts of allelochemicals which were released from the croton residues in the field (Kowthar *et al*., 2011). Interestingly, a greater inhibition of goosegrass emergence and seedling growth was observed when being mixed with a reduced rate of pretilachlor. Rachis at a rate of 2.0 t/ha added with pretilachlor gave the highest inhibition of goosegrass seedling growth and emergence (Fig1).



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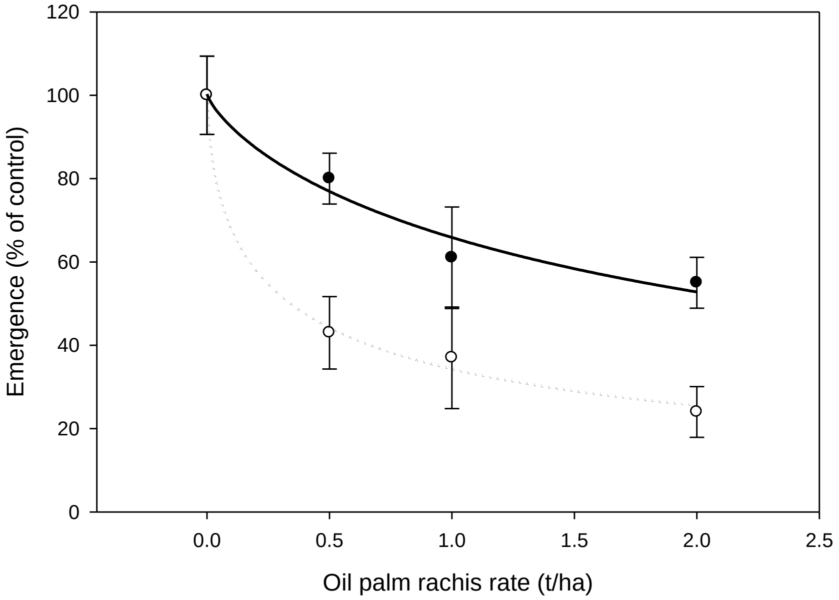


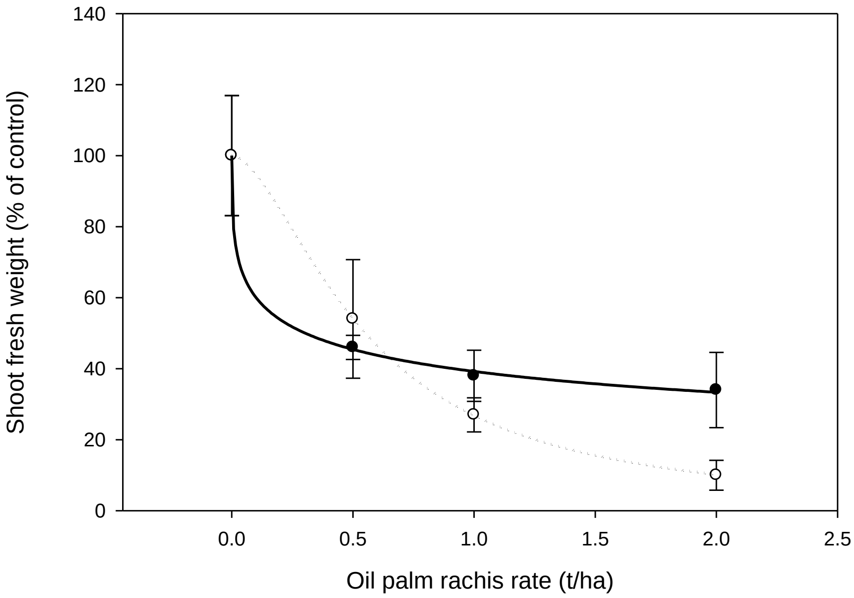


**Fig. 1.** Effects of oil palm rachis residue mulch in combination with (··) or without (-) pretilachlor at a reduced rate on emergence (A) and shoot fresh weight (B) of goosegrass.

The results have shown that rates which give 75% inhibition of emergence ($ED_{75}$) and 90% inhibition of shoot fresh weight ($ED_{90}$) of goosegrass were 16.7 and 37.5 g/ha, respectively, when treated with pretilachor alone. The respective $ED_{75}$ and $ED_{90}$ values were reduced by 70 and 87% when being mixed with OPR mulches at 2t/ha, implying that application of OPR mulches has ability to reduce the pretilachlor rate on control of goosegrass (Table 1 and 2). It is hypothesized that the mulch binds the herbicides and possibly acts as slow release carriers for the herbicides and reduces the leaching potential of the herbicides (Case and Mathers 2003). According to Knight *et al.* (2001) the application of pre-emergence herbicides onto organic mulches reduced herbicide leaching by 35% to 74% compared with bare soil pre-emergence herbicide applications.



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**Table 1**. $ED_{75}$ value of goosegrass emergence in relation to single application and mixture of oil palm rachis residues and pretilachor treatment

| Pretilachlor (g/ha) or oil palm rachis residues (t/ha) treatment alone | | Mixture of pretilachlor (g/ha) and oil palm rachis residues (t/ha) | |
|---|---|---|---|
| Pretilachlor | Rachis | Pretilachor | Rachis |
| 16.7 | >2.0 | 5.0 | 2.0 |

*$ED_{75}$ is the rate that causes 75% inhibition of emergence of goosegrass

**Table 2**. $ED_{90}$ value of goosegrass shoot fresh weight in relation to single application and mixture of oil palm rachis and pretilachor treatment

| Pretilachlor (g/ha) or oil palm rachis residues (t/ha) treatment alone | | Mixture of pretilachlor (g/ha) and oil palm rachis residues (t/ha) | |
|---|---|---|---|
| Pretilachlor | Rachis | Pretilachor | Rachis |
| 37.5 | >2.0 | 5.0 | 2.0 |

*$ED_{90}$ is the rate that causes 90% inhibition of shoot fresh weight of goosegrass

In conclusion, this study has revealed that oil palm rachis residues mulche mixed with a reduced rate of pretilachlor has potential to suppress goosegrass while increasing growth and postharvest quality of Chinese spinach.

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# EVALUATION OF BIO-EFFICACY, WEED CONTROL EFFICIENCY AND YIELD IN HERBICIDE RESISTANT TRANSGENIC-STACKED CORN HYBRIDS (TC1507 x NK603) FOR CROP PRODUCTIVITY

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**ABSTRACT** Weeds are posing a serious problem in maize. The congenial climatic conditions encourage more weed growth in the widely spaced crop like maize and cause yield reduction to the tune of 29 to 70 percent. Advancement in biotechnological research enables genetic engineering to enhance production and minimize crop yield losses through development of crops that are tolerant to insects and herbicides. Transgenic corn hybrids with stacked event, (TC1507X NK603) having both insect protection and herbicide tolerant traits, which will provide protection to the crop against target pests and also provide effective weed management. In this admiration, field experiment was carried out during *kharif*, 2010 at research farm of Tamil Nadu Agricultural University, Coimbatore, India. The experiment was laid out in Randomized Block Design with treatments replicated thrice. The treatments consisted of two transgenic hybrids (30V92 &30B11 HR) resistant to glyphosate, were tried with two different doses of post emergence Glyphosate at 900 and 1800 g a.e $ha^{-1}$ were compared with pre-emergence application of atrazine at 0.5 kg $ha^{-1}$ followed by hand weeding on 40 DAS in non-transgenic maize hybrids like 30V92, 30B11, BIO9681 and COHM5. From the study it is concluded that early post emergence application of glyphosate at 1800 g a.e $ha^{-1}$ recorded lower weed index, weed dry weight and high weed control efficiency at all the intervals compared with other treatments. Higher grain yield was recorded with application of Glyphosate at 1800 g a.e $ha^{-1}$ in transgenic stacked maize hybrid of 30V92HR recorded high productivity and profitability. Unweeded control accounted lesser grain yield which in turn reflected through higher weed index of 58.39 per cent due to heavy competition of weeds for nutrients, space and light.

***Keywords***: Transgenic maize, herbicide tolerance, weed control efficiency and yield

## INTRODUCTION

Maize was an ideal crop due to availability of the plant to acclimate to many soil and climate conditions. It is an important cereal crops cultivated worldwide. Maize not only important human nutrient source, but also a basic element of animal feed and raw material for manufacture of many industrial products. The major yield reducing factors for maize cultivation in India are weeds and insects. Weeds cause considerable yield loss due to competition of resources with maize crop. The development of crop cultivar with resistance to selected herbicides has the positive impact on agricultural production. Wilcut *et al*.,(1996) was reported by selection of proper herbicides is essential for successful weed management in all crop production systems .

Herbicidal weed control seems to be a competitive and promising way to control weeds at initial stages of crop growth. Post emergence herbicides have been achieved adequate weed control programmes, due to its broad spectrum of activity, excellent crop safety, convenience and flexibility was stated by Ferrel and Witt (2002). This necessitates the development and testing of selective early post emergence herbicides for weed control in maize. Glyphosate is a foliar applied, broad spectrum, post emergence herbicide capable of controlling annual,



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perennial grasses and dicotyledonous weeds. The introduction of glyphosate resistant crops has created new opportunities for the use of effective non selective herbicides like glyphosate as selective weed control in crop production. Prior to the introduction of glyphosates resistant crops, glyphosate is being applied to control existing vegetation prior to sowing the crops. Now it can be used as post emergence herbicide in glyphosate resistant crops Herbicide resistant corn plants confer tolerance to glyphosate by production of the glyphosate resistant CP4 5-enolpyruvyl shikimate-3 phosphate synthase (*CP4 EPSPS*) proteins. Transgenic stacked hybrid maize (TC1507 X NK 603) was developed for preventing yield losses for maize crop due to pests and weeds and to improve productivity. The stacked corn crop having both insect protection and herbicide tolerant traits will provide protection to the crop from target pests and also provide tolerance to glyphosate herbicide.

Post emergence application of glyphosate at 1800 g a.e $ha^{-1}$ gave significantly lower weed index, weed dry weight and high weed control efficiency at all the intervals. Post emergence application of glyphosate at 1800 g a.e $ha^{-1}$ in transgenic maize 1800 g a.e $ha^{-1}$ recorded high productivity and profitability. In view of the above facts, an experiment on ‘’Evaluation of bio-efficacy, weed control efficiency and yield in herbicide resistant transgenic stacked corn hybrids’’ was formulated with the following objectives:

i) To evaluate weed control efficiency with K salt of glyphosate formulation in transgenic stacked corn hybrids along with other selective herbicides in conventional hybrids
ii) To study the effect of different weed management practices on corn growth and development

## MATERIALS AND METHODS

Field experiments was laid out during *kharif* season of 2010 in Eastern Block Farm of Tamil Nadu Agricultural University, located at Coimbatore, India. The geographical location of the experimental site is situated in western agro climatic zone of Tamil Nadu at 11°N longitude and 77°E latitude with an altitude of 426.7 m above MSL and the farm receives the total annual rainfall of 674 mm in 45.8 rainy days. The soil of the experimental site was sandy clay loam in texture (32.48% clay, 18.50% silt and 28.96% coarse sand) with low available nitrogen, medium in available phosphorous and high in available potassium.

### Treatment details

$T_1$ - Transgenic stacked 30V92 with POE glyphosate at 900 g a.e/ha*
$T_2$ - Transgenic stacked 30V92 with POE glyphosate at 1800 g a.e./ha*
$T_3$ - Transgenic stacked 30V92 with control (no weed and insect control)
$T_4$ - Transgenic stacked 30B11 with POE glyphosate at 900 g a.e./ha*
$T_5$ - Transgenic stacked 30B11 with POE glyphosate at 1800 g a.e./ha*
$T_6$ - Transgenic stacked 30B11 with control (no weed and insect control)
$T_7$ - Non-transgenic 30V92 with PE atrazine at 0.5 kg $ha^{-1}$ on 3 DAS *fb* HW 40 DAS and insect control
$T_8$ - Non-transgenic 30V92 with no weeding and only insect control
$T_9$ - Non-transgenic 30V92 with no weeding and no insect control
$T_{10}$ - Non-transgenic 30B11 with PE atrazine at 0.5 kg $ha^{-1}$ on 3 DAS *fb* HW 40 DAS and insect control
$T_{11}$ - Non-transgenic 30B11 no weeding and only insect control
$T_{12}$ - Non-transgenic 30B11 with no weeding and no insect control
$T_{13}$ - BIO 9681 with PE atrazine at 0.5 kg $ha^{-1}$ on 3 DAS *fb* HW 40 DAS and insect control
$T_{14}$ - BIO 9681 no weeding and no insect control
$T_{15}$ - CoHM 5 with PE atrazine at 0.5 kg $ha^{-1}$ on 3 DAS *fb* HW 40 DAS and insect control
$T_{16}$ - CoHM 5 no weeding and no insect control

** Glyphosate application at 2 - 4 leaf stage of weeds (approximately 20-25 DAS of maize)*



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**Experiment details**

| | | |
|---|---|---|
| Treatments | : | 16 |
| Replications | : | 3 |
| Design | : | RBD |
| Gross Plot (3.6m x 5.0 m) | : | 18 $m^2$ |
| Rows per plot | : | 6 |
| Row length | : | 5 meter |
| Spacing between rows | : | 60 cm |
| Spacing between plants | : | 25 cm |
| Spacing between replications | : | 1.5 m |
| No. of rows of African tall maize | : | 13 (all four sides of experimental plot) |
| Gross experimental area including African tall | : | 2903 sq m (77.2 m x 37.6 m) |
| Water volume for herbicide spray | : | 250 l/ha for spray of herbicide |
| Spray equipment | : | Knapsack sprayer fitted with flat fan nozzle |

Observations were made on predominant weed flora and weed density and dry weight, corn yield, herbicide residue in the trial.

## RESULTS AND DISCUSSION

### Effect on Weeds

Weed flora of the experimental field consisted of predominantly ten species of broad leaved weeds, five species of grass weeds and a sedge weed. *Trianthema portulacastrum*, *Datura stramonium*, *Cleome gynandra*, *Digera arvensis*, *Physallis minima* and *Corchorus olitorius.* The dominant grass weeds were *Setaria verticillata* and *Cynodon dactylon. Cyperus rotundus* was the only sedge present in the experimental field. During *kharif*, 2010 broad leaved weeds dominated the weed flora (86.09 per cent) and it was followed by sedge (8.48 per cent) and grass (5.41 per cent) at 20 DAS. At 40 DAS, broad leaved weeds dominated among the weed flora (80.49 per cent) followed by sedge (10.89 per cent) and grass (8.61 per cent). At 60 DAS and 80 DAS broad leaved weeds were more (77.76 and 76.95 per cent) compared to sedge (25.00 and 12.82 per cent) and grass (8.50 and 4.36 per cent) respectively.

### Weed Density and Dry Weight

During *kharif*, 2010 season, lower weed density was achieved under non transgenic maize hybrid BIO 9681 and 30B11 with pre emergence application of atrazine at 0.5 Kg $ha^{-1}$ followed by hand weeding at 20 DAS. Relatively, a higher density was observed under unweeded checks and transgenic maize before imposing post emergence application of glyphosate. At 40 DAS and 60 DAS, lower weed density observed under transgenic maize hybrid 30V92 with post emergence application of glyphosate at 1800 g a.e ha⁻1 was 2.04 and 2.35 No's $m^{-2}$. This was comparable with other non-transgenic corn hybrids with same herbicidal treatment resulted in effective control of broad leaved weeds, grasses and sedge due to its broad spectrum action was stated by Kogger and Reddy (2005). This may due to more impressive control of broadleaved weeds like *Trainthema portulacastrum, Datura stramonium, Cleome gynandra and Physallis minima*. Foliar application of glyphosate was readily and rapidly translocated throughout the actively growing aerial and underground portions at active growing stage of broadleaved weeds might have blocked the 5- Enulpyruvyl shikimate-3-phosphate synthase enzyme and arrest the amino acid synthesis which led to complete control was reported by Summons *et al*., (1995).

Weed dry weight is the most important parameter to assess the weed competitiveness for the crop growth and productivity. Pre emergence application of atrazine at 0.5 ka $ha^{-1}$ *fb* HW recorded significantly lesser dry weight of weed under 30V92 maize hybrid and was comparable with same treatment under other non-transgenic hybrids BIO 9681 and COHM5.



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Higher dry weight of total weeds was recorded under unweeded check plots and transgenic hybrids before imposing POE application of glyphosate at different rates.

At 40 and 60 DAS, Considerable reduction in weed dry weight was recorded with the application of glyphosate at 1800 g a.e $ha^{-1}$ in transgenic 30V92 (1.58 and 1.87 g $m^{-2}$ ). This was comparable with other doses of glyphosate under both the transgenic hybrids. Conspicuously, higher dry weight of weeds was registered at unweeded check plots. This might be weed control as achieved by glyphosate. This findings were in accordance with earlier reports that post emergence application followed by pre emergence herbicide reduced total weed dry weight by at least 97 per cent when compared to without glyphosate applied plots was reported by Kumar and Thakur (2005). In non-transgenic maize hybrids, application of atrazine at 0.5 kg $ha^{-1}$ was proved as effective pre-emergence weed control option in maize. Atrazine effectively controlled majority of broad leaved and grassy weeds at earlier stages of maize growth. Mundra *et al.,* (2003) reported that application of atrazine at 0.5 kg $ha^{-1}$ as pre-emergence *fb* inter cultivation at 35 DAS in maize significantly reduced the total weed density and weed dry weight.

**Weed Control Efficiency**

Weed control efficiency which indicates the comparative magnitude of reduction in weed dry matter was highly influenced by different weed control treatments. Pre emergence application of atrazine at 0.5 Kg $ha^{-1}$ followed by hand weeding recorded higher weed control efficiency of 80.28 percent in non-transgenic maize hybrid 30V92 at 20 DAS. Whereas at 40 DAS after spraying of herbicide, higher weed control efficiency of 99.53 per cent was recorded in glyphosate at 1800 g a.e $ha^{-1}$ followed by 30B11was observed 98.97 percent during *kharif*, 2010 (Table 1).

**Effect on Crops**

Among the weed control treatments, post emergence application of glyphosate at 1800 g a.e $ha^{-1}$ in transgenic corn hybrid recorded higher grain yield of 12.21 t·$ha^{-1}$ this was 36.64 percent higher than the unweeded check plot of transgenic 30V92. Lower weed index was recorded with 9.09 and 10.15 per cent in transgenic hybrids of 30V92 and 30B11 (Table 2). Unweeded check plots resulted in higher weed index and performed poorly during the season. This could be achieved control of weeds with non-selective, translocated herbicide, provided the favourable crop growth environment at the establishment stage of the crop itself by minimizing the perennial and annual weeds and increased the seed and stalk yields was stated by Tharp *et al* ., (1999). This might be due to the fact that the perennial weeds like *Cyperus rotundus*, *Cynodon dactylon* and troublesome broadleaved weeds like *Trianthema portulacastrum* were effectively controlled and might increase the maize yield may be due to better light utilization of narrow row zone and faster canopy closure.

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**Table 1.** Effect of weed control methods on weed density, weed dry weight and weed control efficiency in herbicide tolerant transgenic corn*

| Treatments | *Kharif*, 2010 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total weed density (No. $m^{-2}$)* | | | Total weed dry weight (g. $m^{-2}$)* | | | WCE (%) | |
| | 20 DAS | 40 DAS | 60 DAS | 20 DAS | 40 DAS | 60 DAS | 20 DAS | 40 DAS |
| $T_1$ -30V92 POE Glyphosate @ 900 g a.e ha$^{-1}$ | 15.43(236.22) | 2.78 (5.75) | 3.4 (9.63) | 7.61 (55.94) | 1.88 (1.52) | 2.35 (3.54) | - | 98.56 |
| $T_2$ -30V92 POE Glyphosate @1800 g a.e ha$^{-1}$ | 15.33(233.08) | 2.04 (2.15) | 2.35 (3.52) | 7.37 (52.37) | 1.58 (0.49) | 1.87 (1.50) | - | 99.53 |
| $T_3$ - 30V92 POE Glyphosate (Weedy check) | 15.74(245.60) | 14.3(202.93) | 13.81(188.75) | 7.66 (56.62) | 10.39(106.03) | 10.22 (102.43) | - | - |
| $T_4$ -30B11 POE Glyphosate @ 900 g a.e ha$^{-1}$ | 15.78(246.89) | 3.31(8.98) | 3.84(12.74) | 7.40 (52.79) | 2.16 (2.68) | 2.60 (4.75) | - | 97.72 |
| $T_5$ -30B11 POE Glyphosate @1800 g a.e ha$^{-1}$ | 16.06(256.07) | 2.55(4.50) | 3.06 (7.35) | 7.71(57.41) | 1.79 (1.20) | 2.21 (2.87) | - | 98.97 |
| $T_6$ -30B11 POE Glyphosate (Weedy check) | 15.81(248.10) | 14.5 (209.43) | 14.42(205.99) | 8.18(64.92) | 10.94 (117.59) | 10.22 (102.51) | - | - |
| $T_7$ -30V92 PE atrazine 0.5 kg ha$^{-1}$ + HW+ Insect Control | 7.99 (61.85) | 7.81(59.00) | 5.79(31.48) | 3.68 (11.57) | 5.78 (31.43) | 4.52 (18.39) | 80.28 | 72.57 |
| $T_8$ -30V92 No Weed control and only Insect Control | 15.45(236.55) | 13.64(183.99) | 12.7 (160.36) | 7.08 (48.17) | 9.99 (97.79) | 9.42 (86.77) | - | 14.66 |
| $T_9$ -30V92 No Weed control and no Insect Control | 16.05(255.75) | 14.37(204.37) | 14.38(204.69) | 7.79 (58.70) | 10.80 (114.59) | 10.58 (109.99) | - | - |
| $T_{10}$ -30B11 PE atrazine 0.5 kg ha$^{-1}$ + HW+ insect control | 7.55 (55.00) | 8.14(64.34) | 5.87 (32.43) | 3.88 (13.04) | 6.12 (35.48) | 4.72 (20.32) | 79.66 | 70.33 |
| $T_{11}$-30B11No Weed control and only Insect Control | 15.51(238.44) | 13.5 (182.38) | 13.12(170.11) | 7.41(52.92) | 10.3 (105.35) | 9.97 (97.47) | - | 11.92 |
| $T_{12}$ -30B11 No Weed control and no Insect Control | 16.25(262.00) | 15.05(224.47) | 15.0 (224.57) | 8.13 (64.14) | 11.03 (119.61) | 10.98 (118.66) | - | - |
| $T_{13}$ -BIO9681 PE atrazine 0.5 kg ha$^{-1}$ + HW+Insect Control | 7.15 (49.14) | 7.52(54.58) | 5.96(33.49) | 3.74(11.98) | 6.23 (36.77) | 5.12 (24.24) | 77.27 | 68.73 |
| $T_{14}$ -BIO9681No Weed control and no Insect Control | 14.69(213.70) | 13.8 (189.93) | 14.52(208.94) | 7.40(52.71) | 10.94(117.62) | 10.90 (116.82) | - | - |
| $T_{15}$ -CoHM5 PE atrazine 0.5 kg ha$^{-1}$ + HW+Insect Control | 7.83 (59.37) | 8.32 (67.3) | 6.20 (36.44) | 4.08(14.61) | 6.75 (43.55) | 5.69 (30.32) | 79.28 | 68.56 |
| $T_{16}$ -CoHM5 No Weed control and no Insect Control | 16.38(266.19) | 15.24(230.37) | 15.79(247.44) | 8.52(70.54) | 11.8 (138.52) | 12.16 (145.78) | - | - |
| **CD(P=0.05)** | **2.74** | **2.27** | **2.17** | **1.34** | **1.70** | **1.63** | - | - |

*Figures in parenthesis are original values; * Data are square root transformed values



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**Table 2.** Effect of weed control methods on yield and weed index in herbicide tolerant transgenic corn.

| Treatments | *Kharif*, 2010 | |
|---|---|---|
| | Grain Yield (t ha$^{-1}$) | Weed index (%) |
| $T_1$ -30V92 POE Glyphosate @ 900 g a.e ha$^{-1}$ | 11.10 | 9.09 |
| $T_2$ -30V92 POE Glyphosate @1800 g a.e ha$^{-1}$ | 12.21 | 0.00 |
| $T_3$ -30V92 POE Glyphosate (Weedy check) | 8.84 | 27.60 |
| $T_4$ -30B11 POE Glyphosate @ 900 g a.e ha$^{-1}$ | 10.97 | 10.15 |
| $T_5$ -30B11 POE Glyphosate @1800 g a.e ha$^{-1}$ | 11.98 | 1.88 |
| $T_6$ -30B11 POE Glyphosate (Weedy check) | 9.12 | 25.30 |
| $T_7$ -30V92 PE atrazine 0.5 kg ha$^{-1}$ + HW+ Insect Control | 10.23 | 16.21 |
| $T_8$ -30V92 No Weed control and only Insect Control | 8.33 | 31.77 |
| $T_9$ -30V92 No Weed control and no Insect Control | 7.52 | 38.41 |
| $T_{10}$ -30B11 PE atrazine 0.5 kg ha$^{-1}$ + HW+ insect control | 9.76 | 20.06 |
| $T_{11}$-30B11No Weed control and only Insect Control | 8.20 | 32.84 |
| $T_{12}$ -30B11 No Weed control and no Insect Control | 7.35 | 39.80 |
| $T_{13}$ -BIO9681 PE atrazine 0.5 kg ha$^{-1}$ + HW+ Insect Control | 8.00 | 34.47 |
| $T_{14}$ -BIO9681No Weed control and no Insect Control | 6.12 | 49.87 |
| $T_{15}$ -CoHM5 PE atrazine 0.5 kg ha$^{-1}$ + HW+ Insect Control | 7.33 | 39.96 |
| $T_{16}$ -CoHM5 No Weed control and no Insect Control | 5.08 | 58.39 |
| **CD (P=0.05)** | **0.84** | - |

## CONCLUSION

From the results of the field experiment, it could be concluded that post emergence spraying of potassium salt of glyphosate at 1800 g a.e. ha$^{-1}$ in transgenic hybrid of 30V92 enhanced complete control of broad spectrum weeds can keep the weed density, dry weight reasonable at lower level and enhance higher productivity and profitability with higher grain yield.

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# EFFECT OF ESTABLISHMENT METHODS ON SHIFTING OF WEED FLORA IN RICE-WHEAT CROPPING SYSTEM

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**ABSTRACT** Rice-wheat cropping system (RWCS) is a major agricultural production system, which serve as a source of livelihood for millions of people of Indo-Gangetic Plains (IGP). Currently, the productivity of rice-wheat system is under great threat due soil fatigue, declining water availability and a shift in weed flora. Adoption of different establishment methods, in RWCS has resulted in shift in associated weed flora. Research carried out on different establishment methods on RWCS as long term experiment during past ten years at N.E. Borlaug Crop Research Centre, Pantnagar, Uttarakhand (India). Research and prevalent practices show various establishment methods in RWCS *viz.,* transplanting replaced by dry as well as wet seeding (WSR) and zero till seeding in rice (ZTR) crop and shifting of conventional tillage to zero tillage in wheat resulted in shift from grassy to non-grassy weeds and sedges.The shifting from transplanting to direct seeding of rice (DSR) resulted not only an increase in weed growth but also shift in the relative abandunce of particular species. The density of *Echinochloa colona* was higher in WSR followed by DSR and ZTR, however, the maximum density of *Alternanthera sessilis* and *Cyperus rotundus* was recorded in ZTR than in DSR. It was noticed that DSR is accompanied by a rapid shift in weed flora with an increase in abundance of *Echinlchloa crus-galli, Echinochloa colona* and *Ischaemum rugousm*.Shift from conventional tillage to zero tillage resulted in abundance of perennial weeds and reduced infestation of annual weeds. Therefore, there is need to modify the current weed management practices to take care of the shifting weed flora under different establishment systems of RWCS.

***Keywords*****:** RWCS, DSR, WSR, ZTR.

## INTRODUCTION

Rice wheat cropping system (RWCS) is a major agricultural production system, which serve as a source of livelihood for millions of people of Indo Gangetic plains (IGP). These two crops share about 87.5per cent of the total irrigated area (nearly 10.5 mha area in north – west, north – east and central India adjoining Indo – Gangetic Plains) and thus makes it most prominent cropping system in India (Gopal *et al*, 2010). Both crop components of this cropping system are fertility exhaustive and need more water, labour, time, non – renewable energy, heavy farm machineries and costs for their successful cultivation. Rice is grown by transplanting method to realize good yields and manage weed. The land preparation to grow transplanted rice is not only tedious, costly and time consuming, but it is also deteriorates the soil – properties due to formation of compacted hard soil surface.

Alternative technologies of rice establishment – dry and wet seeding – can be used to reduce the water and labour requirement of rice transplanting cultures without yield penalty. (Anonymous, 2005).However, weeds pose great threat to direct seeded rice culture.

Weeds are one of the most important biological constraints in rice production system. Singh *et. al.* (2002) reported 48.9 per cent yield reduction in weedy check as compared two handweeding in transplanted rice at Ludhiana. Transplanted rice encounters with problems of complex weed flora in different regions of the country resulting in 15-76% reduction in grain yield (Mishra 1997, Singh *et. al.* ,2004). In direct seeded crops, high yield depends to a greater extent on effective weed management. Direct seeded systems face a potential threat from



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changes in the competing weeds, with an increase in those species that are difficult to control. These include *Ischaemum rugosum*, *Echinochloa crus-galli*, *E. Colona* and *Leptochloa chinensis.* Shift from TPR to DSR had impacted weed dynamics of wheat too.

There is enough scope to increase the average yield of rice provided that improved package of practices are adopted. Information on weed shift and thus their subsequent control is one of the most important and suggestive practice for better and potential management of rice crop.

Wheat is mostly grown after conventional tillage however, sizable acerage is under zero tilled in IGP. It is most widely adopted resource conserving technology (RCT) in the Indo – Gangetic plains (IGP). Zero tillage of wheat after rice generate significant benefits at the farm level, both in terms of significant yield gains (6-10%, particularly due to timely planting of wheat) and cost savings (5-10% particularly tillage savings) (Anonymous, 2007).

Change in wheat establishment methods has its bearing on wheat weeds with more dominance of perennial weeds like *Cyperus rotundus* and *Cynodon dactylon*. Also shift from conventional tillage to zero tillage had to change in weed flora in rice too.

Therefore an attempt has been made to summarize the work on weed dynamics and shift in different establishment system of rice and wheat and its impact on grain yield of rice- wheat cropping system.

These subheads briefly reviews the research work carried out in Pantnagar from 2000-2008, both at the research station as well as on farmer's field.

## REVIEW OF RESEARCH WORK CARRIED OUT AT PANTNAGAR

### Weed shift and effect on rice yield

A consequence of cropping intensification or change in weed management is often a shift, if not an absolute increase, in the weed population (Mortimer, 1996). These changes can be extremely rapid. Shifts have been reported in *Tarai* ecosystem where attempts were made to replace transplanting with direct seeding.

A field experiment was conducted at research station of Kashipur during Kharif season of 2003 and 2004. By taking four rice establishment method [conventional planting of 21 days old seedlings after soil puddling (TPR), wet seeding- sowing of pre germinated seeds on puddle soil (WSR), dry seeding after conventional tillage (DSR) and dry seeding zero-tillage after flush irrigation (ZTR)] in main plot and four weed management practices (weedy check($WC_1$), pre emergence application of herbicides+ one hand weeding at 30 DAS/DAT($WC_2$), pre emergence application of herbicide followed by two hand weeding done at 30and 60 DAS/DAT($WC_3$) and two hand weeding at 30and 60 DAS/DAT($WC_4$)] in sub plot. The density of *Echinochloa colona* , *Dactyloctenium aegyptium, Leptochloa chinensis* and *Eleusine indica* was higher in wet seeded rice (WSR followed by direct seeded (DSR) and zero tilled rice (ZTR). However, the maximum density of *Alternenthera sessili*s , *Corchorus actutangulus* and *Cyperus rotundus* was recorded in ZTR than in DSR. There were no significant differences between the transplanting and other rice establishment method with respect to density of *E.colona* and *E.indica*,while transplanting caused significant reduction in density of *Dactyloctenium aegyptium* in comparison to other establishment methods. WSR had significant reduction in density of *Leptochloa chinensis* than other establishment methods were as minimum density *A.sessilis* was recorded in ZTR. The maximum reduction of weed species was obtained with application of herbicides as pre emergence supplemented by two hand weeding at 30 and 60 DAS. Rice establishment and weed management both significantly affected grain yield of rice. The higher grain yield (4304 kg $ha^{-1}$) was obtained by transplanting (TPR) than wet seeding (WSR), zero till (ZTR) and direct seeded rice (DSR). Herbicides supplemented with two hand weeding at 30 and 60 DAS/DAT gave significantly higher yield of rice (3929 kg $ha^{-1}$) than the pre emergence application of herbicide and one hand weeding and only two hand weeding in TPR (Singh, *et al*, 2005).



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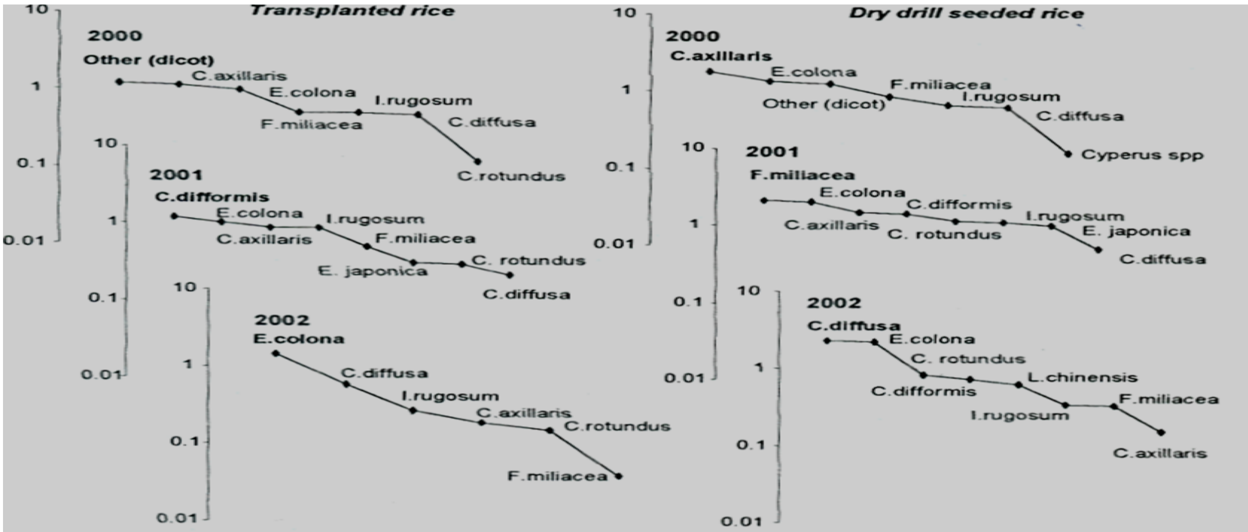


Figure 1. Changes in abundance of weed species in relation to crop establishment method in unweeded plots. Data are the mean (n = 8) abundance of species measured as dry biomass 56 DAS. In each year, species are ranked on a logarithmic scale. Within each year, there were significant differences ($P < 0.01$) in species composition (MANOVA) and across years significant changes in rank order ($P < 0.05$) were evident (Page's test, Page, 1963). The most abundant species, in each year, is emboldened.

*Caesulia axillaris : C. axillaris*
*Commelina diffusa : C. diffusa*
*Cyperus difformis : C. difformis*
*Cyperus rotundus : C. rotundus*
*Echinochloa colona : E. colona*
*Eragrostis japonica : E. japonica*
*Fimbristylis miliacea : F. miliacea*
*Ischaemum rugosum : I. rugosum*
*Leptochloa chinensis : L. chinensis*

Singh *et al.,* (2006) in a long term experiment examine five rice establishment methods and found that weed shift were evident after four cropping seasons with *Ischaemum rugosum* being dominant in wet seeded plots, *Echinochloa colona* dominating in dry seeded plots and *Cyperus iria* and *Echinochloa colona* being abundant under transplanting.

Among different establishment methods and weed management practices, weed free (CW) plots of wet seeded rice were equivalent to those of transplanted rice, while those of dry seeded rice (DS, DSF and ZT) had significantly less yield. Similar yields were obtained from zero tilled (ZT) and conventionally tilled plots (DSF, DS). A single hand weeding was insufficient to prevent to prevent major yield loss to weeds in direct seeded rice culture while it had very less yield reduction under transplanted rice. A similar pattern of results for grain yield was observed in 2002 although yield loss due to weed competition was less severe when a single hand weeding was employed.

**Table 1.** Interaction effect of rice establishment and weed management on grain yield (t $ha^{-1}$) of rice

| **Establishment method** | **2001 Weed management** | | | **2002 Weed management** | | |
|---|---|---|---|---|---|---|
| | CW | HW | TO | CW | HW | TO |
| TP | 7.8 | 7.2 | 5.9 | 6.1 | 5.5 | 4.6 |
| WS | 8.1 | 5.6 | 1.0 | 6.8 | 5.4 | 0.9 |
| DS | 6.1 | 1.0 | 0.0 | 6.7 | 5.1 | 1.0 |
| DSF | 6.6 | 1.6 | 0.0 | 6.1 | 4.7 | 0.4 |
| ZT | 6.6 | 1.5 | 0.0 | 5.9 | 5.4 | 0.2 |
| SE | 0.04 | | | 0.24 | | |

CW, conventional weeding HW, hand weeding TO, weedy



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**Weed shift and effect on yield of wheat**

Singh *et al*,2003 in the same experiment from 2001 to 2002 found that wheat yield were not affected significantly by establishment method of rice viz., conventional transplanting, WS-wet seeding , DS-dry seeding , DSF- dry seeding after conventional tillage but final tillage following a flush irrigation and ZT-zero tillage after flush irrigation and wheat crop itself.

In Pantnagar, average of ten year data revealed that there is less intensity of weeds specially P. minor, Melilotus spp. and Polygonum spp., in ZT wheat as compared to wheat sown by conventional practice at 30 DAS, resulting less infestation of weeds and less competition with crop. The grain yield obtained was also higher in zero tillage wheat over the conventional.

In Pantnagar, the different establishment methods used in a long term rice wheat cropping system (10 years) showed that wheat grain yield was higher in zero tillage practice over the conventional practice (Singh et al., 2010).

Both long-term trial and farmers' field surveys suggest a change in the weed spectrum in ZT wheat field (Singh et al., 2010). Trials conducted at 5 locations in IGP under National Agriculture Technology Project (NATP) of Indian Council of Agricultural Research showed higher grain yield of wheat in zero tillage practice in four locations viz. Pantnagar, Faizabad, Varanasi and Patna and it was at par with conventional practice of wheat growing at Ludhiana location (Fig. 3).

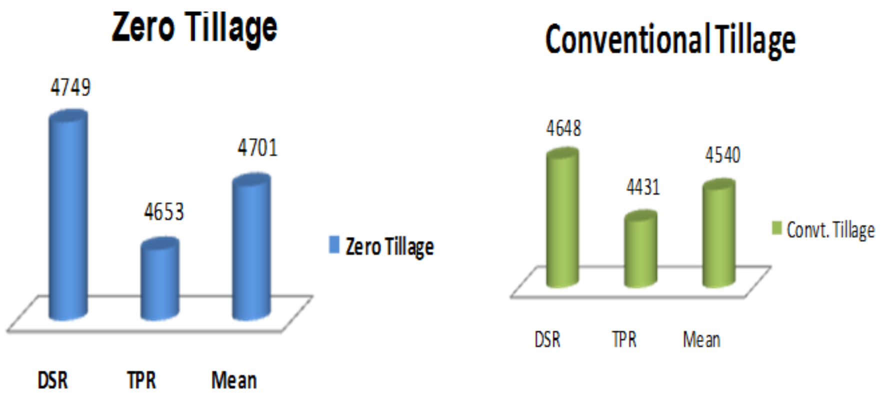


**Fig 2.** Effect of rice wheat establishment methods on wheat grain yield at farmers field (Avg. of 10 yrs.)

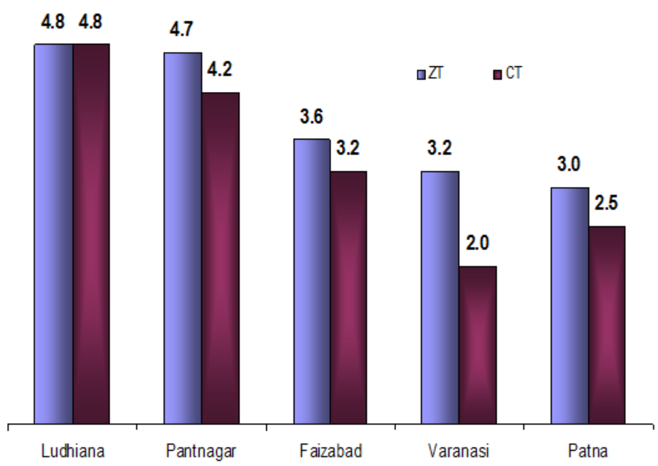


**Fig 3.** Wheat yield (t $ha^{-1}$) under two tillage systems in five different locations.



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**Table 2.** Effect of different establishment methods in rice-wheat system on weeds situation in wheat crop 30 DAS at Pantnagar (Avg. of 10 years)

| Establishment systems | Weed density (no. $m^{-2}$) | | | | | | Grain yield (t $ha^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | ZTW | CTW | ZTW | CTW | ZTW | CTW | ZTW | CTW |
| | *P. minor* | | *Melilotus spp.* | | *Polygonum spp.* | | | |
| Trans Planted Rice | 34 | 34 | 7 | 13 | 30 | 48 | 3.6 | 3.7 |
| Wet Seeded | 11 | 15 | 5 | 15 | 35 | 126 | 3.7 | 3.7 |
| Direct Seeded | 14 | 27 | 6 | 8 | 23 | 117 | 3.9 | 3.8 |
| DSF | 10 | 21 | 4 | 11 | 34 | 69 | 3.8 | 3.7 |
| Zero Tillage Rice | 8 | 8 | 9 | 6 | 20 | 147 | 3.8 | 3.8 |
| Average | 15 | 21 | 6 | 11 | 28 | 101 | 3.8 | 3.7 |

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## *Economics and Weed Management*

# ECONOMIC WEED MANAGEMENT APPROACHES FOR RICE IN ASIA

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**ABSTRACT** Rice farmers in Asia are altering the way they establish crop and manage weeds because of changes in the availability and cost of major resources such as labour, water and the energy. Direct-seeding is being practiced to reduce water and labour costs associated with transplanting rice. Weeds continued to be one of the major impediments for realising optimum rice productivity in rice based cropping systems of Asia. The effective and economical weed management is an important component of technology to improve rice productivity. In spite of reduced availability and increased cost of labor, hand-weeding continued to be used as method of weed control either alone or in combination with herbicides and other methods of weed management in rice. The cost of cultivation of rice and the weed management became major limiting factors for the farmers to realise higher system productivity and net returns. Hence an effort is made in this paper to identify most economic weed management approaches under different methods of rice establishment in Asia. Several economical and effective herbicides based integrated weed management (IWM) options are available for use across Asia for rice grown under varying methods of rice establishment. However, management decisions for selecting the economic IWM options are difficult for rice farmers. The need for developing simple methods or models to help rice farmers to make long-term effective and economic weed management decision is emphasized and discussed.

***Keywords:*** Asia; economics, herbicides; integrated weed management; rice.

## INTRODUCTION

Rice is the staple food of about half of the world’s population, the majority of which is located in Asia. Rice is grown in Asia in 140 million ha with production of 653million tons of paddy rice and an average productivity of 4.5 t/ha (http://ricestat.irri.org). It is estimated that 2.3 billion farmers and their households depend on rice as their main source of livelihood (Mohanty et al., 2010). India has the highest acreage under rice i.e. 44 million ha with a production of 155.7 million tons of rice. However China produces 202.7 million tons from an acreage of 30 million ha. Rice productivity in China is 6.67 t/ha compared to world average of 4.4 t/ha . Rice is an important commercial crop in the other Asian countries like Indonesia, Bangladesh, Combodia, Laos, Pakistan, Thailand, Philippines, Myanmar, Vietnam, Sri Lanka, among others. The development that is occurring in majority of the Asian countries is coupled with the increase in the cost of cultivation of the rice farming community. This in turn is making rice farming economically unviable. Producing rice that costs less to farmers and consumers of the domestic market and increasing income of the farmers are the goals of strategic importance in Asia. Hence, priority needs to be given for the research and extension that makes rice farming economically viable in the Asian countries.

Rice farming will need to produce about 8–10 million tons more paddy per year over the next decade in Asia. Without area expansion this will require an annual yield increase of about 1.2–1.5%, equivalent to an average yield increase of 0.6 t $ha^{-1}$ world-wide. Improving global food security will necessitate concerted efforts to increase the productivity of rice per unit of land, water and labour, which in turn necessitates continued and increased research



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efforts to close yield gaps and raise yield ceilings across rice growing environments while coping with climate change. Increasing the theoretical yield potential of cereals is uncertain, existing yield gaps must be closed and average crop yields may have to approach 80% of the yield potential or more in areas with good quality soils and favorable rainfall or irrigation (Cassman et al., 2003). Productivity in other rice ecosystems also needs to be improved. Yield growth needs to be revitalized and the unit cost of production needs to be lowered to benefit rice farmers and the consumers. Among the research efforts the identification of impediments in attaining optimal yield to bridge the yield gap is the key. Among the biological constraints weeds stand first as the world wide estimated potential and actual rice production losses due to weeds were estimated as 37.1 and 10.2 % respectively (Oerke, 2006). The estimated strong negative impacts that weeds can have on rice yields and farmers profits speaks of the necessity for cost-effective weed management strategies. The choice of weed management should take into consideration the system of rice cultivation, farmer resources, the farmers' managerial capability agronomic feasibility and economic viability of the available technology.

## HOW IMPORTANT ARE WEEDS IN RICE OF ASIA?

Weeds are recognized as major biological constraints that hinder the attainment of optimal rice productivity (Rao et al., 2007; Kumar and Ladha, 2011; Rao and Nagamani, 2013) and quality. In Asia, rice is grown in a wide range of climatic conditions ranging from from river deltas to mountainous regions with domination in irrigated systems. Detailed list of weeds associated with rice in Asia (Moody, 1989; Galinato et al., 1999; Rao et al., 2007; Caton et al., 2010) indicate that weeds adapted to all climatic conditions are associated with rice. Weeds are fundamentally an economic problem in terms of their causes, effects and control. Weeds have been found to reduce rice yield by 20 to 40% in Sri Lanka (Abeysekara et al., 2010), 10-35% in Malaysia ( Karim et al., 2004); 25% of transplanted rice and 46% of direct-seeded rice in Vietnam (Chin and Thi, 2010); 13 to 22% in India (Rao and Nagamani, 2010) and 10 to 20% in China (Zhang, 2003). World-wide losses in rice yields due to weeds have been estimated to be around 10 per cent of total production (Oerke, 2006). This means that production losses due to weeds in rice in Asia are around 15.1 million tons of rice annually, which highlights the strong economic incentives accruing to the employment of effective weed control. Optimum yields can be obtained only when the crop is free from weeds. Hence, weed control has always been a major input in rice production.

## HOW TO MAKE A DECISION FOR ECONOMICAL WEED MANAGEMENT?

The answer to this question is not easy, especially for rice farming community of developing countries in Asia Pacific Region, as the studies on decision making are very limited, even though the need for developing decision making tools was identified (Rao et al., 2007). Integrated weed management (IWM) may be defined as weed population management system for keeping weed infestation levels below those that cause economic losses by combing two or more preventive, cultural, mechanical, and chemical or biological methods (De Datta and Beltazar, 1996). In other words, IWM is a decision making process involving, a) when to apply weed control measures with use of economic threshold levels, and b) which control measures combinations would provide best weed control combined with higher profits. In long term IWM also should aim at preventing harmful effects to environment and non target organisms as well as preventing built up of any particular weed or weed populations (Rao and Nagamani, 2013).

The economic threshold, (i.e. estimation of the weed density at which the benefit derived from herbicide application equals the cost of control), is a well-known but not practical implementation of this fundamental economic principle (Sanyal et al., 2008). Information on economic threshold levels (Chul et al., 2010) is also limiting in many of the Asian developing countries. Cousens (1987) argued that the use of short term economic thresholds as a within



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season management tool has little value for two reasons: (1) wide variation is seen in yield response to the same weed density when inter site comparisons are made and (2) it is difficult to accurately anticipate the future market value of the crop. Long term economic thresholds that take into account how weed control in one year affects future weed infestation densities and how financial decisions made in the present year impact on future expenditures for weed control is a more strong basis on which to develop a weed management plan (Rao et al., 2007).

Based on their location specific experience, farmers normally use their logical thinking and cost of the control measures and implement it in the field. Farmers who implement the control measures during the critical period succeed in managing weeds economically and the rest end up in losses which were estimated to be high. Simple decision making tools for the use of farmers are to be given top priority by researchers of rice.

## WHICH COMPONENTS OF INTEGRATED WEED MANAGEMENT MAKE IT AN ECONOMIC WEED MANAGEMENT APPROACH?

The goals of IWM range from maximizing profit margins to safeguarding natural resources and minimizing the negative impact of weed control practices on the environment (Sanyal et al., 2008; Rao and Ladha, 2011). Depending on the goal, varying non chemical and chemical methods of weed management are used as the components of the IWM (Rao and Nagamani, 2010; 2013).The economics of the components vary with the location and the time. For example, hand weeding was considered cheaper during 1990's is now a non economic method, when used alone, as the labour wages increased (Table: 1) due to their scarcity.

### a) Non chemical methods of weed control

Transplanting of rice seedlings is the traditional way of rice cultivation in many Asian countries. Increasing labor costs, scarcity of irrigation water and currently better technology availability have resulted in a general shift in rice production systems from transplanted rice to direct-seeded rice (DSR) in Asia (Kumar and Ladha 2011). Direct-seeding offers the advantages of reduced labor requirements and drudgery, earlier crop maturity, more efficient water use and higher tolerance of water deficit, lower methane emissions and often higher profit in areas with an assured water supply. Weeds, however, are the main constraint for farmers practicing direct-seeding (Rao et al., 2007; Rao and Nagamani, 2007) as the inherent weed control due to standing water at transplanted rice establishment is lost and weeds emerge concurrently with rice thereby competing with rice for resources. Effective weed control is one of the major requirements to ensure successful economical DSR production (Rao et al., 2007; Rao and Nagamani, 2007; 2013; Kumar and Ladha, 2011).

The traditional methods of weed control practices of smallholders include hand weeding by hoe and hand pulling in Bangladesh (Alam et al., 2002), India (Rao and Nagamani, 2010) and several other Asian countries. Usually, two or three hand weedings are done for growing a rice crop depending upon the nature of weeds, their intensity of infestation and the method of rice establishment. Manual weeding can be implemented only when weeds have reached a size to pull, and it has an inherent opportunity cost. Manual weeding is difficult as weeds (e.g., *Echinochloa crus-galli*) and rice seedlings look similar (Rao and Moody, 1987; 1988), and the labour needed for hand weeding would be too high for the farmer to bear the cost. At the time of the peak period of the labor crisis, weeding sometimes becomes late, causing drastic losses in grain ditions, and the presence of perennial weeds that fragment on pulling may all lead to lowered weeding efficacy and lesser economic returns due to manual weeding usage alone, in developing countries of Asia also. yield (Hasanuzzaman et al., 2009). Labor scarcity, high labor cost, poor weather con

In general, weed population density and total dry weight per unit area decrease as water depth increases (Moody et al., 1986). Many weeds will not germinate under flooded conditions (Yamada, 1959), while others were unaffected by flooding (Bhan, 1983). Even though water is proved to keep several weeds under control, water is a looming crisis due to competition among



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agricultural, industrial, environmental and domestic users and in the future, many rice farmers in Asia, may have limited access to irrigation water (Tuong and Bouman 2003). Continuous ponding required about 1.5-2.0 times more water than intermittent irrigation and hence not economical (Islam and Molla, 2001). The correct water management during the crop establishment stage may effectively lower the economic and possible runoff costs of herbicides without reduction in yield or water productivity (Tuong et al., 2000).

The mechanical weeders also could be used as a component of IWM as usage of rotary weeder saves nearly 57% labour compared with hand weeding (Subudhi, 2004). The cost of weeding for female labours could be reduced by 4.85 times and 5.2 times by using rotary weeder and cono weeder respectively, compared to hand weeding. While for male labours the weeding cost could be reduced by 6.6 times and 7.6 times by using rotary weeder and cono weeder respectively, compared to hand weeding. (Remesan et al., 2007). Preventive measures (Rao and Moody, 1990); fertiliser (De Datta and Barker. 1977), tillage (Bhagat et al., 1999), weed competitive cultivars (Tuong et al., 2000; Anwar et al., 2010), rice population density (Chauhan et al., 2011); biocontrol (Kumar et al., 2007); crop rotations ( Kabir and Rawson, 2011); intercropping (Kar et al., 2006); allelopathy (Xuan et al., 2005); and mulching (Devasinghe et al., 2011) were also considered as the key components for economical and sustainable IWM.

**b) Chemical method of weed management**

Herbicide application has increased significantly over the last decade due to labor shortages, low herbicide prices, increased herbicide effectiveness, and water scarcity. Norton et al. (2010) identified that sales for herbicides applied to rice crops increased by 2% per year over the period 1981–2007. The annual growth of expenditure on herbicide for rice crops globally is estimated to be around US$60 million (M) year−1, exceeding insecticides (US$47 M), and fungicides (US$41 M) (Zhang et al., 2004). Chemical weed control is practiced by nearly 90% of the farmers in countries like Sri Lanka (Abeysekara et al., 2010; Weerakoon et al., 2011),. Major factor behind the increasing use of herbicides in Asia is is the shift in method of establishment from transplanting rice crops to direct-seeding, which utilizes less labor and water. Direct-seeding involves the seeding of rice seed, either on wet soil using drum seeder or by broadcasting (wet-seeded rice) or by broadcasting or seeding in row by seed drill either on zero tilled or tilled soil (dy-seeding) (Rao et al., 2007; Rao and Nagamani, 2011; Kumar and Ladha, 2011). The availability of effective herbicides for weed control is the key to success of direct-seeding by both the methods as the rice and weeds seedlings emerge simultaneously and weed competition is critical factor that decides rice productivity. In Philippines, with increased labor cost, herbicide application was found to be superior to manual weeding even at the lowest weed density by US$25-$54/ha. At the highest weed density and highest labor cost, herbicide application is approximately 80% (about US$200/ha) more profitable than hand weeding (Beltran et al., 2012). The age of the farmer, household size, and irrigation use are significant determinants of the decision of farmers to adopt herbicides as an alternative to manual weeding, while economic variables such as the price of herbicides, total income, and access to credit determine the level of herbicide use (Beltran et al., 2013).

Herbicide use has been shown to be privately profitable for Asian rice farmers, but the question remains as to whether it is socially profitable when environmental costs, health costs, and the societal costs of labour displacement are accounted for (Naylor, 1994).

## SCIENTIFICALLY PROVED ECONOMIC INTEGRATED WEED MANAGEMENT APPROACHES

The fundamental economic principle for weed management is simple: act only if the benefits exceed the costs (Sanyal et al., 2008). Implementation of the principle is difficult with the many and typically uncertain costs and benefits of management.

Majority of the research findings on IWM proved that most economical approach for managing weeds, irrespective of method of establishment is the use of herbicide or their



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combinations in integration with hand weeding in India, Bangladesh, Philippines, Sri Lanka, Korea . The weeding labour input to direct-seeded rice was found unacceptably high for farmers (Mazid et al., 2001). The integration of herbicide with hand weeding was reported to result in 85% reduction in labour and 77.5% in labour cost of WSR; while it was 58%in labour days, 52.9% in labour costdry DSR (Mazid and Johnson, 2010). The IWM proved to be effective at different countries in Asia include the integration of hand weeding (HW) with herbicides such as pretilachlor/chlorimuron + metsulfuron/HW; cyhalofop-butyl/2,4-D/HW; Cyhalofop-butyl/chlorimuron + metsulfuron/HW; pretilachlor with safener fb hand-weeding; pyrazosulfuron ethyl followed by continuous submergence and several combinations of chemical and non chemical methods listed earlier (Banerjee et al., 2008; Rashid et al., 2012; Rao et al., 2007; Rao and Nagamani, 2013).

## WHAT SHOULD BE THE FUTURE RESEARCH APPROACH FOR MAKING WEED MANAGEMENT ECONOMICAL?

**i) Need based use of herbicides as component of IWM**: Weeds normally occur in smaller or bigger patches. It is possible to reduce the cost of herbicide (post emergence) applied by confining herbicide to the weedy areas of the field, keeping in view of the variability of the presence of weeds within the field. This way the cost of herbicide and application is reduced and the chemical pressure on the environment is decreased.

**ii) Educating farmers on proper use of weed management tools**: The acceptance of any economic IWM by farmers will depend on their perceived risk to management, individual management capability, and environmental interactions that will influence the economic viability of the cropping system (Sanyal et al., 2008). With the rotary-hoe weeders, the weeding labor is reduced by up to 60 -65% while the herbicides reduce it by over 80%. However, farmers do not always use the herbicides correctly and this can impact negatively on the economic returns, health and the environment hence the need for the educating the farmers in Asia.

**iii). Understanding weed ecology and biology**: An understanding of weed ecology biology would help to undertake weed management at susceptible period of weed life cycle and there by cost of weed management can be reduced substantially.

**iv). Working closely with the economists and farmers**: Meaningful analysis for economic weed management is currently limited by lack of understanding of weed population and spatial dynamics and problematic communication between weed scientists and agricultural economists (Sanyal et al., , 2008). Intensified efforts must be made to evolve economic approaches for weed management by closer interaction with economists in participation with the farmers.

**v). Preventing herbicide resistance in weeds:**
Herbicide resistance is observed in several of the Asian developing countries also. Substantial loss in the returns and profits may be expected with the increase in the herbicide resistance. Hence, weed scientists in these countries must focus their research efforts and simultaneously educate farmers on the economical ways and means of proper use of herbicides to prevent or delay the onset of herbicide resistance.

**vi). Developing non transgenic herbicide resistant (HR) rice**: In many of the developed countries, herbicide resistant crops have helped growers to manage weeds and produce higher and more profitable yields. Weeds that are closely related to the crop are often the most competitive and difficult to control with selective herbicides or mechanical practices. For example the weedy rice is becoming a thrat to rice production in several of the Asian countries. One approach that may help econom ic management of weedy rice is HR rice. Keeping in view of the hesitation about genetically modified crops, development of non transgenic HR rice



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would allow growers to use a herbicide to control the weeedy rices while not injuring the crop. The main limitation of this approach is currently the likelihood of rapid gene transfer from the crop to the related weed populations. Biotechnology can help to prevent this (Gressel, 2008; Green, 2012).

Adoption of IWM is economically beneficial in rice systems and is essential for the management of herbicide resistance. IWM also plays an important role in minimising the size of weed seedbanks over time, and has clear benefits for managing the environmental risk on efficacy (Rao and Ladha, 2011; Rao and Nagamani, 2013). Using a range of tactics in an IWM plan is essential for the effective, long-term economic management of weeds. In order to effectively manage weeds, for proper policy making, and to set appropriate research priorities, economic analyses are needed. Weed scientists must continuously evolve and extend location specific, cost-effective, ecologically safe methods to manage weeds in rice of Asia.



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**Table 1**. Change in cost of hand weeding and herbicides during ten years period in India.

| **Year** | **Women labour wage Rs/day ($ per day)** | **$ rate / rupee** | **Cost of 1 HW Rs./ha (20 women labour/ha)** | **Two hand weeding (HW) (Rs/ha)**** | **Cost of Pendimethalin (Rs / kg)** | **Oxadiagryl (@ 70 g a.i./ha) (PRE) fb Chlorimuron+metsulfuron methyl (4 g a.i./ha) (POST) (Rs/ha)** | **Oxadiagryl (PRE) fb Chlorimuron+metsulfuron methyl (POST) fb one HW*** (Rs/ha)** |
|---|---|---|---|---|---|---|---|
| 2003-2004 | 58 ($1.3) | 45 | 1161 ($26) | 2322 ($51.6) | 300 ($6.7) | - | - |
| 20013-14 | 150 to 200 ($2.5 to 3.4) | 59 | 3000 to 4000 ($50.8 to 67.8) | 6000 to 8000 ($ 101.7 to 135.6) | 1000 to 1330*($16.9 to $22.6) | 575+425 ($17) | 575+425+3000 ($67.8) |

PRE = Pre emergence treatment; POST = Post emergence treatment.

*Price varies with manufacturing company.

** Minimum number of hand weedings needed in direct-seeded rice

*** One of the currently recommended integrated weed management.



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# INTEGRATED WEED MANAGEMENT IN MAIZE (*ZEA MAYS* L.) FOR SUPPORTING FOOD SECURITY IN ANDHRA PRADESH, INDIA

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**ABSTRACT** In Andhra Pradesh, India maize is cultivated in 7.86 lakh ha with productivity of 5260 kg/ha. Field experiments were conducted during *Kharif* season (June - October) 2010 and 2011 at ANGRAU, Hyderabad, India to identify economic and efficient weed management practices in early growth stages of the maize. The treatments included pre-emergence application of herbicides *viz.,* atrazine 50% WP (1.0 kg/ha), pendimethalin 30% EC (1.0kg/ha) and oxyfluorfen 23.5%EC (0.3kg/ha) either alone or in combination with intercultivation (IC) at 30 DAS and these treatments were compared with hand-weeding at 20 and 40 DAS and weedy check. The predominant weed flora in the experimental field comprised of *Cyperus rotundus, Cynodon dactylon, Digitaria sanguinalis, Dactyloctenium aegyptium, Rottoboellia exaltata, Parthenium hysterophorus, Celosia argentia, Euphorbea hirta, Digera arvensis and Trianthema portulacastrum.* Lowest WDM (weed dry matter) and highest WCE (weed control efficiency) readings were recorded with hand weeding (89.8%), followed by atrazine fb IC (87.5%), oxyfluorfen fb IC (84.2%) and pendimethalin fb IC (81.3%). Highest grain yield recorded in hand-weeding treatment (7450 kg/ha) was significantly superior to singular applications of pendimethalin (5350 kg/ha) or oxyfluorfen (6020 kg/ha) or atrazine (6570 kg/ha) and control and was on par with atrazine fb IC (7310 kg/ha), oxyfluorfen fb IC (6970 kg/ha) and pendimethalin fb IC (6770kg/ha). Oxyfluorfen was more persistent in soil (90 days after application (DAA) with $DT_{50}$ of 36.8 to 43.2 days. Atrazine and pendimethalin residues persisted up to 45 and 60 DAA, respectively. Maize grain and stover were free from herbicide residues at harvest. Different IWM practices in maize revealed that pre-emergence application of either atrazine 1.0 kg/ha or oxyfluorfen 0.3 kg/ha or pendimethalin 1.0kg/ha in combination with IC at 30 DAS provided economical and efficient weed control, indicating the importance of IWM to achieve higher grain yield in maize in support of food security.

***Keywords:*** IWM, intercultivation,, economics, herbicide, persistence, WDM,WCE

## INTRODUCTION

Maize is the world's third most important cereal crop after wheat and rice. In India, it is grown over an area of 8.07 million hectares with total production of 19.73 million tonnes In Andhra Pradesh, maize is cultivated in an area of 7.86 lakh ha with a total production of 4135 thousand tons and with a productivity of 5260 kg/ha. Maize, being a rainy season and widely spaced crop, gets infested with variety of weeds and subjected to heavy weed competition, which often inflects huge losses ranging from 28 to 100 per cent (Patel et al 2006). There are very few herbicides options available for weed control in maize in India. Currently, herbicides used for control of weeds include pre emergence application of Atrazine, Pendimethalin, Alachlor and post emergence application of 2-4, D. Most of these herbicides provide only a narrow spectrum weed control in maize. Uncontrolled growth of weed flora present in the field takes over other and may offer severe competition to crop. Moreover continuous use of single herbicide may lead to evolution of herbicide resistant weed species and shift in weed flora. Under such circumstances, to get effective control of composite weed flora integrated approach of weed management is the best choice. Further the persistence of herbicides in soil and grain is also a major concern. Since information on best approach of integrated weed management in maize and persistence of herbicides in soil and maize grain in Andhra Pradesh is meagre, the present investigation was therefore under taken to design an efficient and economic integrated weed



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management system for maize under agro climatic conditions of Andhra Pradesh for supporting food security.

The specific objectives of the study were to: (i) evaluate the effect and economical integrated weed management on yield and economics of maize; (ii) compare the effectiveness of different weed control measures; and (iii) study the persistence of herbicides in soil and grain.

## MATERIALS AND METHODS

A field experiment was conducted at College farm, Acharya N.G. Ranga Agricultural University, Hyderabad, India during *kharif*, 2010 and 2011. Hyderabad lies between 78º28' E longitude and 17º19' N and latitude. The experiment site had mean soil pH of 6.95 with 65.2, 11.6 and 23.2% of clay, silt and sand respectively. The soil was poor in available N, medium in $P_2O_5$ and $K_2O$.The experiment was laid out in randomized block design (RBD) with three replications. Field was ploughed thrice to make a fine seed bed followed by planking. Experimental field was irrigated as and when needed. Plot size was 5.25mX4.0m. There were eight (8) treatments in the experiment with a spacing of 75cmX20cm. The seed rate was used higher than the required and then thinning was done after complete germination to maintain the required plant population. Maize seed was sown by dibbling. Maize hybrid DHM-117 was used in the experiment. Nitrogen was applied at the rate of 150 kg/ha in the form of Urea in three equal splits (basal, kneehigh and tasseling) and $P_2O_5$ and $K_2O$ were applied in the form of SSP and MOP at the rate of 60 kg /ha.The experiment was comprised of the following treatments, *viz:*1. Oxyfluorfen 23.5%EC @ 0.3 kg a.i./ha as PE ; 2. Atrazine 50% WP @ 1.0 kg a.i /ha as PE; 3. Pendimethalin 30% EC @ 1.0 kg a.i /ha as PE; 4. Oxyfluorfen 23.5% @ 0.3 kg a.i /ha as PE fb intercultivation at 30 DAS; 5. Atrazine 1.0 kg a.i /ha as PE fb intercultivation at 30 DAS; 6. Pendimethalin 1.0 kg a.i./ha as PE fb intercultivation at 30 DAS; 7. Hand weeding at 20 and 40 DAS; and 8. Weedy check.

The herbicides were applied with the help of knapsack sprayer fitted with flood jet nozzle within two days after sowing by maintaining sufficient moisture in the field. Inter cultivation was done at 30 DAS. Hand weeding was done at 20 and 40 DAS as per the treatment. Observations on weed density, weed dry matter, yield attributes and grain yield were recorded. The data collected were analysed statistically by using Fisher's analysis of variance technique and the data were suitably interpreted to draw valid conclusions. Atrazine, oxyfluorfen and pendimethalin residues in soil as well as maize grain were analysed according to procedure outlined by Sankaran et al (1993) using GC – ECD.

## RESULTS AND DISCUSSION

### Effect on weed

The predominant weed flora recorded in the experimental field consisted of *Cyperus rotundus, Cynodon dactylon, Digitaria sanguinalis, Dactyloctenium aegyptium, Rottoboellia exaltata, Chloris barbata, Parthenium hysterophorus, Acalypha indica, Celosia argentia, Euphorbia hirta, Digera arvensis and Trianthema portulacastrum.*

### Weed Density (No./0.25m$^2$):

The weed density recorded in different treatments revealed significant differences when pre emergence herbicides were applied alone or in combination with inter cultivation. Weed density was the lowest when atrazine@1.0kg/ha or oxyfluorfen @0.3kg/ha were applied as pre emergence integrated with intercultivation at 30 DAS (6.1 and 7.3/0.25m$^2$) and were on par with hand weeding twice (5.0/0.25m$^2$) and significantly superior to weed density recorded with lone application of herbicides and control indicating the importance of IWM in efficient weed control. Highest weed density was recorded with control where no weed management treatment was imposed.(Table 1) Reduced weed density under IWM could be due to better efficacy of pre emergence herbicides and removal of the late emerged weeds by intercultivation. Pandey et al(2001)and Seemanthini et al (2013) also reported similar results. Singh et al (2009) also



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reported that the lowest weed indices were recorded with atrazine as pre emergence fb one mechanical weeding at 30 DAS.

**Table 1**. Weed Density (No/0.25m$^2$) as influenced by different IWM practices in maize at 40DAS (Pooled data of two years)

| **Treatment** | **Weed Density (No/0.25m$^2$)** | | | |
|---|---|---|---|---|
| | **Grasses** | **Sedges** | **Broad leaved Weeds** | **Total** |
| Oxyfluorfen @ 0.3 kg/ha as PE | 8.3 | 7.6 | 12.2 | 28.1 |
| Atrazine @ 1.0 kg/ha as PE | 7.5 | 7.1 | 10.6 | 25.2 |
| Pendimethalin @ 1.0 kg/ha as PE | 8.5 | 7.5 | 15.8 | 31.8 |
| Oxyfluorfen @0.3 kg/ha as PE fb IC at 30 DAS | 2.5 | 1.6 | 3.2 | 7.3 |
| Atrazine @ 1.0 kg/ha as PE fb IC at 30 DAS | 2.3 | 1.2 | 2.6 | 6.1 |
| Pendimethalin @1.0 kg/ha as PE fb IC at 30 DAS | 3.0 | 2.5 | 5.8 | 11.3 |
| Hand Weeding at 20 & 40 DAS | 1.5 | 1.7 | 1.8 | 5.0 |
| Weedy check | 22.3 | 10.3 | 31.5 | 64.1 |
| **CD (5%)** | | | | 3.2 |

**Weed Dry Matter (WDM) (g/m$^2$):**

The weed dry matter recorded in different treatments revealed significant differences when pre-emergence herbicides applied alone or in combination with inter cultivation. (Table 2). At 30 and 60 DAS, significantly the lowest weed dry matter was recorded with atrazine at 1.0 kg/ha as PE fb intercultivation at 30 DAS which was on par with hand weeding and oxyfluorfen at 0.3 kg/ha as PE fb intercultivation and all these treatments were significantly superior to pre-emergence application of pendimethalin or oxyfluorfen or atrazine . Pre-emergence application of atrazine at 1.0 kg/ha resulted in weed dry matter which was on par with that of oxyfluorfen @0.3kg/ha and both were significantly superior to pendimethalin @ 1.0 kg/ha. All the weed control treatments were significantly superior to unweeded control. IWM involving intercultivation at 30DAS in pre emergence herbicide applied treatments significantly reduced total population and dry weight of weeds compared to their respective pre- emergence herbicides alone. Reduced weed dry weight under IWM could be due to better control of weeds due to pre emergence herbicides and also control of late emerged weeds through intercultivation that resulted in lower weed density and weed dry weight. Similar results were also reported by Rao et al (2009)

**Table 2.** Effect of different weed management practices on weed dry matter and weed control efficiency in maize (Pooled data of two years)

| **Treatment** | **WDM(g/m$^2$) (30 DAS)** | **WCE (%)** | **WDM(g/m$^2$) (60 DAS)** | **WCE (%)** |
|---|---|---|---|---|
| Oxyfluorfen @ 0.3 kg/ha as PE | 22.5 | 61.5 | 53.5 | 59.5 |
| Atrazine @ 1.0 kg/ha as PE | 20.3 | 65.3 | 49.5 | 62.5 |
| Pendimethalin @ 1.0 kg/ha as PE | 27.0 | 53.8 | 57.5 | 56.4 |



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| | | | | |
|---|---|---|---|---|
| Oxyfluorfen @ 0.3kg/ha as PE fb IC at 30 DAS | 5.7 | 90.3 | 20.8 | 84.2 |
| Atrazine @ 1.0 kg/ha as PE fb IC at 30 DAS | 3.8 | 93.5 | 16.5 | 87.5 |
| Pendimethalin @ 1.0 kg/ha as PE fb IC at 30 DAS | 9.9 | 83.1 | 24.7 | 81.3 |
| Hand Weeding at 20 & 40 DAS | 4.5 | 92.3 | 13.5 | 89.8 |
| Weedy check | 58.5 | - | 132.0 | - |
| **CD (5%)** | 2.1 | | 3.2 | |

**Weed control Efficiency (%)**

Weed control efficiency was highest i.e more than 80% with hand weeding twice , atrazine fb intercultivation , oxyfluorfen fb intercultivation and pendimethalin fb intercultivation where as WCE was less when pre-emergence application of atrazine alone was done followed by oxyfluorfen and pendimethalin indicating the importance of integrated weed management in maize for efficient weed control. Higher weed control efficiency with atrazine as PE followed by intercultivation in maize was also reported Aravind Kumar and Ramesh Babu (2009)

**Effect on Crop**

Highest grain yield was recorded with hand weeding at 20 and 40 DAS (7450 kg/ha) which was significantly superior to only pre-emergence application of pendimethalin (5350 kg/ha) or oxyfluorfen (6020 kg/ha) or atrazine (6570 kg/ha) and control (3190 kg/ha) and was on par with the Atrazine fb intercultivation (7310 kg/ha) and oxyfluorfen fb intercultivation (6970 kg/ha) and pendimethalin fb intercultivation (6770kg/ha). (Table 3). Grain yield recorded with Atrazine fb intercultivation (7310 kg/ha) was also on par with Oxyfluorfen fb inter cultivation (6970 kg/ha) and pendimethalin fb inter cultivation (6770kg/ha) and significantly superior to all the other treatments involving pre-emergence herbicide application alone and also control. Significantly lowest grain yield was recorded with unweeded control (3190 kg/ha). Lower values of Weed Index reported were also indicative of superiority of IWM in maize. Higher yield under IWM may be due to reduced competition for nutrients, moisture and light between maize crop and weeds that can be attributed to checked weed growth due to application of pre emergence herbicides during early stages and due to post emergence weed control measures (intercultivation) at later stages resulting in reduced crop –weed competition and thereby providing superior values of yield attributes. The crop utilized the entire soil and environmental resources for growth and development and as a result various yield contributing characters were affected favourably leading to higher grain yield. These results are in line with the findings of Muhammad and Mahboob(2001) and Prabhakaran et al (2009)

**Table 3.** Yield attributes, grain yield and weed Index in maize as influenced by integrated weed management (Pooled data of two years)

| Treatment | No of Cobs/ha | Cob length (cm) | Cob width (cm) | Grains/ Cob | 100 grain wt (g) | Grain Yield (kg/ha) | Weed Index (WI)(%) |
|---|---|---|---|---|---|---|---|
| Oxyfluorfen @ 0.3 kg/ha as PE | 64.90 | 16.5 | 13.1 | 300 | 30.1 | 6020 | 19.2 |
| Atrazine @ 1.0 kg/ha as PE | 65.23 | 18.1 | 13.6 | 315 | 32.1 | 6570 | 11.8 |
| Pendimethalin a@ 1.0 kg/ha as PE | 64.10 | 15.5 | 12.1 | 280 | 28.3 | 5350 | 28.2 |



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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Oxyfluorfen @ kg/ha as PE fb IC at 30 DAS | 66.22 | 18.5 | 14.1 | 320 | 32.3 | 6970 | 6.4 |
| Atrazine @ 1.0 kg/ha as PE fb IC at 30 DAS | 66.31 | 19.6 | 14.2 | 325 | 32.4 | 7310 | 1.9 |
| Pendimethalin @ 1.0 kg/ha as PE fb IC at 30 DAS | 66.26 | 17.9 | 13.7 | 318 | 29.5 | 6770 | 9.1 |
| Hand Weeding at 20 & 40 DAS | 66.32 | 19.5 | 14.3 | 323 | 32.5 | 7450 | - |
| Weedy check | 61.21 | 13.8 | 12.0 | 230 | 25.6 | 3190 | 57.2 |
| C.D (5%) | 1.1 | NS | 0.6 | 18 | 2.5 | 710 | |

**Economics of weed management**

All the weed control treatments were superior in terms of monetory returns compared to hand weeding and unweeded control. IWM involving pre-emergence application of either atrazine or oxyfluorfen or pendimethalin fb intercultivation had more monetory advantage in terms of higher net returns and Benefit : Cost ratio than respective pre-emergence herbicide alone.(Table 4) Lowest net returns and Benefit :Cost ratio was recorded under hand weeding and control that could be due to efficient weed control with lesser cost of herbicides and lesser use of manual labour under integrated weed management. Prabhakaran et al (2009) also reported higher yield and economic returns with pre-emergance application of herbicides followed by intercultivation due to efficient weed control in irrigated maize

**Table 4.** Economics of different weed management practices in maize (Pooled data of two years)

| Treatment | Cost of Cultivation (Rs/ha) | Gross Returns (Rs/ha) | Net Returns (Rs/ha) | B:C ratio |
|---|---|---|---|---|
| Oxyfluorfen @ 0.3 kg/ha as PE | 17,100 | 60,200 | 43,100 | 3.52 |
| Atrazine @ 1.0 kg/ha as PE | 16,500 | 65,700 | 49,200 | 3.98 |
| Pendimethalin @ 1.0 kg/ha as PE | 17,100 | 53,500 | 36,400 | 3.13 |
| Oxyfluorfen @ 0.3kg/ha as PE fb IC at 30 DAS | 18,600 | 69,700 | 51,100 | 3.75 |
| Atrazine @ 1.0 kg/ha as PEfb IC at 30 DAS | 18,000 | 73,100 | 55,100 | 4.06 |
| Pendimethalin @ 1.0 kg/ha as PE fb IC at 30 DAS | 18,600 | 67,700 | 49,100 | 3.64 |
| Hand Weeding at 20 & 40 DAS | 26,500 | 74,500 | 48,000 | 2.80 |
| Weedy check | 14,000 | 31,900 | 17,900 | 2.28 |

*Cost of maize grain Rs 10.00/kg

**Herbicide Residues in maize grain and soil:**

Mean recoveries of the herbicides from soil, studied through fortification with technical standards (2.0 ppm to 0.001 ppm) varied from 85-92 % for atrazine, 96-102 % pendimethlin and 86-90 % for oxyfluorfen. During both the years, dissipation of the all the herbicides was biphasic, with first 50% of the initial detected amount dissipating more rapidly than the remaining soil residue. Among the three herbicides, oxyfluorfen was more persistent compared to the other two other herbicides and the residues of atrazine persisted for shortest period. The $DT_{50}$for atrazine was varied from 19.2 to 24.2 days and the residues persisted up to 45 days beyond which they reached BDL. Pendimethalin residues persisted up to 60 days after application, when 4.9 % of the initial detected amount was noticed. (Table 5& Fig.1)



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**Table 5.** Atrazine, pendimethalin, oxyfluorfen residues in soil

| Days after application (DAA) | Atrazine | | Pendimethalin | | Oxyfluorfen | |
|---|---|---|---|---|---|---|
| | 0-10 cm | 10-20 cm | 0-10 cm | 10-20 cm | 0-10 cm | 10-20 cm |
| 0 | 0.668 | BDL | 0.612 | BDL | 0.286 | BDL |
| 5 | 0.556 | 0.096 | 0.561 | 0.082 | 0.210 | 0.03 |
| 15 | 0.424 | 0.054 | 0.402 | 0.064 | 0.178 | BDL |
| 30 | 0.242 | BDL | 0.191 | BDL | 0.146 | BDL |
| 45 | 0.138 | BDL | 0.088 | BDL | 0.134 | BDL |
| 60 | 0.056 | BDL | 0.056 | BDL | 0.086 | BDL |
| 90 | BDL | BDL | BDL | BDL | 0.052 | BDL |
| Harvest (120) | BDL | BDL | BDL | BDL | BDL | BDL |

Oxyfluorfen's the $DT_{50}$ was 36.8 to 43.2 days during two years and the residues could be detected up to 90 days after application. Residues of pendimethalin and oxyfluorfen could not be detected in the samples of 20-30 layers indicating their limited leaching potential. Residues of all the three herbicides were below the detectable limit in grain and straw at the time of harvest.

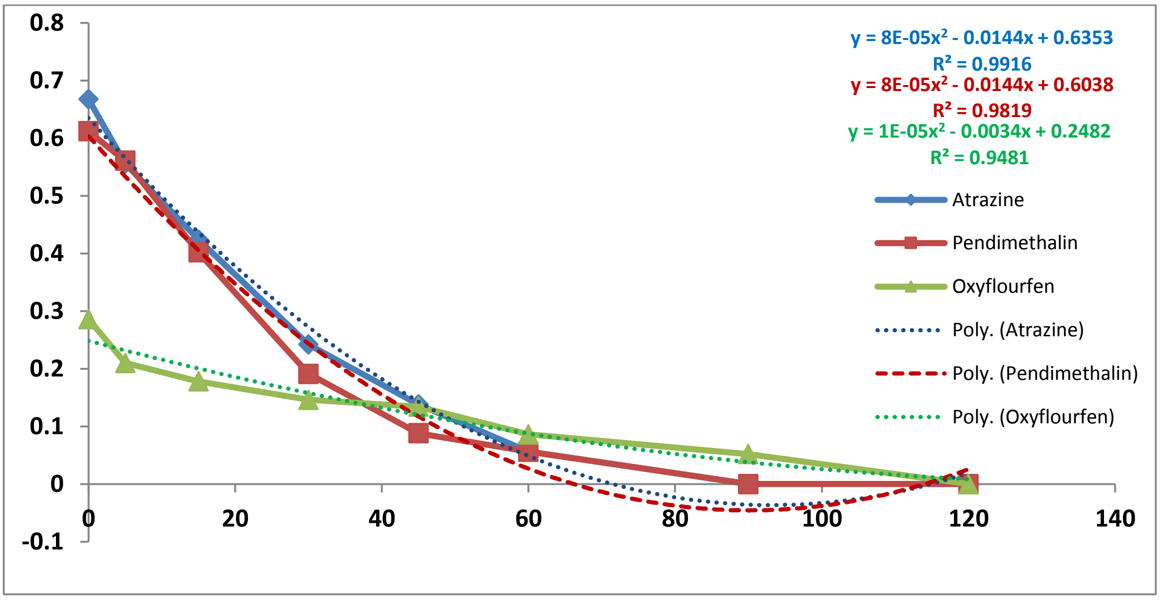


**Fig.1.** Degradation patterns of atrazine, pendimethalin and oxyfluorfen in soil

## CONCLUSION

From the study it can be concluded that integrated weed management strategy consisting of pre-emergence application of either atrazine@1.0kg/ha or oxyfluorfen@0.3kg/ha or pendimethalin @1.0kg/ha followed by intercultivation at 30 DAS is the economical and efficient method for maize under the agroclimatic conditions of Andhra Pradesh for supporting the food security without any residual effect on soil as well as maize grain.



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# *Posters*

## *Weed Invasives and Ecology*

### WEED INVASION IN AIRPORT CONCRETE PAVEMENT AND WEED CONTROL ISSUES

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**ABSTRACT** The airport pavement, which ensures safe high-speed taxiing of aircrafts, plays a role in the interruption from FOD (Foreign Object Damage: the engine sucking debris on the ground). Weedy plants usually invade the gaps of the concrete pavement joints over which aircraft wheels do not pass. In the absence of weeding in an airport heightens the risks of FOD, but weeding of paved runways, taxiways and aprons is actually difficult during aircraft operation. Therefore, proper weed control of airport pavement is an important issue for aircraft safety in an international airport under 24-hour operation. In order to obtain a fundamental knowledge of how to carry out weed control on the airport concrete pavement, we monitored weed species in the gaps of pavement joints on the guard concrete slab that have been constructed with materials from the airport pavement. The results indicated a distinct relationship between the occurrence of weeds, joint width, and the texture of concrete pavement. Thirty-five species invaded the concrete pavement in the spring, and the parallel figure was 25 species in the summer. Three hundred and forty four plants including 4 dominant weeds, *Vicia sativa*, *Vulpia myuros*, *Paspalum notatum*, and *Vicia hirsuta* were found in the joints surveyed in the spring. Two hundred and twenty five plants including three dominant weeds, *Paspalum notatum*, *Imperata cylindrica*, *Conyza sumatrensis* were found in the joints surveyed in the summer. There were few weeds in the gaps of pavement joints whose width were 10mm or less. Although the type of damage to pavement joints by a specific weed is unknown, the results revealed that the appropriate control design and management skill for weedy species on airport pavement, and a suitable weed control might help to reduce the need for weeding and maintenance of airport concrete pavement joints.

***Keywords*:** Airport pavement, Alien plant, Gap of pavement, Pavement joint, Seed length

### INTRODUCTION

Airplanes are a popular means of delivering commodities and carrying passengers. Due to the increase in the volume of air transportation, plant propagules and/or seeds are being disseminated at the globally. Invasion and colonization of alien plants are due to the long haul of airplanes where the original ecosystem has collapsed. However, there is a paucity of information on species composition and growth of vegetation in airports, although weed species reside in concrete pavement on the runway and apron.

The airport pavements are required to be structurally safe for the taxing and parking of aircrafts. These pavements comprise of the runway, taxiway and apron (Fig. 1) (Japan Civil Aviation Bureau 2013a). The runway is for taking off and landing of the aircraft; the apron is for parking the aircraft, boarding air passengers and loading air cargo; the taxiway is for



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taxing of the aircraft between the runway and apron. Normally, the runway and taxiway are composed of asphalt pavement, and the concrete pavement to the apron. Runway and taxiway pavements require easy maintenance and low roughness, and apron pavement requires high toughness for concentration of aircraft gear load and for oil leaking of the fuel supply.

The concrete pavement requires pavement joints at 5m to 10m intervals for the transverse and longitudinal directions for prevention of random cracking by shrinkage and expansion after placing by temperature changes (Figs. 2, 3) (Japan Civil Aviation Bureau 2013b). Airport pavement requires certain functions that interrupt FOD, Foreign Object Damage, which is due to the engine sucking debris, such as gravel and weeds, from the ground (Japan Civil Aviation Bureau 2012). Sometimes, the concrete pavement joints incur damage due to peeling of sealant material and/or increasing of joint width by weed growth. Consequently, the damage of concrete pavement joints by weed invasion in the apron increases the risk of FOD (Figs. 4, 5).

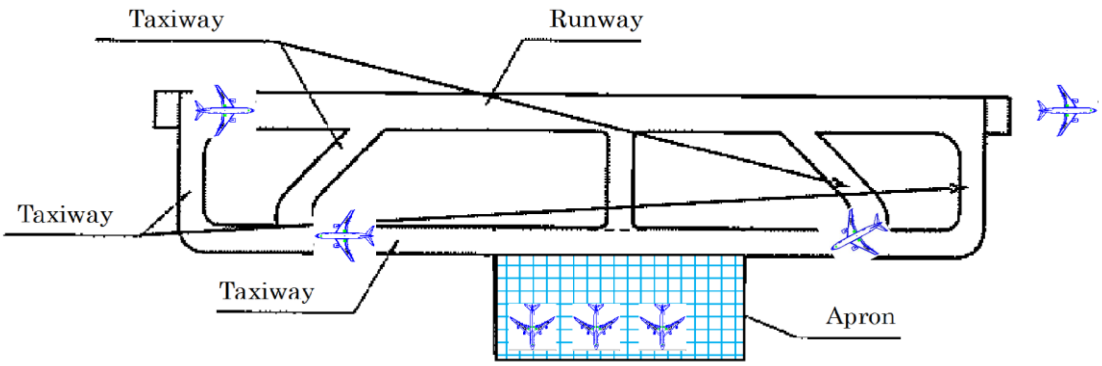


**Fig. 1.** Airport facilities and pavement.

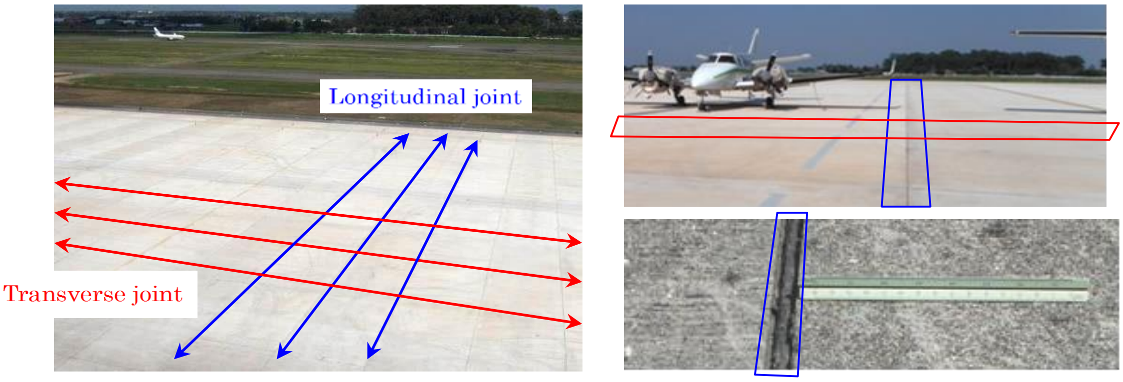


**Fig. 2**. Apron pavement and pavement joint.



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## STUDY SITE AND METHODS

This study was conducted in Phase-2 of Kansai International Airport (KIX), "Monument of the Millennium," the first man-made island for an airport in the world and the biggest and busiest airport in Western Japan, located at 135 E, 34 N, 4 km from the coast of the Osaka gulf. The sea depth around the man-made island is *ca.* from 18m to 20m. The total reclamation island (Phase-1&2) took 430 million cubic meters of soil and rock, and the construction period was 8 years for Phase-1 and 7 years for Phase-2. The airport operation started in 1994 for Phase-1 and 2007 for Phase-2.

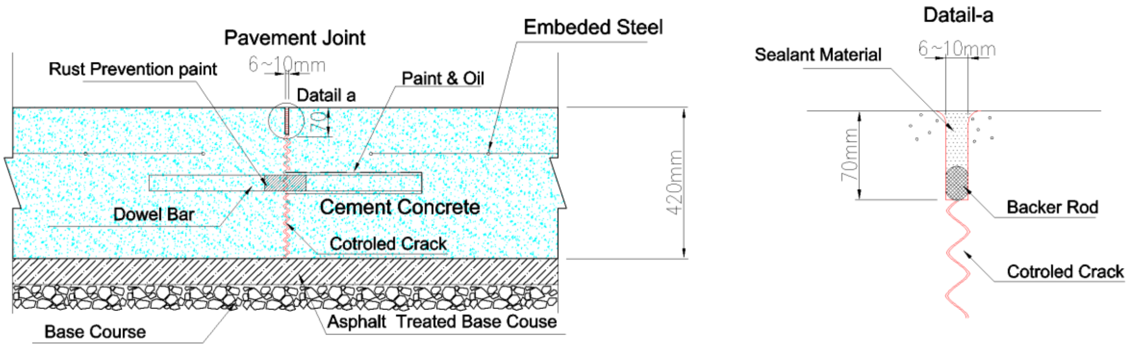


**Fig. 3**. Concrete pavement cross section (left) and pavement joint (right)

**Fig. 4.** Weed invasion to concrete pavement in airport apron

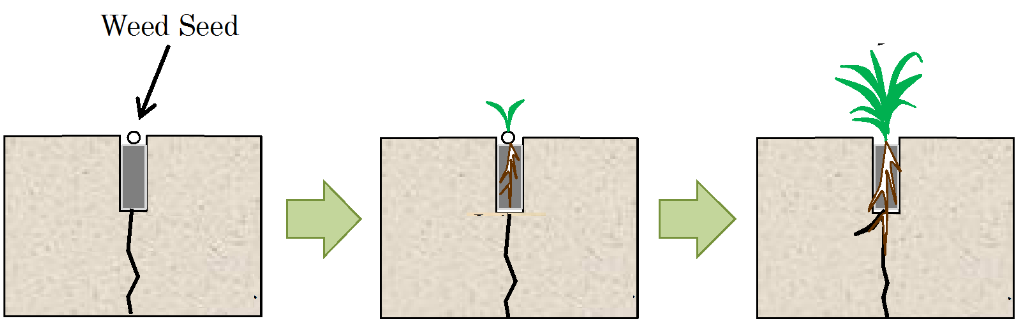


**Fig. 5**. Theoretical invasion process of weeds into concrete pavement joint

Due to the difficulty in surveying the concrete pavement in the operational area, we examined weeds on the concrete blocks that formed a remote apron of Phase-1 by cutting them into small pieces and settling them along the fence beside airport perimeter for security reasons (Fig. 6). The growth of weeds in the concrete blocks and the conditions of the concrete blocks were monitored, and four types of gaps or cracks were measured in length and width (Fig. 7). Joint width of surface (3point for each block), crack width of section



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(depth of 10cm/20cm/30cm) and dimensions of concrete block (width, length and thickness) were measured.

Weed growth was monitored on 300 blocks including on four gaps, longitudinal and transversal joints, cracks and on cutting tracks; measurement of crack size, taxonomic name, number of plants, and plant size were recorded on April 18, 27 (Spring) and on July 28 (Summer) in 2013. The vegetation was analyzed in the 1m square quadrat on the green belt per every 10 block-interval along a block array for comparison. Scientific name and seed size (longitudinal length) were referred to published botanical illustration books (Nakayama *et al*. 2000, Osada 1976, Satake *et* al. 1981, 1982a, 1982b, Shimizu 2003, Shimizu et al. 2001, Uemura et al. 2010).

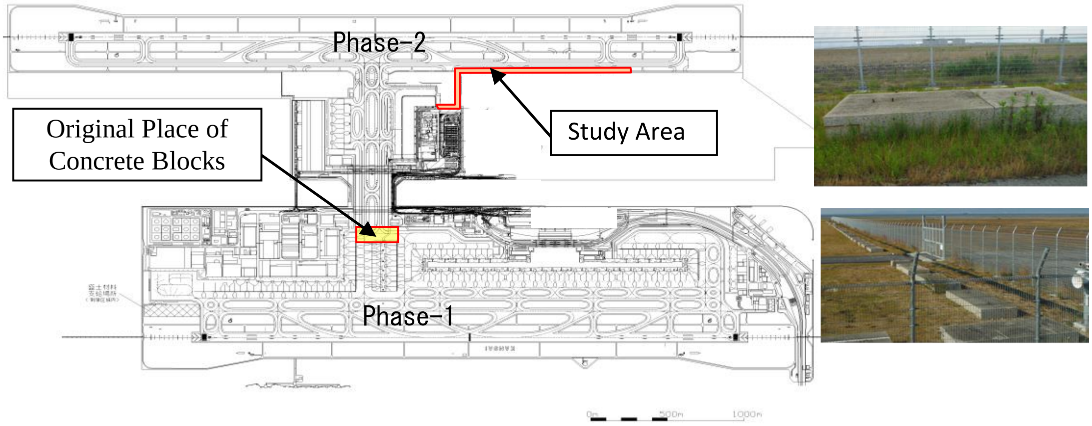


**Fig. 6**. Study site (Left) and monitored blocks and green belt along airport fence (Right).

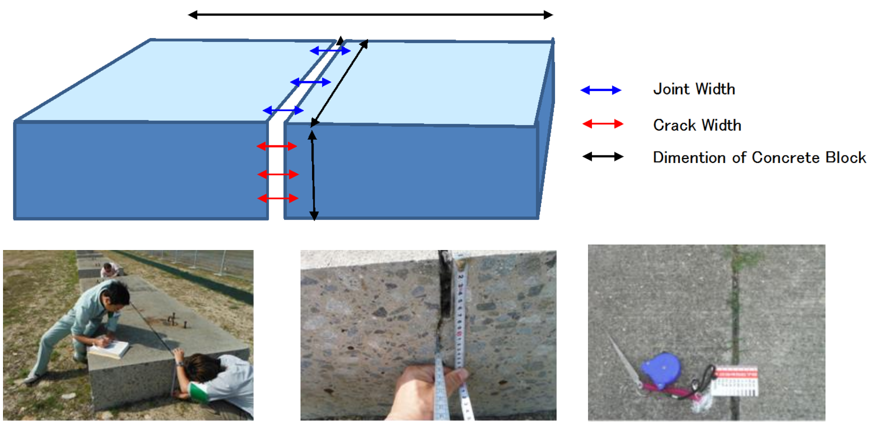


**Fig. 7.** Gap terminology (Up) for pavement and measurement of joint width on concrete block (Bottom)

## RESULTS AND DISCUSSION

Species composition of weeds in the green belt of airport perimeter and gap of concrete blocks: There were 53 species in the spring and 39 species in the summer (total 68 species) in the green belt around the airport runway (Table 1). Although most airport weeds were alien, *ca*. 22% (15 species) of them were weeds true indigenes of Japan. Among them, *ca*. 53% (36 species) were annual and *ca*. 38% (26 species) were perennial. Thirty-five species were registered in the spring and 25 species in the summer on concrete blocks (total 45 species), which were composed of 24 annual species (53%) and 15 perennials (33%), which are similar to those of green belt vegetation (Tables 2, 3). Their seed length ranged from 0.4mm to 3.0mm. Twenty-two species in spring and 14 species in the summer inhabited the green belt and concrete blocks. In contrast, 18 species in the spring and 14 species resided in the summer



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only in the green belt; 13 species in the spring and 11 species in the summer only in concrete blocks.

**Table 1.** Floral composition of airport weeds

| Species | Spring | Summer | Ecological feature |
|---|---|---|---|
| *Agrostis gigantea* | | ○ | |
| *Artemisia indica* | ◎ | ◎ | N, p |
| *Aster subulatus* | ◎ | ◎ | a |
| *Briza minor* | ○ | | a |
| *Cerastium glomeratum* | ○ | | a |
| *Chamaecrista nomame* | | ○ | N ,a |
| *Chamaesyce maculata* | △ | △ | a |
| *Chenopodium album* | | △ | a |
| *Chenopodium ambrosioides* | | ◎ | a |
| *Conyza canadensis* | | ○ | a |
| *Conyza sumatrensis* | ◎ | ◎ | a |
| *Cynodon dactylon* | ◎ | ◎ | p |
| *Dianthus armeria* | ○ | | p |
| *Dianthus* sp. | ○ | | p |
| *Digitaria ciliaris* | | △ | a |
| *Elymus tsukushiensis* | | △* | p |
| *Erigeron annuus* | ○ | ○ | a |
| *Festuca pratensis* | | ○ | p |
| *Festuca rubra* | △ | ◎ | p |
| *Festuca* sp. | ○ | | p |
| *Geranium carolinianum* | ○ | | a |
| *Gnaphalium pensylvanicum* | ◎ | | a |
| *Imperata cylindrica* | △ | ◎ | N, p |
| *Kummerowia striata* | | ◎ | N, a |
| *Lepidium virginicum* | △ | ○ | a |
| *Lespedeza cuneata* | ○ | ◎ | N, p |
| *Lolium multiflorum* | ◎ | ◎ | a |
| *Lysimachia japonica* | △ | | N, p |
| *Nuttallanthus canadensis* | ○ | | a |
| *Oenothera perennis* | ○ | ○ | a |
| Ophioglossum sp. | ○ | | p |
| *Oxalis corniculata* | △ | △ | N, p |
| *Parentucellia viscosa* | | △ | a |
| *Paspalum notatum* | ◎ | ◎ | p |
| *Petrorhagia nanteuilii* | ◎ | | a |
| *Phyllanthus urinaria* | △ | | N, a |
| *Plantago lanceolata* | ○ | ○ | a |
| *Plantago virginica* | ○ | | a |
| *Poa annua* | ◎ | | a |
| *Poa* sp. | △ | | p |
| *Poa sphondylodes* | ◎ | | p |
| *Polygonum aviculare* | | △ | a |
| *Senecio madagascariensis* | | ○ | p |
| *Silene gallica* | ○ | | a |
| *Silene nocturna cf.* | ◎ | ◎ | a |
| *Sisyrinchium rosulatum* | ◎ | ◎ | a |
| *Solanum americanum* | | △ | a |
| *Solidago altissima* | ◎ | | p |
| *Sonchus oleraceus* | ○ | ○ | a |
| *Spiranthes sinensis* | ○ | ○ | N, p |
| *Stellaria media* | ◎ | △ | N, a |
| *Trifolium arvense* | | ○ | p |
| *Trifolium dubium* | ◎ | ○ | a |
| *Trifolium repens* | ○ | | p |
| *Verbena brasiliensis* | ◎ | | p |
| *Veronica arvensis* | ◎ | | a |
| *Vicia hirsuta* | ◎ | | N, a |
| *Vicia sativa* | ◎ | △ | N, p |
| *Vicia tetrasperma* | ◎ | | N, a |
| *Vulpia myuros* | ◎ | | a |
| *Zoysia japonica* | ◎ | ◎ | N, p |
| *Zoysia pacifica* | ○ | ○ | N, p |
| unknoun (A) | △ | | - |
| unknoun (B) | △ | | - |
| unknoun (C) | △* | | - |
| unknoun (D) | △ | | - |
| unknoun (E) | △* | | - |
| unknoun (F) | | △ | - |

-, unknown N, native (domestic)
◎, found both in green and gap a, annual
○, found in green p, perennial
△, found in concrete gap or slit *, in cutting track

Weed species frequency in relation to gap width on concrete blocks: There were 35 species and 344 plants/unit area? in the spring, and 25 species and 225 plants/ unit area? in the summer in the gap of concrete blocks (Tables 4, 5). Although many weeds existed in the gaps and cracks of the cutting track, transversal and longitudinal pavement joints are still a major element in the invasion gaps. The gap width was larger in the cutting track, and many more weeds were present during two seasons (Tables 4, 5). In cases where the gaps width were less than 10mm, 7 plants (3.2% to total 222 plants except for in the crack of the cutting track) were found in the spring and 6 plants (4.3% to total 139 plants except for in the crack of the cutting track) in the summer. The first five dominant species were *Vicia sativa*, *Vulpia myuros*, *Paspalm notatum*, *Vicia hirsuta*, and *Cynodon dactylon* in the spring, while *Paspalm notatum*, *Imperata cylindrica*, *Conyza sumatrensis*, and *Chamaesyce maculata* were dominant in the summer. Concerning the gap width and seed length, invasion seemed more frequent among smaller seeds rather than in larger ones.

Almost all airport weeds are alien, and there are more annual plants than perennial (Table 1). Since the area surveyed was a reclaimed area in the foreshore prior to greening, many alien plants originated from seed sources in adjacent areas and plants. The ratio of alien plants in the residential district, paddy field and paved road is *ca*. 40 % (Suto et al. 2004,



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2005). The ratio in the airport is much higher than the average in Japan. About 35% of species registered only in the green belt in each season, hence it assumed that plants in the gap need particular conditions in order to invade and colonize. A larger gap size indicates where the plants with larger seeds invaded. Therefore, seed size is one of the factors that needs to take into account when considering how weeds invade pavement gaps.

Disseminating behavior of weed seeds, water requirement for germination and juvenile growth are some of the eco-physiological features may determine the success of invasion by weeds. Since block height is *ca*. 40cm, it is difficult for seeds to fall from the tops of plants. However, any species that has smaller seeds than gap width does not invade, but a particular species such as *Vicia sativa* with relatively large seeds (2.5-3mm in length) tends to invade the gaps. One of the possible factors for seed disseminations in this case is due to hard wind from sea and aircraft. The gap feature may select weed species establishments from potential seed sources of weeds in the green belt including in far distance seed disseminating species, which are almost alien to Japan.

**Table 2**. Life form and seed size of weeds found in the green belt of airport perimeter

| Season | No. of species | Life form | | | No. of native species | Seed length (mm) |
|---|---|---|---|---|---|---|
| | | Annual | Perennial | Unknown | | |
| Spring | 53 (4) | 27 | 21 | 5 | 13 | 0.4–3.0 |
| Summer | 39 (1) | 22 | 16 | 1 | 11 | 0.4–4.3 |
| Total | 68 (3) | 36 | 26 | 6 | 15 | 0.4–4.3 |

In parentheses, not found in cutting track

**Table 3.** Life form and seed size of weeds found in the gap of concrete blocks

| Season | No. of species | Life form | | | No. of native species | Seed length (mm) |
|---|---|---|---|---|---|---|
| | | Annual | Perennial | Unknown | | |
| Spring | 35 (6) | 17 | 13 | 5 | 10 | 0.4–3.0 |
| Summer | 25 (3) | 14 | 10 | 1 | 9 | 0.4–3.0 |
| Total | 45 (0) | 24 | 15 | 6 | 12 | 0.4–3.0 |

In parentheses, found in only cutting track

Although the damage to pavement joints by a specific weed is unknown in our evaluation, the result may provide an appropriate control design and management skill for weedy species at airports, and a suitable weed control may reduce the needs for weeding and maintenance of airport concrete pavement joints. However, easy application of herbicide causes biodiversity loss in the airport environment, where human habitats affect those of animals and plants. Establishment of weed control skill at the airport is an enterprise issue to be overcame for FOD problems and invasive plant protection during this era of globalization. Basic information on weeds in airports may provide us with an Eco-airport-conserving natural conventional environment.



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**Table 4.** Number of plants occurring in the gap of concrete blocks in spring

| | Gap of pavement joints | | | | | | Crack | Cutting track | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Longitudinal | | | Transverse | | | | | |
| Gap width (mm) | Less than 10 | 10–12 | More than 12 | Less than 10 | 10–12 | More than 12 | | 6–7 | |
| No. of gaps | 35 | 34 | 39 | 17 | 18 | 18 | 5 | 75 | 241 |
| No. of species | 5 | 15 | 11 | 2 | 13 | 16 | 2 | 18 | 35 |
| No. of plants | 8 | 26 | 56 | 2 | 47 | 83 | 8 | 114 | 344 |
| Seed length (mm) | 1.0–3.0 | 0.4–3.0 | 0.4–3.0 | 0.4–0.8 | 0.4–3.0 | 0.4–3.0 | | 0.4–3.0 | 0.4–3.0 |
| Species | | | | | | | | | |
| *Vicia sativa* | 2 | 2 | 16 | | 8 | 18 | | 30 | 76 |
| *Vulpia myuros* | | | 10 | 1 | 15 | 9 | | 12 | 47 |
| *Paspalum notatum* | | 1 | 4 | 1 | 4 | 17 | 1 | 16 | 44 |
| *Vicia hirsuta* | 4 | | 13 | | 5 | 5 | | 13 | 40 |
| *Cynodon dactylon* | | 3 | | | 1 | 5 | | 13 | 22 |
| *Conyza sumatrensis* | 1 | 1 | 1 | | 2 | 7 | | 1 | 13 |
| *Festuca rubra* | | | 4 | | | | 7 | | 11 |
| *Artemisia indica* | | | 3 | | 3 | | | 3 | 9 |
| *Lolium multiflorum* | | 1 | 1 | | | 3 | | 4 | 9 |
| *Imperata cylindrica* | | | | | | | | 7 | 7 |
| Others (25 species) | 1 | 18 | 4 | | 9 | 19 | | 15 | 66 |

**Table 5.** Number of plants occurring in the gap of concrete blocks in summer

| | Gap of pavement joints | | | | | | Crack | Cutting track | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Longitudinal | | | Transverse | | | | | |
| Gap width (mm) | Less than 10 | 10–12 | More than 12 | Less than 10 | 10–12 | More than 12 | | 6–7 | |
| No. of gaps | 35 | 34 | 39 | 17 | 18 | 18 | 5 | 75 | 241 |
| No. of species | 2 | 11 | 11 | 2 | 11 | 12 | | 14 | 25 |
| No. of plants | 2 | 23 | 25 | 4 | 29 | 56 | | 86 | 225 |
| Seed length (mm) | 0.9–1.0 | 0.6–1.5 | 0.4–3.0 | 0.8–0.9 | 0.7–1.2 | 0.7–3.0 | | 0.7–3.0 | 0.4–3.0 |
| Species | | | | | | | | | |
| *Paspalum notatum* | | 1 | 5 | 1 | 4 | 16 | | 13 | 40 |
| *Imperata cylindrica* | | | | | | | | 31 | 31 |
| *Conyza sumatrensis* | 1 | 2 | 1 | | 2 | 10 | | 8 | 24 |
| *Chamaesyce maculata* | | 2 | 3 | 3 | 6 | 8 | | 1 | 23 |
| *Cynodon dactylon* | | 3 | | | 1 | 9 | | 5 | 18 |
| *Lolium multiflorum* | 1 | | 1 | | 3 | 4 | | 7 | 16 |
| *Artemisia indica* | | | 2 | | 3 | | | 7 | 12 |
| *Kummerowia striata* | | | 1 | | 4 | 2 | | 5 | 12 |
| *Vicia sativa* | | | 9 | | | 1 | | 1 | 11 |
| *Aster subulatus* | | 3 | | | 1 | 3 | | 3 | 10 |
| Others (15 species) | | 12 | 3 | | 5 | 3 | | 5 | 28 |


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# RHIZOPHERE FUNCTIONAL MICROORGANISMS ANALYSIS OF *FLAVERIA BIDENTIS* (L.) KUNTZE

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**ABSTRACT** Pot experiments in greenhouse were carried out to investigate the influence of yellow top [*Flaveria bidentis* (L.) Kuntze] on the functional microorganisms in rhizophere. After planting yellow top, the amount of potassium bacteria, amonifying bacteria and phosphate-solubilizing bacteria significantly increased by 40%, 103% and 48% in the soil in which invasive yellow top grew, and by 13%, 314% and 17% in the soil in which native plant grew. While the amount of desulphurizing bacteria significantly decreased by 21% and 16% in the two kinds of soil, respectively. The effects of yellow top on the quantities of Organic phosphate-dissolving bacteria, denitrifying bacteria, aerobic azotobacter and sulfate-reduction bacteria were not significant. The results preliminarily explained why the contents of some elements (e.g. P, S) in the soil changed after yellow top invasion, and it provided theoretical basis for the invasion mechanism of *Flaveria bidentis* (L.) Kuntze in the micro-ecology level.

***Keywords:*** *Flaveria bidentis*; functional microorganisms; rhizophere

## INTRODUCTION

Invasive alien species cause serious harm to the development of economy and even to human health by reducing the native species diversity and altering natural ecosystems. Since first discovered in China in 2001, yellow top [*Flaveria bidentis* (L.) Kuntze] has become a very dangerous invasive plant. The invasion mechanism of this species is one of the hotspots in recent years. And the interaction between yellow top and rhizosphere microorganisms is a very important research direction. Many reports showed that yellow top invasion changed the amounts of bacteria, fungi and actinomycetes in the soil. However, detailed effects of yellow top on the functional microorganisms need to be demonstrated, so that the changes of soil physicochemical properties and nutrient contents after invasion could be better explained.

## MATERIALS AND METHODS

Pot experiments in greenhouse were carried out to investigate the influence of yellow top on the functional microorganisms in rhizophere. Two kinds of soils, yellow top invasive soil (Invaded soil) and native plant soil (Non-invaded soil), were collected and used in this experiment. Yellow top was planted to the different treatments through transplant seedlings. Soil samples were obtained before planting yellow top (Before invasion) and after the whole growth cycle of it (After invasion). Potassium bacteria, amonifying bacteria, inorganic phosphorus dissolving bacteria, organic phosphate dissolving bacteria, denitrifying bacteria, aerobic azotobacter, desulphurizing bacteria and sulfur bacteria were selected as the representative functional microorganisms and the variation of the amounts were analyzed.

## RESULTS AND DISCUSSION

The results showed that, after planting yellow top, the amount of potassium bacteria, amonifying bacteria, organic phosphate dissolving bacteria significantly increased by 40.0%, 103.0% and 129.0% in the soil in which invasive yellow top grew, while the amount of aerobic azotobacter, denitrifying bacteria and desulphurizing bacteria significantly decreased by 13.3%, 53.4% and 96.8%, respectively. But in the soil in which native plant grew, the amount of amonifying bacteria, potassium bacteria, aerobic azotobacter, inorganic phosphorus



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dissolving bacteria, organic phosphate dissolving bacteria and denitrifying bacteria significantly increased by 314.0%, 13.0%, 131.0%, 17.0%, 8.0% and 49.0%, respectively. Desulphurizing bacteria and sulfur bacteria were inhibited in this soil, of which the amounts decreased by 20.0% and 44.4%, respectively (Table 1).

The results preliminarily explained why the contents of some elements (e.g. P, S) in the soil changed after yellow top invasion, and it provided theoretical basis for the invasion mechanism of *Flaveria bidentis* (L.) Kuntze in the micro-ecology level.

**Table 1**. Effects of *Flaveria bidentis* invasion on the quantity of functional bacteria in soil

| Soil types | | Potassium bacteria /($\times10^6$cfu·g$^{-1}$) | Amonifying bacteria /($\times10^6$cfu·g$^{-1}$) | Aerobic azotobacter /($\times10^5$cfu·g$^{-1}$) | Inorganic phosphorus bacteria /($\times10^6$cfu·g$^{-1}$) | Organic phosphorus bacteria /($\times10^6$cfu·g$^{-1}$) | Denitrifying bacteria /($\times10^5$cfu·g$^{-1}$) | Sulphur bacteria /($\times10^4$cfu·g$^{-1}$) | Desulphurizing bacteria /($\times10^5$cfu·g$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| Invaded soil | Before invasion | 12.90±0.47* | 9.5 7±0.79* | 20.00±2.65 | 11.83±2.54 | 3.83±0.79* | 15.77±0.32* | 1.5 | 95 |
| | After invasion | 18.07±0.81* | 19.40±1.90* | 17.33±3.93 | 17.57±0.62 | 8.77±0.38* | 7.33±1.57* | 1.5 | 3.0 |
| Non-invaded soil | Before invasion | 8.03±0.87 | 3.73±0.27 | 13.00±1.15 | 8.03±0.90 | 5.80±0.21 | 8.43±1.74 | 25 | 45 |
| | After invasion | 9.07±1.64 | 15.47±3.52 | 30.00±7.23 | 9.43±2.11 | 6.27±0.61 | 12.60±2.18 | 20 | 25 |

Values are mean ± SE,* indicate that means are significantly different at $p < 0.05$ (T-test)



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# UNDERSTANDING HOW WEED SPECIES, DIVERSITY AND COMMUNITIES VARY WITH WEATHER, CLIMATE, SOIL, LAND USE AND TIME IN SOUTH-WEST WESTERN AUSTRALIA

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**ABSTRACT** Successful weed management depends on a sound understanding of the factors affecting the distributions of weed species and communities and how these change over time. To determine these patterns, the prevalence of weeds in fields in the south west of Western Australia was surveyed in 1997 and again in 2008 across a total of 956 sites from both surveys. Altogether 194 weed species (or groups of species within a genus) were identified. The majority of survey sites were used for cropping, and 152 weed species were identified within these cropped fields. Between 1997 and 2008, noticeable decreases in incidence (in cropped fields) were observed for *Vulpia* spp. (-25.3%), *Aira caryophyllea* (-20.5%), *Bromus diandrus* (-19.9%), *Avena fatua* (-17.6%) and *Austrostipa* spp. (-12.8%), with only *Raphanus raphanistrum* (11.3%) and *Arctotheca calendula* (7.1%) significantly increasing in frequency. A community analysis approach was then used to investigate potential environmental drivers of changes in community composition, including annual and seasonal temperature and rainfall over both the survey years and the preceding decades, as well as soil characteristics such as thickness, water-holding-capacity, organic carbon, bulk density and pH. This analysis showed that a wide range of edaphic and meteorological factors were significantly related to differences in weed community composition across time and space.

***Keywords:*** Weed diversity, land-use, community composition, distribution

## INTRODUCTION

Successful weed management depends on a sound understanding of the factors affecting the distributions of weed species and communities and how these change over time. Geographical distribution surveys identify problem weeds within an area, and if done repeatedly, can help demonstrate the spread and incidence of such weeds over time and their link to changes in agricultural and land-use practices (Hyvonen *et al.*, 2003; Andreasen & Stryhn, 2008). As land-use and herbicide application practices have changed over time there have been documented shifts in the weed species diversity and abundance (Andreasen *et al.*, 1996; Benton *et al.*, 2003, Hyvonen *et al.*, 2003; Andreasen & Stryhn, 2008). In southern Australia, there have been many widespread changes within agricultural systems that have had a direct impact on the patterns of weed abundance and distribution, including conservation tillage, increased crop diversity and intensity, and an increasing dependence on herbicides (Llewellyn *et al.*, 2002; D'Emden *et al.*, 2006).

## MATERIALS AND METHODS

To determine these patterns, the prevalence of weeds in fields in the south west of Western Australia was surveyed in 1997 and again in 2008 across a total of 956 sites from both surveys. Weed incidence and abundance were scored along a transect, (in Spring, which is the reproductive stage for Western Australian weeds) and land use recorded as different types of either crop or pasture. Detailed soil and met data for each field in each year was gathered. Farmers of the fields were surveyed and asked what they perceived to be the most abundant and problem weeds. Analyses were carried out using R and its vegan Community Ecology



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package. Species richness and Simpson and Shannon diversity indices were calculated, and we looked to see whether there were spatial patterns in these and whether they could be explained by environmental drivers. We then calculated the Bray-Curits distance between pairs of sites and a distance matrix constructed. A community analysis approach was then used to investigate potential environmental drivers of changes in community composition, including annual and seasonal temperature and rainfall over both the survey years and the preceding decades, as well as soil characteristics such as thickness, water-holding-capacity, organic carbon, bulk density and pH.

## RESULTS AND DISCUSSION

Altogether 194 weed species (or groups of species within a genus) were identified. The majority of survey sites were used for cropping, and 152 weed species were identified within these cropped fields. Between 1997 and 2008, noticeable decreases in incidence (in cropped fields) were observed for *Vulpia* spp. (-25.3%), *Aira caryophyllea* (-20.5%), *Bromus diandrus* (-19.9%), *Avena fatua* (-17.6%) and *Austrostipa* spp. (-12.8%), with only *Raphanus raphanistrum* (11.3%) and *Arctotheca calendula* (7.1%) significantly increasing in frequency.

Results of the diversity analysis indicate that crop type, met variables, soil, space and time were all significant and that 37% of diversity differences were explained by the 'best' model. There were also significant spatial patterns in diversity (Fig 1.). Results of the composition analysis showed that a wide range of edaphic and meteorological factors were significantly related to differences in weed community composition across time and space.

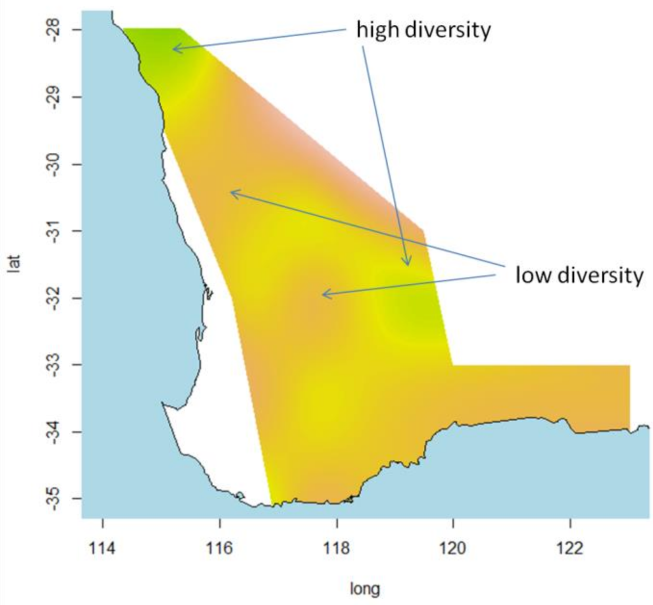


**Fig. 1.** Our analysis revealed significant spatial patterns in diversity with distinct areas of low and high diversity.

Crop type was the most important, explaining 14% of the variation in composition. There was a significant difference between the two survey years, even when other factors such as changes in cropping practices were accounted for. Our best model explained 44% of observed variation. The results from the farmer surveys sometimes matched results of the weed surveys; for example, *Raphanus raphanistrum* was increasing in both in abundance and in levels of concern according to farmers. However, in some cases farmer surveys gave different results to the weed surveys; for example *Conyza* species were a big concern but not yet common in the region.

From our results, we conclude that farmer surveys do not always match the results of field surveys, so researchers should consider both to achieve a complementary understanding.



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There were significant changes in weeds in south-west Western Australia between 1997 and 2008 at the level of individual species, community composition and overall diversity. These are likely largely due to changes in management and farming systems, with smaller impacts from changing climate and the unfolding of invasion dynamics. Much variation was not explained by the variables we were able to investigate; this could be due to details of the way that fields were managed. In future work, we would like to investigate these management factors more deeply. We would also like to use predictive simulation modelling of seed bank and population dynamics and spatial invasion dynamics to better understand the causes of weed distributions, community composition and diversity (Renton *et al.*, 2008; 2011).

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# SALT ACCUMULATION CAPACITY COMPARISSION BY THE PARTS OF THE PLANT OF THE FORAGE BARNYARD GRASS UNDER THE SALT STRESS

Cho Kwang-Min[1 *], Lee Sang-Bok[1], Back Nam-Hyun[1], Moon Yae-Jin[1], Yang Chang-Hyu[1], Shin Pyung[1], Lee Kyung-Bo[1], Park Ki-Hun[1] and Jung Nam-Jin[2]
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**ABSTRACT** This study has been conducted by cultivating the barnyard grass and utilizing them as a bulky feed which were once recognized as weeds in South Korea's reclaimed lands. The barnyard grass is highly adaptive to the environment having poor drainage with high salt concentration and was recognized as a plant that can be cultivated in the reclaimed land which has not been desalinated. Therefore in this study, 7 types of barnyard grass identified as a salt tolerant species with high biomass were seeded in the port and 5 weeks after the emergence of the plant body, each day for 30 days 0.1% saline was irrigated to cultivate by maintaining the soil salinity from 0.4 to 0.6% and the salinity accumulation ability of the forage barnyard grass was compared for the use with the future salt decontamination research. 60 days after seeding the measurement for the stem height + ear length was 95 ~ 145cm with the Jeju barnyard grass(JBG) being the tallest. After collecting the plant body and separating the stem, roots, leaves and seeds for drying and analyzing the salt concentration using the ICP-OES, each stem showed 0.92 ~ 3.92% amounts of the salt with JBG and wild barnyard grass showing very high amounts at 3.92%, 3.19% and the leaves showed 0.44 ~ 3.11% and the roots showed 0.77 ~ 3.94% amounts. Even in the seeds showed 0.11~0.32% contents of the salt which showed that the seeds are also affected by the movement of the salt. As a result, the barnyard grass having the salt tolerance accumulates a lot of salt into the plant body with especially the stem and the leaves storing the most and even the roots to the seeds showed a movement of the salt. Also as it contains high amounts of salinity, it is not affected by it therefore it has proven the possibility of being used as crops that can be cultivated on the reclaimed land.

***Keywords:*** Salt stress, salt accumulation, barnyard grass

## INTRODUCTION

Bulky feed cultivation is induced (Park *et al.,* 2012) instead of rice cropping that is available to cultivate if fresh water is provided from reclaimed land which is caused by reduce of rice consumption recently in Korea. Among those, barnyard grass is possible for growth in high salinity area and is known to have excellent biomass output (Lee *et al.* 2003). Therefore, if barnyard grass is able to grow with accumulating salt, biological desalinization effect is possible and possible to use as roughage. This research was performed to find out the possibility of barnyard grass growth in reclaimed land of Korea.

## MATERIALS AND METHODS

In order to find out the possibility of growth of barnyard grass in reclaimed land, the research was accomplished by purchasing from RDA-Gene bank Information Center in Korea. Life stage test was performed in rectangular port(50x30x25cm, WxLxH) filled with soil of reclaimed-land(Table 1) inside of greenhouse in National Institute of Crop Science. The temperature of greenhouse was 25~28 °C on day, 18~20 °C at night, and over 100,000 LUX light was illuminated during 18~21 o'clock. The amount of applied fertilizer was 10kg of N,



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5kg of P and 5kg of K per 10a, and 50% of N was used as basal fertilization and the other 50% was used as additional fertilizer one month after seeding. In order to check salt tolerance, the plant was watered with 0.1% saline water for three weeks from plant germination. The form of plant length, tiller number, seed was checked 60 days after seeding. Lastly, salinity in the plant was checked using ICP-OES after grinding with dividing into shoot, leaf, root and grain.

**Table 1**. The chemical characteristics of soil before experiment**.**

| Division | pH (1:5) | OM g $kg^{-1}$ | Av.$P_2O_5$ mg $kg^{-1}$ | Ex.-cations(cmol $kg^{-1}$) | | |
|---|---|---|---|---|---|---|
| | | | | K | Ca | Mg |
| Reclaimed land | 7.8 | 1.8 | 35 | 2.4 | 1.0 | 2.5 |

## RESULTS

The stem length+ear length 60 days after seeding was 95~145 cm and Jeju traditional barnyard grass was the tallest. The Maxa race had the most tiller number with 23. All races had seed after ear emergence, but barnyard grass in Saemangeum reclaimed land almost did not show ripening (Fig. 1).

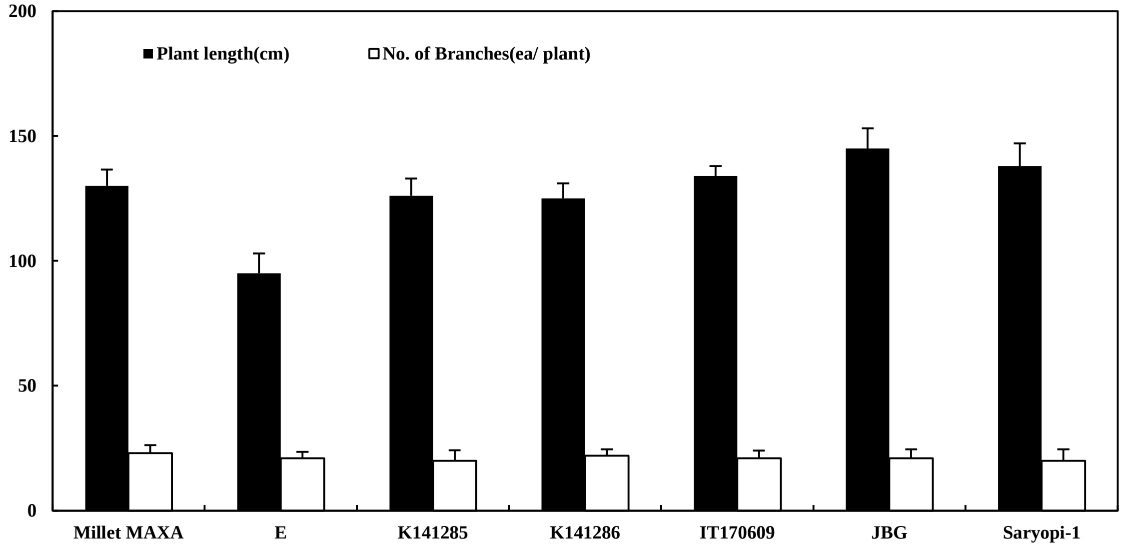


**Fig. 1.** Growth of barnyard grass under salt stress. JBG: Jeju barnyard grass, E: *Echinochloa oryzicola*

After collecting the plant body and separating the stem, roots, leaves and seeds for drying and analyzing the salt concentration using the ICP-OES, each stem showed 0.92 ~ 3.92% amounts of the salt with JBG and wild barnyard grass(*Echinochloa oryzicola*) showing very high amounts at 3.92%, 3.19% and the leaves showed 0.44 ~ 3.11% and the roots showed 0.77 ~ 3.94% amounts. Even in the seeds showed 0.11~0.32% contents of the salt which showed that the seeds are also affected by the movement of the salt(Fig. 2).



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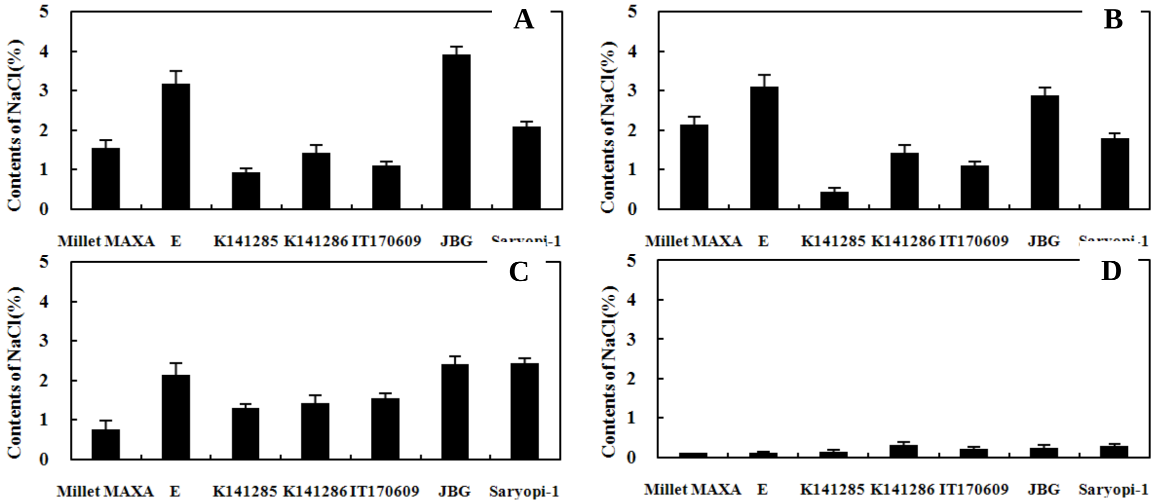


**Fig. 2.** Salt accumulation of barnyard grass of salt tolerance by part under salt stress. A: Shoot, B: Leaf, C: Root, D: Grain, JBG: Jeju barnyard grass, E: *Echinochloa oryzicola*

## CONCLUSIONS

The barnyard grass having the salt tolerance accumulates a lot of salt into the plant body with especially the stem and the leaves storing the most and even the roots to the seeds showed a movement of the salt. Also as it contains high amounts of salinity, it is not affected by it therefore it has proven the possibility of being used as crops that can be cultivated on the reclaimed land.

## ACKNOWLEDGEMENTS

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# SEED GERMINATION OF THE FORAGE BARNYARD MILLET COLLECTED FOR SEEDING UNDER THE SALT STRESS

Cho Kwang-Min[1 *], Lee Sang-Bok[1], Back Nam-Hyun[1], Moon Yae-Jin[1], Yang Chang-Hyu[1], Shin Pyung[1], Lee Kyung-Bo[1], Park Ki-Hun[1] and Jung Nam-Jin[1]
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**ABSTRACT** Recently in South Korea, in order to improve the self-sufficiency of the bulky feed, there has been a big interest by the farmers to cultivate the barnyard millet as a crop in the large reclaimed land. However, there have been many difficulties because the supply of superior barnyard millet species is not smooth. Therefore this study is used for seeding and cultivating the barnyard millet and the research for safely supplying the seeds to the farmers has been conducted. 7 types of barnyard millet was cultivated for 120 days in the soil having a high salt concentration of 0.5% or more and the seeds were gathered to analyze the germination, seed vitality measurement and solubility proteins to check on the stability of the seeds. After checking the germination of the gathered seeds, each types of the barnyard millet showed a big difference of 3~72% and from this Jrju barnyard millet(JBM) was the highest with 72%. After conducting the tetrazolium chloride (TTC) test in order to check the vitality of the seeds, the seeds gathered at the general farmland had red staining on the TTC reagents for most but the seeds gathered at the reclaimed land had lower or no degree of staining compared to the seeds collected at the general farmland. The protein contents of the gathered seeds from the general farmland and the reclaimed lands had little difference of 11.69% to 12.19% but for the seeds gathered at the reclaimed land showed a difference of contents by 11.5% and thought to affect the germination. As a result, for the JBM, even if it is cultivated at the reclaimed land with high salt concentration showed a high biomass for being used as a bulky feed and even if the seeds are gathered from the reclaimed land, it ripened well with high germination rate and thought to be an excellent genetic resource that can be utilized from the reclaimed land.

***Keywords:*** Salt stress, germination, barnyard millet

## INTRODUCTION

Korea performs the research on barnyard grass to produce roughage in the reclaimed land (Shin *et al*., 2006). In addition, farmers have great interest in barnyard grass cultivation for difficulties in traditional crop cultivation due to high salt and poor drainage in large reclaimed land. However, barnyard grass is easy to be weed and difficult to cultivate in ordinary farmland for high competition with rice(Kwon *et al*., 2007). Therefore, this study was performed to cultivate barnyard grass and seed production in reclaimed-land

## MATERIALS AND METHODS

The safety of seed was checked with analyzing germination rate, seed viability measurement, and soluble protein by harvesting seed after cultivating 7 types of barnyard grass for 120 days including JBG in the soil with over 0.5% of salinity of Samangeum reclaimed land. Germination rate was measured by 100 seeds in petri-dish for 7 days and seed viability was measured by comparing the degree of dyeing to red with using TTC dye. The protein was quantified by multiplying protein conversion factor after analyzing nitrogen with using element analyzer after grinding samples of each seed according to the report of Bewely &



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Black (1982) that the amount of protein longitudinally increase in the early stage of germination.

## RESULTS AND DISCUSSION

After checking the germination of the gathered seeds, each types of the barnyardgrass showed a big difference of 3~72% and from this Jeju barnyard grass (JBG) with the highest germination rate of 72%(Fig. 1).

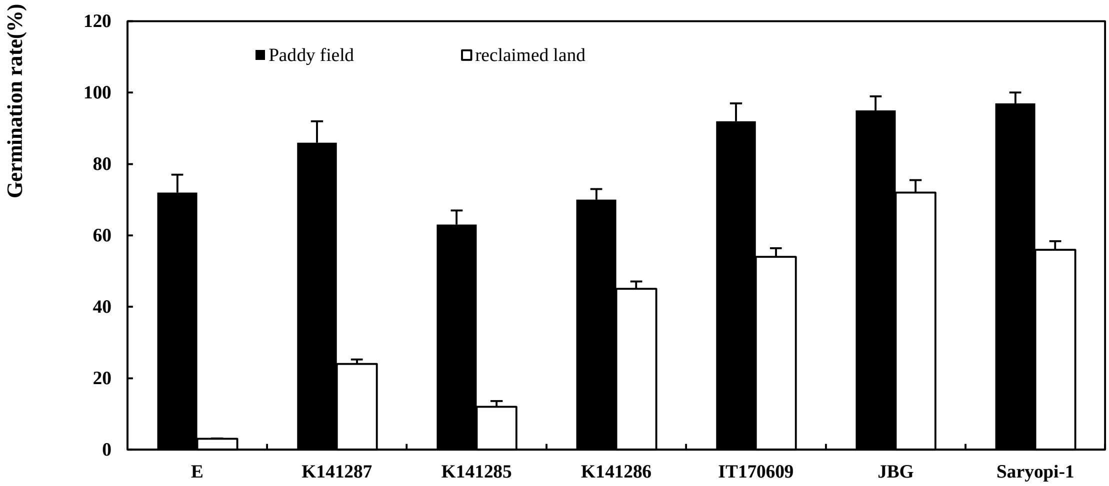


**Fig. 1.** Germination rate of barnyard grass by seed harvesting under salt stress.E: *Echinochloa oryzicola,* JBG: Jeju barnyard grass.

After conducting the tetrazolium chloride (TTC) test in order to check the vitality of the seeds, the seeds gathered at the general farmland had red staining on the TTC reagents for most but the seeds gathered at the reclaimed land had lower or no degree of staining compared to the seeds collected at the general farmland (Fig. 2).

The protein contents of the gathered seeds from the general farmland and the reclaimed lands had little difference of 11.69% to 12.19% but for the seeds gathered at the reclaimed land showed a difference of contents by 11.5% and thought to affect the germination (Fig. 3).

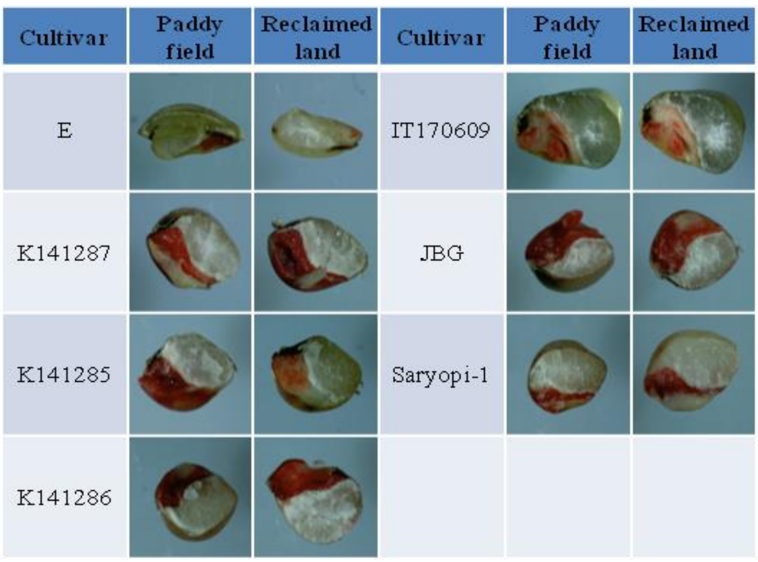


**Fig 2. Tetrazolium** test of seeds by seed harvesting under salt stress.



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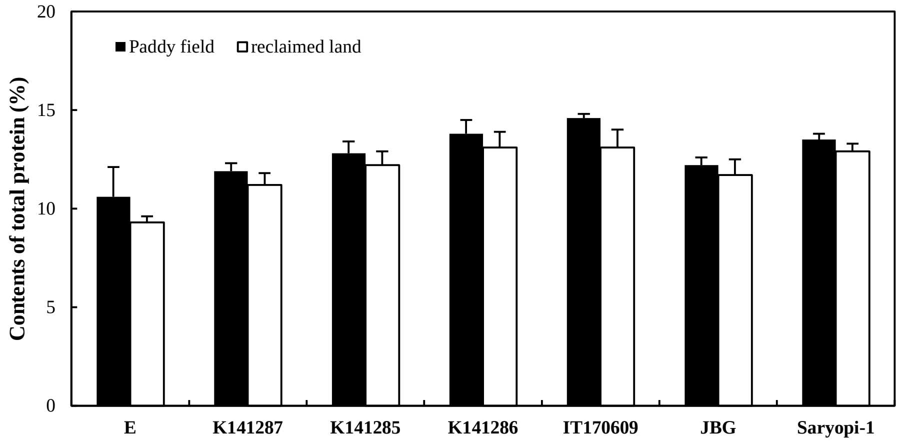


**Fig. 3**. Amounts of total protein barnyard grass by seed harvesting under salt stress.

## CONCLUSIONS

The result was verified with excellent seed viability and germinability compared with other breeds, which is possible to grow in 0.5% of salinity and harvested seed in the case of Jeju traditional barnyard grass and it is expected to be helpful in producing roughage with barnyard grass which is possible for seed harvesting and cultivation in the reclaimed land.

## ACKNOWLEDGEMENTS

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# CHANGE OF VEGETATION CHARACTERISTICS AND SOIL CHEMICAL PROPERTIES AT SAEMANGEUM RECLAIMED LAND GE-HWA AREA IN KOREA

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**ABSTRACT** This study was conducted to obtain to observe vegetation succession in line with changes in various soil amendments in tidal land. Vegetation succession were surveyed and soil characteristics were analyzed at the natural plot in tidal land from 2010 to 2012 in Saemangeum reclaimed land in Korea. Soil salinity were 0.16~22.3 *dS* $m^{-1}$ in first survey, when the three years later decreased as 0.12~4.22 *dS* $m^{-1}$. Vegetations were classified as 6 families and 26 species as increased 8 families and 31 species after three years. Numbers of average species in survey site were increased from 7.1 species to 10.6 species. Numbers of vegetation were increased at each survey sites except for survey site 7(survey site 1-13): there was decreased halophyte according to decrease the soil salinity. Biomass production was increased at low production site, and showed highest production in area of dominant vegetation as *Phragmites communis*. Simpson's dominance ratio (SDR) of main vegetation as *Phragmites communis*, *Calamagrostis epigeios* were increased and *Suaeda maritima*, *Salicornia europaea*, *Puccinellia nipponica*, *Zoysia sinica* were decreased. Therefore, *Phragmites communis*, *Calamagrostis epigeios* are the best vegetation species to improve and ameliorate soil's physical properties and however in the future it is predicted that they will threaten to crop on new arable land after the desalinization tidal period.

***Keywords:*** Halophyte, reclaimed tidal land, soil salinity, vegetation.

## INTRODUCTION

There is an increasing interest in the reclaimed tidal land (here will being the best for a stable food supply), in spite of this, it has high salt content levels and poor physical properties with low level organic matter contents. Occurrence of diverse vegetation and species succession is known to have salt contents in their roots from soil (Kim, 2005). The vegetation and species dynamics are useful factors to improve and ameliorate soil's physical properties. However, the vegetation change was a result of weed colonization in new arable land as the salt land is transitioning into farmland. Therefore the objective of this study was to observe vegetation succession in line with changes in various soil amendments.

## MATERIALS AND METHODS

The study took place at Saemangeum reclaimed tidal land, Gye-hwa area in Korea, in mid-September of 2010 and 2012. The land concentrated on within the survey was formed in 2008 as a result of a sea wall being built. Vegetation succession was surveyed at 13 different sites guided by GPS at the naturally maintained plot, and soil samples were picked at the same point- 15cm under the soil surface. Vegetation was classified based on literature written by Lee (2006) and the summed dominance ratio was based on works by Kim (1998); in addition to this, soil EC and pH were analyzed in reference to a conductive meter (Switzerland) and organic content was analyzed using an elementary analysis method.

## RESULTS AND DISCUSSION

Readings on soil salinity, organic soil content, pH etc. saw temporal improvements from 2010 to 2012. Soil salinity in the first survey was 0.16-22.3 dSm-1, this later decreased to 0.12-4.22



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dSm-1 after three years. Soil pH also decreased, however, organic soil content increased in 10 (out of 13) sites. The decrease in soil salinity and soil pH were the results of decreased ground water level and the flowing stream from rainfall.

**Table 1.** Changes of soil salinity, organic matter and pH at survey sites in Saemangeum Gye-hwa area in Korea.

| Soil chemical properties | Year | Survey sites | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Soil chemical properties | | 1♩ | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | Mean |
| Salinity : $dS\ m^{-1}$ | 2010 | 22.3 | 14.0 | 12.0 | 6.72 | 5.0 | 0.3 | 10.0 | 2.97 | 1.1 | 3.44 | 0.16 | 3.13 | 2.97 | 6.46 |
| Salinity : $dS\ m^{-1}$ | 2012 | 4.22 | 2.80 | 2.02 | 3.64 | 0.9 | 0.1 | 1.0 | 0.12 | 2.4 | 0.62 | 0.12 | 0.44 | 0.62 | 1.46 |
| Organic content : % | 2010 | 0.14 | 0.09 | 0.06 | 0.08 | 0.09 | 0.08 | 0.16 | 0.08 | 0.26 | 0.24 | 0.28 | 0.27 | 0.35 | 0.16 |
| Organic content : % | 2012 | 0.16 | 0.10 | 0.10 | 0.12 | 0.11 | 0.14 | 0.21 | 0.23 | 0.24 | 0.30 | 0.33 | 0.27 | 0.28 | 0.19 |
| pH | 2010 | 8.95 | 8.49 | 8.59 | 8.76 | 9.26 | 9.11 | 8.01 | 8.74 | 7.33 | 7.96 | 7.4 | 6.7 | 7.01 | 8.17 |
| pH | 2012 | 7.68 | 8.27 | 8.78 | 8.34 | 9.16 | 8.81 | 8.64 | 7.97 | 6.45 | 7.12 | 6.83 | 6.66 | 6.58 | 7.79 |

♩: Survey sites (longitude and latitude), 1♩ N35˚ 46' 10.2", E126˚ 37' 07.4". 2: N35˚ 46' 09.3", E126˚ 36' 10.9". 3: N35˚ 46' 07.2", E126˚ 36' 15". 4: N35˚ 46' 05.5", E126˚ 36' 26.9". 5: N35˚ 46' 04.6" , E126˚ 36' 30.0". 6 : (N35˚ 46' 03.8" , E126˚ 36' 35.2. 7 : N35˚ 46' 03.3" , E126˚ 36' 50.6". 8 :N35˚ 46' 01.7", E126˚ 37' 09.5". 9 : N35˚ 46' 09.5" , E126˚ 37' 32.5". 10 : N35˚ 46' 03.1" , E126˚ 37' 33.4". 11 : N35˚ 46' 56.7" , E126˚ 37' 34.6". 12 : N35˚ 45' 50.5", E126˚ 37' 35.2". 13 : N35˚ 45' 44.4", E126˚ 37' 61.4"

The number of species increased from 7.1 to 10.6. The survey registered especially large increase in species number at sites: 1, 2, 3, 7 and 13. These sites had higher salt content levels compared to the other sites from the first survey. The survey also were founded that areas with low salt content levels saw no change in vegetation species. In conclusion it appears the increase in vegetation species was a direct result of decreased salt levels in those particular areas.

Simpson's dominance ratio (SDR) values decreased for species such as *Suaeda maritima*, *Salicornia europaea*, *Puccinellia nipponica*, *Zoysia sinica*, whereas for *Phragmites communis*, *Calamagrostis epigeios*, these were increased. Therefore, *Phragmites communis*, and *Calamagrostis epigeios* are the best vegetation species to improve and ameliorate soil's physical properties and however in the future it is predicted that they will be threatening to crops on new arable land after the desalinization tidal period has lapsed.

**Table 2.** Change of species numbers of vegetation at each survey sites of reclaimed land in Saemangeum Gye-hwa area in Korea.

| Year | Number of plant species per ea $m^{-2}$ | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | 1♩ | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | average |
| 2010 | 0 | 3 | 4 | 11 | 14 | 9 | 3 | 7 | 12 | 6 | 3 | 7 | 6 | 7.1 |
| 2012 | 11 | 13 | 11 | 12 | 14 | 10 | 10 | 7 | 9 | 14 | 6 | 8 | 13 | 10.6 |
| Difference | **11** | **10** | **7** | **1** | **0** | **1** | **7** | **0** | **-3** | **8** | **3** | **1** | **7** | **3.5** |

1♩ to 13♩: survey site



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**Table 3.** Distributions of main vegetation Simpson's dominance ratio (SDR) values in the survey sites at Saemangeum Gye-hwa area in Korea.

| Species | SDR (%) | |
|---|---|---|
| Vegetation | 2010 | 2012 |
| *Phragmites communis* | 16.0 | 24.5 |
| *Suaeda maritima* | 15.7 | 5.1 |
| *Salicornia europaea* | 14.4 | 3.0 |
| *Aster subulatus* | 12.8 | 14.9 |
| *Calamagrostis epigeios* | 7.3 | 17.4 |
| *Zoysia sinica* | 6.7 | 2.9 |
| *Aeschynomene indica* | 5.3 | 7.8 |
| *Puccinellia nipponica* | 4.5 | 1.3 |
| *Conyza canadensis* | 3.1 | 0.7 |
| *Suaeda asparagoides* | 2.8 | 2.5 |
| *Echinochloa* spp | 2.0 | 3.5 |
| *Aster tripolium* | 1.5 | 0.3 |
| *Carex pumila* | 1.4 | 0.6 |
| *Kochia scopariavar.littorea* | 1.1 | 0.9 |
| *Suaeda japonica* | 1.0 | 0.3 |

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# WEED IDENTIFICATION ON RICE CULTIVATION OF SOBARI SYSTEM

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**ABSTRACT** The experiment was aimed to identify the dominant weed species in order to determine the weed control program on rice cropping System Organik Base Controlled Aerob Rice Intensification Technology (SOBARI). Aplication of combination four water level management on two plant spacings were performed. The water level managements were - 20 cm, -15 cm, -10 cm, - 5 cm water level below the soil surface and plant spacing were 30 x 25 cm and 30 x 30 cm. The weed control in each treatment was carried out both mechanic and manually by hand . The result of the weed inventory indicated that six species weeds were found at the land prior to the treatment, there were of four species of broad leave groups, namely: *Monochoria vaginalis*, *Ludwigia octovalvis*, *Alternanthera* sp dan *Marsilea crenata* and two species of the grasses groups namely : *Ischaemum rugosum*, and *Cynodon dactylon*. At the beginning of the experiment the land was dominated by *Ischaemum rugosum* (12.62%), *Cynodon dactylon* (31.55%), *Monochoria vaginalis* (32.62%), 30 days after planting (DAP) in all treatment combinations of level water management and plant spacing showed that the dominant weed were, *Monochoria vaginalis*, *Ludwigia octovalvis*, *Alternanthera sessilis* (L) from the class of broad leaves, *Echicocloa cruss-galli*, *Ischaemum rugosum*, and *Panicum repens* from the class of the grass.

***Keywords:*** Water level management, plant spacing, Sobari

## INTRODUCTION

System Organic Based on Controlled Aerobic Rice Intensification Technology (SOBARI) is a holistic rice production system by using and integrating the soil biological power, plant, fertilizers and water management according to the plan and design (by design) (Simarmata 2008). SOBARI also implements a wide spacing plant. The more extensive root zone, and field studies based on minimum spacing is 30 cm X 30 cm and a maximum of 50 cm x 50 cm spacing on the other hand can play an important role in minimizing weed pressure on the rice in aerobic soil conditions. Plant spacing showed a negative correlation with weed biomass but positively associated with grain yield, because according Norhidayati Bt. Sunyob, et al., (2012), narrower spacing can be adopted to obtain a high yield of rice and better weed suppression in an integrated weed management program for aerobic rice. Therefore in this experiment the aim is to look for weed control system that can be applied to rice cultivation in SOBARI.

Most of the population of weed species decreases with increasing water depth. At the flooding weed growth will be depressed but will not disturb the growth of rice plants, it shows that the flooding significantly affected to the rice crop and weed growth, whereas in rice cultivation technology based SOBARI the muddy level water only, this is sure to cause more weed growth either type or amount. If unchecked it will lead to a relatively large yield losses, because the weeds grow since the beginning of the growth of rice plants, so that the competition started earlier and longer. Rice plants are not necessarily aquatic plants, but can grow in flooding conditions because it has the ability to supply oxygen to the root system. In flooded conditions, plants utilize the energy to supply oxygen to the root system.



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One of the factors that causes low rice yields both quality and quantity is a competition of weed. Weeds as crop pests is an important problem to be overcome in increasing rice production in Indonesia (Pitoyo, 2006).

Weed is one limiting factor of rice production , because weeds can absorb nutrients and water faster than the main crop (Gupta, 1984).In rice production systems, weed control costs can reach 50% of the total cost of production (IRRI, 1992).

When compared with the control of pests and diseases, weed management is often overlooked, because they seem do not harm the growth nor reduce the yields of rice. Yet in reality weeds can reduce the rice yield considerably. According Madkar et al. (1989) weeds can reduce the yield by 20-40% if not weeded. Fryer and Matsunaka (1977) in Nawilda et al., (2006) stated that the decline in yield due to the weeds in Japan ranged from 10 to 70%. According to Rahman (1995) the percentage of decline in agricultural output because of the weed was directly proportional to the density of weeds per unit area, such as *Echinocloa crusgalli* that could reduce rice yield by 57%.

According to Rijn (2000), weeds reduced crop yields in the competition for light, oxygen, and $CO_2$, as well as nutrients. The decrease was attributable to yield because weeds reduced the plant growth, inflicting chlorosis, nutrient deficiency, as well as a reduction in the number and size of plant organs. Symptoms of nutrient deficiency in rice may lead to total failure of seed production, and dwarfted plants. Nutrient deficiency showed characteristic symptoms on the leave for each mineral nutrient, followed by tissue disorders (Sukman and Yakup, 2002).

Information about identification the dominant weed species in order to determine the weed control program on rice cropping System Organik Base Controlled Aerob Rice Intensification Technologi (SOBARI) system was still limited, so the writer reported the results of our experiment on that point to fill the existing information gap.

## MATERIALS AND METHODS

In this experiment, seeds of rice varieties used were Sidenuk, where planted on the field, , basic fertilizer used in the form of straw compost, Urea, SP 36 and KCl. To prevent pests and diseases of plants used pesticides.

The experiment was conducted in a paddy field, District Ciherang, Banjaran Regency, West Java, with a randomized block design, consisting of combination four water level managements on two plant spacings, there were eight treatments consisting of : A = water level - 20 cm below the soil surface with a spacing (30 x 25) cm, B = -15 cm water level below the soil surface with a spacing (30 x 25) cm, C = water level - 10 cm below the soil surface with a spacing (30 x 25) cm, D = -5 cm water level below the soil surface with a spacing (30 x 25) cm, E = water level - 20 cm below the soil surface with a spacing (30 x 35) cm, F - water level -15 cm below the soil surface with a spacing (30 x 35) cm, G = height of water level - 10 cm below the soil surface with spacing (30 x 35) cm, and H = height of water level -5 cm below the soil surface with a spacing (30 x 35) cm, each plot takes an area of 4 x 5 m, at each sample plot were determined for observation weeds whice located in the diagonal direction respectively. Weed control in each treatment was carried out manually by hand. The treatments were randomized in blocks.

The seed was transplanted into the area after 10-15 days after sowing, by using twin seedling (2 plant) per hill and in 5cm spacing plants, transplant quickly, shallow (2-3 cm). Water conditions at planting time is wet but not flooded at first, then the water supply is stopped and the water level is allowed to fall naturally depending on the treatment, when the ground water level has gone down and reached the specified depth limits on treatment (-5, -10, -15, and -20 cm from ground level). Water level depth measurements were performed using PVC (diameter 20 cm, length 35 cm) with a perforated wall mounted rice terraces related.



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Water level management was done since the plant was 7-10 days up to maximum vegetative phase. In the mature phase of the milk until harvest soil allowed to dry naturally (not the addition of water) for the entire treatment. The pest and disease control was done mechanically, physically or using the pesticides in accordance with the target.

The variables collected were species and value total domination of weeds. Observations on samples made of 2 samples in each plot by using the square (50 cm x 50 cm) (Tjitrosudirdjo dkk, 1998). Any weeds in the sample plots removed with roots and put into envelopes which have been labeled for easy identification. The collected data was used to calculate of the species dominance ratio (SDR).

## RESULTS AND DISCUSSION

### Species and Value Total domination of weeds

The result of the calculation of the value of total dominance at the age of rice plants 30 days after transplanting (DAT) showed that the dominant pattern of weeds is not so different from the treatment. Weeds that grown on the land before the treatment there are six species of weeds. Which consists of six dominant species, four species of broad leave groups, namely: *Monochoria vaginalis*, *Ludwigia octovalvis*, *Alternanthera* sp dan *Marsilea crenata*, two species of grass groups, namely: *Ischaemum rugosum*, and *Cynodon dactylon*. At the beginning of the experiment area was dominated by weeds *Ischaemum rugosum* (12.62%), *Cynodon dactylon* (31.55%), *Monochoria vaginalis* (32.62%) , after the rice plants were 30 days after transplanting (DAT) land is still dominated by the same weed species (Table 1) , in all treatment combinations of level water managements and plant spacing showed that the dominant weed were, *Monochoria vaginalis*, *Ludwigia octovalvis*, *Alternanthera sessilis* (L) from the class of the broad leave, *Echicocloa cruss-galli*, *Ischaemum rugosum*, and *Panicum repens* from the class of the grasses.

This happens because the weeds reproduce by seeds and seedbank that existed in the experiment fields are of this class. While tillage weed seeds in the soil will be lifted to the surface and develop into new plants (Pons et al., 1997), except that after weeding either manually or with herbicides the dead weedswas used as mulch or buried on the land so the pieces remain of weeds have a chance to breed again, because besides the weeds quickly adapt to the environment and has high competitiveness so that it can grow well especially in matters that are not flooded.

In this experiment, using the plant spacing is relatively wide (30 x 25) cm (30 x 35) cm and not flooded so that light is needed for seed germination can be freely taken by the weed seeds that are already on the ground, while the weed seeds are buried in the soil due to tillage will only germinate if the seeds are again on the surface due to tillage in the next season.

Dominance of weeds in each plot of land will affect the recommendations implemented controls. The conditions can have implications for the control of different ways.

**Table 1.** Species of weeds and value total domination (Species Dominance Ratio/SDR) in the treatment combinations of level water management and plant spacing age at 30 DAT

| No. | Name of Weeds | SDR | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H |
| 1 | *Echicocloa cruss-galli* | 8.12 | 28.99 | - | 10.76 | 18.94 | 36.82 | 16.74 | 32.51 |
| 2 | *Ischaemum rugosum* | - | 9.45 | - | - | - | 12.41 | 25.66 | 5.37 |
| 3 | *Cyperus iria* | - | - | - | - | - | - | 7.29 | - |
| 4 | *Fimbristylis miliacea* | 8.12 | - | - | - | - | - | - | - |
| 5 | *Monochoria vaginalis* | 26.74 | 32.21 | - | 3.26 | - | 33.10 | - | 9.08 |
| 7 | *Ludwigia octovalvis* | 23.80 | - | - | - | 42.07 | 8.38 | 10.29 | - |
| 8 | *Alternanthera sessilis (L)* | 18.77 | - | 52.13 | 44.01 | 22.89 | - | 18.48 | 3.55 |



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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 11 | *Marsilea crenata* | - | 10.18 | - | 17.99 | - | - | - | - |
| 12 | *Panicum repens* | 14.44 | 19.16 | 47.87 | 50.75 | 16.09 | 9.28 | 21.53 | 22.82 |
| Total | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |

Note: - Not found; A – H, Treatment

## CONCLUSION

Based on the experiment results, it can be conclused : in all treatment combinations of level water management and plant spacing at 30 Days After Transplanting (DAT) showed that the dominant weed were, *Monochoria vaginalis*, *Ludwigia octovalvis*, *Alternanthera sessilis* (L) from the class of broad leave, *Echicocloa cruss-galli*, *Ischaemum rugosum*, and *Panicum repens* from the class of the grasses.

## ACKNOWLEDGEMENTS

Thanks to the Advisor, Post Graduate Program, of Padjadjaran University, Chairman Gapoktan Al Mukhlis and The Village Agricultural Extension District Ciherang Bandung regency, for the facilities needed and supported in the research activity. This article is part of Merry Antralina's disertation with guidance of second and third author.

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# VEGETATION CHANGES AND WEEDS MANAGEMENT OF FARMLAND AFTER THE TSUNAMI OF GREAT EAST JAPAN EARTHQUAKE IN MIYAGI PREFECTURE

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**ABSTRACT** In Miyagi Prefecture, 14,000 hectares of farmland were flooded with seawater, and covered with a large amount of sludge and debris flowed by the Tsunami of Great East Japan Earthquake, March 2011. The most of the affected paddy fields were not able to crop, and was covered with a large amount of weeds, mainly *Echinocloa spp*. Weed management is an important issue for farmland restoration; thus, multiple strategies were used including a grass cutter and boom sprayer for herbicide. As disaster area was very large, access to farmlands were restricted as a result of rubble presence and partial cave-in of fields, aerial application of burn-down herbicide with radio control helicopter was considered and utilized by October 2011.

***Keywords:*** Great East Japan Earthquake, vegetation changes, weed management, *Bolboschoenus koshevnikovi*

## INTRODUCTION

In Miyagi Prefecture, 14,000 hectares of farmland were flooded with seawater, and covered with a large amount of sludge and debris flowed by the Tsunami of Great East Japan Earthquake, March 2011. Most of the affected paddy fields were not able to crop, and was covered with a large amount of weeds. Appropriate weed management strategies were desired for farmland restoration; bush cutters, boom sprayers and radio control helicopters for application of burn-down herbicide were totally enforced. We surveyed weed vegetation of the affected farmlands to identify the problematic species during the restoration period. Simultaneously, field experiments were conducted to evaluate the effective use of various herbicides on *Bolboschoenus koschevnikovi* control in fallow paddy field. In paddy rice crop, pyraclonil and acetolactatesynthase inhibiters ware proved be effective on *B.koschevnikoi*, but unknown about effect under salinity condition. Greenhouse experiments were also conducted to evaluate the herbicide performance in the condition with salty water.

## MATERIALS AND METHODS

### Vegetation changes between 2011 and 2012

From July to August of 2011 and 2012, weed species found in the paddy field or on the levee were identified. Thirty-six sites with 30 fallow paddies and 6 re-cropping fields in 2011, and 48 sites with 18 fallow and 30 re-cropping in 2012 were investigated. Twenty-six sites including 3 cropping fields were identical in both years.

### Bulrush management in fallow paddy field

Field experiments were conducted to *B. koshevnikovi* control in the Tsunami attacked paddy field in Natori city, where *B. koshevnikovi* dominated. Two formulations of glyphosate potassium salt (2,235 ga.i./ha and 2,400 ga.i./ha), diquat-paraquat (1,400 ga.i./ha diquat bromide and 1,000 ga.i./ha paraquat dichloride) and bush cutter as a control were treated at May 30 and June 27 respectively, each 20 square meter per plot with two replications. Plant height and cover degree of the plot were measured to estimate shoot volume at the end of the July 2011 and July 10 of next year. Tuber produced in the soil of 30cm x 60cm x 20cm depth was recovered at November 22 of 2011 to determine fresh weight.



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### Bulrush control in rice re-cropping salty field

Greenhouse experiments were conducted as a model system of re-cropping rice filed in Furukawa experimental station at Ouksaki city. Both tubers of *B. koshevnikovi* and rice seedlings were planted in Wagner pot (1/2,000a) contained with the soil which salt content as 0, 0.1, 0.3 % (w/w) of NaCl per dry soil adding seawater, in May 21, 2012. Fentrazamide-pyroxasulfone granule (50 ga.i./ha fentrazamide and 300 ga.i./ha pyroxasulfone), pyraclonil-propyrisulfuron floable (200 ga.i./ha pyraclonil and 90 ga.i./ha propyrisulfuron) and an unregistered herbicide for *B. koshevnikovi* as control were treated 15 days after planting at May 5. Plant height and stem number of *B. koshevnikovi* of each pot at July10, to estimate shoot volume. Shoot and tuber weight were also measured at September 10.

## RESULTS AND DISCUSSION

### Vegetation changes

In the year of Tsunami, *Echinochloa crus-galli*, *Bolboschoenus koshevnikovi* and *Chenopodium album* were dominant in the affected farmlands in fallow (Fig.1). Further, spreading of invasive alien species such as, *Ambrosia trifida, Sicyos angulatus* on levees and around the fields was concerned. Associated with farmland restoration, including appropriate weed management practices to rice cropping again, the many weed species found during 2011 decreased in the following year. However, *Echinochloa spp.* (including *E.crus-galli* and *E.oryzicola*) and *Schoenoplectus juncoides* was frequently found in re-cropping rice fields in 2012, it is a same level of unaffected inland area. On the other hand, sequential occurrence of *B. koshevnikovi* which has been problematic in coastal lowlands suggesting that continuous management of the species must be required for rice farming even after the restoration.

### Bulrush management at fallow field

Application of glyphosate potassium salt or diquat-paraquat formulation by the end of May effectively suppressed both *B. koshevnikovi* shoot growth and tuber production (Fig. 2). In the case of glyphosate potassium applied at end of June, shoot sprouting in following spring strongly suppressed, despite some tubers to produce. These results suggest that splaying glyphosate potassium by end of June is recommended to *B. koshevnikovi* control, considering to following rice cropping.



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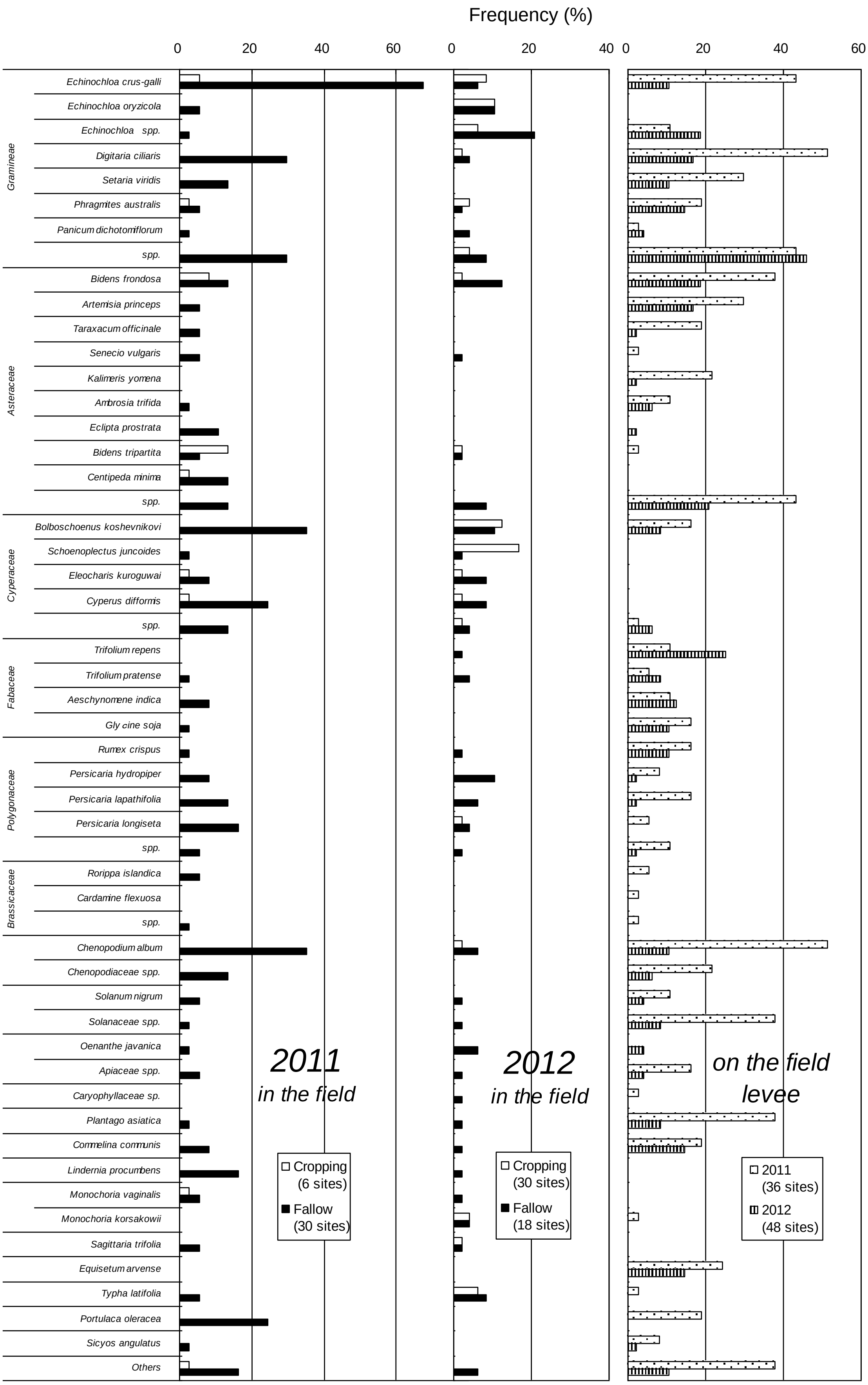


**Fig.1.** Weed species frequency of occurrence in fields of the rice cropping, fallow and levees of Tsunami affected paddy fields. Frequency (%) based on the total number of surveyed site in each year; 36 sites in 2011 and 48 sites in 2012, respectively.



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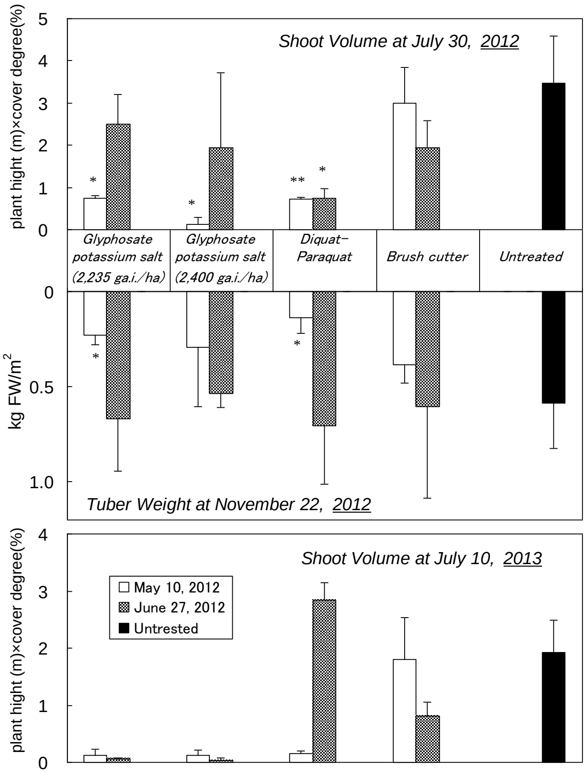
**Bulrush control in salty rice re-cropping field**

About the herbicides expected to spread recently, that pyraclonil and/or novel acetolactatesynthase inhibiter (e.g. pyroxasulfone, propyrisulfuron) containing agent exhibited high efficiency to *B. koshevnikovi*. Herbicide performance was stable by 0.1% NaCl, under this content rice plants able to normally grow (Fig. 3).



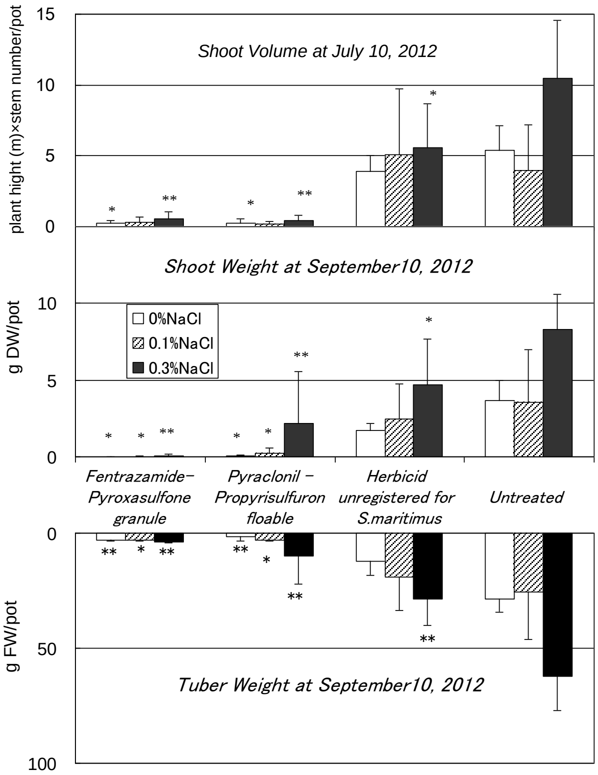




**Fig.2.** Effect of treatments and the phase on shoot and tuber reproduction of *B. koshevnikovi* in fallow field; May 10 was flowering, June 27 was shoot maturing stage.

**Fig.3.** Effect of soil salinity on herbicides efficacy to *B. koshevnikovi* planted with rice in a pot. Salinity prepared with seawater.

** and * indicate significance to each untreated plot at 0.01 and 0.05 levels , respectively

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# WEEDS DIVERSITY IN BAMBOO BASED AGROFORESTRY SYSTEM IN SEMI-ARID CENTRAL INDIA

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**ABSTRACT**: This study was conducted under semi-arid conditions of Jhansi (Uttar Pradesh), India during 2012-13 in an ongoing experiment (since 2007) to understand the weed diversity in a bamboo (*Dendrocalamus strictus*) based agroforestry system. Blackgram and chickpea crops were grown during *kharif* (rainy) and *rabi* (winter) seasons as intercrops. The diversity indices *viz*., Shannon Index H', Simpson Index 'D', were calculated to understand the variation in weed diversity. During *kharif* season *Cyperus rotundus* (137.6 plants $m^{-2}$) and *Echinochloa crusgalli* (74.8 plants $m^{-2}$) were the most dominant weeds, however *Commelina benghalensis* recorded high 1.78 H' and '5.97' Simpsons index at 60 days after sowing (DAS). At harvest stage *Digitaria sanguinalis* recorded the highest H' value of 1.75; whereas *Commelina diffusa* recorded 5.75 Simpsons index value. Total weed population in *kharif* season was 390 weeds $m^{-2}$ (60 DAS) and 342.8 weeds $m^{-2}$ (at harvest stage). During *rabi* season *Chenopodium album* (13 plants $m^{-2}$) and *Anagallis arvensis* (11.33 plants $m^{-2}$) were the most dominant weeds and *Chenopodium* recorded highest 1.77 H' value at 60 DAS. At harvest stage also *Chenopodium album* (18.67 plants $m^{-2}$) had highest H' value of 1.73 and Simpsons index D of 5.78. Weed biomass was 21.16 g $m^{-2}$ (DW) at 60 DAS and 45.38 g $m^{-2}$ (DW) at harvest stage. Total weed density was 27.6 and 17.8% higher in case of pure crops of blackgram and chickpea as compared to bamboo based agroforestry system. Therefore, bamboo based agroforestry system not only results in higher system productivity supporting food security but also supports rural livelihood.

***Keywords:*** Agroforestry system, Bamboo, Weed density, Weed diversity

## INTRODUCTION

Agroforestry plays a significant role in maintaining the resource base and increasing overall productivity in arid and semi-arid regions. It has been a way of life and livelihood in this region contributing significantly to meet the demand of fuel, fodder, timber and livelihood support. The microclimate changes caused by trees in tree–crop associations may have negative, positive, or neutral impacts on weeds. Complementary effect of shading, mulch from prunings, and potential allelopathy from hedgerow species reduced weed populations in alley cropping (Rao *et al*., 1991). Dhyani *et al.* (2008) found that weed infestation could be greatly reduced under suitable Agroforestry systems in the north-eastern India. Integration of bamboo under agroforestry systems help in soil & water conservation, yields value added products, timber, forages, edible shoots, fibre and craft wood etc. Bamboo which is a fast growing, perennial, renewable natural resource can be harvested every year and hence regular income will start much earlier than expected from any other woody component. Importance of There are more than 1500 recorded uses of bamboo. Measurements of weed diversity have been of historical significance and their importance still remains today. Species richness and relative abundance of a weed species is important to understand the weed diversity. In this study an attempt has been made to understand the species richness, evenness, abundance and biomass gained by weeds at different crop growth stage in the bamboo based Agroforesty system.



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## MATERIALS AND METHODS:

This study was carried out at the research farm of National Research Centre for Agroforestry, Jhansi (Uttar Pradesh), India during 2012-13 in an ongoing experiment (since 2007 on bamboo (*Dendrocalamus strictus*) based agroforestry system. The bamboo plants were spaced at 10m x 10m having plant population of 100 clumps $ha^{-1}$. Blackgram and chickpea crops were grown during *kharif* (rainy) and *rabi* (winter) seasons as intercrops. The site of experimental field was situated at $25^0$ 30' - 25' $32^0$ N latitude and $78^0$ 32' – $78^0$ 34' E longitudes at an altitude of 272 m above msl. Annual rainfall of the area ranges from 700-1150 mm. The potential evaporation is quite high in the range of 1400-1700 mm with moisture index value of – 40 to – 50. Maximum temperature during May and June goes up to $48^0$ C and it goes down up to $6^0$ C during December and January. The soil of experimental site was intermixed red and black soil group of Bundelkhand regions of Uttar Pradesh covered under the order of Alfisols. The soil pH was 6.5. Observations on weed density and biomass were taken by selecting six quadrates measuring 50cm x 50cm randomly at 60 days after sowing (DAS) and at harvest stage. This experiment explores two methods for measuring weed species diversity "Shannon-Weiner Index" 'H' and the "Simpson's Index" D'. Diversity value (H') ranging between 0 (indicating low community complexity) and 4 (indicating high community complexity). "Shannon-Weiner Index" 'H' measures the probability that two individuals randomly selected from a sample will belong to the same species (or some category other than species). Diversity indices were derived using the diversity and richness calculations software developed by P.A. Anderson and R.M.H Seaby, PISES Conservation Ltd.

## RESULTS AND DISCUSSION

### Observations on Bamboo

Observations recorded on *D. strictus* during September/October reveals that average number of culms/clump and internodes/culm were 32, 10.5, respectively. The internodal length was 19.14 cm and average diameter at breast height (DBH) was 3.75 cm. The height gained by bamboo varied in the range of 2.04 to 3.24 m.

### Weeds infestation during *kharif* season:

Data presented in Table 1 reveals that in bamboo + blackgram agroforestry system at 60 DAS it was observed that *Cyperus rotundus* (137.6 $m^{-2}$), *Echinochloa crusgalli* (74.8 $m^{-2}$), *Commelina benghalensis* (74.8 $m^{-2}$) and *Digitaria sanguinalis* (50.8 $m^{-2}$) were most dominant weeds species. At harvest stage also *Cyperus rotundus* (104.8 $m^{-2}$) was the most dominant weed followed by *Digitaria sanguinalis* (100.8 $m^{-2}$), *Echinochloa crusgalli* (50 $m^{-2}$), *Commelina benghalensis* (39.2 $m^{-2}$), *Celosia argentea* (23.2 $m^{-2}$). On an average weed population was observed to be 390 weeds $m^{-2}$ (60 DAS) and 342.8 weeds $m^{-2}$ (at harvest stage) indicating heavy infestation of weeds. Biomass gained by weeds was 85.72 g $m^{-2}$ (DW) at 60 DAS and 112.42 g $m^{-2}$ (DW) at harvest stage. Data reveals heavy infestation of weeds in the system. A perusal of the data also reveals that at 60 DAS *Commelina benghalensis* recorded the highest H' value of 1.78 followed by *Cyperus rotundus* (1.77), *Echinochloa crusgalli* (1.77) and the least was noticed in *Digitaria sanguinalis* (1.61). In case of Simpsons Index 'D', *Commelina benghalensis* (5.97) showed highest diversity followed by *Echinochloa crusgalli* (5.81). The lowest was found in *Digitaria sanguinalis* (4.67). At the time of harvest *Digitaria sanguinalis* recorded highest diversity (1.75) followed by *Commelina benghalensis* (1.73) *and Cyperus rotundus* (1.73). The lowest was noticed in *Celosia argentea* (1.64). *Commelina benghalensis* recorded the highest 'D' value of 5.75 followed by *Digitaria sanguinalis (5.64) and Cyperus rotundus* (5.50), the lowest was observed in *Dactyloctenium aegyptium* (4.56). Rank abundance of weeds at 60 DAS is depicted in Fig. 1.



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**Table 1.** Average weed density, H' and D' in Bamboo+ blackgram agroforestry system

| Weed species | At 60 DAS | | | At Harvest | | |
|---|---|---|---|---|---|---|
| | Density m$^{-2}$ | H' | 'D' | Density m$^{-2}$ | H' | 'D' |
| *Celosia argentea* | 36 | 1.75 | 5.79 | 23.2 | 1.64 | 4.78 |
| *Commelina benghalensis* | 74.8 | 1.78 | 5.97 | 39.2 | 1.73 | 5.47 |
| *Commelina diffusa* | 0 | 0 | 0 | 4 | 1.67 | 5.75 |
| *Cyperus rotundus* | 137.6 | 1.77 | 5.79 | 104.8 | 1.73 | 5.50 |
| *Dactyloctenium aegyptium* | 0 | 0 | 0 | 6 | 1.49 | 4.56 |
| *Digitaria sanguinalis* | 50.8 | 1.61 | 4.67 | 100.8 | 1.75 | 5.64 |
| *Echinochloa crusgalli* | 74.8 | 1.77 | 5.81 | 50 | 1.70 | 5.29 |
| *Oldenlandia diffusa* | 0 | 0 | 0 | 14.8 | 1.69 | 5.37 |
| Unidentified broad leaves | 16 | 1.67 | 4.99 | 0 | 0 | 0 |

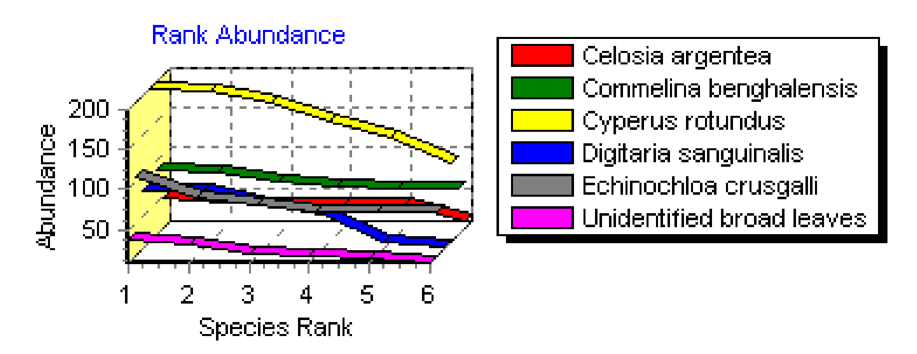


**Fig. 1.** Rank abundance at 60 DAS during *kharif*

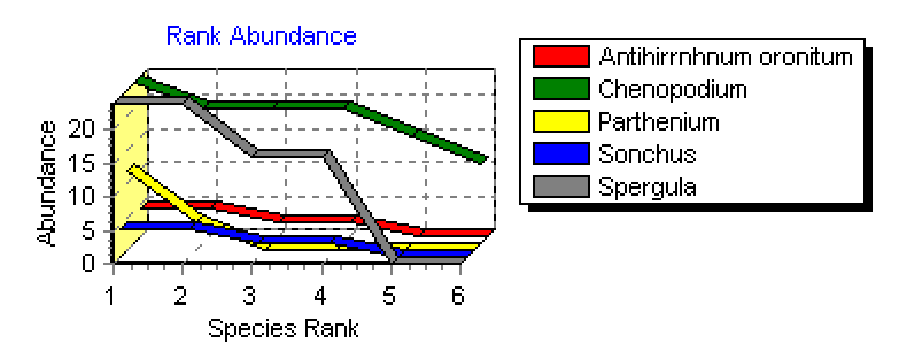


**Fig. 2.** Rank abundance at 60 DAS during *rabi*

**Weeds infestation during *rabi* season**

Data presented in Table 2 reveals that during *rabi* season in bamboo + chickpea agroforestry system at 60 DAS it was observed that the density of *Chenopodium album* was highest (112 m$^{-2}$) followed by *Spergula arvensis* (80 m$^{-2}$), *Parthenium hysterophorus* (16 m$^{-2}$), *Sonchus oleraceous* (12 m$^{-2}$) and *Antirrhinum orontium* (12 m$^{-2}$). At harvest stage also *Chenopodium album* (80 m$^{-2}$) was the most dominant weed followed by *Anagalis arvensis* (68 m$^{-2}$) and *Antirrhinum orontium* (52 m$^{-2}$). Average density of weeds was observed 232 weeds m$^{-2}$ at 60 DAS and 306 weeds m$^{-2}$ at harvest stage. It was observed that biomass gained by the weeds was 16.12 g m$^{-2}$ (DW) at 60 DAS and 45.38 g m$^{-2}$ (DW) at harvest stage. The data indicated that there was comparatively less infestation during the *rabi* season. At 60 DAS *Chenopodium album* recorded highest H' value of 1.77 followed by *Spergula arvensis* (1.36) and *A. orontium* (1.32) and *Sonchus oleraceous* (1.32). *Parthenium hysterophorus* recorded lowest diversity value of 0.56. Similarly in case of Simpsons diversity index 'D',



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*Chenopodium album* recorded the highest diversity (6.02) followed by *A. orontium* (4.71), *Sonchus oleraceous* (4.71) and the lowest was noticed in *P. hysterophorus* (1.66). At harvest stage a few new weeds *viz*., *Anagalis arvensis, Fumaria parviflora, Rumex acetosella, Vicia sativa* had emerged. At harvest stage also *Chenopodium album* dominated all other species w.r.t H' (1.56), followed by *Fumaria parviflora* (1.52). The lowest was observed in *Antirrhinum orontium* (0.69). *Chenopodium album* showed highest diversity 'D' (3.43), however *Fumaria parviflora, Vicia sativa* were found on par. The lowest diversity was observed in *Antirrhinum orontium* (2.01). Rank abundance of weeds at 60 DAS is depicted in Fig. 2.

**Weed infestation under sole crop situation:** By and large the weeds prevalent in the bamboo based agroforestry system were also observed in the sole crop situation, however under overall situation weed density was 27.6 and 17.8% higher in case of sole crops of blackgram and chickpea as compared to bamboo based agroforestry system thereby indicating that presence of tree component under agroforestry system reduces the weed infestation.

**Table 2.** Average weed density, H' and 'D' in Bamboo+ chickpea agroforestry system

| Weed species | At 60 DAS | | | At Harvest | | |
|---|---|---|---|---|---|---|
| | Density $m^{-2}$ | H | 'D' | Density $m^{-2}$ | H | 'D' |
| *Anagalis arvensis* | 0 | 0 | 0 | 68 | 1.44 | 3.20 |
| *Antirrhinum orontium* | 12 | 1.32 | 4.71 | 52 | 0.69 | 2.01 |
| *Chenopodium album* | 112 | 1.77 | 6.02 | 80 | 1.56 | 3.43 |
| *Fumaria parviflora* | 0 | 0 | 0 | 39 | 1.52 | 3.42 |
| *Rumex acetosella* | 0 | 0 | 0 | 26 | 1.38 | 3.20 |
| *Spergula arvensis* | 80 | 1.36 | 3.98 | 30 | 1.44 | 3.36 |
| *Vicia sativa* | 0 | 0 | 0 | 11 | 1.32 | 3.39 |
| *Parthenium hysterophorus* | 16 | 0.56 | 1.66 | 0 | 0 | 0 |
| *Sonchus oleraceous* | 12 | 1.32 | 4.71 | 0 | 0 | 0 |

Therefore, it can be concluded that bamboo based agroforestry system can result in harvesting of bamboo culms every year that gives additional income and livelihood opportunities to the farmers. Bamboo grown under agroforestry system can be a rich source of energy, conserves soil and water, provide fodder to the animals during the lean period. Due to the micro-climate changes the tree component decreases weed infestation and thereby improves the overall productivity, supporting food security as well as rural livelihood.

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# EVIDENCE-BASED IMPACT ASSESSMENTS: BENEFITS AND THREATS OF WEEDS FOR NATIVE FAUNA

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Biological invasions are a major threat to native ecosystems globally, yet in some landscapes they can also have important positive effects on native biodiversity. For example, invasive non-native plants have the potential to act as ecological engineers in highly degraded novel ecosystems by 'creating' habitat where it is otherwise lacking, thereby increasing the diversity and abundance of native fauna. Yet little is known of their net effect on population persistence. Understanding the impact of non-native plants on native fauna is becoming increasingly urgent for conservation management, particularly in degraded and novel ecosystems where the broad-scale removal of weeds could threaten native fauna populations and the ecological processes they contribute to.

This study takes a local and global view to investigate the conservation conundrum that sometimes exists between native fauna and non-native plants. We present a case study and decision-making framework to evaluate several methods for impact assessments where weeds may provide critical habitat for native fauna.

## MATERIALS AND METHODS

The research was undertaken in the Mount Lofty Ranges of South Australia, a biodiversity hotspot that is considered a 'canary landscape' for temperate woodlands. The environmental decline seen here is expected to follow similar trends elsewhere. Blackberry (*Rubus anglocandicans*) is a non-native and highly invasive environmental weed that has been reported to provide habitat for native birds and mammals in the region.

The research was conducted as a multi-species study of small mammal responses to blackberry, with a particular focus on the nationally endangered southern brown bandicoot (*Isoodon obesulus*). Small mammal communities were surveyed for 11 consecutive seasons across 13 sites (7500 hectares) that represented native, mixed and blackberry-dominated novel ecosystems of the region. Blackberry and native vegetation characteristics were surveyed at five randomly selected 10 x 10 m plots per site. We used a levy pole to record over 15 characteristics of the native and non-native plant communities, including floristic richness, vegetation density and structure.

A mixed modelling approach was used to quantify the net effect and thresholds of blackberry for fauna responses at multiple scales, including: individual (reproduction); population (abundance, adult female density, and productivity); and community (species richness and diversity). To the best of knowledge, this is the first study on the impact of weeds on the recruitment and population persistence of native mammals.



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## RESULTS AND DISCUSSION

Ten species of small mammals, including six native, were captured across 12,235 captures and 31,407 trap sessions. Blackberry was identified as an ecological engineer in blackberry-dominated novel ecosystems, where it retains diverse native mammal communities of yellow-footed antechinus (*Antechinus flavipes;* vulnerable), bush rat (*Rattus fuscipes*), brush-tail possum (*Trichosurus vulpecula;* rare), short-beaked echidna (*Tachyglossus aculeatus*) and southern brown bandicoot (*Isoodon obesulus;* endangered). The abundance, density, dispersal and recruitment of bandicoots were also greatest in blackberry, indicating that blackberry is facilitating persistence of this endangered bandicoot population across the region.

The consistent modelling approach enabled us to critique the usefulness of individual, population and community responses in quantifying the impact of non-native plants on native fauna, and to use this as a foundation to develop an assessment framework. We present here an evidence-based decision making framework to assess the negative and beneficial impacts of non-native plants on native fauna.

### Implications for weed management

Interactions between non-native and native species are increasing worldwide, and quantifying these complex dynamics is essential if we are to successfully tackle the conservation challenges of the future. Our results confirm that non-native plants can act as ecosystem engineers in novel ecosystems and create critical habitat that supports mammal communities where they would otherwise become locally extinct. Further, they highlight the importance of using evidence-based frameworks to assist decision making where weed management could have detrimental effects on threatened fauna populations.



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# A COMPARISON OF ARTHROPOD COMMUNITIES IN HERBICIDE-TOLERANT AND CONVENTIONAL SOYBEAN

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**ABSTRACT** Herbicide-tolerant (HT) soybean has been planted widely and provided an effective tool for weed control. One ecological concern is regarding the potential effects of HT soybean on biodiversity of non-target organisms. We investigated on the abundance and diversity of arthropods in field plots of HT soybean with sprayed with glyphosate were investigated over one growing season. There were no significantly differences in arthropod community structure parameters among soybean treatments. The species richness (S) values were 17.45, 17.95, 16.70, and 17.40, separately in HT with glyphosate, HT without glyphosate, recipient cultivar and conventional soybean. The Shannon-Wienner diversity (H) values were1.43, 1.55, 1.51, and 2.02, dominant index (C) values were 0.43, 0.41, 0.40, and 0.20, evenness index (J) values were 0.50, 0.54, 0.53, and 0.71 individually in different soybean treatments. The species composition structure of non-target pest sub-community in HT with glyphosate, were similar to other treatments. The herbicide-tolerance transgenic soybean has no effect of arthropod communities in short term.

***Keywords:*** Arthropod communities, herbicide-tolerant soybean

## INTRODUCTION

Herbicide-tolerant (HT) soybean has been planted widely and provided an effective tool for weed control. However, one ecological concern regarding the potential effects of HT soybean on biodiversity of non-target organisms has been debated. HT transgenic soybean “FG72”, which expresses 2mepsps protein for glyphosate tolerance and hppdPf w336 protein for Isoxaflutole tolerance, has been developed by Bayer CropScience Company.

## OBJECTIVES

In order to evaluate the potential risks to non-target arthropods and to investigate whether the transgenic soybean line can be applied in China, preliminary experiments were conducted to measure the impacts on arthropod community.

## MATERIALS AND METHODS

The abundance and diversity of arthropods in field plots of HT soybean with spraying glyphosate were investigated over one growing season by direct observation sampling method, comparing with those of other soybeans treated including HT soybean without spraying glyphosate, recipient cultivar “Jack” and conventional soybean “Zhonghuang 13”. The investigations were performed weekly from the mid August to the late October. At each of replicated plot we selected five points in diagonal, and surveyed 10 soybean plants of each point. We identified the insect species and counted the numbers of all arthropods to calculate arthropod community structure parameters such as dominant index (C), Shannon-wienner diversity (H), evenness index (J), and species richness(S).

## RESULTS AND DISCUSSION

There were no significantly differences of arthropod community structure parameters among soybean treatments. S values were17.45, 17.95, 16.70, and 17.40, separately in HT with glyphosate, HT without glyphosate, recipient cultivar and conventional soybean. H values



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were1.43, 1.55, 1.51, and 2.02, C values were 0.43, 0.41, 0.40, and 0.20, J values were 0.50, 0.54, 0.53, and 0.71 individually in different soybean treatments. The species composition structure of non-target pest sub-community in HT with glyphosate, were similar to other treatments. Lepidopteran insects and sucking insects were the main functional pest groups. The number of lepidopteran pests in HT soybeans sprayed glyphosate with 6.05 per 50 plants, was not significantly different from those of HT soybean without glyphosate (6.40 per 50 plants), recipient cultivar (11.00 per 50 plants) and conventional soybean (9.20 per 50 plants). The number of silverleaf whitefly*Bemisia tabaci* populations in HT with glyphosate was 15.40 per 50 plants, and was not significantly different from those of HT without glyphosate (15.50 per 50 plants), recipient cultivar (14.50 per 50 plants) and conventional soybean (16.95 per 50 plants). The numbers of soybean aphids *Aphis glycines* in HT with glyphosate, HT without glyphosate and recipient cultivar, were 133.50, 158.70, and 138.15 per 50 plants separately and there were no significant difference among three treatments. While 56.00 per 50 plants soybean aphids in conventional soybean was significant lower than those of HT soybeans. This difference may be due to different soybean variety. In natural enemy sub-community, green lacewing *Chrysoperla sinica* was the dominant group. The numbers of green lacewing *C. sinica* was not reduced in HT with glyphosate comparing to other treatments. 14.56, 21.45, 19.50 and 21.30 per 50 plants were in HT with or without glyphosate, recipient cultivar and conventional soybean.

## CONCLUSION

The herbicide-tolerance transgenic soybean has no effect of arthropod communities in short term.



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# BIOSECURITY THREATS FROM ASIAN WEED INCURSIONS IN NEW ZEALAND

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***Keywords:*** Asian weeds, Chinese knotweed, *Persicaria chinensis*, weed incursion, weed control and eradication, molecular diagnostics.

## INTRODUCTION

*Persicaria chinensis* was first detected by Auckland Council (AC) on eight properties in the Auckland suburb of Glenfield in November 2009. It was subsequently declared an Unwanted Organism under the Biosecurity Act 1993, thereby making available the full suite of Biosecurity Act powers to allow management of invasive species. Another detection of *P. chinensis* was made by the Waikato Regional Council (WRC) on two properties in the Hamilton suburb of Chartwell in June 2011, followed by detection by AC on another Auckland property (Glen Eden) in August 2011.

In July 2011, Ministry for Primary Industries (MPI) and WRC made a media release seeking publics assistance in locating any further infestations of Chinese knotweed. Many calls and samples were received but all of them turned out to be negative for Chinese knotweed. Members of public frequently misidentified bindweed (*Convolvulus arvensis, Calystegia silvatica*) and climbing dock (*Rumex sagittatus*). Morphologically, Asiatic knotweed (*Fallopia japonica*) can be easily confused as Chinese knotweed and sometimes Chinese knotweed can remain unnoticed in the early phases of incursion. Restricted Place (RP) notices were served by MPI under s130 of the Biosecurity Act 1993 preventing the movement of Chinese knotweed material on all the properties with confirmed Chinese knotweed infestation. Organism management activities were undertaken by AC (Auckland incursions) and MPI (Hamilton incursions). These are likely to continue until eradication of Chinese knotweed is achieved. Each site will be monitored for five years after the last observation to confirm eradication has been successful.

## MATERIALS AND METHODS

We describe recent incursions of weeds of Asian origin and their implications for biosecurity risk management in New Zealand. Chinese knotweed (*Persicaria chinensis* (L.) H.Gross (1913) is considered to be native of Asia and South East Asia (USDA Agricultural Research Service, 2013) and an introduced invasive species in Hawaii Islands (Wagner et al., 1999; Imada et al., 2008), Singapore (Chong et al., 2009) and French La Réunion Island (Lavergne, 2006). In Australia, it is occasionally cultivated as an ornamental plant; naturalised in one place in Glenrock State Conservation Area, Newcastle and considered to be potentially weedy in coastal New South Wales and Queensland (Wilson, 2012).

## RESULTS AND DISCUSSION

An interesting detection came from a garden outside Hamilton in March 2012 that became a diagnostic challenge. The sample from this detection was originally identified as *P. chinensis* based on the morphological features alone. The leaf shapes and flowers of the plants were similar to that of Chinese knotweed, but the plant had a relatively erect growth habit as opposed to the climbing and prostrate growth habit of Chinese knotweed. The garden owner



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advised MPI the plants had been sold in New Zealand for almost two decades as an ornamental garden plant ‘Red Dragon’ (*P. microcephala*) and these plants haven’t exhibited any invasive habits in the past. Voluntary movement controls were imposed until the diagnostic uncertainty associated with this detection could be resolved. Two more detections of suspect Chinese knotweed at Auckland properties (Waitakere and Glendowie suburbs) came from AC in Nov/Dec 2012. At the time, these were morphologically identified as Asiatic knotweed (*Fallopia japonica*).

DNA diagnostic tests (chloroplast, nuclear and AFLP fingerprinting techniques) were conducted on samples from Auckland and Hamilton incursions. Chloroplast DNA data divided the samples into two groups that differed by a single nucleotide substitution with the commercial variant ‘Red Dragon’ being placed into a different group than *P. chinensis*. This allowed an identification of samples within the genus

*Persicaria*/*Polygonum* but not a clear species differentiation between *P. chinensis* and *P. microcephala*. The chloroplast dataset indicated a close relatedness of all samples in the maternal lineage, as chloroplasts are maternally inherited in Polygonaceae (Hollingsworth et al., 1999). In contrast, ITS sequence (nuclear DNA) data showed a partially new grouping, probably due to the nature of nuclear ribosomal DNA that can undergo different scenarios after a hybridization event. However, the *P. microcephala* samples were placed in a different group than *P. chinensis* using the ITS sequencing technique, either by single nucleotide substitution or by different chloroplast type to the others in the ITS group. The partially new grouping formed by using the ITS sequencing technique may be due to the nature of nuclear ribosomal DNA that can undergo different scenarios after a hybridization event. Topological disagreement between chloroplast DNA and nuclear DNA is likely to be seen when nuclear ribosomal DNA is homogenized to the paternal type.

AFLP fingerprinting technique was used to compare the likelihood of the different collections (incursions) being either clonal or very similar to each other. The AFLP fingerprinting conclusively placed the Auckland and Hamilton city incursions of *P. chinensis* into one group and the Hamilton garden incursion and a commercial *P. microcephala* sample purchased from local nursery into another group, thereby resolving the diagnostic uncertainty that arose from morphological diagnostic tests. *Persicaria* is known to form numerous hybrids. *Persicaria microcephala* (Red Dragon) is likely to be a commercial hybrid variant of *P. chinensis* that is not as invasive as *P. chinensis*. It was probably commercialised as a garden ornamental plant in New Zealand some time ago. As a result, a decision was made to not serve the RP notices at the Hamilton garden site. However, voluntary movement control measures were kept in place while the plants were observed for change in morphological characters during the growing season and to observe the invasiveness of the plants. Asiatic knotweed sample from one (Glendowie) of the two Auckland properties was the most distinctive group by AFLP type. However, the Asiatic knotweed sample from the other Auckland property (Waitakere) had similar AFLP traces with those of confirmed *P. chinensis* incursions in Auckland (Glenfield) and Hamilton (Chartwell). While these plants were not identical, they were similar enough to conclude that they were all close relatives of similar nuclear constitution (all sharing at least 22 of the 26 peaks scored for these samples in the AFLP tests).

The diagnostic tests on samples from different incursion sites indicate that the history of the plants from the genus *Persicaria* found in New Zealand environments had involved some degree of commercial breeding in the past. This hypothesis is supported by the evidence from DNA tests as the presence of different chloroplast types with variable nuclear regions indicate that crossing to form may have been to more than one maternal parent i.e. the observed variants (natural or commercial) are a product of bi-directional crossing in commercial breeding programmes. Due to the complexities mentioned above and almost similar morphologies of *P. chinensis*, *P. microcephala* and *F. japonica*, further studies are



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needed at the molecular level. This includes development of suitable polymorphic markers to enable discrimination between the three species with higher confidence.

*Persicaria chinensis* or *P. microcephala* are not known to set seeds in New Zealand growing environments indicating that propagation is likely to be by vegetative means in New Zealand. It is possible that *P. chinensis and P. microcephala* (and possibly *Fallopia japonicai*) were originally bred overseas a few decades ago and then imported (legally or illegally) in New Zealand for personal (e.g. use as Chinese medicine) or commercial reasons (to sell as ornamental plants), respectively. The *P. chinensis* vs. *P. microcephala* is a classic example of uncertainties associated with plant variants from the same genus that sometimes makes biosecurity risk assessment and management a challenging task. Taxonomists may place these biological variants into the same box and this determines their biosecurity risk nature and the corresponding regulatory actions on their movements and control. These variants may be the same, morphologically, and in some cases like the current one, more than 99% identical genetically but exhibits contrasting growth characteristics and different invasive habits.

The invasiveness of a weed species is a critical factor in determining the level of management required to prevent its spread and distribution, and to successfully eradicate it in good time. However, the invasiveness of a variant (biological form) of a weed species is seldom taken into consideration when determining its taxonomic nomenclature. Internationally, a biosecurity risk organism (including weeds) is referred to and related on the basis of taxonomic classification. However, despite the fact that taxonomy enables us to recognise plants and the relationships between them, it may be considered a science of opinion. Nomenclature provides us a form of communication via scientific names. It is common to consider morphological forms/features in taxonomic determinations, but these morphological features may not necessarily reflect its functional form (e.g. invasiveness in particular environments). From a biosecurity point of view, the functional characteristic (invasiveness) is of relative importance and must be considered when discriminating between the variants for accurate biosecurity risk assessment and management. As seen in the current case, Chinese knotweed (*P. chinensis*) can be easily confused with *P. microcephala* c.v. Red Dragon based on morphology alone for most of the growing season. Both species are almost similar in their morphological and genetic makeup. The only time they can look different to an untrained eye is when the foliage of *P. microcephala* turns red during spring in New Zealand, whereas *P. chinensis* foliage remains green at that time of the year. *P. chinensis* does not have hair on the ochrea while *P. microcephala* possess hair on the ochrea. The *P. microcephala* samples examined in the current case did have some hair on the ochrea, but these were not abundant enough to easily discriminate between the two species in initial morphological tests.

*Persicaria chinensis* is an unwanted organism and a prohibited species under Biosecurity Act 1993, whereas *P. microcephala* is not an unwanted organism, implying lesser or no regulatory requirements for its propagation and distribution in New Zealand. If it was misdiagnosed as *P. chinensis*, regulatory tools such as RP notices would have been served, thereby exposing MPI to potential compensation claims. Prior to this study, *P. microcephala* was considered to be absent from New Zealand and may be deemed as a new organism under the Hazardous Substances and New Organisms Act 1996 (HSNO Act). This investigation determined that *P. microcephala* had been sold as a commercial ornamental variety Red Dragon, and the species may have been present in New Zealand since the 1980s. However, only the Environmental Protection Authority (EPA) can provide a definitive determination on the new organism status of *P. microcephala*. There are plants in New Zealand that are not listed on the MPI Plant Biosecurity Index (PBI[1]) and *P. microcephala* is one of those unlisted plants. In some cases the plants are still widely grown, or there have been changes in



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taxonomy which may have caused confusion, while in other instances they may be historical plantings in that they were here prior to provisions in the HSNO Act relating to new organisms. The HSNO Act took effect in July 1998 but some of the plant species not listed on PBI may or may not still be grown in New Zealand by smaller plant enthusiasts and various plant societies, and by botanical gardens such as the Auckland Domain, Christchurch Botanic Gardens and Dunedin Botanic Garden.

Morphological diagnostics on plant samples may sometimes be difficult as all natural variants or commercial hybrids in the genus *Persicaria* are not lodged in New Zealand plant herbariums and so there are not many herbarium samples available for comparisons. DNA diagnostics on the collected samples were relatively informative and added confidence to the morphological identifications and saved MPI from potential compensation issues and reputational risks. A practical and pragmatic approach enabled MPI to make informed biosecurity decisions for management and eradication of these Asian invasive weeds from New Zealand environment. The poster highlights the diagnostic uncertainties associated with plant variants from the same genus, the need to deploy and develop suitable molecular diagnostic tools for accurate identification, and the importance of determining the invasiveness and history of a biological variant for accurate biosecurity risk assessment and management.

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# WEED MAPPING IN TWO CORN *(ZEA MAYS)* PRODUCTION CENTER IN WEST JAVA PROVINCE OF INDONESIA

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**ABSTRACT** The purpose of this study was to map weeds in corn and to determine the spread of weeds in corn in two central areas of corn production in west java Indonesia (district of Sumedang and Garut) and this information can be used to control weed in corn earlier. This study was conducted from March to June 2011. The experimental design used was descriptive with survey methods. Six observation areas were determined in each district, so there were 12 observation areas that have different in soil types, altitude and micro-climate, but with the age of corn that are almost similar between the age of 5 to 8 weeks. The spread of vegetation was determined through analysis of all weed species, summed of dominant ratio (SDR) and dry weight of weeds. Questionnaire was given to the farmer to find out the history of the studied area of corn, such as crop varieties used, cropping patterns used, kind of fertilizer used, weed control technique, age of corn, row spacing and type of tillage. The results showed that the dominant broad leaf weed species that found both in Garut and Sumedang research areas were *Ageratum conyzoides and Oxalis berrelieri L* (broad leaves), *Imperata cylindrica* (L.) Beauv. and *Cynodon dactylon* (L.) (grass) and *Cyperus rotundus* (sedge). The dominant weed that found in Sumedang district are five broad leaves species :*Ageratum conyzoides L, Comelina, L, Lantana camara, L, Muscari botryoides (L) Willd and Oxalis berrelieri*, L, four species of grasses *: Cynodon dactilon* (L.) Pers, *Digitaria setigera* (Roem & Schult.), *Borreria laepikaulis* Schum and *Imperata cylindrica,* whereas sedge species found is *Cyperus rotundus.* The dominant weed species that found in Garut district are four species of broad leaves weeds (*Ageratum conyzoides L, Amaranthus L, Ephorbia hirta* L and *Oxalis berrelieir L*), two species of grasses weed (*Imperata cylindrica* (L.) Beauv and *Cynodon dactilon* (L.) Pers), and one sedge species (*Cyperus rotundus).*

***Keywords:*** Corn, dominant weed species, weed mapping

## INTRODUCTION

Corn is one of the agricultural food commodities that have good prospects in Indonesia, because domestic demand is likely to increase from year to year as a result of increasing of consumption, growth of population and income (Rizal and Purnomowati, 2007). The efforts to improve corn productivity can be achieved by planting high yielding varieties, control of pest, plant diseases and weeds. Weeds can reduce crop yields through competition of nutrients and water in the soil, sunlight for photosynthesis, and space. The presence of weeds in cultivated crops decrease both quantity and quality of crop, so weeds in corn should be controlled to prevent yield losses (Rukmana and Saputra, 2003). Yield loss due to weeds can exceed the yield losses due to pests and diseases. Several studies have shown a negative correlation between weed dry weight and yield of corn, with a decrease in the yield up to 95% (Violic, 2000). According to Clay and Aquilar (1998) corn that planted with monoculture system and use low input does not give a high yield as a result of competition with weeds. The amount of reduction in corn due to weed competition was ranged from 16 to 62% (Sasmita, *et al*., 2003). Therefore, weed control is necessary to reduce weed infestation and crop losses.

In Indonesia there are 140 types of broadleaved weeds, 36 species of grassy weeds, and 51 species of sedges weeds (Laumonier *et al*., 1986). Weeds that commonly found in corn



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from class of gramineae are *Eleusine indica*, *Echinochloa colonum*, *Paspalum vaginatum*, *Cynodon dactylon*, *Panicum spp.*, *Imperata cylindrica* and *Polytrias amaura*, whereas from class of cyperaceae are: *Cyperus rotundus*, *Cyperus Iria* and *Cyperus compressus*, and from class of broad leaf weeds are: *Mimosa invisa*, *Ageratum conyzoides* and *Althernanthera sessilis* (Warisno, 1998).

Mapping of weeds in corn in various ecosystems and environmental conditions in two production centers of corn in West Java province is required in order to design the control method of weed in corn effectively. Corn production centers in West Java Province in accordance with West Java corn belt program, is concentrated in the Kuningan District, Majalengka, Sumedang, Ciamis, Tasikmalaya, Garut, Bandung and Sukabumi.

At this present time in Indonesia there is no specific information regarding the type of weed species that exist on the corn field at various stadium, location, soil type, altitude, agro-climate and previous cropping patterns. Weed mapping in corn in a production center of Sumedang and Garut Districts are necessary for planning weed control efforts systematically. The purpose of this study was to determine the spread of corn weeds in Sumedang and Garut districts and this information can be used as a strategy to control weed in corn earlier

## MATERIALS AND METHODS

The experiment was conducted in the corn field owned by local farmers in Sumedang and Garut District. The experiment areas were located at the different altitude, soil type, agro-climate condition and different cropping pattern. The experiment was carried out from March 2011 to June 2011.

The experimental design used was descriptive with survey methods. In each district, six areas of corn field were determined that spread in a variety of different environmental conditions, but with the age of corn that are almost similar between the age of 5 to 8 weeks. The vegetation analysis was carried out on each corn crop area by using the method of minimum plot technique. The spread of vegetation was determined through analysis of all weed species, summed of dominant ratio (SDR), and dry weight of weeds. Questionnaire was given to the farmer to find out the history of the studied area of corn, such as crop varieties used, cropping patterns used, kind of fertilizer used, weed control technique, age of corn, row spacing and type of tillage.

The observations used are percentage of summed dominance ratio (SDR), and total dry weight of the dominant weed species (broad leaves, grasses and sedges).



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## RESULTS AND DISCUSSION

### Characteristics of study areas

**Table 1.** Characteristic of study areas in Districts of Sumedang and Garut

| Category | District of Sumedang | District of Garut |
|---|---|---|
| Altitude | Sub district Jatinangor 700 m<br>Sub district Tanjungsari 850 m<br>Sub district Pamulihan 1050 m | ▪ Sub district Karangpawitan 750 m<br>▪ Sub district. Banyuresmi 800 m<br>▪ Sub district Wanaraja 650 m |
| Crop cultivar used | NK - 99 , Bisi 16, Nusantara NT 10 | Pioneer 23, Bioseed India B – 89, Bisi 2 |
| Cropping pattern | ▪ Sub district Jatinangor: Polyculture<br>▪ Sub district Tanjungsari: Monoculture<br>▪ Sub district Pamulihan: Polyculture | ▪ Sub district Karangpawitan<br>▪ Monoculture<br>▪ Sub district Banyuresmi :<br>▪ Monoculture & polyculture<br>▪ Sub district Wanaraja:<br>▪ Monoculture |
| Soil tillage | Minimum tillage | Maximum tillage |
| Spacing | 100 x 60 cm | 70 x 20 cm |
| Previous crop used | Corn | Corn and soybean. |
| Climatic condition at year 2009 | ▪ Sub district Jatinangor<br>▪ Annual rainfal is 1.776 mm and days of rainfal is 119 days.<br>▪ Sub district Tanjungsari<br>▪ Annual rainfal is 1.972 mm and days of rainfal is 144 days<br>▪ Sub district Pamulihan<br>▪ Annual rainfal is 2.036 mm and days of rainfal is 159 days | ▪ Sub district Karangpawitan<br>▪ Annual rainfal is 1510 mm and days of rainfal is 136 days.<br>▪ Sub district Banyuresmi<br>▪ Annual rainfall is 1516 mm and days of rainfall is 126 days<br>▪ Sub district Wanaraja<br>▪ Annual rainfal is 917 mm and days of rainfal is 107 days. |
| Soil condition | • N content : moderate<br>• $P_2O_5$ content : moderate<br>• $K_2O$ content: low | • N content : moderate<br>• $P_2O_5$ content : moderate<br>• $K_2O$ content: very low |

### Vegetation analysis

**Table 2.** Weeds dominant in six corn fields in district of Sumedang

| No | Species | Summed Dominance ratio (SDR ) % | | | | | |
|---|---|---|---|---|---|---|---|
| | | A* | B | C | D | E | F |
| 1 | *Ageratum conyzoides* L | | 15.5 | 18.1 | 12.5 | 10.3 | |
| 2 | *Comelina* L | | | | | 14.4 | |
| 3 | *Lantana camara* L | | | | | | 10.5 |
| 4 | *Muscari botryoides (L) Willd* | | 11.5 | | | | |
| 5 | *Oxalis berrelieri* L | | | 13.3 | 13.4 | 12.1 | |
| 6 | *Cynodon dactilon* (L.) Pers. | | | | 10.5 | | 10.2 |
| 7 | *Digitaria setigera* (Roem & Schult.) | | | 11.4 | 10.2 | | |
| 8 | *Imperata cylindrica* (L.) Beauv | 10.6 | | | 10.2 | | 10.0 |
| 9 | *Borreria laepikaulis* Schum | 10.6 | | | | | |
| 10 | *Cyperus rotundus* | | | 11.2 | | | |
| | Others weeds species | 78.8 | 73.0 | 46.0 | 43.2 | 63.2 | 69.3 |

*Note: A = village of Cileles, B = village of Cilayung, C = village of Tanjungsari, D = village of Gudang, E = village of Cimarias, F = village of Cijeruk



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The dominant weed species found in Sumedang district research area were five species of broad leaf weeds: *Ageratum conyzoides* L., *Commelina* sp., *Lantana camara* L., *Muscari botryoides* (L.) Willd. and *Oxalis berrelieri* L., four species of grasses: *Cynodon dactilon* (L.) Pers, *Digitaria setigera* (Roem & Schult.), *Borreria laepikaulis* Schum. and *Imperata cylindrica* and one species of sedge: *Cyperus rotundus* (Table 2). It also showed that the dominant of weed species found in the research area of Sumedang District vary, but generally dominated by broad leaf weeds. Broad leaf weed of *Ageratum conyzoides* L is the most dominant weed and is found in four research areas, namely Cilayung, Tanjungsari, Gudang and Cimarias. The *I. cylindrica* (L) Beauv belongs to the most dominant grass weed species found in three research areas namely Cileles, Gudang, and Cijeruk, while the other grass weeds species *Cynodon dactilon* (L.) only found in research area of Cijeruk. *Cyperus rotundus* is a dominant sedge weed that found in Tanjungsari.

**Table 3.** Weeds dominant in six corn fields in district of Garut

| **No** | **Species** | **Summed Dominance Ratio ( SDR ) %** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **A** | **B** | **C** | **D** | **E** | **F** |
| 1 | *Ageratum conizoides* L | 12.5 | 15.6 | 10.0 | | 14.9 | |
| 2 | *Amaranthus* L | | | | 15.5 | | 12.2 |
| 3 | *Ephorbia hirta* L | | | | | | 11.8 |
| 4 | *Oxalis berrelieri* L | | | 11.6 | | | |
| 5 | *Cynodon dactilon* (L.) Pers. | | 12.5 | | 22.8 | | 12.8 |
| 6 | *Imperata cylindrica* (L.) Beauv | | | | | | 15.3 |
| 7 | *Cyperus rotundus* | 13.5 | | | | 12.0 | |
| Other weeds species | | 74.0 | 71.9 | 78.4 | 61.7 | 73.1 | 47.9 |

Note: A = village of Karangpawitan, B = village of Situgede, C = village of Karyasari,
D = village of Cimareme, E = village of Wanaraja, F = village of Wanamekar

The dominant weed species that found in the research area of Garut district is four broad leaf weed species: *Ageratum conyzoides L, Amaranthus L, Ephorbia berrelieir Oxalis hirta L*, while the dominant grass weeds species are *Imperata cylindrica* (L.) Beauv and *Cynodon dactilon* (L.) Pers. Sedge weed species (*Cyperus rotundus)*, was found in two research areas of Karangpawitan and Wanaradja (Table 3). The weed of *Ageratum conyzoides* L is the most dominant broad leaf weed species that found in four research areas of:Karangpawitan, Situgede, Karyasari and Wanaradja. The dominant grass weeds species of *Cynodon dactilon* (L) was found in three research areas: Situgede, Cimareme and Wanamekar (Table 3).

Table 2 and 3 showed that the dominant weed that found in research areas of Sumedang and Garut District were different, but generally dominated by ten species of broad leaf weeds, two species of grass weed species and one species of sedge weed species. The dominant broad leaf weed species that found in Garut and Sumedang research areas were *Ageratum conyzoides and Oxalis berrelieri L* and L, while the dominant grass species are *Imperata cylindrica* (L.) Beauv. and *Cynodon dactylon* (L.). *Cyperus rotundus* was the dominant sedge weed species that found both in Sumedang and Garut districts. According to Mercado (1979) the difference of weed species is due to different crop management such as water control, type of fertilizer used and different cropping system.



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## CONCLUSSIONS

Two prinicpal conclusions can be derived from this study, *viz*. (i) The dominant broad leaf weed species that found both in Garut and Sumedang research areas were *Ageratum conyzoides and Oxalis berrelieri L* (broad leaves), *Imperata cylindrica* (L.) Beauv. and *Cynodon dactylon* (L.) (grass) and *Cyperus rotundus* (sedge); and (ii) Weed composition in corn in districts of Sumedang and Garut are different. The dominant weed that found in Sumedang district are five broad leaves species :*Ageratum conyzoides L, Comelina, L, Lantana camara, L, Muscari botryoides (L) Willd,* and *Oxalis berrelieri*, L, four species of grasses *: Cynodon dactilon* (L.) Pers, *Digitaria setigera* (Roem & Schult.), *Borreria laepikaulis* Schum and *Imperata cylindrica,* whereas sedge species found is *Cyperus rotundus.* The dominant weed species that found in Garut district are four species of broadleaves weeds (*Ageratum conyzoides L, Amaranthus L, Ephorbia hirta* L and *Oxalis berrelieir L), two species of grasses weed* (*Imperata cylindrica* (L.) Beauv and *Cynodon dactilon* (L.) Pers), and one sedge species (*Cyperus rotundus).*

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## *Weed Management in Economic Crops*

# INTEGRATED WEED MANAGEMENT IN AEROBIC RICE

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**ABSTRACT** Despite high efficacy, sustainability issue is one of the driving forces behind the reconsideration of herbicide dependent weed management strategy in rice. This demands reappearance of physical and cultural management options combined with judicious application of herbicides in an integrated way. Keeping those in mind, different manual weeding and herbicides combinations were evaluated for their weed control efficacy in rice under aerobic soil conditions. Weed competitive rice variety AERON 1, higher seeding rate of 300 seeds/$m^2$ and seed priming by Zappa® were incorporated to provide a more competitive environment in favor of rice. As a consequence of integrating different agronomic practices, lower weed pressure, higher weed control efficiency (WCE) and higher yield were evident in this study. Aapplication of Cyhalofop-butyl + Bensulfuron or Propanil/Thiobencarb or Bispyribac-sodium at 10 DAS followed by Bentazon/MCPA or manual weeding at 30 DAS, and Pretilachlor/safener at 1 DAS followed by Propanil/Thiobencarb at 10 DAS followed by Bentazon/MCPA or manual weeding at 30 DAS resulted in high WCE and grain yield, which was similar to season-long weed-free yield. Treatments comprising only herbicides produced higher net benefit; on the contrary, treatments comprising both herbicide and manual weeding resulted in lower net benefit. Application of Cyhalofop-butyl + Bensulfuron or Bispyribac-sodium or Propanil/Thiobencarb at 10 DAS followed by Bentazon/MCPA at 30 DAS appeared as highly remunerative. Despite less economic return, spraying with any of the aforesaid early-post emergence herbicides in rotation at 10 DAS followed by a manual weeding at 30 DAS may be recommended from sustainability view point.

***Keywords:*** Aerobic soil, competitive variety, herbicide, seeding density, seed invigoration, weed management.

## INTRODUCTION

Rice (*Oryza sativa* L.) is the leading cereal of the world and more than half of the world population depends on rice for their daily sustenance. By tradition, rice had been cultivated in flooded conditions, and rice alone consumes about 30% of world freshwater consumption and more than 45% of total freshwater used in Asia. For the last few decades, sustainability of water resources has been a global challenge (Juraimi *et al*., 2010). Therefore, flood-irrigated rice cultivation is no more feasible, and finding ways of living with water scarcity deems necessary. A new concept of rice cultivation termed as ‘aerobic rice’ involves growing rice in well-drained, nonpuddled and non-saturated soils, which minimizes water use up to 50% and boosts up water productivity by around 200% (Tuong and Bouman, 2003) compared to lowland rice. But, aerobic rice is subject to much higher weed pressure compared with flood-irrigated rice. In tropic, average rice yield losses from weeds is 35%, while in aerobic rice, yield penalty is as high as 50-91% (Rao *et al*., 2007). Dry tillage, aerobic soil conditions and absence of standing water layer make aerobic rice highly vulnerable to weeds (Anwar *et al*, 2010). Therefore, an efficient, cost- effective and eco-friendly weed management strategy is crucial for the sustainability of this potential technology.



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Different options are available for weed management in rice. Physical control are eco-friendly but tedious and labor-intensive. Chemical control, on the contrary is the most effective, economic and practical way of weed management. Many researchers working on weed management in direct seeded rice opined that herbicide may be considered to be a viable alternative/supplement to hand weeding (Anwar *et al*., 2012a; Juraimi *et al*., 2013). But, intensive use of herbicides may result in development of resistant weed biotypes and public health hazard. The other option left is cultural weed control through adoption of different agronomic practices including tillage (Rao *et al*., 2007), competitive cultivar (Anwar *et al*., 2010), seeding density (Anwar *et al*., 2011), water management (Rao *et al*., 2007), seed invigoration (Anwar *et al*., 2012b) and so on. A single weed control approach may not be able to keep weeds below the threshold level of economic damage which demands adoption of diverse technology for weed management. Therefore, all the methods that are ecologically and economically justifiable should be integrated in a comprehensive way- known as integrated weed management (IWM). The IWM involves the selection, integration, and implementation of effective weed control means with due consideration of economics, environmental, and sociological consequences. A substantial impact of IWM on rice farming has been documented by many researchers (Azmi and Baki, 2002), who concluded that integration of different agronomic practices in conjunction with limited herbicide use managed weeds efficiently and ensured high yield. So far, however, little attention has been paid to sustainable weed management in aerobic rice which demands research on integrated weed management to make aerobic rice technology a popular as well as sustainable one. The present study was, therefore, conducted to find out suitable herbicide and manual weeding combination(s) simultaneously incorporated with different agronomic practices to provide a comprehensive integrated weed management system for aerobic rice variety AERON 1.

## MATERIALS AND METHODS

The field trial was conducted in main season 2010/2011 (November – January) at Universiti Putra Malaysia, Malaysia ($3^o$00' 21.34" N, $101^o$42' 15.06" E, 37 m elevation). The experimental soil (Serdang series) was sandy clay loam in texture and slightly acidic in reaction. The local climate was hot humid tropic with plentiful rainfall. The experiment was laid out in a randomized complete block design with three replications. Fourteen different herbicides and manual weeding combinations were evaluated for their weed control efficacy; weed-free check and weedy check were also included in the trial (Table 1). Herbicides used in this experiment were selected based on their performances in the earlier study (Anwar *et al*., 2012a). Different agronomic tools were integrated in this study to achieve higher weed control efficiency. Aerobic rice variety AERON 1, sourced from International Rice Research Institute, was used as the plant material since it was the most weed competitive and productive variety under aerobic soil conditions as found in the previous study (Anwar *et al*., 2010). A seeding rate of 300 seeds/$m^2$ was used for better weed competitiveness as evident in the earlier study (Anwar *et al*., 2011). Based on the findings of the preceding study (Anwar *et al*., 2012b), rice seeds were primed by soaking in 1% Zappa® solution for 24 hours followed by air drying for 12 hours to boost weed competitiveness through faster and higher emergence rate and increased seedling vigor. Timing of herbicide application and manual weeding was adjusted to match with the predetermined critical period of weed control of 20-43 days after seeding (Anwar *et al*., 2012c).The soil was dry-ploughed and harrowed but not puddled during preparation. Rice seeds were directly dry-seeded at 2 cm depth in rows with 25 cm inter-row and 15 cm intra-row spacing. Soil was maintained under non-saturated aerobic conditions throughout. The trial was primarily rain-fed, but supplemental sprinkler irrigation was given when needed. Overflow canals were kept to facilitate drainage following heavy rainfall to avoid ponding.

A 25 cm × 25 cm quadrate was randomly placed at four spots for recording of weed data at harvest. Weeds were clipped to ground level, identified, counted and oven dried at $70^o$ C for 72 h.



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Dominant weed species were identified by summed dominance ratio (SDR). Weed control efficiency (WCE) of different treatments was calculated as WCE (%) = (DWC – DWT) / DWC x 100, where, DWC = dry weight of weeds in weedy check plots and DWT = dry weight of weeds in treated plots. At maturity, central 3 $m^2$ area of each plot was hand harvested to record grain yield expressed as t/ha (adjusted to14% moisture content). Percent relative yield loss (RYL) due to weeds was calculated as: [100 × (weed-free yield - weedy yield)/weed-free yield]. All data were subjected to ANOVA by using SAS statistical software package version 9.1 (SAS, 2003). Significant differences among means were adjudged by using Fisher's protected Least Significant Difference (LSD) test at $p \leq 0.05$. Economic analysis was done based on the local market price.

## RESULTS AND DISCUSSION

**Weed Composition:** The experimental field was infested with broadleaf weeds, sedges and grasses, and mostly dominated by broadleaf weeds. The relative composition of the broadleaf, sedges and grasses were about 60, 22 and 18%, respectively. The weed community had a wide spectrum of 17 species representing 10 different families. Based on the SDR values, the most prevalent weed species were *Physalis heterophylla, Scoparia dulcis, Cyperus rotundus, Eleusine indica and Leptochloa chinensis* (data not shown). As documented in earlier studies, weed community in the aerobic rice is mostly dominated by broadleaf weeds followed by sedges and grasses (Jayadeva *et al*., 2011; Anwar *et al*., 2012c). Jaya Suria *et al*., (2013), on the contrary, reported from their study with aerobic rice that sedges was the most dominant group followed by grasses and broadleaf weeds.

**Table 1**. List of weed control treatments.

| Label | Treatments | Application rate | Time (DAS) |
|---|---|---|---|
| T1 | Pretilachlor /safener fb Bentazon/MCPA | 0.5 kg a.i./ha fb 0.6/0.1 kg a.i. /ha | 1 fb 30 |
| T2 | Pretilachlor /safener fb MW | 0.5 kg a.i./ha | 10 fb 30 |
| T3 | Pretilachlor /safener fb Propanil/ Thiobencarb | 0.5 kg a.i./ha fb 1.2/2.4 kg a.i. /ha | 1 fb 10 |
| T4 | Pretilachlor /safener fb Propanil/ Thiobencarb fb Bentazon/MCPA | 0.5 kg a.i. /ha fb 1.2/2.4 kg a.i. /ha fb 0.6/0.1 kg ai /ha | 1 fb 10 fb 30 |
| T5 | Pretilachlor /safener fb Propanil/ Thiobencarb fb MW | 0.5 kg a.i. /ha fb 1.2/2.4 kg a.i. /ha | 1 fb 10 fb 30 |
| T6 | Propanil / Thiobencarb | 1.2/2.4 kg a.i. /ha | 10 |
| T7 | Propanil / Thiobencarb fb Bentazon/MCPA | 1.2/2.4 kg a.i. /ha fb. 0.6/0.1 kg a.i. /ha | 10 fb 30 |
| T8 | Propanil / Thiobencarb fb MW | 1.2/2.4 kg a.i. /ha | 10 fb 30 |
| T9 | Cyhalofop-butyl + Bensulfuron | 0.1 kg a.i./ha + 0.06 kg a.i./ha | 10 |
| T10 | Cyhalofop-butyl+ Bensulfuron fb Bentazon/MCPA | 0.1 kg a.i./ha + 0.06 kg a.i./ha fb 0.6/0.1 kg a.i. /ha | 10 fb 30 |
| T11 | Cyhalofop-butyl + Bensulfuron fb MW | 0.1 kg a.i./ha + 0.06 kg a.i./ha | 10 fb 30 |
| T12 | Bispyribac-sodium | 0.03 kg a.i./ha | 10 |
| T13 | Bispyribac-sodium fb Bentazon/MCPA | 0.03 kg a.i./ha fb 0.6/0.1 kg a.i. /ha | 10 fb 30 |
| T14 | Bispyribac-sodium fb MW | 0.03 kg a.i./ha | 10 fb 30 |
| T15 | Season long weed-free | | Season long |
| T16 | Season long weedy | | - |



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/ means that the herbicides were formulated as a proprietary mixture, + means that the herbicides were tank-mixed and applied at the same time,
All herbicides were applied as per manufacturers' recommended rates in 300L of water per hectare by knapsack sprayer. DAS = days after seeding; fb = followed by

**Weed Dry Weight and Density:** Weed dry weight and density were significantly influenced by weed control treatments (Table 2). All the treatments effectively suppressed weed growth. Weed dry weight in different weed control treatments ranged from 4.77 to 62.58 g/m$^2$ and weed density from 73 to 130 plants/m$^2$; the respective values in weedy check were recorded as high as 326 g/m$^2$ and 295plants/m$^2$. It is evident that an early post emergence application (at 10 DAS) followed by a manual weeding (at 30 DAS) or a pre-emergence application (at 1 DAS) trailed by an early post emergence application (at 10 DAS) followed by a manual weeding/post-emergence application of Bentazone/MCPA (at 30 DAS) produced lowest and similar weed biomass and density. This finding negates the inevitability of a pre-emergence application of herbicide in aerobic rice. It is notable that the consequence of manual weeding and Bentazone/MCPA application was similar with respect to weed biomass and density reduction irrespective of early-post emergence application. But, manual weeding was found more effective compared with Bentazone/MCPA application when pre-emergence application of Pretilachlor/safener was not followed by an early-post emergence application. The weed density and dry weight as observed in this trial are respectively 36 and 14 % lower than those recorded in herbicide screening trial (Anwar *et al*., 2012a) and 39 and 20% lower than those recorded from critical period study (Anwar *et al*., 2012c). All the three studies were conducted with the same rice variety (AERON 1) at the same site and agro-climatic conditions. Despite the similarity in spatio-temporal aspects among the three sites, much lower weed pressure in the present study as compared to those of previous studies might be the contribution of integrating higher seed rate ( 300 instead 200 seeds/m$^2$) and seed invigoration (Zappa® primed seeds instead unprimed ones). Sowing primed seeds at higher rate enhanced rice competitiveness against weeds resulted in better weed suppression and ultimately reduced weed density and dry weight.

**Weed Control Efficiency (WCE):** Different weed control treatments significantly influenced the WCE (Table 2). All the weed control treatments exhibited high performance (>80% WCE), and few treatments performed excellent (>95% WCE). Pretilachlor /safener fb Propanil / Thiobencarb fb manual weeding resulted in the highest WCE (96.93%) closely followed by Cyhalofop-butyl + Bensulfuron fb manual weeding (96.60%). On the other hand, Bispyribac-sodium performed the worst (79.34% WCE) closely followed by Propanil / Thiobencarb (81.75% WCE). Our findings corroborate that spraying with any of the early post emergence herbicides under study at 10 DAS followed by a manual weeding/ post-emergence application of Bentazone/MCPA at 30 DAS can provide excellent weed control. Pre-emergence application fb early-post emergence application fb post emergence application or manual weeding, and early-post emergence application fb post emergence application or manual weeding resulted in higher WCE as compared with a single early-post emergence application. This might be due to that, when pre-emergence or early-post emergence application was not followed by post emergence application or manual weeding, late emerged weeds remained uncontrolled. These results closely resembles to that of Jaya Suria *et al.* (2011), who documented that for achieving high WCE in aerobic rice, a pre-emergence application should be followed by a manual weeding at later growth stage. The WCEs achieved in this study is much higher than those obtained from the previous study done at the same site with same herbicides (Anwar *et al*., 2012a). Pooled merits of higher seeding density and seed invigoration offered more competitive surroundings in favor of rice which might augment herbicide efficacy.



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**Rice Yield and Relative Yield Loss (RYL):** Rice grain yield was significantly influenced by weed control treatments (Table 3). All the treatments produced significantly higher yield than weedy check did, and a number of treatments resulted in yield as high as weed-free yield. The RYL due to weed varied widely (2.79 - 23.18%) among the weed control treatments (Table 3). In weedy check, RYL was recorded as high as 62.44%. Pretilachlor/safener fb Propanil/Thiobencarb fb manual weeding allowed the least yield penalty of only 2.79%. A single application of Bispyribac-sodium or Propanil/Thiobencarb or Cyhalofop-butyl + Bensulfuron resulted in high RYL (>20%). The WCE was ultimately reflected in grain yield. All the weed control treatments significantly out-yielded weedy check, and some treatments performed very close to weed-free check because of their high WCE. In contrast, weed control treatments with low WCE resulted in poor yield. The WCE was also translated into RYL. It is evident from this study that higher the WCE, lower the RYL. The increase in rice grain yield by increasing WCE has also been reported by many workers (Jaya Suria *et al*., 2011; Anwar *et al*., 2012a). Reasonably, weed-free check enjoyed the highest RYL. This result may be explained by the fact that use of primed seeds at higher rate resulted in faster and higher emergence rate along with vigorous stand offering rice plants an advantage to outcompete weeds, which ultimately translated into better growth, allometry, yield components and finally increased yield.

**Table 2.** Weed dry weight, weed density and weed control efficiency of different weed control treatments.

| Treatment | Weed dry weight ($g/m^2$) | Weed density ( $no./m^2$) | Weed control efficiency (%) |
|---|---|---|---|
| T1 | 50.89bc | 120.89bc | 82.90 |
| T2 | 26.66ef | 94.66ef | 90.28 |
| T3 | 37.68de | 105.68de | 86.92 |
| T4 | 9.63g-j | 77.63gh | 95.46 |
| T5 | 4.77ij | 72.77h | 96.94 |
| T6 | 54.65bc | 122.65bc | 81.76 |
| T7 | 19.87fg | 87.87fg | 92.34 |
| T8 | 9.98g-j | 77.98gh | 95.35 |
| T9 | 46.72cd | 114.72cd | 84.17 |
| T10 | 16.73f-i | 84.73f-h | 93.30 |
| T11 | 5.87h-j | 73.87h | 96.63 |
| T12 | 62.58b | 130.58b | 79.34 |
| T13 | 17.37f-h | 85.38f-h | 93.10 |
| T14 | 8.36g-j | 76.36gh | 95.84 |
| T15 | 00.00j | 00.00i | 100 |
| T16 | 326.51a | 295.50a | 0 |
| LSD | 12.59 | 13.05 | - |

LSD = least significant difference

Within a column, means sharing same alphabets are not significantly different at P=0.05 probability level according to least significant difference (LSD) test.

## Economics

Weed control treatments showed a wide range of economic return (Table 3). Cost analysis revealed that the highest net benefit of Ringgit Malaysia (RM) 4066/ha was recorded with Cyhalofop-butyl + Bensulfuron fb Bentazon/MCPA. On the other hand, when manual weeding was integrated with herbicides, net benefits were found lower (ranging from RM 2693/ha to RM 3010/ha) as compared with those obtained from only herbicide application. The season-long weed-free plots resulted in a



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net benefit of only RM 2160/ha which was not much higher than that obtained from season-long weedy plots (RM 1750/ha), and comparatively lower than that of any of the treatments. Despite the highest gross income (RM 4660/ha), season-long weed-free plots resulted in very low net benefit, and replacement of manual weeding by Bentazon/MCPA increased gross income but decreased net benefit because of high cost involvement in manual weeding. Even a single early-post emergence application generated more net benefit as compared to early-post emergence application followed by manual weeding. Consequently, manual weeding is less remunerative compared to herbicidal control, and practicing manual weeding throughout the season is a losing concern, confirming the view of many others (Mahajan *et al*., 2009; Sunil *et al*., 2010). Thus, integrated weed management resulted in lower economic return compared to herbicide based management. Compared to earlier study (Jaya Suria *et al*., 2011) higher gross income and higher net benefit were encountered in the present study, which might be due to better yield and higher WCE as a consequence of more competitive cropping system in favor of rice.

**Table 3**. Grain yield, relative yield loss of AERON 1 and economics of different herbicide treatments.

| Ttreatment | Grain yield (t/ha) | Relative yield loss (%) | Total Weeding cost (RM /ha) | Gross income (RM /ha) | Net benefit (RM /ha) |
|---|---|---|---|---|---|
| T1 | 4.03e | 13.51 | 277 | 4030 | 3753 |
| T2 | 4.11de | 11.80 | 1417 | 4110 | 2693 |
| T3 | 4.21c-e | 9.65 | 469 | 4210 | 3741 |
| T4 | 4.47a-c | 4.07 | 579 | 4470 | 3891 |
| T5 | 4.53ab | 2.79 | 1719 | 4530 | 2811 |
| T6 | 3.65f | 21.68 | 302 | 3650 | 3348 |
| T7 | 4.38a-d | 6.01 | 412 | 4380 | 3968 |
| T8 | 4.41a-c | 5.36 | 1552 | 4410 | 2858 |
| T9 | 3.75f | 19.52 | 274 | 3750 | 3476 |
| T10 | 4.45a-c | 4.51 | 384 | 4450 | 4066 |
| T11 | 4.51ab | 3.20 | 1524 | 4510 | 2986 |
| T12 | 3.58f | 23.18 | 170 | 3580 | 3410 |
| T13 | 4.34b-d | 6.87 | 280 | 4340 | 4060 |
| T14 | 4.43a-c | 4.93 | 1420 | 4430 | 3010 |
| T15 | 4.66a | 00.00 | 2500 | 4660 | 2160 |
| T16 | 1.75g | 62.44 | 0.0 | 1750 | 1750 |

RM= Ringgit Malaysia; 1 US$=3 RM (approx). Gross income = paddy yield (t/ha) × market price (RM t/ha); Net benefit = gross income – total weeding cost.

## CONCLUSIONS

A more competitive cropping system in favor of rice as a consequence of combined use of competitive variety, higher seeding rate and seed priming is evident from the study, which was reflected in lower weed pressure, higher weeds control efficiency and better yield. Weed control only during critical period of competition is also justified as some weed control treatments produced yield similar to weed-free yield. Herbicide and manual weeding combinations resulted in lower net benefit compared to herbicidal control because of high cost involvement in manual weeding. From economic view points, application of Cyhalofop-butyl + Bensulfuron or Bispyribac-sodium or Propanil/Thiobencarb at 10 DAS followed by Bentazone/MCPA at 30 DAS can be recommended; while for the sustainability of long-term weed management, Cyhalofop-butyl



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+ Bensulfuron or Bispyribac-sodium or Propanil/Thiobencarb should be applied in rotation at 10 DAS followed by a manual weeding at 30 DAS.

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# PLANT SPACING INFLUENCE ON WEED COMPETITIVENESS IN AEROBIC RICE

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**ABSTRACT** Growing rice in aerobic soil conditions is a potential water-wise rice production system. But aerobic rice is subject to severe weed infestation and, therefore, adoption of a sustainable weed management strategy is crucial for its wide spread adoption. Cultural weed control is a key component of integrated weed management. Manipulation of different cultural practices could increase the competitiveness of a crop against weeds. Spacing of a crop determines solar radiation interception, canopy coverage and biomass accumulation which have cumulative effect on its weed suppressive ability. This research was therefore initiated to reveal the influence of planting geometry and spacing on weed suppression towards reducing yield loss due to weed competition in aerobic rice. The study was conducted at Pinang, Malaysia with aerobic rice variety AERON 1 using 5 different plant spacings such as 10 x 10 cm, 15 x 15 cm, 20 x 20 cm, 25 x 25 cm and 30 x 30 cm under both weedy and weed-free conditions. The experiment was laid out in split-plot design with four replications assigning weeding regime in main plots and plant spacing in sub-plots. Aerobic rice field was infested with 12 weed species having *Echinochloa colona*, *Leptochloa chinensis*, *Digitaria aescendens* and *Cyperus iria* as predominant weeds. Rice spacing exerted significant influence on both weed pressure and yield performance of AERON 1. With the decrease in plant spacing weed dry matter decreased but rice yield increased. The closest spacing resulted in maximum weed suppression which ultimately produced highest rice yield. Weed inflicted relative yield loss was also minimized by the closest spacing. The present findings imply rice spacing mostly determines rice-weed competition, and can play a decisive role to minimize weed pressure. Therefore, closer spacing could be considered as a vital tool in integrated weed management program for aerobic rice.

## INTRODUCTION

Rice is grown on a large scale in Asia and is a main source of food for more than half of the world's population, especially in South and Southeast Asia (Chauhan and Johnson, 2010). However, the increasing scarcity of water threatens the sustainability of food production from irrigated agriculture (Gleick, 1993; Juraimi *et al.,* 2010). Rice grown under upland conditions, where the crops are not flooded at any time during the growth period is an effective way to save water and to reduce methane emissions produced by flooded rice paddies. Aerobic rice is a direct seeding system in which dry rice seeds are sown in non-puddled dry or wet soil and subsequent irrigations are applied to keep the soil moist but not saturated (Tuong *et al*., 2005).

Commonly used weed control strategies are water management, hand weeding, mechanical weeding and chemical herbicides (Juraimi *et al*., 2013). Chemical weed control represents a practical and economical alternative to hand weeding (Akobundu, 1987; Anwar *et al*., 2012). However, there are concerns over the evolution of herbicide resistance in weeds, weed species population shifts, increased cost of chemical control measures and concern about the environment (Primot *et al*., 2006). If crop cultivars can tolerate weeds, it may reduce the need of synthetic



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herbicides, allow the use of less costly and more environmentally sound herbicides, decrease the number of cultivations or improve yield stability (Lemerle *et al*., 2001; Anwar *et al*., 2010). Competition between crops and weeds can be modified by crop density because increasing crop density can enhance the crop's share of the total resource pool and reduce resource availability for weeds. Therefore, the objective of these experiments was to determine the optimum plant spacing for rice that can substantially reduce rice yield loss caused by weed competition under aerobic conditions.

## MATERIALS AND METHODS

The studies were conducted in two cropping seasons at experimental field at Malaysian Agriculture Research and Development Institute (MARDI), Pulau Pinang, Malaysia. The soil series was classified as Sogomona series with average pH of 4.32. The organic matter (OM) content and cation exchange capacity (CEC) of the soil were 1.1 % meq/100 g soil, The experiment was laid out in split-plot design with four replications. Weeding regimes (weedy and weed free) was assigned in the main plot while plant spacing was allocated as the sub plot. In the first trial, there were five different plant spacing including 10x 10cm, 15 x 15cm, 20 x 20cm, 25 x 25 cm and 30 x 30 cm. And in the second trial, slight modification in the spacing was made, which were 15 x 15 cm, 15 x 20 cm, 20 x 20 cm, 20 x 25 cm and 25 x 25 cm. The data obtained were analyzed for analysis of variance (ANOVA) and significant mean comparison was tested by using Least Significant Difference (LSD) at 5% probability level.

## RESULTS AND DISCUSSION

### Weed composition

The first trial field was infested with 12 weed species .Weed species consisted of 5 grasses (77.56%), one sedge (12.63%) and 6 broadleaf weeds (9.83%). The dominant weeds were *Echinochloa colona* (46.21%), *Digitaria ascendens* (18.13%), *Cyperus iria* (L.) (12.63%), *and Eleusine indica* (12.53%) (Table 1).

**Table 1. Sum dominance ratio (SDR) of different weed species in aerobic rice field**

| Weed Species | Family name | Weed type | SDR (%) |
|---|---|---|---|
| *Echinochloa colona* (L.) Link | Poaceae | Grass | 46.21 |
| *Digitaria ascendens* (H.B.R.Henr.) | Poaceae | Grass | 18.13 |
| *Cyperus iria* (L.) | Cyperaceae | Sedge | 12.63 |
| *Eleusine indica* (L.) gaertn. | Poaceae | Grass | 12.53 |
| *Mimosa invisa* Mart. | Fabaceae | Broadleaf | 5.12 |
| *Melochi achorcorifolia* L. | Sterculiaceae | Broadleaf | 2.01 |
| *Ludwigia hyssopifolia* (G. Don) | Onagraceae | Grass | 1.32 |
| *Cleome rutidosperma* DC. | Capparaceae | Broadleaf | 0.71 |
| *Leptochloa chinensis* (L.) Nees | Poaceae | Grass | 0.67 |
| *Croton hirtus* (L.) Herit | Euphorbiaceae | Broadleaf | 0.38 |
| *Calopogonium mucunoides* Desv. | Fabaceae | Broadleaf | 0.27 |
| *Phyllantus niruri* (L.) | Euphorbiaceae | Broadleaf | 0.03 |

### Weed dry weight

During first trial, weed dry weight was significantly affected by plant spacing (Table 2). On average, weed dry weight varied from 26.0 g/m2 up to 174.1 g/m2. At 25 DAS, 10 x 10 cm spacing produced lesser weed dry weight (30.7 g/$m^2$) while widest spacing (30 x 30 cm) gave the highest weight (74.7 g/$m^2$). However, 15 x 15 cm spacing did not differ significantly with 20 x 20 cm and



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25 x 25 cm. At 50 DAS, similar trend was observed for lowest and highest weed dry weight. However, 15x15cm and 20 x 20cm spacing also 25 x 25cm and 30 x 30 cm spacing did not differ significantly to each other, respectively. At harvest, 10 x 10cm gave the minimum weed dry weight (46.6 g/m$^2$) while 30 x 30cm produced the maximum weight (127.5 g/m$^2$). 15 x 15cm spacing produced significantly lower weed dry weight compared to 25 x 25 cm but not with 20 x 20 cm spacing.

Generally, the highest weed dry weight was recorded at the widest spacing while the lowest weight produced by closest spacing. The result was in line with Chauhan and Johnson et al, 2010 where greater rice yield and lower yield and lower weed biomass has been reported for 20 cm rows compared to 30 cm rows. Reductions in weed biomass related to crop density occurred earlier and were larger than those related to cultivar (Champion *et al*., 1998). Ghadiri and Bhayat (2004) stated that decreasing plant spacing with rows significantly reduced weed dry weight and the interaction of row and plant spacing with weed dry weight was significant.

**Table 2. Weed dry weight of weedy treatment under different plant spacing**

| Spacing | Weed dry weight (g/m$^2$) | | |
|---|---|---|---|
| | 25 DAS | 50 DAS | At harvest |
| 10 x 10 cm | 30.7c | 46.7c | 46.6d |
| 15 x 15 cm | 51.7b | 100.9b | 79.4c |
| 20 x 20 cm | 60.7ab | 115.3b | 100.9bc |
| 25 x 25 cm | 63.9ab | 144.4a | 122.5ab |
| 30 x 30 cm | 74.7a | 156.6a | 127.5a |
| LSD (0.05) | 17.82 | 21.97 | 24.11 |

Means within a column with the same letter are not significantly different at P= 0.05 (LSD), DAS; days after sowing

**Plant height**

Table 3 shows the effect of weed competition on plant height at five different plant spacings. The range of plant height was 32.9 cm to 157.1 cm. Weeding regimes was not influenced the plant height at 25 DAS, at 50 DAS and at harvest. Plant height was affected by plant spacing during only at harvest but not at 25 DAS and 50 DAS and increased as the increasing of observational dates. There was no significant effect of interaction between weeding regimes and plant spacing in term of plant height. Hasanuzzaman *et al*., (2009) reported that maximum plant height was observed with closest spacing where the shortest plant was observed with the widest spacing.

**Yield components**

Yield components comprised of filled grain per panicle, thousand grain weight and panicle/m$^2$. There was significant difference in weeding regimes and plant spacing, but not in plant spacing at thousand grain weight (Table 4). Panicle/m$^2$ was the highest at 10 x 10 cm (968.1) and the lowest at 30 x 30cm (194.8). The spacing of 15 x 15 cm, 20 x 20 cm, and 25 x 25 cm produced intermediate panicle number with 504.5, 332.1, and 251.9, respectively.Thousand grain weights in weedy condition were significantly lower than weed free condition. Thousand grain weights were ranging from 28.2 g to 29.8 g (Table 4).

The yield attributes were decreased with decreasing plant spacing both under weedy and weed free conditions. This hypothesis could be supported by (Madonni *et al*.,2006) who reported that the decrease in grain number and grain weight could have been related to lower availability of metabolites in leaves at higher densities as a result of high competition for light, water and minerals.



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There was highly significant difference in weeding regimes and plant spacing (Figure 1). Range of mean grain yield was between 0.88 ton/ha and 3.13 ton/ha. Grain yield in weedy condition was significantly lower than grain yield in weed free condition. As the plant spacing increased, grain yield was decreased. In weedy condition, the maximum yield was produced by 10 x 10 cm spacing (2.0 ton/ha) followed by 15 x 15 cm (1.6 ton/ha). The minimum grain yield was produced by 30 x 30 cm (1.0 ton/ha) which did not differ significantly with 25 x 25 cm (1.1 ton/ha). In weed free condition, the maximum yield was produced by 10 x 10 cm (3.00 ton/ha) which significantly than other four plant spacing.



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**Table 3.** The effect of weed competition on plant height at different plant spacing

| Treatment | Plant height at 25 DAS# | | | Plant height at 50 DAS# | | | Plant height at harvest# | | |
|---|---|---|---|---|---|---|---|---|---|
| | W | WF | Mean | W | WF | Mean | W | WF | Mean |
| 10 x 10 cm | 41.5[a] | 37.9ab | 39.7ab | 113.8a | 116.7b | 115.2b | 118.5a | 119.3c | 118.9 c |
| 15 x 15 cm | 41.8a | 39.7a | 40.7a | 117.0a | 117.5ab | 118.2ab | 121.0a | 121.6bc | 121.3bc |
| 20 x 20 cm | 39.5a | 35.2b | 37.4b | 114.8a | 118.4ab | 116.6ab | 120.4a | 125.9abc | 123.2abc |
| 25 x 25 cm | 40.4a | 34.8b | 37.6b | 116.7a | 117.1b | 118ab | 121.8a | 128.5ab | 125.1ab |
| 30 x 30 cm | 39.3a | 37.6ab | 38.4b | 117.1a | 124.3a | 120.7a | 122.3a | 130.3a | 126.3a |

**Table 4.** The effect of weed competition on yield component under different plant spacing

| Treatment | Panicle number /m2# | | | Thousand grain weight (g) | | |
|---|---|---|---|---|---|---|
| | W | WF | Mean | W | WF | Mean |
| 10 x 10 cm | 784.5a | 1151.8a | 968.1a | 28.2 | 29.6 | 22.4e |
| 15 x 15 cm | 434.0b | 575.0b | 504.5b | 28.6 | 29.6 | 23.2d |
| 20 x 20 cm | 260.3c | 404.0c | 332.1c | 28.7 | 29.7 | 23.7bc |
| 25 x 25 cm | 222.8c | 281.0d | 251.9d | 29.0 | 29.8 | 24.3b |
| 30 x 30 cm | 144.0d | 245.5d | 194.8e | 29.4 | 29.8 | 25.5a |



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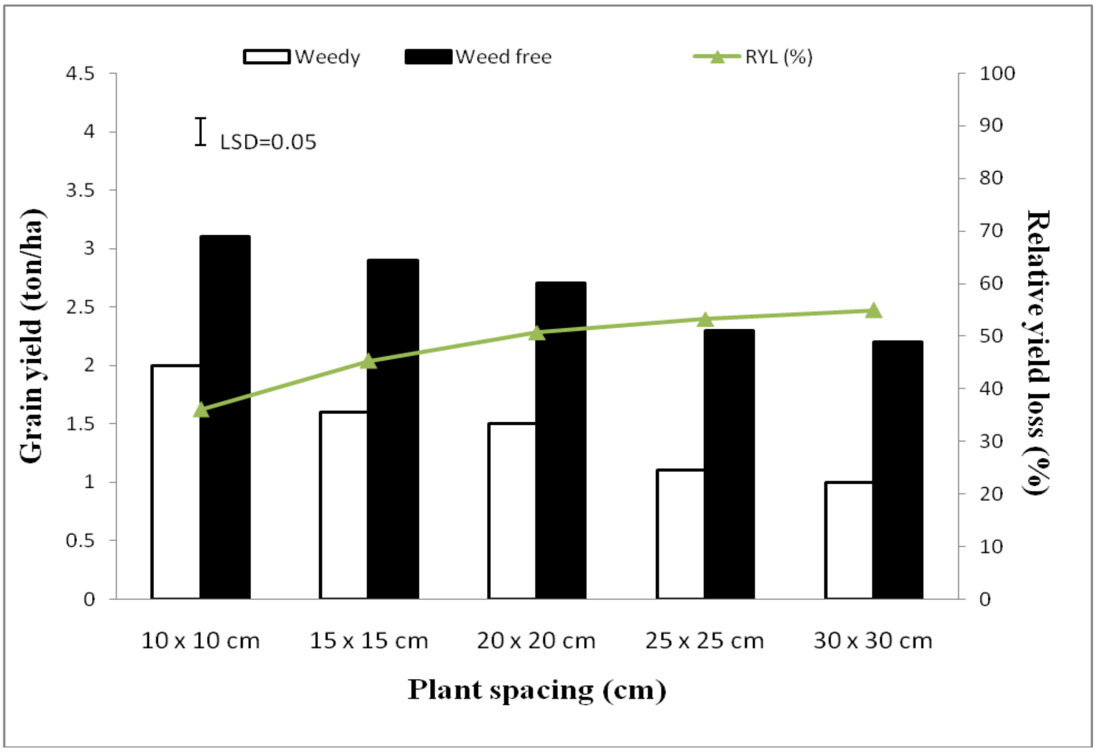


**Fig 1**. Grain yield of AERON 1 under weedy and weed free condition in aerobic systems

## Grain Yield

Statistically, both weeding regime and plant spacing significantly influenced the rice grain yield (Fig. 1). . There was no interaction effect detected between weeding regime and plant spacing in term of grain yield. As expected, grain yield in weedy condition was lower than weed free condition. Grain yield decreased as the plant spacing increased. In weedy condition, 15 x 15 cm (1.25 ton/ha) produced the highest grain yield while 25 x 25cm (0.90 ton/ha) produced the lowest grain yield. Grain yield of 25 x 25 cm (0.90 ton/ha) and 20 x 25 cm (0.95 ton/ha) did not differ significantly from each other. 15 x 20 cm (1.10 ton/ha) and 20 x 20 cm (1.05 ton/ha) also did not differ significantly from each other.

Relative yield loss was ranging from 50.7 % to 57.2 %. 15x15cm spacing gave the lowest relative yield loss where weed competition has reduced the yield for about 50.7% while the highest relative yield loss was at 25 x 25 cm spacing. Relative yield loss of 15 x 20 cm, 20 x 20 cm, 20 x 25cm spacing was 54.3%, 55.8% and 56.6 %, respectively. Wider plant spacing resulted in lower rice grain yield in both weedy and weed free conditions. This result is supported by Chauhan and Johnson (2011) where yield of aerobic rice were lower under wider spacing (30 cm) than narrow spacing (15 cm to 20 cm) in weed free condition. Row spacings between 15 and 45 cm had little impact on grain yield in the absence of weed in direct seeded rice, but in competition with weeds, the lowest grain yield was observed with wider row spacing (Akobundu and Ahissou, 1985).

## CONCLUSIONS

Our study reveals that planting spacing can play a vital role in minimizing weed pressure in rice under aerobic soil conditions. Weed dry weight and weed density were decreased with the decreasing plant spacing, and the closest spacing of 10 X 10 cm resulted in the highest grain yield. Therefore, 10 X 10 cm spacing could be adopted for higher yield and better weed suppression. However, before making decision regarding plant spacing as a tool for weed management, weed competitiveness of the rice cultivar and weed composition and degree of infestation of the site must be taken into consideration for better result.



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# BROADLEAVED WEEDS IN TURF GRASS BLOCKS OF CIBODAS BOTANIC GARDEN, CIANJUR, INDONESIA

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**ABSTRACT**: Since the establishment of Cibodas Botanic Garden (CBG) in 1852, the attention was drawn to the plant collections. No research or even an inventory was made on the prevailing weed flora. A survey was conducted in 11 turfgrass blocks in CBG in June 2012 to identify the most common broadleaved weeds associated with turfgrass. This survey provides a list of weeds to be evaluated for future turfgrass blocks and collections management in CBG. The turf blocks were covered by carpet grass (*Axonopus compressus*). Point method was used for these blocks survey along the roadside that marks a particular block. Coverage, frequency and relative abundance were enumerated. A total of 58 species from 30 different families of broadleaved weeds were identified. *Athyrium javanicum* and *Plantago major* recorded as the highest frequency readings among blocks, occurring in 10 out of 11 blocks. *Centella asiatica* and *Hydrocotyle sibthorpioides* registered as the highest coverage among blocks surveyed with only a slight difference between them, hence representing the two most abundant weeds of the surveyed blocks.

***Keywords:*** broadleaved weeds, Cibodas Botanic Garden, turfgrass

## INTRODUCTION

In Cibodas Botanic Garden (CBG), the area used to plant the collections are mostly covered with turfgrass (*Axonopus compressus*). There are also areas covered by turfgrass only that meant to be used by visitors of CBG, no plant collections in some distances. A turfgrass field need to be maintained in order to keep its function. The presence of weeds here will reduce its attractiveness to the visitors. Weed here is defined as any plant growing where it is not wanted. The weed can also compete with the turfgrass and act as a host to pest which can harm the plant collections. Therefore weeds must be eliminated. To be eliminated, one must first make an inventory of weeds occurred, documenting information such as the presence, coverage, frequency and abundance. This kind of information was not available yet in CBG. Thus, the object of this study was to identify the most abundant weeds in CBG, including the coverage and frequency of other weeds recorded.

## MATERIALS AND METHODS

A survey was conducted in turf grass blocks in Cibodas Botanic Garden to identify the most common broadleaved weeds. The blocks surveyed were 1250-1300 m asl. The turf grass blocks were covered by *Axonopus compressus* (carpet grass). Point method was used using a wooden stick, where the distance between points was 65 cm. Any part of plant that touches the stick was counted as one occurrence. The points themselves were run along an imaginary line transect, one meter away into the turf grass area from the border between the tarmac road and the turf grass block. There were different numbers of points, depended upon the length of the border of each block. All broadleaved weeds were identified, counted and recorded. Blocks that were just mowed were excluded from this survey.

The data were summarized using four quantitative measures; coverage, relative coverage, frequency and relative frequency. Coverage was calculated as the percentage of the



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total number of individual plant species of the total number of points. Relative Coverage was calculated as the percentage of the coverage value of a certain species of the total coverage values for all species. Frequency was calculated as the percentage of the occurrence of a species of the total number of points. Relative frequency is calculated as the percentage of the frequency value of a certain species of the total frequency values for all species. Relative Abundance was calculated as the sum of relative coverage and relative frequency for particular species. The naming of the weed species and families followed those names accepted in The Plant List (theplantlist.org) wherever possible.

## RESULTS AND DISCUSSION

The weed species represented 30 families of which the Compositae family had the highest number (16) of species. Rubiaceae family had the second highest number with seven species, followed by Oxalidaceae (3). Araliaceae, Lamiaceae, Leguminosae, Selaginellaceae, and Urticaceae families had two species each, while the rest of the families were represented by one species only (Table 1).

**Table 1**. Identification of broadleaved weeds in Cibodas Botanic Garden.

| Family Name | Species Name |
|---|---|
| Acanthaceae | *Rostellularia procumbens* (L.) Nees |
| Adiantaceae | *Adiantum capillus-veneris* L. |
| Apiaceae | *Centella asiatica* (L.) Urb. |
| Araliaceae | *Hydrocotyle javanica* Thunb. |
| | *Hydrocotyle sibthorpioides* Lam. |
| Balsaminaceae | *Impatiens platypetala* Lindl. |
| Brassicaceae | *Nasturtium officinale* R.Br. |
| Campanulaceae | *Lobelia angulata* G.Forst. |
| Caryophyllaceae | *Drymaria cordata* (L.) Willd. ex Schult. |
| Commelinaceae | *Commelina diffusa* Burm.f. |
| Compositae | *Acmella ciliata* (Kunth) Cass. |
| | *Acmella uliginosa* (Sw.) Cass. |
| | *Ageratina riparia* (Regel) R.M.King & H.Rob. |
| | *Ageratum conyzoides* (L.) L. |
| | *Artemisia vulgaris* L. |
| | *Bidens pilosa* L. |
| | *Conyza bonariensis* var. *leiotheca* (S.F.Blake) Cuatrec. |
| | *Dichrocephala integrifolia* (L.f.) Kuntze |
| | *Emilia sonchifolia* (L.) DC. ex DC. |
| | *Galinsoga parviflora* Cav. |
| | *Gnaphalium affine* D.Don |
| | *Mikania micrantha* Kunth |
| | *Pseudelephantopus spicatus* (B.Juss. ex Aubl.) Rohr ex Gleason |
| | *Synedrella nodiflora* (L.) Gaertn. |
| | *Taraxacum officinale* Webb |
| | *Youngia japonica* (L.) DC. |
| Euphorbiaceae | *Homalanthus populneus* (Geiseler) Pax |
| Lamiaceae | *Ocimum* sp. |
| | *Salvia coccinea* Buc'hoz ex Etl. |
| Leguminosae | *Desmodium triflorum* (L.) DC. |



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| | |
|---|---|
| | *Calliandra calothyrsus* Meisn. |
| Malvaceae | *Sida rhombifolia* L. |
| Melastomataceae | *Clidemia hirta* (L.) D. Don |
| Moraceae | *Ficus punctata* Thunb. |
| Oxalidaceae | *Oxalis barrelieri* L. |
| | *Oxalis corniculata L.* |
| | *Oxalis latifolia* Kunth |
| Passifloraceae | *Passiflora ligularis* Juss. |
| Piperaceae | *Peperomia tetraphylla* (G.Forst.) Hook. & Arn. |
| Plantaginaceae | *Plantago major* L. |
| Polygalaceae | *Polygala paniculata* L. |
| Polygonaceae | *Persicaria nepalensis* (Meisn.) Miyabe |
| Rosaceae | *Rubus rosifolius* Sm. ex Baker |
| Rubiaceae | *Mitracarpus hirtus* (L.) DC. |
| | *Paederia foetida* L. |
| | *Richardia brasiliensis* Gomes |
| | *Spermacoce alata* Aubl. |
| | *Spermacoce articularis* L.f. |
| | *Spermacoce exilis* (L.O.Williams) C.D.Adams ex W.C.Burger & C.M.Taylor |
| | *Spermacoce laevis* Lam. |
| Selaginellaceae | *Selaginella belangeri* (Bory) Spring |
| | *Selaginella opaca* Warb. |
| Solanaceae | *Solanum americanum* Mill. |
| Urticaceae | *Elatostema strigosum* Hassk. |
| | *Maoutia setosa* Wedd. |
| Violaceae | *Viola pilosa* Blume |
| Woodsiaceae | *Athyrium javanicum* Copel. |
| Xanthorrhoeaceae | *Dianella javanica* (Blume) Kunth |

Kamal-Uddin *et al*. (2009) found 32 broadleaved weeds in 50 different turfgrass areas. Compared to his study, CBG has more weed species. This could be caused by the different nature of turfgrass surveyed. Although the turfgrass field surveyed by Kamal-Uddin *et al*. have also some similar nature with the turfgrass blocks in CBG e.g. recreational park area, somehow there were more weed species here in CBG. Another possibility was the method used to conduct the survey. His method covers more areas including the middle, while the method used here focused on the road side areas where the edge effect could take place. Edge effects result from the interplay between two spatially contiguous ecosystems (Murcia, 1995) and edge effect hypothesis states that diversity is higher in ecotones than in adjacent assemblages (Odum, 1971).

Among the weed species recorded, *Hydrocotyle sibthorpioides* and *Centella asiatica* had the highest relative cover with the value of 9.66 and 9.46, respectively (Table 2). The two species counts were 241 and 236 individual plants out of 2495 in total. It seems that the turfgrass blocks in CBG is a very suitable place for both species. In term of coverage (relative cover), the top five weeds were *Hydrocotyle sibthorpioides*, *Centella asiatica*, *Synedrella nodiflora* (7.13), *Ageratina riparia* (6.93) and *Rostellularia procumbens* (5.93), respectively.



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**Table 2.** Percent cover, relative cover, percent frequency, relative frequency, and relative abundance of broadleaved weeds in Cibodas Botanic Garden.

| Species Name | % cover | relative cover | % frequency | relative frequency | relative abundance |
|---|---|---|---|---|---|
| *Hydrocotyle sibthorpioides* | 9.35 | 9.66 | 81.82 | 3.70 | 13.36 |
| *Centella asiatica* | 9.15 | 9.46 | 81.82 | 3.70 | 13.16 |
| *Synedrella nodiflora* | 6.90 | 7.13 | 63.64 | 2.88 | 10.01 |
| *Ageratina riparia* | 6.71 | 6.93 | 45.45 | 2.06 | 8.99 |
| *Rostellularia procumbens* | 5.74 | 5.93 | 81.82 | 3.70 | 9.64 |
| *Galinsoga parviflora* | 5.04 | 5.21 | 72.73 | 3.29 | 8.50 |
| *Oxalis corniculata* | 4.97 | 5.13 | 81.82 | 3.70 | 8.83 |
| *Plantago major* | 4.93 | 5.09 | 90.91 | 4.12 | 9.21 |
| *Ageratum conyzoides* | 4.73 | 4.89 | 63.64 | 2.88 | 7.77 |
| *Athyrium javanicum* | 4.46 | 4.61 | 90.91 | 4.12 | 8.72 |
| *Ocimum* sp. | 3.65 | 3.77 | 72.73 | 3.29 | 7.06 |
| *Selaginella opaca* | 3.53 | 3.65 | 54.55 | 2.47 | 6.12 |
| *Drymaria cordata* | 2.64 | 2.73 | 45.45 | 2.06 | 4.78 |
| *Impatiens platypetala* | 2.52 | 2.61 | 27.27 | 1.23 | 3.84 |
| *Polygala paniculata* | 2.33 | 2.40 | 54.55 | 2.47 | 4.87 |
| *Commelina diffusa* | 1.94 | 2.00 | 54.55 | 2.47 | 4.47 |
| *Clidemia hirta* | 1.71 | 1.76 | 36.36 | 1.65 | 3.41 |
| *Artemisia vulgaris* | 1.63 | 1.68 | 72.73 | 3.29 | 4.98 |
| *Taraxacum officinale* | 1.59 | 1.64 | 72.73 | 3.29 | 4.94 |
| *Acmella uliginosa* | 1.40 | 1.44 | 63.64 | 2.88 | 4.32 |
| *Viola pilosa* | 1.20 | 1.24 | 54.55 | 2.47 | 3.71 |
| *Richardia brasiliensis* | 1.05 | 1.08 | 36.36 | 1.65 | 2.73 |
| *Youngia japonica* | 1.01 | 1.04 | 63.64 | 2.88 | 3.92 |
| *Mitracarpus hirtus* | 0.97 | 1.00 | 63.64 | 2.88 | 3.88 |
| *Dichrocephala integrifolia* | 0.89 | 0.92 | 27.27 | 1.23 | 2.16 |
| *Oxalis latifolia* | 0.78 | 0.80 | 54.55 | 2.47 | 3.27 |
| *Desmodium triflorum* | 0.66 | 0.68 | 45.45 | 2.06 | 2.74 |
| *Emilia sonchifolia* | 0.54 | 0.56 | 63.64 | 2.88 | 3.44 |
| *Selaginella belangeri* | 0.43 | 0.44 | 27.27 | 1.23 | 1.68 |
| *Adiantum capillus-veneris* | 0.39 | 0.40 | 18.18 | 0.82 | 1.22 |
| *Pseudelephantopus spicatus* | 0.35 | 0.36 | 18.18 | 0.82 | 1.18 |
| *Maoutia setosa* | 0.31 | 0.32 | 9.09 | 0.41 | 0.73 |
| *Conyza bonariensis* var. *leiotheca* | 0.27 | 0.28 | 36.36 | 1.65 | 1.93 |
| *Hydrocotyle javanica* | 0.27 | 0.28 | 27.27 | 1.23 | 1.52 |
| *Spermacoce articularis* | 0.27 | 0.28 | 27.27 | 1.23 | 1.52 |
| *Oxalis barrelieri* | 0.23 | 0.24 | 27.27 | 1.23 | 1.48 |
| *Dianella javanica* | 0.23 | 0.24 | 27.27 | 1.23 | 1.48 |
| *Rubus rosifolius* | 0.19 | 0.20 | 27.27 | 1.23 | 1.43 |
| *Bidens pilosa* | 0.16 | 0.16 | 27.27 | 1.23 | 1.39 |
| *Acmella ciliata* | 0.16 | 0.16 | 18.18 | 0.82 | 0.98 |
| *Calliandra calothyrsus* | 0.16 | 0.16 | 18.18 | 0.82 | 0.98 |



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|---|---|---|---|---|---|
| *Persicaria nepalensis* | 0.16 | 0.16 | 18.18 | 0.82 | 0.98 |
| *Spermacoce alata* | 0.16 | 0.16 | 18.18 | 0.82 | 0.98 |
| *Paederia foetida* | 0.16 | 0.16 | 9.09 | 0.41 | 0.57 |
| *Lobelia angulata* | 0.12 | 0.12 | 18.18 | 0.82 | 0.94 |
| *Nasturtium officinale* | 0.08 | 0.08 | 9.09 | 0.41 | 0.49 |
| *Mikania micrantha* | 0.08 | 0.08 | 9.09 | 0.41 | 0.49 |
| *Salvia coccinea* | 0.08 | 0.08 | 9.09 | 0.41 | 0.49 |
| *Ficus punctata* | 0.08 | 0.08 | 9.09 | 0.41 | 0.49 |
| *Passiflora ligularis* | 0.08 | 0.08 | 9.09 | 0.41 | 0.49 |
| *Peperomia tetraphylla* | 0.08 | 0.08 | 9.09 | 0.41 | 0.49 |
| *Solanum americanum* | 0.08 | 0.08 | 9.09 | 0.41 | 0.49 |
| *Gnaphalium affine* | 0.04 | 0.04 | 9.09 | 0.41 | 0.45 |
| *Homalanthus populneus* | 0.04 | 0.04 | 9.09 | 0.41 | 0.45 |
| *Sida rhombifolia* | 0.04 | 0.04 | 9.09 | 0.41 | 0.45 |
| *Spermacoce exilis* | 0.04 | 0.04 | 9.09 | 0.41 | 0.45 |
| *Spermacoce laevis* | 0.04 | 0.04 | 9.09 | 0.41 | 0.45 |
| *Elatostema strigosum* | 0.04 | 0.04 | 9.09 | 0.41 | 0.45 |

The most frequent weed species encountered were *Plantago major* and *Athyrium javanicum* with 4.12 value (relative frequency). In term of frequency, the top five weeds were *Plantago major* and *Athyrium javanicum* (4.12); *Hydrocotyle sibthorpioides*, *Centella asiatica*, *Rostellularia procumbens* and *Oxalis corniculata* (3.70); *Galinsoga parviflora*, *Ocimum* sp., *Artemisia vulgaris*, *Taraxacum officinale* (3.29); *Synedrella nodiflora*, *Ageratum conyzoides*, *Acmella uliginosa*, *Youngia japonica*, *Mitracarpus hirtus* and *Emilia sonchifolia* (2.88); *Selaginella opaca*, *Polygala paniculata*, *Commelina diffusa*, *Viola pilosa* and *Oxalis intermedia* (2.47), respectively. Of the 16 member of Compositae family, eight species were on the top five frequencies. This is possible due to the Compositae family way of seed dispersal which can be flown away easily by mean of wind. *Hydrocotyle sibthorpioides* (13.36) and *Centella asiatica* (13.16) counted as the most abundant weed in this survey, followed by *Synedrella nodiflora* (10.02), *Rostellularia procumbens* (9.64), and *Plantago major* (9.21), respectively. These five species were the top five dominant weeds in CBG.

## CONCLUSIONS

Among 58 species recorded, *Hydrocotyle sibthorpioides* and *Centella asiatica* were the most abundant weeds in turgrass blocks surveyed. Further periodic surveys are needed to gain more information on weed dynamics in CBG.

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# WEED MANAGEMENT IN INTEGRATED RICE-BASED CULTIVATION MODELS IN TENGGARONG SEBERANG KUTAI KARTANEGARA, INDONESIA

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**ABSTRACT** An experiment on weed management in rice (*Oryza sativa* L.) - based cultivation integrated with ducks (*Anas* spp.) alabio type, tilapia (*Oreochromis mossambicus* W.K.H Peters), and mungbeans (*Vigna sinensis* L.) was conducted in Tenggarong Seberang, Kutai Kartanegara, Indonesia in 2010. The objectives of this experiment were to (1) determine the effective model of rice cultivation to minimize weed growth, (2) establish rice cultivation models that provide the best optimal results, and (3) determine the economic contribution of ducks, tilapia and mungbeans on the income of farmers when integrated with rice cultivation. Rice cropping models tested were Legowo 20cm x10cm (M1) with 30cm free space, 20cm x 20cm Legowo (M2) with 40cm free space, and 30cm x 30cm SRI (SRI). During the experiment weed traditionally controlled with 'gasrok' or 'landak' at 30, 45, and 60 days after transplanting. The experimental results showed that (1) the average dry weight of weeds at Legowo (M1), Legowo (M2) and SRI 25.63 g m$^{-2}$, 34.27 g m$^{-2}$ and 36.10 g m$^{-2}$, respectively, (2) the highest rice yield obtained at the Legowo models (M1) 5.04 ton ha$^{-1}$, followed by SRI 4.73 ton ha$^{-1}$, and Legowo (M2) 4.61 ton ha$^{-1}$ and (3) the average contribution of ducks, tilapia fish, and mungbeans equivalent to 15.27%, 8.33%, and 10.12% of the rice production when planted in monculture, as the economic returns to the farmer’s income.

***Keywords:*** Rice-based cropping models, weed management, economy.

## INTRODUCTION

Rice is an important food for the Indonesian people, including people in Kutai Kartanegara. Weeds remain a deleterious limiting factor in rice production. As a biotic confounding factor, weed control is important because weeds compete with rice for nutrients, water, and solar radiation, and growing space (Pane and Jatmiko , 2009). Some weed species in transplanted rice include *Eleocharis kuroguwai*, *Sagittaria trifolia*, *S. pygmaea*, *Echinochloa crus-galli*, and *Monochoria vaginalis* (Chul and Goo, 2005). Various attempts have been made to control weeds in rice fields is essentially by enlarging farm inputs, and thus minimize the chance of profit or even detrimental (Moody, 1990). To minimize weed control cost, agronomic practices and farm activities are integrated with the technical culture of rice (Prambudyani and Djufry, 2006). Weed control in technical culture basically not directly aimed at suppressing weed population growth but rather prepare rice for the better field conditions, with the resultant lower weed population. Among the weed control techniques in the technical culture of rice spacing is most easily engineered.

Integrated farming with a variety of commodities in sympatric with each other have been practiced in Indonesia for a long time, however, its existence in terms of supporting the economy of the farmers as well as security and food security are still outside the academic justification. Hossain, *et al*., (2005) reported that the evaluation of the activities carried out in Bangladesh showed that the rice-duck system is not only feasible, but also economically rewarding for the farmers. The yield of rice is, on average, 20% higher in the rice-duck system than the traditional rice system (sole rice), thereby ensuring about 50% higher net return and rice-provisioning ability. The ducks in the rice-duck fields control weeds and insects very effectively; as a consequence, labor and pesticide costs for controlling weeds and insects are likewise minimized and the soil health is improved. Integrated farming is a 'reduction' in the



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principal crop acreage in this case is rice, to provide flexibility for other integrated components in order to live and thrive in the same area. From the point of view and principle of sharing, integrated farming provides additional income if the input commodities possess a more working economic and ecological value, so the key word is the selection of commodities. Integrated agriculture is based on the utilization of agricultural resources optimally so that the maximum benefit farmers in sustainable production systems that integrate technology components according to the capacity of the land.

The present study of rice-based integrated farming was undertaken with the following objectives, viz. (1) To determine the effective models of rice cultivation to minimize weed growth, (2) To establish a rice-based cultivation models which provide the optimal economic returns, and (3) to determine the economic contribution of ducks, tilapia fish and mungbeans to the income of farmers.

## MATERIALS AND METHODS

This experiment was conducted in the village of Karang Tunggal Tenggarong Seberang Kabupaten Kutai, in the first growing season (rainy season) from September up to December 2010 with Randomized Completely Block Design, with six replications and three rice-based models of cropping, comprising Legowo 20cm x 10cm ($M_1$) with 30cm free space , Legowo 20cm x 20cm ($M_2$) with 40cm free space , and 30 x3 0cm SRI (SRI). Rice variety used was Mekongga, fertilized by Urea:SP-36:KCl=150:150:100 kg $ha^{-1}$, and 1 $kgha^{-1}$ by chicken dung manure. Paddy farmers put up with the technology that is Tillage done when a little water using a hand tractor, then fertilized and incubated for a week. Rice seed is sown on a wet seedbed and rice seedlings at 10 days old, were planted. Weed control is done with traditional tools 'gasrok' or 'landak' in 30, 45, and 60 days after transplanting (DAT). Duck was released every day in the field as the first weed control operation at 30 DAT. Duck is species of Alabio on stadia ‘barah’ (one month after the eggs will be maintained) and stocking of Nile Tilapia (size 8-10 cm) one month after transplanting rice with standing water. Mungbeans were planted in the embankment of paddy field along with the time of planting rice. Baby bean fruit harvested 2-3 times during the growth period of rice as a distraction activity of the farmer.

Rice is harvested with 80 % panicles of rice ripening. Harvesting is done gradually adapted to the available workforce. The data collected include weed dry weight, dry weight of grain, farmed ducks, tilapia and results mungbean (Rp). The LSD-test (5%) was used to compared between treatment means.

## RESULTS AND DISCUSSION

Rice-based cultivation models showed a significant effect on weed dry weight at 30, 45, and 60 DAT (Table 1). At 30 DAT the Legowo (M2) model encouraged more growth of weeds compared, albeit fairly small differences, with Legowo (M1) or SRI counterparts. The same patterns of weed infestation were observed at 45 DAT. At 60 DAT SRI registered significantly higher infestation of weeds than Legowo M1 or Legowo M2 planting models.

Rice yield components were measurably affected by the rice planting or spacing models. These were exemplified by significantly higher number of prodcutive tillers in Legowo M2 and SRI planting models *vis-à-vis* the Legowo M1 model. The number of grains/panicle was fairly similar in the three planting models, albeit slightly higher value registered in the SRI model. Interestingly, the Legowo M2 model displayed higher 1000 grain-weight compared with either the Legowo M1 or the Sri planting model. (Table 2). Arguably, these three planting models encouraged freely rice root development, which mimics in rice root development by the rice plants in the available inter-row spaces. Despite these differences in terms of the degree of weed infestation and yield components among the three cropping (planting space), the overall yields were fairly similar (Table 3).

With integration of duck, tilapia and mungbeans with rice cultivation, better retruns to the rice farmers were observed. Based on a simple economic analysis, these returns with the



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integration of ducks, tilapia fish, and mungbeans were 15.27 , 8.33 and 10.12 % higher respectively when compared with rice planted in monoculture devoid of such integration (Table 4).

**Table 1.** Average of weed dry weight at 30, 45, and 60 days after transplanting (DAT)*

| Rice Cropping Models | Weed dry weight ($g.m^{-2}$)(DAT) | | |
|---|---|---|---|
| | 30 | 45 | 60 |
| Legowo (M1) | 17.63b | 22.52b | 25.63c |
| Legowo (M2) | 20.45a | 27.15a | 34.27b |
| SRI (SRI) | 19.50a | 29.50a | 36.10a |
| LSD (5%) | 1.21 | 2.65 | 2.31 |

*Figures followed by the same letter in each column are not significant by LSD (5%)

**Table 3.** Some rice yield components as influenced by planting distance*.

| Rice Cropping Model | Productive tillers | No. grain /panicle | 1000 grains weight |
|---|---|---|---|
| Legowo (M1) | 11c | 104b | 32.58b |
| Legowo (M2) | 28a | 103b | 36.52a |
| SRI (SRI) | 25b | 107a | 32.81b |
| LSD (5%) | 2.92 | 1.06 | 2.04 |

*Figures followed by the same letter in each column are not significant by LSD (5%)

**Table 3.** Yields of rice under rice cropping models.

| Rice Cropping Models | Tonnes/ha* |
|---|---|
| Legowo (M1) | 5.04a |
| Legowo (M2) | 4.61a |
| SRI (SRI) | 4.73a |

*Figures followed by the same letter in the same column means not significant by LSD 5%

**Table 4.** Respective economic returns of incorporation of duck, tilapia fish, and mungbean to rice farm.

| Commodity integrated | % increase* |
|---|---|
| Duck | 15.27 |
| Tilapia Fish | 8.33 |
| Mungbean | 2 |

*When rice planted in monoculture devoid of integration, based on rice price/unit area.

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# CHEMICAL WEED MANAGEMENT OF GROUNDNUT AND ITS RESIDUAL EFFECT ON SUCCEEDING YELLOW SARSON UNDER LATERITIC SOIL OF WEST BENGAL, INDIA

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**ABSTRACT** Groundnut is one of the most important oilseed crops in India. Infestation of weed is considered as one of the major constraints in groundnut production. A field experiment was conducted during wet season of 2012 and winter season of 2012-13 at Agricultural Farm, Institute of Agriculture, Visva-Bharati University, Sriniketan,India to study the effect of chemical weed management on weeds and productivity of groundnut (*Arachis hypogaea* L.) var. ICGV 91114 and its residual effect on succeeding yellow sarson var. B-9. Ten treatments comprising imazethapyr at 50 and 75 g/ha alone and with surfactants Cyspred at 1.0 ml $lit^{-1}$ and Mainboost (Ammonium sulphate) at 2.0 g $lit^{-1}$ each, two doses of imazethapyr at 100 and 125 g $ha^{-1}$ alone, quizalofop-p-ethyl at 50 g $ha^{-1}$, fenoxaprop-p-ethyl at 60 g ai $ha^{-1}$, hand weeding twice (20 and 40 DAS) and unweeded control were laid out in randomized complete block design with three replications. The experimental field was infested with 13 weed species out of which *Cyanotis axilaris* was the most predominant among monocot weeds followed by *Fymbristylis miliacea, Digitaria sanguinalis* and *Cyperus iria* and *Ludwigia parviflora* and *Oldenlandia corymbosa* among broadleaved. Imazethapyr at 75 g $ha^{-1}$ with Cyspred at 1.0 ml $lit^{-1}$ and Mainboost at 2.0 g $lit^{-1}$ recorded lower weed population and dry weight at 45 and 60 DAS and registered higher values of yield attributes as well as higher pod and haulm yields of groundnut. The treatment also gave maximum net returns, return $rupee^{-1}$ invested as compared to other treatments. But all the treatments comprising imazethapyr showed residual toxicity on succeeding yellow sarson resulting poor germination, plant stand, reduced values of yield attributes and very low yield as compared to other treatments, devoid of imazethapyr.

***Keywords:*** Groundnut, weed growth, imazethapyr, residual toxicity, yellow sarson

## INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is the most important oilseed crop in India and accounts for 33% area and 45% production of total oilseeds in the country. Among the groundnut producing states in the country Gujarat is the largest producer contributing about 25 per cent of the total production followed by Tamil Nadu, Andhra Pradesh, Karnataka and Maharashtra. It is also a promising oilseed crop in West Bengal. Groundnut contains on an average 40.1 per cent fat and 25.3 per cent protein and is a rich source of calcium, iron and vitamin B complex like thiamine, riboflavin, niacin and vitamin A. It plays a pivotal role in the oilseed economy of India. Around 75 per cent of the crop is produced in wet season (June-September) and remaining 25 per cent in winter season (November-March). But weed infestation is one of the major constraints that limit the productivity of groundnut especially in wet season. Hot and humid weather and ample moisture available in the soil during this season favour their luxuriant growth, which smothers crop and reduces yield. Groundnut crop suffers from high intensity and heavy infestation of wide spectrum of weeds including grasses, broadleaved and sedges from early stage of crop growth. Weeds compete for resources and reduce the yield of groundnut to varied magnitude depending upon the density and type of weed flora. The yield reduction of groundnut due to weed infestation ranges from 17 to 96 % (Rajendra and Lourduraj, 1999; Pandey *et al.*, 2000; Prasad, 2002 and Jhalal *et al.*, 2005). If the weeds are not kept under control during the critical period, 70-80 per cent yield is lost and even cent per cent crop failure in groundnut may occur



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(Singh and Oswalt, 2005). Besides, pegging of groundnut is inhibited and its harvesting becomes difficult due to weeds. Therefore, weeds also cause damage at later stage and maintaining weed free environment in groundnut is difficult. Timely and effective management of weeds in groundnut has great importance as the crop suffers heavily due to weed competition. Groundnut production in India under the present system of cultivation is costly because of weed. In this perspective, improvement in production of groundnut by adopting low cost, effective technologies suited to resource poor farmers assumes greater importance. Timely weed management by mechanical or manual means is uncertain due to unfavourable soil and climatic conditions in wet season. Therefore use of herbicide has become a necessity to manage weeds. Several herbicides like trifluralin, pendimethalin, alachlor, fluchloralin, etc. are being used for controlling grassy weeds in groundnut. But these have not been found much effective against broad spectrum weeds. Again application of pre-emergence herbicides in rain-fed wet season is uncertain. Post emergence herbicides may play a great role under this situation. So, there is urgent need of new herbicide molecules which could manage all types of weeds effectively and in time. In India, imazethapyr has been reported to give good control of weeds in groundnut when applied as post emergence between 14-20 days after sowing at 1-2 leaf stage of weeds (Singh, 2009 and Dubey *et al.*, 2010). However, residual toxicity of herbicides on the succeeding crops is one of the main considerations in use of chemicals for weed management. But very meagre information is available on the efficacy of this herbicide against weeds in groundnut and its residual effect on succeeding yellow sarson particularly in West Bengal. With this perspective, the present investigation was carried out to study the effect of chemical weed management on weeds and productivity of groundnut and its residual effect on succeeding yellow sarson.

## MATERIALS AND METHODS

A field experiment was conducted at Agricultural Farm, Institute of Agriculture, Visva-Bharati University, Sriniketan, West Bengal, India, during wet season of 2012 and winter season of 2012-13 to study the effect of chemical weed management on weeds and productivity of groundnut (*Arachis hypogaea* L.) var. ICGV 91114 and its residual effect on succeeding yellow sarson var. B-9. The Agricultural Farm is located at the heart of the sub-humid, sub-tropical belt of western part of West Bengal, India and is situated at about $23^0 39'$ N latitude and $87^0 42'$ E longitude with an average altitude of 58.9 m above the mean sea level. The total rainfall received during crop growing season in July, 2012 to February 2013 was 869 mm. The long-term average rainfall was 1022.4 mm. So, the rainfall received during the cropping season of 2012 was slightly lower than the normal rainfall**.** The maximum and minimum temperature varied from 21.48 to 36.23 ºC and 7.13 to 27.09 ºC respectively during the crop growing period. The soil of the experimental site was sandy loam in texture with pH 6.2, low in organic carbon (0.41%), available nitrogen (145.6 kgha$^{-1}$), available phosphorus (25.42 kg ha$^{-1}$), and medium in available potassium (124.85 kg ha$^{-1}$). Ten treatments comprising imazethapyr at 50 and 75 g ha$^{-1}$ alone and with surfactants Cyspred at 1.0 ml lit$^{-1}$ and Mainboost (Ammonium sulphate) at 2.0 g lit$^{-1}$ each, two doses of imazethapyr at 100 and 125 g ha$^{-1}$ alone, quizalofop-p-ethyl at 50 g ha$^{-1}$, fenoxaprop-p-ethyl at 60 g ha$^{-1}$, hand weeding twice (20 and 40 DAS) and unweeded control were laid out in a randomized complete block design with three replications. Groundnut was sown on last week of June. The recommended dose of fertilizers *viz*. 20 kg N, 60 kg $P_2O_5$ and 40 kg $K_2O$ ha$^{-1}$ were applied in the form of diammonium phosphate (DAP), single super phosphate (SSP) and muriate of potash (MOP) respectively, in each plot as basal on the day of sowing. To study the residual effect of herbicides applied in groundnut after its harvest on succeeding yellow sarson, the individual plots were prepared manually without disturbing the original layout. The plot size was 3m × 4m, and the crop was sown at 30 ×10 cm spacing with 100 kg seed rate of groundnut. Yellow sarson crop was grown following the recommended package of practices. Weed population and dry matter of weeds were recorded at 45 and 60 DAS. Weed control efficiency (WCE) of individual treatment and weed index was also



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determined. In succeeding yellow sarson, germination percentage, plant population, yield attributes and yield were also recorded. The economic analysis of each treatment was done on the basis of prevailing market price of inputs used and output obtained under each treatment. The experimental data relating to each character of crop and weed were analyzed statistically by the technique of "Analysis of variance" using MSTAT statistical package in the computer and significance was tested by variance ratio i.e. value at 5% level of significance as described by Gomez and Gomez (2003).

## RESULTS AND DISCUSSION

**Effect on weeds:** The experimental field was infested with 13 weed species, out of which *Cyanotis axilaris* was the most predominant among monocot weeds followed by *Fymbristylis miliacea*, *Digitaria sanguinalis* and *Cyperus iria* and *Ludwigia parviflora* and *Oldenlandia corymbosa* among broadleaved. Imazethapyr at 125 g $ha^{-1}$ registered significantly the lowest number of total weeds at 45 DAS and was statistically at par with that of imazethapyr at 75 g $ha^{-1}$ + Cyspread at 1.0 ml/litre of water + 2.0 g Cyboost/ litre of water, imazethapyr at 75 g $ha^{-1}$, hand weeding twice at 20 and 40 DAS and imazethapyr at 100 g $ha^{-1}$ (Table 1). Imazethapyr at 125 g $ha^{-1}$ also recorded significantly lower dry weight of total weeds but it was at par with imazethapyr at 100 g $ha^{-1}$, 75 g $ha^{-1}$ + Cyspread at 1.0 ml/litre of water + 2.0 g Cyboost/ litre of water and hand weeding twice at 20 and 40 DAS. The highest weed control efficiency (WCE) was registered with imazethapyr at 125 g $ha^{-1}$ closely followed by imazethapyr at 100 g $ha^{-1}$ and imazethapyr at 75 g $ha^{-1}$ + Cyspread at 1.0 ml/litre of water + 2.0 g Cyboost/ litre of water (Table 1). All the weed control treatments recorded more than 75% WCE at 45 DAS except quizalofop-p-ethyl at 50 g $ha^{-1}$ and imazethapyr at 50 g $ha^{-1}$. At 60 DAS weed control efficiency was highest in imazethapyr at 125 g $ha^{-1}$ closely followed by imazethapyr at 100 g $ha^{-1}$ and imazethapyr at 75 g $ha^{-1}$ + Cyspread at 1.0 ml/litre of water + 2.0 g Cyboost/ litre of water. The results are in consonance with Kumar *et al.* (2004), Kushwah and Vyass (2006) and Dubey *et al.* (2010). Application of imazethapyr at 75 g $ha^{-1}$ + Cyspread at 1.0 ml/litre of water + 2.0 g Cyboost/ litre of water ($T_4$), imazethapyr at 100 g $ha^{-1}$ ($T_5$), hand weeding twice at 20 and 40 DAS ($T_9$) and imazethapyr at 125 g $ha^{-1}$ ($T_6$) registered lower values of weed index (Table 1).

**Table 1.** Effect of weed management treatments on weed density, dry weight, weed control efficiency (WCE) and weed index (WI)

| Treatments | Weed density (No. $m^{-2}$) at 45 DAS | Weed dry wt. ($gm^{-2}$) at 45 DAS | WCE (%) | | WI (%) |
|---|---|---|---|---|---|
| | | | 45 DAS | 60 DAS | |
| $T_1$ - Imazethapyr at 50 g $ha^{-1}$ | 38.67 | 2.45 | 74.7 | 17.2 | 51.58 |
| $T_2$ - Imazethapyr at 50 g $ha^{-1}$ + Cyspread at 1.0 ml + Cyboost at 2.0 g $litre^{-1}$ of water | 38.67 | 1.85 | 80.8 | 50.0 | 55.23 |
| $T_3$ - Imazethapyr at 75 g $ha^{-1}$ | 27.67 | 2.32 | 76.0 | 60.4 | 23.46 |
| $T_4$ - Imazethapyr at 75 g $ha^{-1}$ + Cyspread at 1.0 ml + Cyboost at 2.0 g $litre^{-1}$ of water | 23.00 | 1.37 | 85.8 | 82.3 | 0 |
| $T_5$ - Imazethapyr at 100 g $ha^{-1}$ | 30.33 | 1.35 | 86.1 | 82.6 | 4.36 |
| $T_6$ - Imazethapyr at 125 g $ha^{-1}$ | 22.67 | 1.14 | 88.2 | 85.13 | 15.83 |
| $T_7$ - Quizalofop-p-ethyl at 50 g $ha^{-1}$ | 68.00 | 4.05 | 58.2 | 21.6 | 28.16 |
| $T_8$ - Fenoxaprop-p-ethyl at 60 g $ha^{-1}$ | 50.33 | 2.10 | 78.3 | 43.2 | 27.01 |
| $T_9$ - Hand weeding twice at 20 and 40 DAS | 29.67 | 1.82 | 81.2 | 83.5 | 5.42 |



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| | | | | | |
|---|---|---|---|---|---|
| $T_{10}$ - Unweeded check | 126.33 | 8.56 | - | - | 65.06 |
| CD at 5% | 10.82 | 1.05 | - | - | - |
| CV (%) | 13.85 | 22.77 | - | - | - |

**Effect on crop:** The number of pods plant$^{-1}$ (27.7) and seeds pod$^{-1}$ (3.5) of groundnut was highest in the imazethapyr at 75 g ha$^{-1}$ + Cyspread at 1.0 ml/litre of water + 2.0 g Cyboost/ litre of water which were statistically at par with that of imazethapyr at 100 g ha$^{-1}$ and hand weeding twice. The 100 seed weight did not vary significantly. The pod yield, kernel yield and haulm yield were highest (Table 2) in the plot treated with imazethapyr at 75 g ha$^{-1}$ + Cyspread at 1.0 ml/litre of water + 2.0 g Cyboost/ litre of water, which was statistically at par with that of imazethapyr at 100 g ha$^{-1}$ and hand weeding twice at 20 and 40 DAS. Higher harvest index (Table 2) was also registered in these treatments. Imazethapyr at 75 g ha$^{-1}$ + Cyspread at 1.0 ml/litre of water + 2.0 g Cyboost/litre of water gave the highest gross return (Rs. 63,455/ha) and net return (Rs. 40,056/ha) and return rupee$^{-1}$ invested (Rs. 2.71) and was statistically on par with those of imazethapyr at 100 g ha$^{-1}$. Similar results were also reported by Singh (2009) and Bhale *et al.* (2011).

**Effect on succeeding crop:** Various doses of imazethapyr applied in groundnut had adverse residual effect on the growth of the succeeding crop yellow sarson. In general, the plant population of yellow sarson was lower than recommended one. The plant population did not vary significantly in the plots where fenoxaprop-p-ethyl, quizalofop-p-ethyl, two hand weeding and lowest dose of imazethapyr (50 g ha$^{-1}$) were applied in groundnut. However, it was drastically reduced with corresponding increase in dose of imazethapyr and ultimately to a lower level of plant population almost nil in the plots treated with imazethapyr at 125 g ha$^{-1}$.

**Table 2.** Effect of treatments on yield and harvest index (HI) of groundnut

| Treatments | Pod yield (kg ha$^{-1}$) | Kernel yield (kg ha$^{-1}$) | Haulm yield (kg ha$^{-1}$) | HI (%) |
|---|---|---|---|---|
| $T_1$ - Imazethapyr at 50 g ha$^{-1}$ | 1009 | 724 | 2352 | 30.09 |
| $T_2$ - Imazethapyr at 50 g ha$^{-1}$ + Cyspread at 1.0 ml + Cyboost at 2.0 g litre$^{-1}$ of water | 933 | 700 | 2383 | 28.21 |
| $T_3$ - Imazethapyr at 75 g ha$^{-1}$ | 1595 | 1229 | 3597 | 30.67 |
| $T_4$ - Imazethapyr at 75 g ha$^{-1}$ + Cyspread at 1.0 ml + Cyboost at 2.0 g litre$^{-1}$ of water | 2084 | 1596 | 3789 | 35.50 |
| $T_5$ - Imazethapyr at 100 g ha$^{-1}$ | 1993 | 1495 | 3567 | 35.88 |
| $T_6$ - Imazethapyr at 125 g ha$^{-1}$ | 1754 | 1315 | 3583 | 32.89 |
| $T_7$ - Quizalofop-p-ethyl at 50 g ha$^{-1}$ | 1497 | 1123 | 3370 | 30.70 |
| $T_8$ - Fenoxaprop-p-ethyl at 60 g ha$^{-1}$ | 1521 | 1151 | 3333 | 31.36 |
| $T_9$ - Hand weeding twice at 20 and 40 DAS | 1971 | 1380 | 3733 | 34.57 |
| $T_{10}$ - Unweeded check | 728 | 547 | 1237 | 37.18 |
| CD at 5% | 202.5 | 144 | 292.9 | 4.7 |
| CV (%) | 7.82 | 7.48 | 5.52 | 8.4 |

The number of siliquae plant$^{-1}$, number of seeds siliqua$^{-1}$, test weight and seed yield of yellow sarson were drastically reduced with gradual increase in the dose of imazethapyr in groundnut. Imazethapyr at 125 g ha$^{-1}$ in groundnut showed highest residual toxicity on succeeding yellow sarson as evident from the data on yield attributes and yield (Table 3). The yield reduction in the plots with imazethapyr was due to the reason of adverse effect of this herbicide on plant stand and yield attributes which resulted in poor seed yield. This is in



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conformity with Punia *et al.* (2011) who also reported severe injury to mustard caused by applied imazethapyr in preceding crop.

**Table 3.** Residual effect of treatments applied in groundnut on yield components and yield of succeeding yellow sarson

| Treatments | Plant population (No. $m^{-2}$) | No of siliquae $plant^{-1}$ | No of seeds $siliqua^{-1}$ | Test wt. (g) | Seed yield (kg $ha^{-1}$) |
|---|---|---|---|---|---|
| $T_1$ - Imazethapyr at 50 g $ha^{-1}$ | 21.3 | 18.0 | 13.7 | 1.90 | 119 |
| $T_2$ - Imazethapyr at 50 g $ha^{-1}$ + Cyspread at 1.0 ml + Cyboost at 2.0 g $litre^{-1}$ of water | 14.0 | 10.7 | 13.3 | 1.93 | 38 |
| $T_3$ - Imazethapyr at 75 g $ha^{-1}$ | 2.0 | 12.0 | 11.7 | 1.90 | 7 |
| $T_4$ - Imazethapyr at 75 g $ha^{-1}$ + Cyspread at 1.0 ml + Cyboost at 2.0 g $litre^{-1}$ of water | 3.3 | 7.0 | 11.0 | 1.87 | 5 |
| $T_5$ - Imazethapyr at 100 g $ha^{-1}$ | 2.3 | 6.7 | 9.0 | 1.77 | 5 |
| $T_6$ - Imazethapyr at 125 g $ha^{-1}$ | 1.3 | 1.7 | 6.0 | 1.33 | 0.2 |
| $T_7$ - Quizalofop-p-ethyl at 50 g $ha^{-1}$ | 23.3 | 31.3 | 16.7 | 2.73 | 552 |
| $T_8$ - Fenoxaprop-p-ethyl at 60 g $ha^{-1}$ | 22.7 | 32.3 | 15.7 | 2.53 | 561 |
| $T_9$ - Hand weeding twice at 20 and 40 DAS | 24.7 | 35.0 | 17.3 | 3.03 | 577 |
| $T_{10}$ - Unweeded check | 22.7 | 31.3 | 15.3 | 2.77 | 546 |
| S. Em (±) | 1.27 | 1.58 | 1.31 | 0.14 | 10.6 |
| CD at 5% | 3.77 | 4.71 | 2.76 | 0.44 | 31.3 |
| CV (%) | 15.99 | 14.76 | 12.40 | 11.90 | 7.58 |

## CONCLUSIONS

Imazethapyr at 75 g $ha^{-1}$ along with 1.0 ml Cyspread + 2.0 g Cyboost $lit^{-1}$ of water or imazethapyr alone at 100 g $ha^{-1}$ considerably reduced the weed infestation- registering lower weed density, dry weight, weed index, higher weed control efficiency and increased the values of growth attributes and yield of groundnut, which were comparable with two hand weeding and appeared as promising for managing broad spectrum weeds and obtaining higher yield, net return of groundnut in lateritic belt of West Bengal. However, yellow sarson should be avoided as succeeding crop of groundnut treated with the herbicide imazethapyr.

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# EFFECTS OF WEEDING REGIMES ON SEED QUALITY OF SOYBEAN (*GLYCINE MAX* (L) MERILL.) IN AMBON, MALUKU, INDONESIA

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**ABSTRACT** This study aimed at determining the optimum time of weeding on the growth and seed production of soybean. The study was conducted in two phases, consisting of a field experiment, conducted in the village of Passo for three months, and testing of seeds for one week in the greenhouse the Faculty of Agriculture, Pattimura University.The experimental design used was completely randomized design (CRD) with WAP (weeks after planting) per block with treatments of weeding time consisting of PO=No weeding, P1 = Weeding at 2 and 5 WAP, P2 = Weeding at 3 and 6 WAP, P3 = Weeding at 4 and 7 WAP, P4 = Weeding at 5 and 8 WAP. Each treatment was replicated three times. The results of this study indicated that the treatment gave very significant effect on plant height, and significant effects on stem diameter, the number of fitted pods, seed weight per plant, germination ability, germination rate, and in synchrony of seedling growth. Treatment P2 or weeding treatment at 3 and 6 WAP resulted in good weed control and higher soybean seed yield productions. P2 treatment gave highest production and germination rate at 18.8 gram/plant and 99.33%, respectively. The dominant weed species at the beginning of the research was *Cleome viscosa* with SDR value 12.57 and at the end of the research this weed remained dominant at every treatment with average SDR 23.368. At the early stage of this study, the number of weed species found were 29 species, and this increased to 36 species at the end of experimentation..

***Keywords:*** Weed, soybean, seed quality, weeding time

## INTRODUCTION

Soybean (*Glycine max* [L.] Merrill.) is an important crop because of its high economic value. The demand for sobean in Indonesia increases every year such demad exceeds domestic production. The government has to import more than 1.2 million tons in 2008 to meet local consumption (Paripurno, 2008).

Karama and Sumardi (1990) reported that low productivity of soybean in Indonesia was due to low quality of soybean seeds. Only about 23% of farmers planted national standardized varieties of soybean. This is possbily due to the limited supply of soybean seeds, and if the supply is enough, the quality of the seed is relatively low (Nasoetion 1990).

In order to achieve effective weed control, this should be done at the critical period of the crop-weed competition, where the presence of weeds decreases the quantity and quality of soybean seed. The present study was conducted primarily with the objective of assessing the optimum weeding regime and its effect on soybean growth rate and seed production.

## MATERIALS AND METHODS

This research was conducted in two stages; namely, a field experiment in Passo village, District of Teluk Ambon Baguala, and seed test experiment for two weeks in the greenhouse at the Faculty of Agriculture, Pattimura University, Ambon. The whole experimentation was from November 2010 to February 2011.

The soil type is regosol, and soybean variety was Willis. The area of each plot was 2 x 5 m as many as 15 plots. The total area of land used for the research was 200 m2. The weeds were cleared away based on the research aim, pest control was used Dursban 20 EC which the



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dosage was 2 cc/l water, spraying once a week. Average rainfall was 213 mm/month and the average temperature was 28.3 oC.

Planting was done at 40cm x 20cm with two uniform healthy soybean plants per hole. Fertilizer was applied at 25 kg $ha^{-1}$ N; 85 kg $ha^{-1}$ P; dan 65 kg $ha^{-1}$ K. TSP and KCl were administrated a day prior to planting and urea was applied twice when planting and after the plants reached one month old. Planting of soybean seedlings or seeding rate? was done at 40cm x 20cm with two good plants per hole. Fertilizer dose used was 25 kg $ha^{-1}$ N; 85 kg $ha^{-1}$ P; dan 65 kg $ha^{-1}$ K. TSP and KCl were administrated a day prior to planting and urea was applied twice when planting and after the plants reached one month old.

The experimental design used was Complete Randomized. Design where the treatment consisted of five weeding regimes, and each treatment was replicated three times. These treatments were P0 = No weeding; P1 = weeding at 2 and 5 weeks after planting (WAP); P2 = weeding time at 3 and 6 WAP; P3 = weeding time at 4 and 7 (WAP); and P4 = weeding time at 5 and 8 (WAP) . The mathematical model used was : $Yij=\mu+\tau i+\beta j+\varepsilon ij$

$Y_{ij}$ = observation values at the treatment from i and block to j; $\mu$ = average value;
$\tau_i$ = treatment influence from i ; $\beta_j$= block influence from j; $\varepsilon_{ij}$= trial error

The analysis SDR of weed was done in two steps, in the beginning and during the research period. The data obtained were used to calculate the Sum Dominance Ratio (SDR) for each treatment (Tjitrosoedirjo, 1984). Soybean seeds used in this study were stock seed type.

Watering was done twice in the morning and in the afternoon. Weed control was done manually depending on the treatments.

The data were analyzed statistically using ANOVA and treatment means were tested for significance with Least Significant Difference (LSD) test where appropriate.

## RESULTS AND DISCUSSION

**Table 1**. Summary of ANOVA for All Observed Parameters

| Observed Parameter | Weeding Period | Observed Parameter | Weeding Period |
|---|---|---|---|
| **Growth** | | **Seed Quality** | |
| a. Plant height | ** | 1. Viability | * |
| b. Numbers of branches | ns | a. Germinating ability | * |
| c. Stem diameter | * | b. Germinating rate | |
| **Production** | | 2. Vigor | |
| a. Weight of 100 seeds | ns | a. Germinating uniformity | ns |
| b. Numbers of beans | * | b. Growth rate | * |
| c. Numbers of filled beans | ns | | |
| d. Weight of seedling | * | | |

** = significant at 1% level, * = Significant at 5% level, ns = not significant

**Tabel 2. Results of LSD test for research parameters**

| Parameters | Treatments | | | | | (*p*= 0.05) |
|---|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | P4 | |
| Plant Height (cm) | 93.423 a | 82.967 b | 81.453 b | 77.557 b | 75.543 b | 8.103 |
| Numbers of Branches | 3.467 b | 5.053 a | 4.910 a | 5.240 a | 4.500 ab | 1.395 |
| Stem Diameter (cm) | 0.243 b | 0.363 ab | 0.477 a | 0.2567 b | 0.370 a | 0.128 |
| Seed Weight per Plant (g) | 13.503 b | 14.660 b | 18.800 a | 16.290 ab | 15.120 b | 2.892 |
| Weight of 100 Seeds (g) | 12.860 b | 13.110 ab | 13.193ab | 13.157 ab | 13.630 a | 0.626 |



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| | | | | | | |
|---|---|---|---|---|---|---|
| Numbers of Filled Bean | 39.367 bc | 59.090 ab | 66.653 a | 51.807 b | 61.807 ab | 14.479 |
| Numbers of Empty Bean | 2.943 a | 4.833 a | 3.937 a | 5.880 a | 5.133 a | 3.802 |
| Germinating Ability (%) | 95.333 b | 96.667 ab | 99.333 a | 94.000 b | 97.333 ab | 3.506 |
| Germinating Rate | 3.567ab | 3.727 ab | 3.367 b | 3.347 b | 4.107a | 0.720 |
| Germinanting Uniformity (%) | 48.667 a | 40.000 a | 30.000 a | 42.000 a | 36.667 a | 21.649 |
| Growth Rate (%) | 25.257 b | 26.960 ab | 29.267 a | 25.890 b | 26.347 b | 2.857 |

*Figures followed by the small letters in the same row are not siginificantly at the level of 5% (LSD tests)

**Table 3.** The sum dominant ratio [SDR] values of weed species accurding to treatments.

| No | Weed Species | SDR of the Treatment | | | | | | | | | SDR * |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P0 | P1 | | P2 | | P3 | | P4 | | |
| | | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | |
| 1 | *Mimosa pudica* | 7.54 | 2.81 | 2.29 | - | 8.62 | - | 12.56 | 6.90 | 10.93 | 6.05 |
| 2 | *Cyperus tenuiculmis* | | - | - | - | - | - | - | - | - | 13.35 |
| 3 | *C. rotundus* | 12.02 | 8.40 | 3.48 | 12.85 | 8.05 | 15.59 | 11.27 | - | - | 1.16 |
| 4 | *C. distans* | | - | - | - | - | - | - | - | - | 1.06 |
| 5 | *C. brevifiolus* | 11.70 | 20.60 | 8.88 | 20.50 | 17.35 | 20.69 | 14.42 | 10.77 | 12.28 | 5.06 |
| 6 | *C. compressus* | 4.84 | 3.46 | 7.43 | - | 7.50 | - | - | 15.98 | 12.25 | 1.36 |
| 7 | *C. kyllinga* | | - | - | - | - | - | - | - | - | 1.68 |
| 8 | *C. spechalatos* | | - | - | - | - | - | - | - | - | 5.02 |
| 9 | *C. flavidius* | | 10.71 | 5.99 | - | - | - | - | - | - | 1.26 |
| 10 | *C. elatus* | | - | - | - | - | - | - | - | - | 4.39 |
| 11 | *Synedrella nodiflora* | | - | - | - | - | - | - | - | - | 4.72 |
| 12 | *Ageratum conyzoides* | | - | - | - | - | - | - | - | - | 1.41 |
| 13 | *Phyllanthus debilis* | 6.51 | 5.93 | 3.98 | 10.65 | 10.10 | 8.69 | 11.67 | 8.61 | 14.89 | 6.63 |
| 14 | *Scoparia dulcis* | | - | - | - | - | - | - | - | - | 0.92 |
| 15 | *Corton hirtus* | 10.99 | - | 2.42 | - | - | 3.94 | 8.75 | 4.63 | 7.54 | 9.41 |
| 16 | *Ischaemum tintensis* | | - | - | - | - | - | - | - | - | 0.89 |
| 17 | *Leucas lavandulaefolia* | 3.33 | - | 5.72 | - | - | 1.63 | - | 9.90 | 3.15 | 7.95 |
| 18 | *Euphorbia prunifolia* | 6.93 | 6.95 | 9.88 | 14.62 | 11.46 | - | - | - | - | 6.86 |
| 19 | *Ludwiqia parennis* | | 5.79 | 2.87 | - | - | 3.51 | - | - | - | 1.24 |
| 20 | *Pasapalum conjugatum* | | - | - | - | - | - | - | - | - | 1.36 |
| 21 | *Eleusine indica* | 4.10 | - | 7.57 | - | - | 1.99 | - | 7.10 | 5.56 | 3.35 |
| 22 | *Amaranthus spinosus* | | - | - | - | - | - | - | - | - | 1.18 |
| 23 | *Murchannia spirata* | | - | - | - | - | - | - | - | - | 1.05 |



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| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | *Stachythopyta indica* | | - | - | - | - | - | - | - | - | 2.49 |
| 25 | *Artanema longiflora* | | 2.01 | - | - | - | 5.79 | - | - | - | 1.07 |
| 26 | *Imperata cylindrical* | | - | - | - | - | - | - | - | - | 5.39 |
| 27 | *Fimbristis milliceae* | | - | - | - | - | - | | - | - | 0.93 |
| 28 | *Digitaria longiflora* | | - | - | - | - | 1.99 | - | - | - | 1.45 |
| 29 | *Commelina diffusa* | 3.30 | 6.22 | 2.57 | 10.03 | 8.83 | 5.01 | 7.53 | - | - | 1.40 |
| 30 | *Boreria alata* | 10.38 | - | 8.77 | - | 7.96 | 6.09 | 10.37 | 4.15 | 10.84 | - |
| 31 | *Boreria distans* | - | - | 6.26 | - | - | - | - | - | - | - |
| 32 | *Cleome viscose* | 12.58 | | | | | | | | | - |
| 33 | *Ipomea triloba* | 5.94 | 2.81 | - | - | - | 1.53 | 32.17 | - | - | - |
| 34 | *Digitaria ciliaris* | - | - | 3.44 | 2.71 | - | - | - | 2.1 | - | - |
| 35 | *Melochia corconifolia* | - | 2.01 | - | - | - | - | - | - | - | - |
| 36 | *Echinochloa colonus* | - | - | - | - | - | - | - | 1.82 | - | - |
| T O T A L | | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |

*n=13 plots

Table 2 shows that treatment P0 (no weeding) gave the highest value for plant height (93.423 cm). Plant density with weed caused the plant undergo elongation. In treatment P0 (3.4667) and P4 (4.5000), numbers of branches were less than those of treatments P3, P2, and P1, which were not different, however, these treatment did not affect numbers of branches.

Table 2 shows that treatment significantly effected the numbers of filled beans and seed weight per plant but was not significant on the weight of 100 seeds and the numbers of empty beans. Treatment P2 and P3 had high numbers of beans, thus, weight of seed per plant was 18,800 g and 16.290 g, respectively. Weeding treatment was not significant on the weight of 100 seeds. Treatment P0 and P3 gave less filled beans than P1, P2, and P4 did, where weed density was low, therefore, nutrients, water and light dan be absorbed well. Low light intensity decreases photosynthesis rate, which affects the formation (filling) of beans and seeds (Jumin, 2002).

Table 2 shows that treatment P2, P4, and P1 gave high percentage of germinating ability but were not different from P0 and P3. The highest germinating rates were obtained by treatment P4 (4.107) and followed by P1 (3.727), P0 (3.567), P2 (3.367), and P3 (3.347).

According to Kuswanto (1996) it is prudent to know seed quality aside from viability test, hence it is necessary to do vigor test with respect to germinating uniformity and seedling growth rate. Sadjad (1993) explained that vigor indicates good seeds grow in sub-optimum condition and resist to be stored in non-ideal condition.

Table 3 shows that in intial vegetation analysis on the onset of the experiment, this was about 29 weed species and after treatment, the number of species increased to 36 species. This is related probably to the succession of weeds or species shifts occurring, possbily due to soil cultivation.

## CONCLUSIONS AND RECOMENDATION

Based on research results, it can be concluded that treatment P2 at 3 and 6 weeks after planting could increase production and quality of soybean seeds. To improve the production of good quality soybeens seeds, weed control should be done twice at the third and sixth week after



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planting. The next research should also be conducted under field conditions infested by other weeds such as grasses or sedges.

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## *Innovative Technology*

# DNA BARCODE OF THE AGRICULTURAL WEEDS OF KOREAN PANICOIDEAE, POACEAE

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## INTRODUCTION

Poaceae Barnhart including approximately 700 genera and 11,000 species in all regions of the world is the fourth largest plant family after the Asteraceae, Fabaceae, and Orchidaceae (Chen et al., 2006). Korean Poaceae, a family of economic importance, includes the major weeds of Korean agriculture. It is described with six subfamilies, 19 tribes, 96 genera, and 252 taxa. Of Korean Poaceae, subfamily Panicoideae contains some of problematic weedy grasses.

It is of importance to be able to detect quickly for effective control of the weeds, especially when invasive species occurred. DNA barcoding is a powerful tool to identify for morphologically difficult plants. This study was, therefore, conducted to make the DNA barcode references of major Panicoideae weeds in Korean Agriculture.

## MATERIALS AND METHODS

133 individuals of 37 taxa across 22 genera of Korean Panicoideae were submitted for sequencing of the cpDNA *rcb*L and *mat*K regions and nrDNA ITS region. We chose more than three individuals of each taxon captured from the six different provincial regions of Korea. The sequences were aligned using clustalW and Neighbor-joining trees were constructed in PAUP* 4.0b 10 (Swofford, 1998). The pairwise sequences divergences were calculated and interspecific and intraspecific sequence divergences for each barcode region were estimated using the MEGA 5.2

## RESULTS AND DISCUSSION

Among the samples attempted for analysis, successful sequences amounted to 92.5% (124 individuals) for *rbc*L, 85.6% (116 individuals) for *mat*K and 90.3% (121 individuals) for ITS. No sequence variation among the taxa was observed in the 22, 23, and 15 taxa for *rbc*L, *mat*K, and ITS, respectively. Of the remaining sequenced taxa, pairwise divergence within taxa was varied from 0 to 1.1% for *rbc*L, from 0.01 to 7.9% for *mat*K and from 0 to 4.3% for ITS. The pairwise divergence between taxa were 0~3.9% for *rbc*L, 0~7.2% for *mat*K, and 0~25.4% for ITS. In the genera such as *Digitaria*, and *Penicum* the mixed clustering was observed in the neighbor-joining trees of all examining regions. The phylogenetic relationship of these genera should be better understood by the morphological study. All other genera were clustered together. DNA barcode could be one of powerful techniques for identifying difficult species such as Poaceae. All vouchers were deposited in HCCN and the barcodes were submitted to GenBank.

**Table 1**. The name of taxa and the number of samples used in this barcode study.

| Taxa name | No. of samples | Taxa name | No. of samples |
|---|---|---|---|
| *Arthraxon hispidus* | 4 | *Oplismenus undulatifolius* | 3 |
| *Arundinella hirta* | 5 | *Panicum bisulcatum* | 5 |
| *Cymbopogon goeringii* | 3 | *Panicum dichotomiflorum* | 4 |
| *Digitaria ciliaris* | 5 | *Paspalum dilatatum* | 3 |



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| | | | |
|---|---|---|---|
| *Digitaria radicosa* | 3 | *Paspalum distichum* var. *indutum* | 3 |
| *Digitaria violascens* | 5 | *Paspalum thunbergii* | 5 |
| *Echinochloa crus-galli* var. *crus-galli* | 6 | *Pennisetum alopecuroides* | 3 |
| *Echinochloa crus-galli* var. *echinata* | 4 | *Pseudoraphis sordida* | 2 |
| *Echinochloa oryzicola* | 5 | *Sacciolepis indica* | 2 |
| *Eriochloa villosa* | 5 | *Schizachyrium brevifolium* | 2 |
| *Hemarthria sibirica* | 3 | *Setaria chondrachne* | 3 |
| *Imperata cylindrica* | 3 | *Setaria faberi* | 5 |
| *Isachne globosa* | 2 | *Setaria pumila* | 8 |
| *Ischaemum anthephoroides* | 3 | *Setaria viridis* var. *pachystachys* | 1 |
| *Ischaemum aristatum* | 2 | *Setaria viridis* var. *viridis* | 5 |
| *Microstegium japonicum* | 2 | *Sorghum halepense* | 3 |
| *Microstegium vimineum* | 3 | *Spodiopogon cotulifer* | 4 |
| *Miscanthus sacchariflorus* | 2 | *Themeda triandra* | 4 |
| *Miscanthus sinensis* var. *purpurascens* | 3 | | |

**Table 2.** Pairwise sequence divergence among species and between species.

| | Interspecific | Intraspecific |
|---|---|---|
| *rbc*L | 0~3.9% | 0~1.1% |
| *mat*K | 0~7.2% | 0.01~7.9% |
| ITS | 0~25.4% | 0~4.3% |



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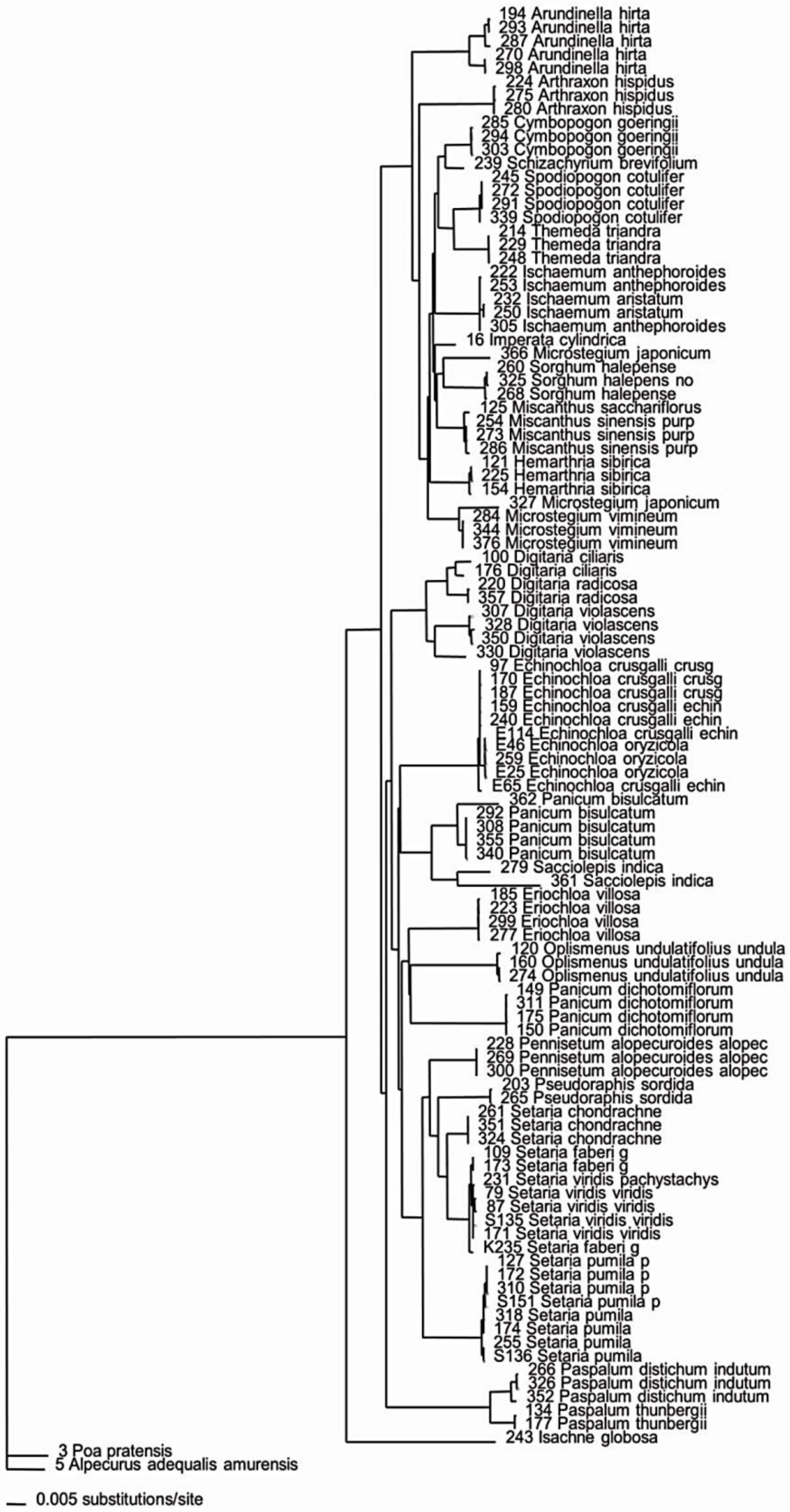


**Fig. 1.** Neighbor-joining tree inferred from the analysis of *rbc*L+matK+ITS from Panicoideae. Branch lengths are proportional to distances estimated from the two-parameter method of KIMURA. *Poa pratensis* and *Alpecurus adequalis* var. *anurensis* are outgroups. The genus *Panicum* seemed not monophyletic.



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# LOW MOLECULAR PEPTIDE-BASED BIOMARKER DISCOVERY FOR CLASSIFICATION OF BARNYARDGRASS SPECIES USING SURFACE ENHANCED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY

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**ABSTRACT** Biomarker of barnyard grass species was important for classification of wild type weed species. We identified some peptide-based biomarkers for barnyard grass (*Echinochloa* spp.) using SELDI-TOF, a mass spectrometry (MS) technique. The proteomic profiling using SELDI-TOF MS techniques could be a useful and powerful tool to discover peptide biomarker for discrimination and to assess weed species, especially under 20 kDa.

In twelve barnyard grass species, 85 and 62 peptides were detected on CM10 (strong anion exchanger) and Q10 (weak cation exchanger) arrays, respectively. Sixteen peptides of CM10 and 12 peptides of Q10 were selected as peptide bio-markers. We have also found that the hierarchical heat map analysis from peptide based biomarkers has clearly coincided with the simple cluster analysis from SDS-PAGE.

***Keywords:*** Barnyard grass, low molecular peptide, biomarker, proteomic analysis, SELDI

## INTRODUCTION

Although two-dimensional gel electrophoresis can detect thousand of proteins, it dese not well represent low-molecular-mass proteins (< 10 kDa) (Giobanni et al., 2010). But surface-enhanced laser desorption ionization time of-flight mass spectrometry (SELDI-TOF MS) is effective at profiling of low molecular weight proteins (< 20 kDa) and requires smaller amounts of sample (Issaq et al, 2002). Because of its simplicity, high throughput capacity and relatively low cost, SELDI-TOF MS is widely studied for discovery of diagnostic markers in medical ailments such as asthma, breast, ovarian cancer and urinary (Altamura et al., 2009; Zhang et al., 2006). But SELDI-TOF MS was not commonly used in study of other field. In the field of plant science, very few reports have been published. It has been demonstrated that the proteomic profiling using SELDI-TOF MS is useful and powerful to discover of biomarkers of cereal crop (Lee, 2011; Park et al., 2013). The objectives of this study are low molecular weight protein profiling and biomarker discovery for identification of cultivars in different barnyard grass seeds using SELDI-TOF MS.

## MATERIALS AND METHODS

Twelve wild barnyard grass seeds (*Echinochloa* spp.) were collected in Korean Peninsula or obtained from Seed Bank of Wild Plant Resources in Korea University, Korea.

**Sample Preparation** For the preparation of total protein soluble protein, 100 mg barnyard grass flour was suspended in 1 ml of extraction buffer [1.25 mM Sodium Borate, pH 10.0, 2% (v/v) 2-mercaptoethanol, 1% (v/v) Triton X-100] mixed on a shaker within an ice box for 1 hr before being centrifuged at 12,000 x g for min. Total soluble protein content of the extract



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was quantified by the Bradford assay (Marion, 1976). Protein extract was used immediately in SELDI-TOF MS analysis.

**Surface Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Peptide Profiling**

**Protein Chip Array Processing**: Two kinds of ProteinChip arrays were conducted with; Q10 (strong anion exchanger) and CM10 (weak cation exchanger) arrays (Bio-Rad Laboratories). Protein samples (1 mg / ml) were diluted by 1:10 with binding/washing buffer. One hundred ( ㎕) of binding solution was added to each well and incubated at room temperature for 5 min with vigorous shaking (250 rpm). This procedure was repeated once. After the second incubation, the buffer was removed and 100 ㎕ of each sample was added to individual well. Samples were incubated at room temperature with vigorous shaking for 30 min. Sample solutions were removed and the wells were washed three times with 200 ㎕ binding for 5 min. with agitation. After washing, wells were washed three time with 200 ㎕ distilled water. The arrays were air-dried for 20 min, and the 1 ㎕ of CHCA ProteinChip energy absorbing molecule (EAM) solution was added to each well. Additional 1 ㎕ of EAM was added to each well. The finally the array surfaces were allowed to dry completely before SELDI-TOF MS.

**Statistical Analysis** ProteinChip Data Manager Software was used for statistical analysis (Bio-Rad Laboratories). Once the peaks were clustered, univariate analysis with a nonparametric test was performed, calculating the *p*-values associated with each cluster (and using the Kruskal-Wallis test for comparision of the twelve barnyardgrass cultivars). Peaks with a $p$-value $< 0.01$ were examined to evaluate their potential as biomarkers. Multivariate analyses with a supervised hierarchical heat map and principal component analysis (PCA) were performed with peaks ($p$-value $< 0.000001$) selected following univariated analysis.

## RESULTS AND DISCUSSION

Peptide profiles using SELDI-TOF MS in twelve barnyard grass species, 85 and 62 peptides were detected on CM 10 and Q 10 arrays, respectively. The twelve barnyard grass species are divided into two different groups by quantitatively differential expression of peptide peaks.

One group included

① *Echinochloa crus-galli* (L.) var. *Oryzicola* (Vasing) Ohwi
② *Echinochloa crus-galli* (L.) var. *Echinata* (Wild). Honda
③ *Echinochloa colonum* (L.) Link
④ *Echinochloa crus-galli* (L.) P. Beauv var. *praticola* Ohwi
⑤ *Echinochloa crus-galli* (L.) P. Beauv. Var. *frumemtacea* (Roxb.) Wight

and the other group included

① *Echinochloa glabrescens* Munro ex Hook. F. & Kossnko
② *Echinochloa crus-galli* (L.) Beauv.
③ *Echinochloa utilis* Ohwi & Yabuno
④ *Echinochloa utilis* Ohwi & Yabuno (awnless)
⑤ *Echinochloa crus-galli* (L.) P. Beauv. Var. *frumemtacea* (Roxb.) Wight
⑥ *Echinochloa crus-galli* (L.) P. Beauv. Var. *frumemtacea* (Roxb.) Wight (awnless)
⑦ *Echinochloa crus-galli* (L.) P. Beauv. Var. *frumemtacea* (Roxb.) Wight (awn).



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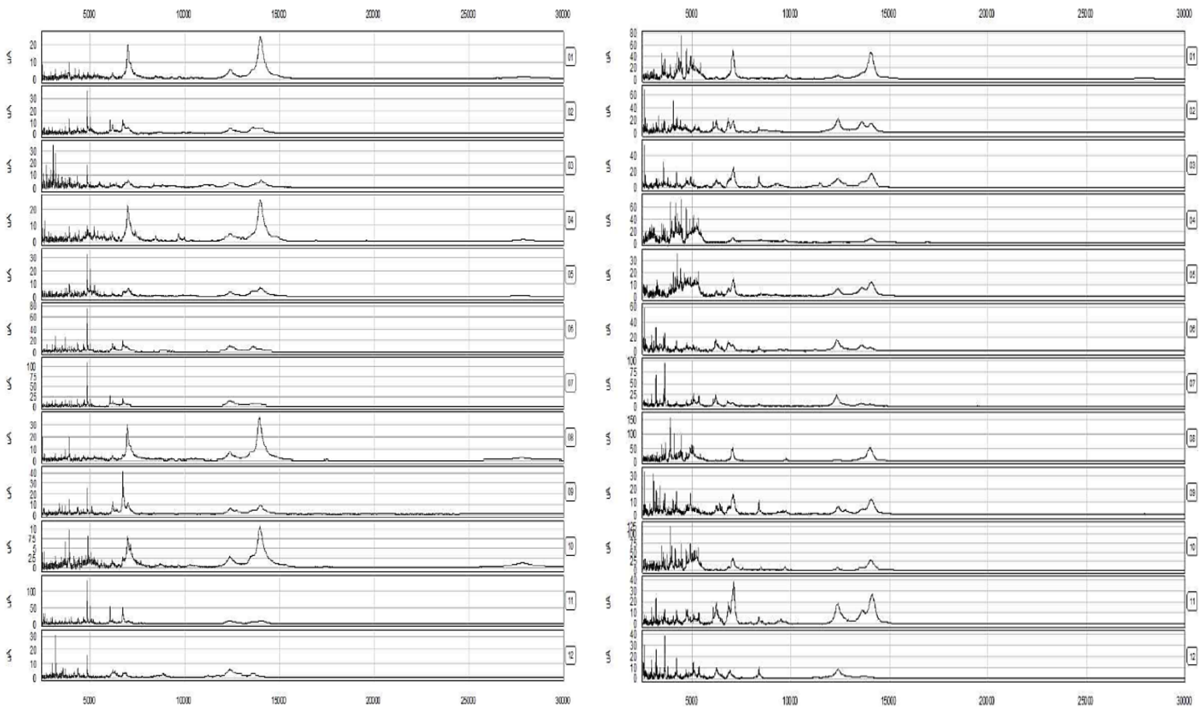
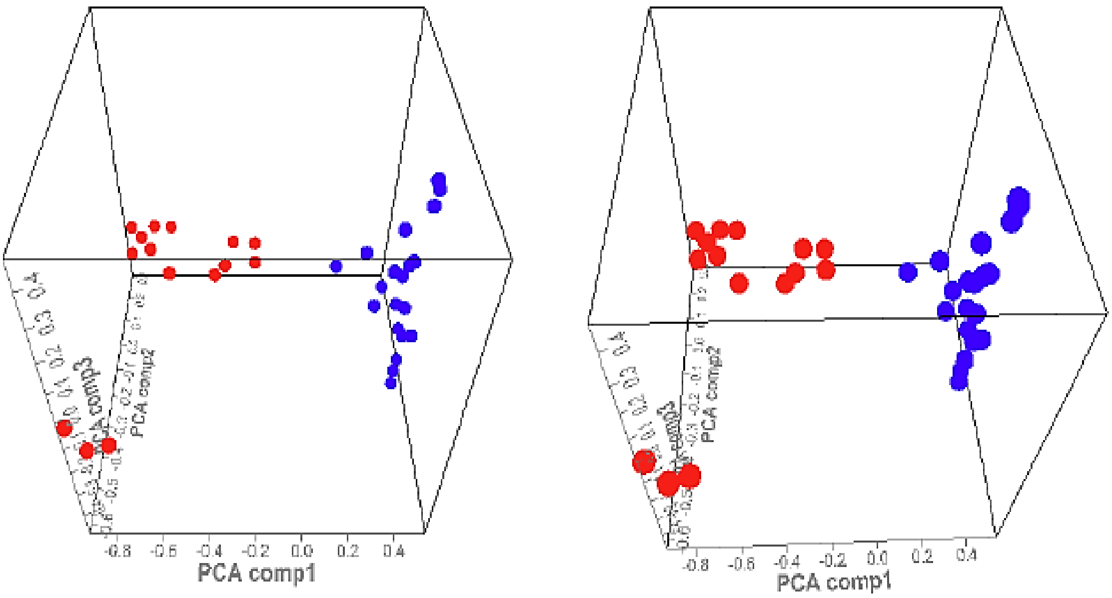
Moreover, 16 Peptide of CM 10 and 12 peptide of Q10 were selected as peptide bio-markers. The proteomic profiling using SELDI-TOF MS techniques could be a useful and powerful tool to discover peptide biomarker for discrimination and assess crop species, especially under 20 kDa.



CM 10 Q 10

**Fig. 1.** SELDI-TOF MS spectra on CM 10 and Q 10 protein chips barnyard grass species.




CM 10 Q 10

**Fig. 2.** Principle component analysis using significantly different peak clusters detected at CM 10 and Q 10 among barnyard grass at $p < 0.000001$ (red, Ⅰ group; blue, Ⅱ group).



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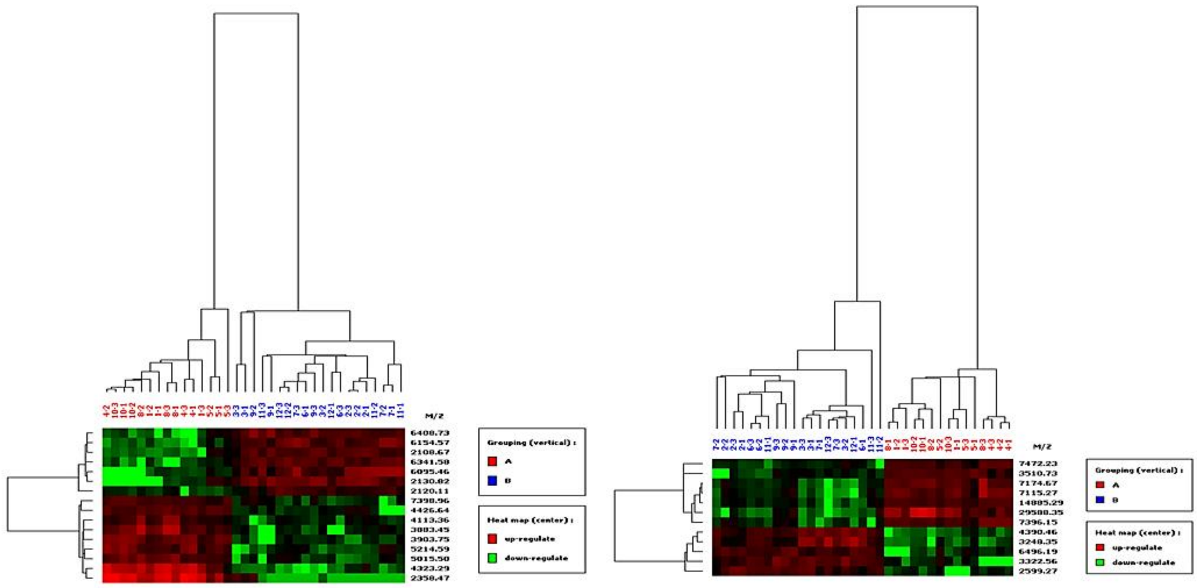

CM 10 Q 10

**Fig. 3.** Supervised hierarchical clustering and heat map using significantly different peaks on CM 10 and Q 10 of SELDI-TOF MS at $p < 0.000001$ in *Echinochloa* spp.

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# RICER™ 60OD: A NOVEL PENOXSULAM + CYHALOFOP-BUTYL FORMULATION FOR USE IN DIRECT-SEEDED RICE IN CHINA

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**ABSTRACT** Ricer™ 60OD is a pre-mix rice herbicide product containing 10 g ai penoxsulam + 50 g ai cyhalofop-butyl/liter. Penoxsulam, a triazolopyrimidine sulfonamide herbicide, is sold in many countries for broad-spectrum control of broadleaf weeds, sedges and some grasses in rice. Cyhalofop-butyl, an aryloxyphenoxy propionate herbicide, is used globally for controlling grass weeds in rice. In China, Ricer™ 60OD efficacy and crop tolerance field trials were conducted in direct-seeded rice from 2005 to 2011 and results demonstrated good to excellent control of many important weed in rice. Ricer™ 60OD at 2.25 and 2.5 L pr/ha (22.5 + 169 and 25 + 188 g ai/ha, penoxsulam + cyhalofop-butyl, respectively) as a post-emergence foliar application at 10 to 15 days after rice seeding provided excellent control of barnyardgrass (*Echinochloa crus-galli)*, Chinese sprangletop (*Leptochloa chinensis*), knot grass (*Paspalum distichum*), annual sedge (*Cyperus difformis/C. iria*), bulrush (*Scirpus juncoides*), monochoria (*Monochoria vaginalis/M. korsakowii*), annual arrowhead (*Sagittaria* spp), water plantain (*Alisma plantago-aquatica*) and Indian rotala (*Rotala indica*). Ricer™ 60OD provided 2 to 4 weeks residual weed control and demonstrated no visual phytotoxicity in direct-seeded rice. This product is being registered in China from 2012 and will be used in transplanted and nursery rice markets in 2014.

***Keywords:*** Penoxsulam, cyhalofop-butyl, direct-seeded rice, transplanted rice, nursery rice, grasses, sedges, broadleaf weeds.

## INTRODUCTION

This report was summarized on Ricer™ 60OD development in directed-seeded rice trials in China from 2006 to 2011. Penoxsulam, a triazolopyrimidine sulfonamide herbicide, is sold in many countries for broad-spectrum control of broadleaf weeds and some grasses in rice. Cyhalofop-butyl, an aryloxyphenoxy propionate herbicide, is used globally for controlling grass weeds in rice. In China, Ricer™ 60OD efficacy and crop tolerance field trials were conducted in direct-seeded rice from 2005 to 2011 and results demonstrated good to excellent control of many important rice weeds. This product is being registered in China for use in transplanted and nursery rice in 2012."

## MATERIALS AND METHODS

Ricer™ 60OD was conducted for field trials started in China from 2006 (**Table 1**). Official trials of Ricer™ 60OD for product registration were started in Anhui, Hubei, Hunan, Jiangsu and Shanghai in 2007 and completed in the same locations in 2008.

**Table 1.** Field trials information of Ricer™ 60OD development in China in 2006-2011.

| Year | Protocol Number | Trial Number | Trial Location |
|---|---|---|---|
| 2006 | PA06E9A024 | CN06H031 | Shanghai |
| 2006 | PA06E9A024 | CN06H032 | Shanghai |
| 2007 | PA07R8E001 | CN07H014 | Anhui |
| 2007 | PA07R8E001 | CN07H015 | Hubei |



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| | | | |
|---|---|---|---|
| 2007 | PA07R8E001 | CN07H016 | Hunan |
| 2007 | PA07R8E001 | CN07H017 | Jiangsu |
| 2007 | PA07R8E001 | CN07H018 | Shanghai |
| 2008 | PA08R8E002 | CN08H018-R8E-OT-AH | Anhui |
| 2008 | PA08R8E002 | CN08H019-R8E-OT-SH | Shanghai |
| 2008 | PA08R8E002 | CN08H020-R8E-OT-JS | Jiangsu |
| 2008 | PA08R8E002 | CN08H021-R8E-OT-HN | Hunan |
| 2008 | PA08R8E002 | CN08H022-R8E-OT-HB | Hubei |
| 2009 | PA09R8E001 | IM09R8E-DIGSA-RICE-JS-CN | Jiangsu |
| 2009 | PA09R8E001 | IM09R8E-ELEIN-RICE-JS-CN | Jiangsu |
| 2010 | PA10E9A018 | WM10E9A-TTTTT-RICE-HLJ-2LF | Heilongjiang |
| 2010 | PA10E9A018 | WM10E9A-TTTTT-RICE-HLJ-4LF | Heilongjiang |
| 2010 | PA10E9A018 | WM10E9A-TTTTT-RICE-HUN-CN-2LF | Hunan |
| 2010 | PA10E9A018 | WM10E9A-TTTTT-RICE-HUN-CN-4LF | Hunan |
| 2010 | PA10E9A018 | WM10E9A-TTTTT-RICE-JS-CN-2LF | Jiangsu |
| 2010 | PA10E9A018 | WM10E9A-TTTTT-RICE-JS-CN-4LF | Jiangsu |
| 2010 | PA10E9A018 | WM10E9A-TTTTT-RICE-ZJ-CN-2LF | Zhejiang |
| 2010 | PA10E9A018 | WM10E9A-TTTTT-RICE-ZJ-CN-4LF | Zhejiang |
| 2010 | PA10J4A003 | WM10J4A-GRASS-RICE-HUN-CN | Hunan |
| 2010 | PA10J4A003 | WM10J4A-GRASS-RICE-JS-CN | Jiangsu |
| 2010 | PA10J4A003 | WM10J4A-GRASS-RICE-ZJ-CN | Zhejiang |
| 2010 | PA10R8E003 | PA10R8E003-2 | Anhui |
| 2011 | PA11R8E001 | CN11H008-DE-638C | Jiangsu |
| 2011 | PA11R8E001 | CN11H009-DE-638C | Jiangsu |

## RESULTS AND DISCUSSION

Ricer™ 60OD was pre-mixed by penoxsulam and cyhalofop-butyl which both chemical structures were listed below (**Figs. 1** and **2**). Penoxsulam inhibits the plant enzyme acetolactate synthase (ALS) which is essential for the synthesis of branched-chain amino acids valine, lecine and isoleucine. Inhibition of amino acid production subsequently inhibits cell division. Cyhalofop-butyl Inhibits acetyl CoA carboxylase enzyme (ACCase) which is selective inhibitor of fatty acid biosynthesis, specifically inhibiting the first committed step in fatty acids biosynthesis.

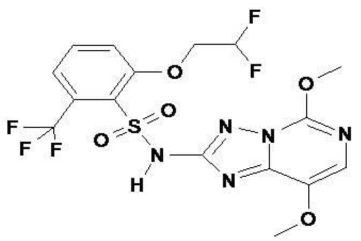

**Fig. 1**. Penoxsulam: 2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5c] pyrimidin-2-yl)-6-(trifluoromethyl) benzene sulfonamide.



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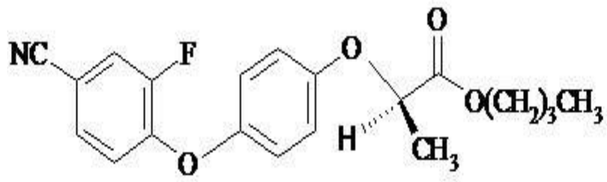

**Fig. 2**. Cyhalofop-butyl: 2-(4-(4-cyano-2-fluorophenoxy)phenoxy)propanoic acid, butyl ester, (R).

Ricer™ 60OD demonstrates as typical ALS and ACCase inhibitor herbicide that shows immediate growth inhibition of susceptible plants and a chlorotic growing point with some vein reddening. Ricer™ 60OD at 2.25 to 2.5 L pr/ha (22.5 to 25 + 169 to 188 g ai/ha, penoxsulam + cyhalofop-butyl, respectively) as a post-emergence foliar application at 10 to 15 days after rice seeding provided excellent control of barnyardgrass (*Echinochloa crus-galli*, ECHCG*)*, Chinese sprangletop (*Leptochloa chinensis*, LEFCH), knot grass (*Paspalum distichum*), annual sedge (*Cyperus difformis/C. iria*, CYPDI), bulrush (*Scirpus juncoides*), monochoria (*Monochoria vaginalis/M. korsakowii*, MOOVA), annual arrowhead (*Sagittaria* spp), water plantain (*Alisma plantago-aquatica*) and Indian rotala (*Rotala indica*). Ricer™ 60OD provided 2 to 4 weeks residual weed control and demonstrated no visual phytotoxicity in direct-seeded rice.

For barnyardgrass (ECHCG) control, Rice™ 60OD was needed at 120 gai/ha for competitive efficacy that there were 74.0% frequency if a weed control level at >95% (**Fig. 3**). When Rice™ 60OD was on Chinese sprangletop (LEFCH), it was needed at 90 gai/ha. It had 82.5% frequency if a weed control level at >90% (**Fig. 4**).

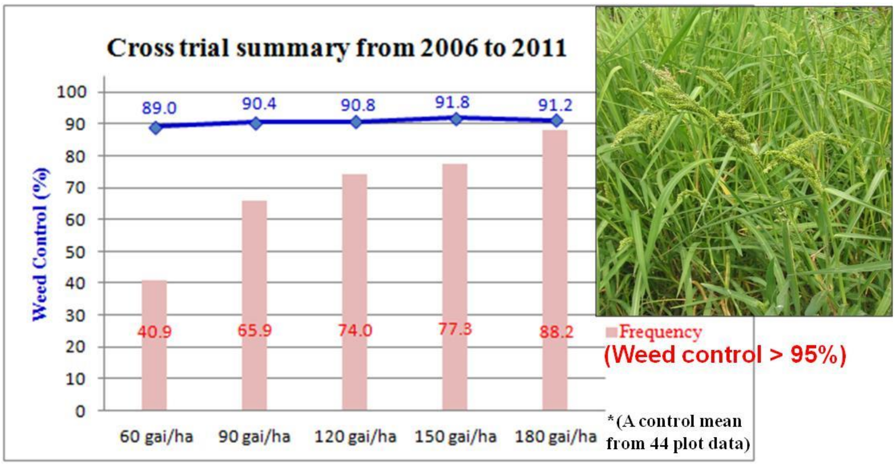


**Fig. 3.** Weed control (%) and control frequency of Rice™ 60OD at different rates on barnyardgrass.



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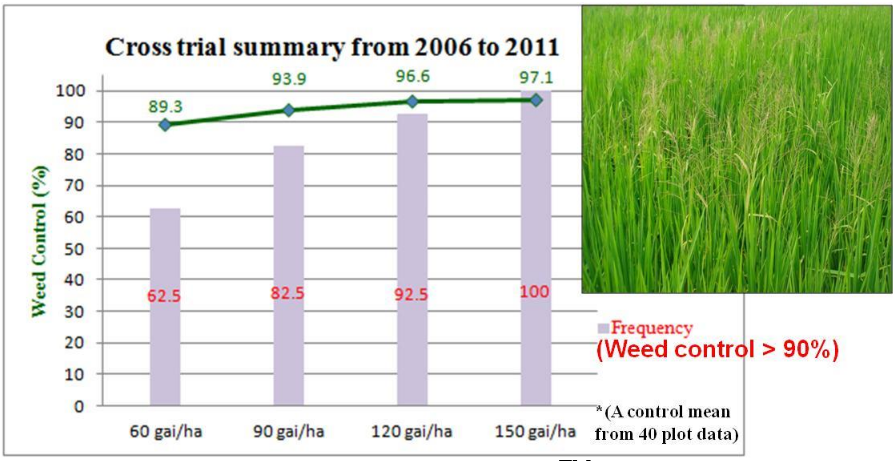


**Fig. 4.** Weed control (%) and control frequency of Rice™ 60OD at different rates on Chinese sprangletop.

For annual sedge (CYPDI), Rice™ 60OD was needed at 90 gai/ha for competitive control. There were 96.5% frequency if a weed control level at >90% (**Figure 5**). For one of broad leaf weeds, monochoria (MOOVA), it was only needed at 60 gai/ha. When a weed level control was at >90%, the frequency was 91.7% (**Figure 6**)

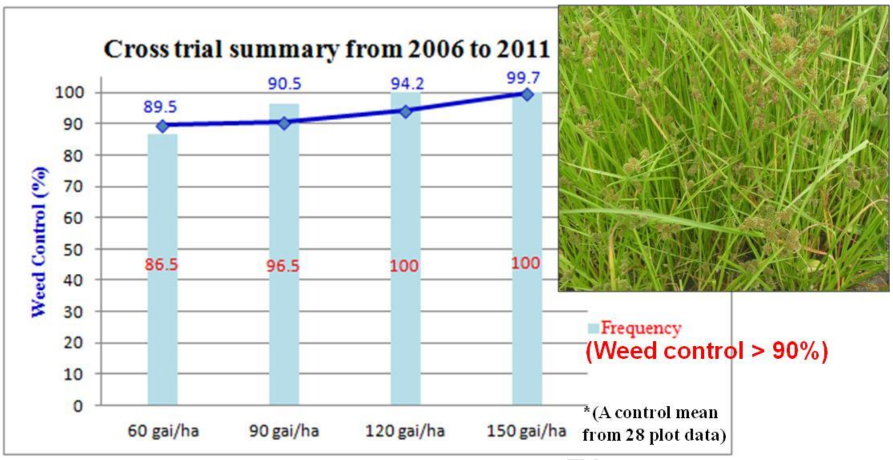


**Fig. 5.** Weed control (%) and control frequency of Rice™ 60OD at different rates on annual sedge CYPDI.

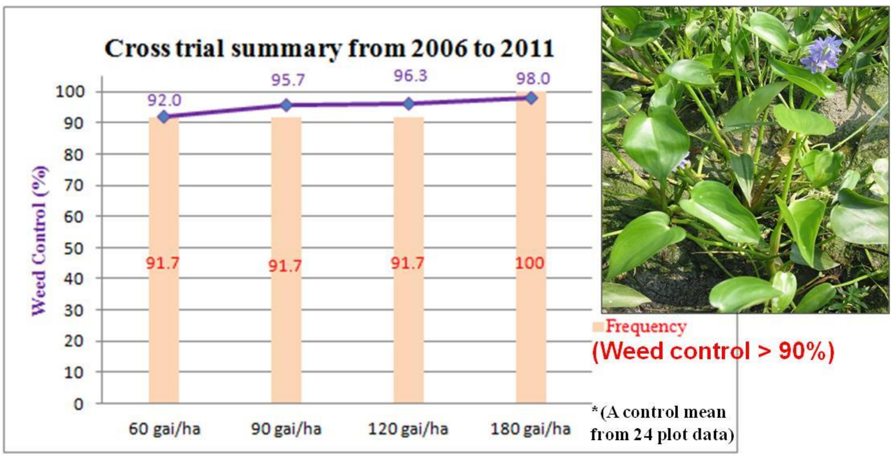


**Fig. 6.** Weed control (%) and control frequency of Rice™ 60OD at different rates on monochoria MOOVA.



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When Rice™ 60OD was launched in China in 2009, there was limited sale volume in rice market. However, Rice™ 60OD was sold 330 KL in 2011 and 550 KL in 2012.

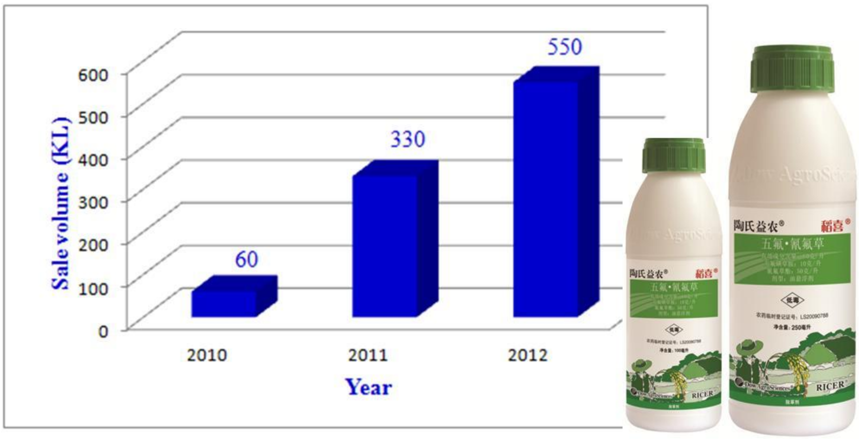


**Fig. 7**. Sale volumes of Ricer™ 60OD in rice market after product launching in China.

## ACKNOWLEDGMENTS

We were really appreciated for the China Biology team members who made great contributions on field trials, the global Biology Team Leader who delivered knowledge and technology, and the RCU R&D Leader who provided full supports.

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# UTILITY OF *LESPEDEZA CUNEATA* AS A FUNCTIONAL PLANT

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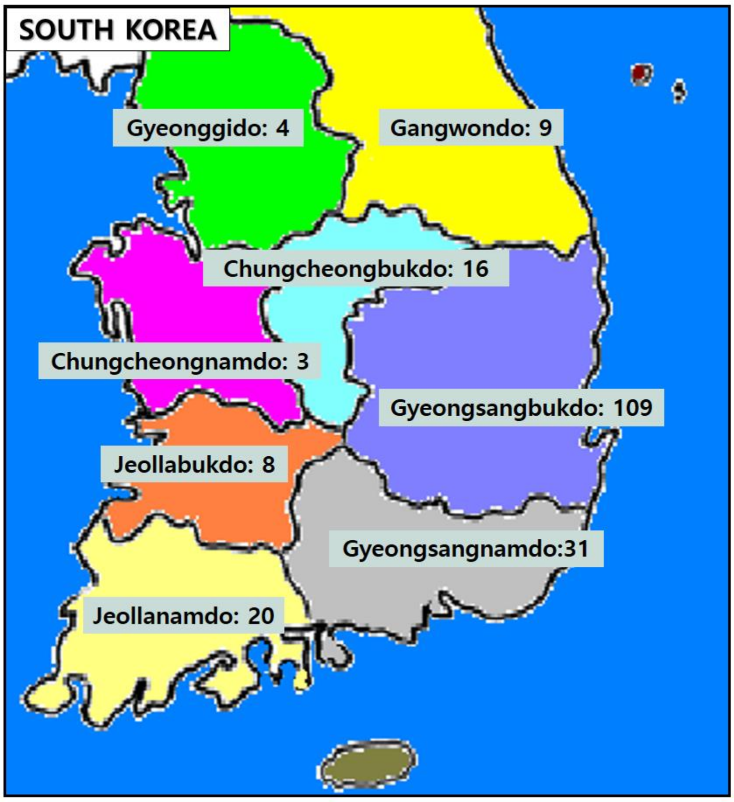
## INTRODUCTION

Lespedeza cuneata is a species of perennial plant in the legume family and it is distributed in Korea, Japan, China, Taiwan and India, etc as an introduced species and sometimes an invasive plant. This plant is considered medicinal in Korea and has been used for protecting enuresis and strengthening the lungs and eye and known as having excellent effects on asthma, mastitis, boils. The bioactive substances such as pinitol, flavonoid, tannin, β-sitosterol, etc. are known to exist in the roots and leaves of Lespedeza cuneata. Different types of flavonoids have been reported. In addition, the hot water and ethanol extracts have been used as a healthy drink which includes minerals, amino acids, vitamins, etc. This study was conducted to collect the plants from different province in Korea and to screen lines which have higher contents of functional compounds so that the plants could be used as a functional plant.

## MATERIALS AND METHODS

**1. *Lespedeza cuneata* collection**




- **Collection sites and plant number**

  The plant collection was made from all over the country in Korea from October 2 to October 24, 2011

  **2. *Lespedeza cuneata* cultivation**

- **Experiment field of Kyungpook National University**



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- **Yeongcheon area**

**3. *Lespedeza cuneata* component analysis**

- **Pinitol content**

  Dried sample (0.2g/50mesh) → sonicated extract (50℃/1hr) → 0.45μm syringe filter → HPLC analysis (Waters 1525 series HPLC, Waters 410 Differential Refractometer, Waters high performance carbohydrate column, 35℃, mobile phase: 85% Acetonitrile, flow rate: 0.8ml/min, injection volume: 20μℓ)

- **Isoflavone content**

  Dried sample (0.2g/50mesh) + 80% methanol (10ml) → sonicated extract (50℃/1hr) → shaking incubator (50℃/150rpm/20hr) → 0.45μm syringe filter → HPLC analysis(PerkinElmer series 200, Browlee choic C18 150*4.6mm, flow rate: 1.0ml/min, injection volume: 10μℓ, UV detector: 260nm, colum temp: 30℃,mobile phase:

  A) Acetonitrile; 5 → 35% (50min), 35 → 5% (20min)

  B) HPLC water (0.1% acetic acid); 95 → 65% (50min), 65 → 95% (20min)

- **Polyphenol content**

  Dried sample (10g) + methanol (20ml) → overnight → centrifuge (3000rpm) → 0.2 μm syringe filter

  ↓

  96 well-pate: extract (50μℓ) + 2% $Na_2CO_3$ (1ml) + 50% Folin-Ciocalteau reagent (50μℓ)

  ↓

  ELISA reader (750nm)



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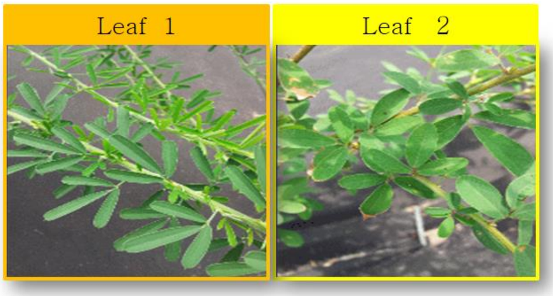
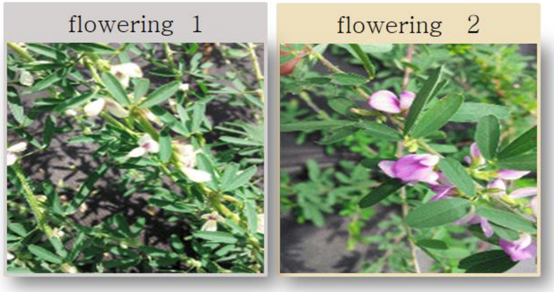
## RESULTS AND DISCUSSION

### 1. Morphology of *Lespedeza cuneata*

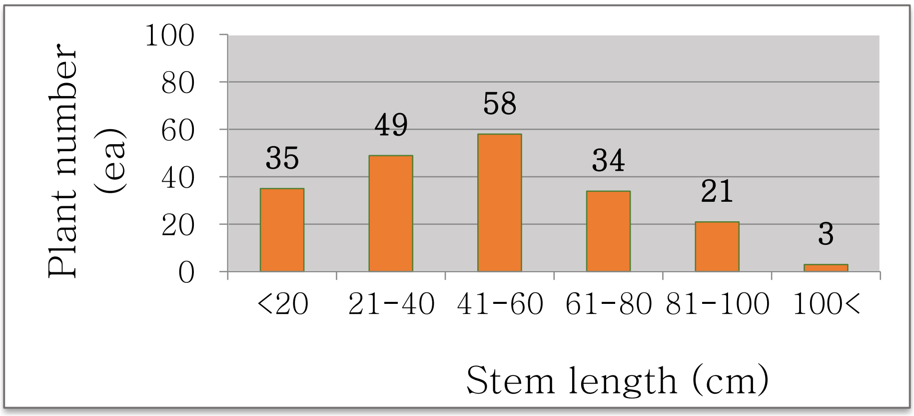
- Leaf




- Flower




- Stem length




Two types of leaf shape were observed in the collected variety of *Lespedeza cuneata*. Lanceolate and oval shape of the leaves was found. Three kinds of flower colors were found and most of the collected varieties showed purple color. The stem length was ranged from 20 to 100 cm, showing the highest plant number in near 50 cm of stem length.



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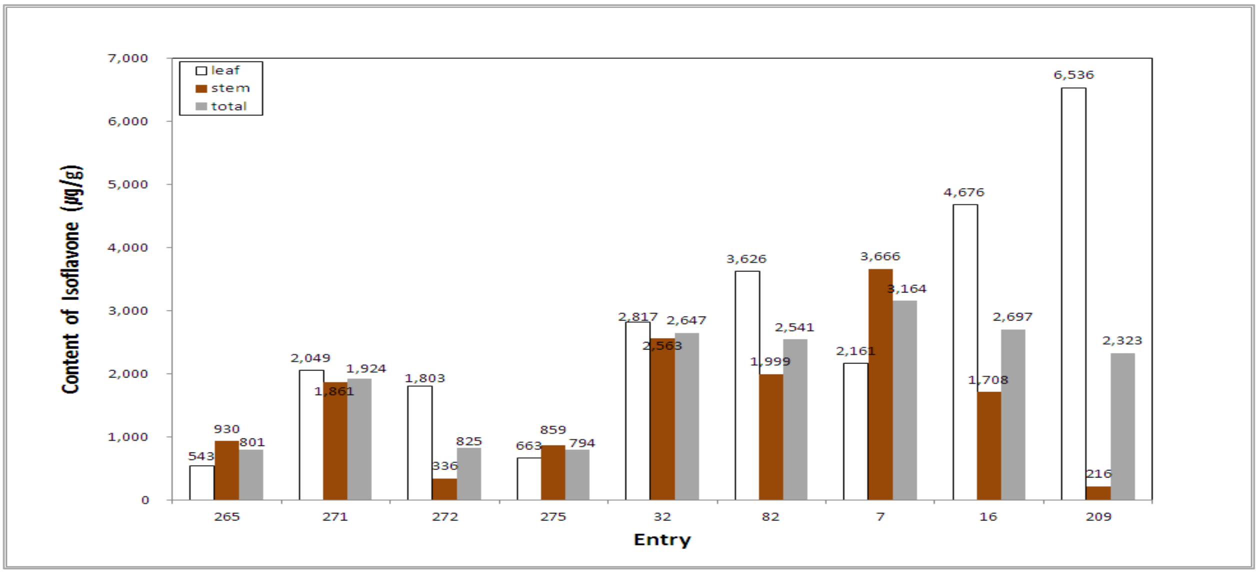
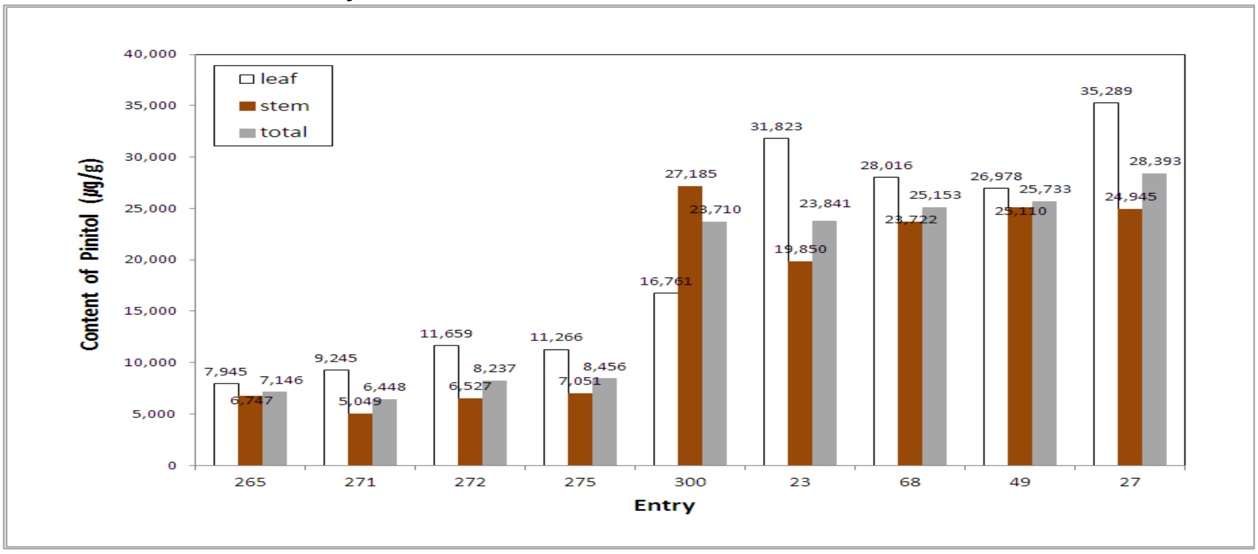
## 2. Compound analysis of *Lespedeza cuneata*

- Isoflavone content analysis




- Pinitol content analysis



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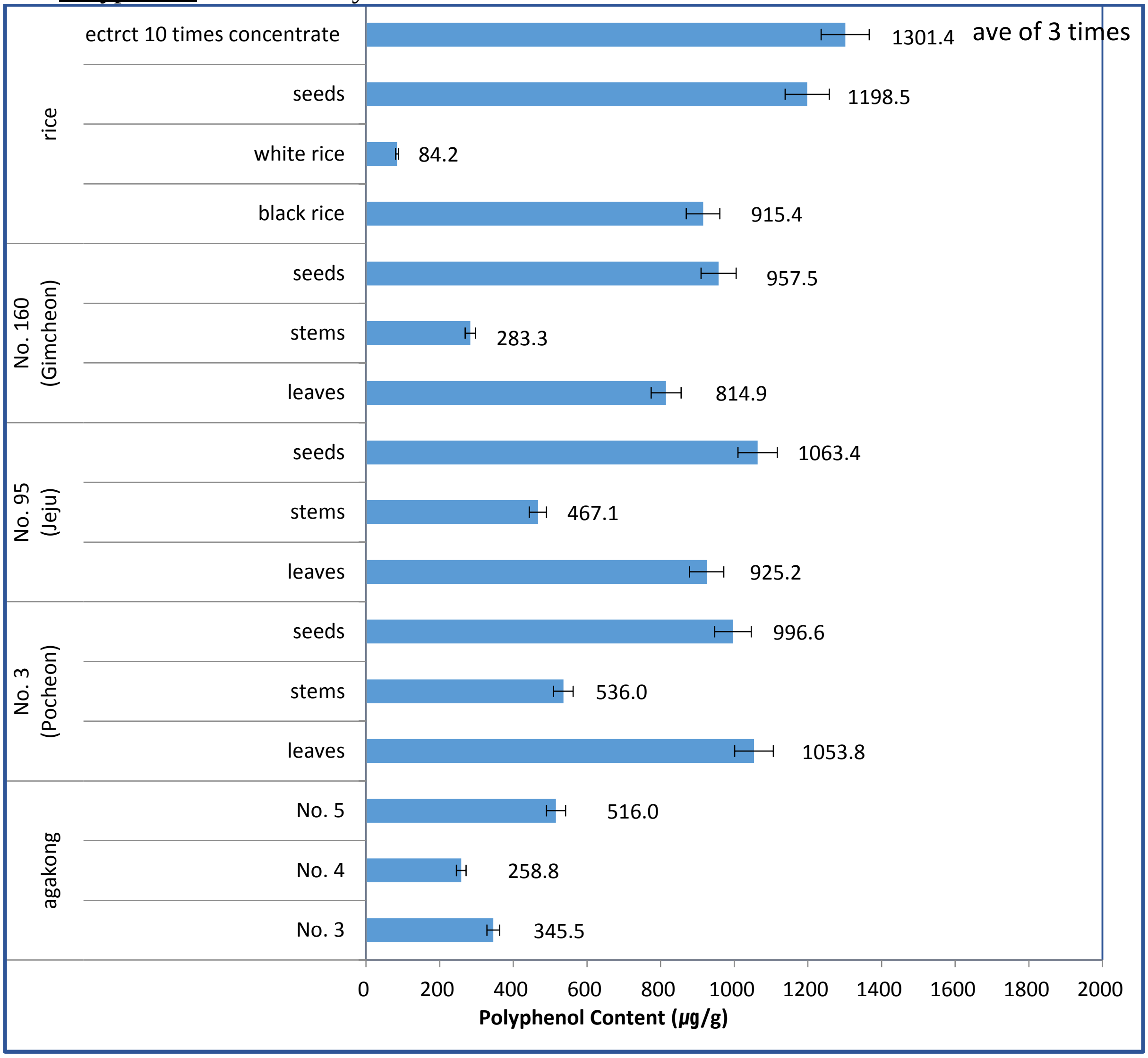
- <u>Polyphenol</u> content analysis




There were significant differences in the contents of functional compounds such as pinitol and isoflavone from the collected variety of *Lespedeza cuneata*. Higher contents of the compounds were detected in the leaf than stem or seed. In addition, the polyphenol content was similar or more compared to the black rice known as a higher carrier of polyphenol.

## CONCLUSIONS

The collected *Lespedeza cuneata* from all over the country in Korea showed varietal differences in the leaf shape and color, flowering date, growth speed, contents of functional compounds such as pinitol and isoflavones. The contents of functional compounds are matters of our concerns because the plants can be utilized as functional resources. Generally the contents are higher in leaf than stem or seed. For the further study, isolation and characterization of the functional compounds should be carried out to exploit the plant as a healthy food. And cultivating system should be established simultaneously for increasing farmer's income.

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# NANOSTRUCTURAL DIFFERENCE OF STARCH GRANULE OF BARNYARDGRASS SPECIES STUDIED BY HIGH RESOLUTION NON-CONTACT ATOMIC FORCE MICROSCOPE

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**ABSTRACT** During gelatinization of wild and cultivated barnyard grass (*Echinochloa crus-galli* L.), nanostructural behaviours were observed by a high resolution non-contact atomic force microscopy (AFM). New nano- structural analysis of barnyard grass starch in an aspect of food processing and industry has rekindled interest in its structure-function relationship. Surface studied at boiling conditions revealed some details of the starch granule nanostructure. The oblong granules of approximately 35.58 to 48.17 nm diameter have been detected at the surface of barnyard grass starch within 20 min after gelatinization.

Gelatinization almost inevitably involves the heating and moisturizing processes. When starch granules are heated in excess water, starch granules swell and dramatically change their size and shape. As blocklet-like structures are dispersed, crystalline regions are irreversibly disrupted. Continuously, the birefringence is evoked, following an unwind of double helices. The occurrence of hair-like structure on the surface of the barnyard grass starch granules is visualized. These are extensions of the glucan polymers from either amylose or amylopectin that are free to origin starch granules in boiling water. Finally, starch granules are solubilized. We obtained stable images and found that the roughness of outmost surface of starch granule blocklet was altered with gelatinization. The roughness (Ra) of the cultivated barnyard grass starch granule is rougher than barnyard grass. In cultivated barnyard grass, the starch blocklet became denser with heating for 60 min compared to wild barnyard grass. We discuss the change in the surface topography in relation to the inner structure observed with scanning electron microscope (SEM).

***Keywords:*** Barnyard grass, starch granules, amylose, gelatinization, nano-structure

## INTRODUCTION

Varieties of the *E. crus-galli* complex are among the world's most serious agricultural weeds (Holm *et. al* 1977) Barnyard grass are particularly abundant in flooded rice fields where it reduce yields by up to 40% (Smith *et al.*, 1977). Since early 1998, the crop rotation like paddy-upland rotation cropping system in Korea has been widely examined using corn, sesame, barley and wheat before and after rice transplanting in order to reduce barnyard grass occurrence in Korea (Kim *et al.*, 2011). Although barnyard grass is one of the most hazardous species in paddy field, it was historically a main food in Korea. Some species of them were traditionally cultivated for food.

The flours of barnyard grass seed has been used to the additives like gruel, cake and liquor with rice flours. The seeds of barnyard grass contain about 13.2% protein, 2.6% fat, 9.5% ash, 7.2% lignin and 19.6% amylose (Fujimoto *et al.*, 1986).

Until now, no an attempt to evaluate starch properties of the barnyard grass was for edible used. Gelatinization is one of the important properties of starch. The starch granule can absorb water and irreversible swelling takes place in the process (Sandhu and Singh, 2007).



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New nano- structural analysis of barnyard grass starch in an aspect of food processing and industry has rekindled interest in its structure-function relationship.

## MATERIALS AND METHODS

Barnyard grass (*Echinochloa crus-galli* L.) were grown and harvested in the experimental fields of Hankyong National University, Anseong, Korea. Their seeds were ground and pulverized. The starch granules were extracted according to Hongxiu's method (1996).

**Gelatinization treatment** The 0.3 mg of starch granules sample was placed into a 2.0 ml eppendorf tube and third distilled water (2 ml) added. Then the tube was placed in a water bath at 100℃ for 10, 20, 40 and 60 min (Hongxiu and Zhaotan, 1996).

**Atomic Force Microscopy (AFM)** Fixed samples onto slide glass are observed using the Atomic Force Microscopy (AFM, PSIA, XE-100). After mounting of gelatinized samples onto slide glass, the samples were measured with the AFM (Head mode: NC-AFM, X, Y Scan size: 0.5, 1, 5㎛, Scan Rate: 0.5Hz).

## RESULTS AND DISCUSSION

After treated with heating, gelatinized samples were observed using atomic force microscopy. The AFM image were to biophysically observe the changes of starch nano-unit chains extending out of granules. After 20 min of heating, the whole nano-unit chains have nearly completed gelatinization process. After 40 min of heating, whole nano-unit chains became clear. Non-treated original starch granule sizes of the wild barnyard grass and cultivated barnyard were 35.58 nm and 25.34 nm, respectively.

The roughness (Ra) of the cultivated barnyard grass starch granule is rougher than barnyard grass. In cultivated barnyard grass, the starch blocklet became denser within heating 60 min compared to wild barnyard grass. The occurrence of hair-like structure on the surface of the barnyard grass starch granules is visualized. These are extensions of the glucan polymers from either amylose or amylopectin that are free to origin starch granules in boiling water.

Finally, starch granules are solubilized. We obtained stable images and found that the roughness of outmost surface of starch granule blocklet was altered with gelatinization. These results lead to conclusion that some gelatinization and pasting properties are able to be affected by the specific starch granules with different amylose contents.



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(A) (B)

Photograph

Stereoscopic microscope

(×100)

Scanning Electron Microscope

(×2,000)

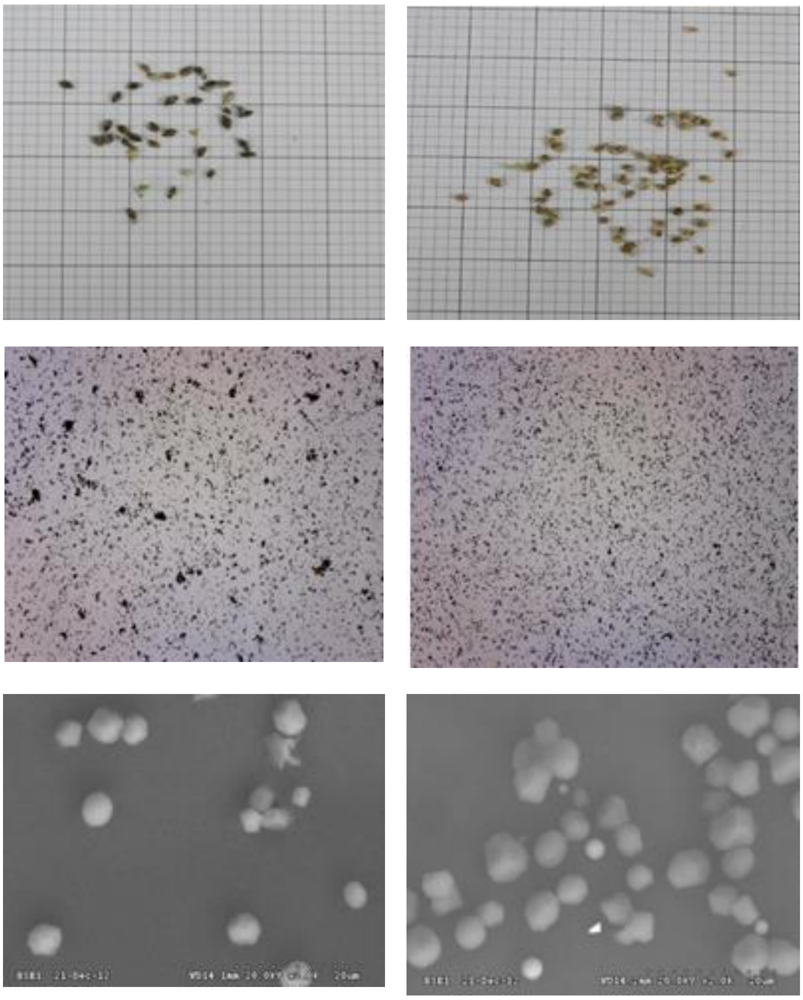
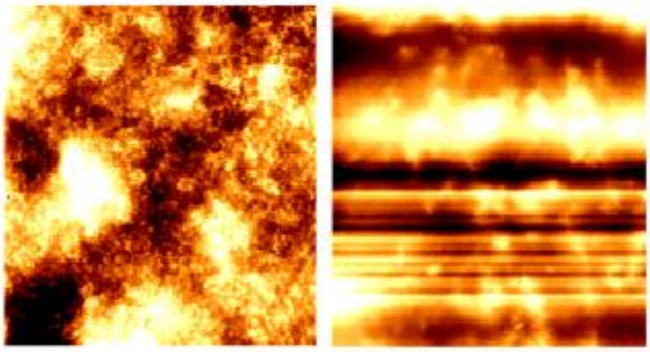


**Fig. 1.** Morphological character of seeds (A) Wild barnyard grass (B) Cultivated barnyard grass.



(A)



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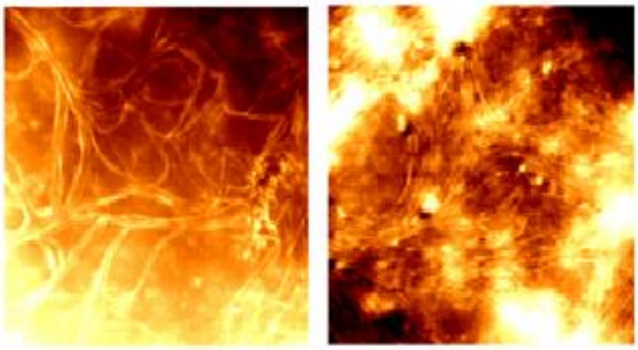
(B) 

Control (0 min) Heating (40 min)

**Fig. 2.** The nano-structural analysis of starch granule of wild barnyard grass and cultivated barnyard grass by AFM non-contact mode after heating starch granule for 0 min (control) and 40 min. Scan size: 5 ㎛. Wild barnyard grass (B) Cultivated barnyard grass.

**Table 1.** The size (nm) of starch granules size (nm) in wild barnyard grass and cultivated barnyard grass until 60 min after the treatment of heating.

| Treatment duration(min) | 0 min | 20 min | 40 min | 60 min |
|---|---|---|---|---|
| Wild Barnyard grass | 35.58 ± 1.39 | 39.69 ± 1.40 | 48.17 ± 0.94 | 37.47 ± 4.75 |
| Cultivated barnyard grass | 25.34±3.47 | 29.63 ± 1.09 | 32.43 ± 1.86 | 31.98 ± 3.51 |

**Table 2.** The roughness (Ra) value (nm) in wild barnyard grass and cultivated barnyard grass until 60 min after the treatment of heating.

| Treatment duration(min) | 0 min | 20 min | 40 min | 60 min |
|---|---|---|---|---|
| Wild barnyard grass | 0.391 ± 0.15 | 0.718 ± 0.16 | 0.645 ± 0.08 | 0.612 ± 0.20 |
| Cultivated barnyard grass | 0.604 ± 0.18 | 0.965 ± 0.54 | 0.254 ± 0.09 | 0.867 ± 0.17 |

## ACKNOWLEDGEMENTS

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# CONTROL OF *MONOCHORIA VAGINALIS*, AN ANNUAL PADDY WEED: EFFECT OF TIMING OF PADDLING ON ITS EMERGENCE PATTERN AND EVALUATION OF AN AUTONOMOUS WEEDING MACHINE "AIGAMO-ROBOT"

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## BACKGROUND OF RESEARCH

Recently, trials have been carried out all over Japan to reduce the use of herbicides. To promote these trials, paddy weed problems need to be solved. So we studied; (1) effect of the timing of paddling on the emergence of *M. vaginalis*, (2) effect of an automatic weeding machine "Aigamo" (crossbreed duck) robot.

## MATERIALS AND METHODS

### (1) Paddling time experiment

On April 17, 2012, a paddy field infested with *M. vaginalis* was paddled in the Kobe University Rokko Campus (34.725°N, 135.233°E). After paddling, the weed plants emerged within three areas of 0.25 m × 0.50 m each were counted and pulled every three days until the emergence was end. The same procedure was repeated for the measurements starting on May 17, June 15, and July 17, 2012. Water depth and water temperature were monitored.

### (2) Aigamo-robot experiment

Transplanting was carried out on June 1, 2012 in Hyogo Prefectural Institute for Agriculture, Forestry and Fisheries (34.918°N, 134.893°E). From three days after transplanting, the robot was operated twice or three times per week for three weeks. Weed sampling was carried out 40 days after transplanting, where weight of the weed was measured. Fig. 1 illustrates the Aigamo-robot with intra- and inter-rows rice planting experiment.

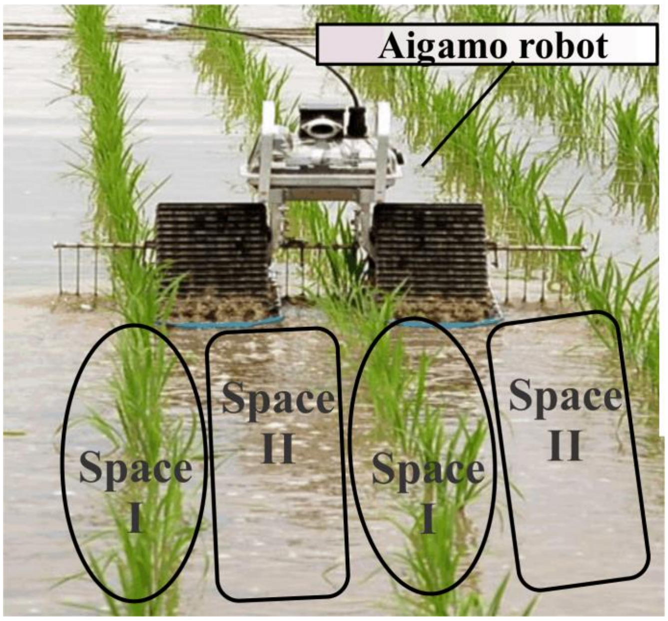


**Fig 1.** Operation of the Aigamo-robot and definition of intra-row spacing (I) and inter-row spacing (II). SpaceI is on planting row; Space II is between planting



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## RESULTS

### (1) Paddling time experiment

The later the timing of paddling, the shorter the duration of the emergence of *M. vaginalis*, which can be partly explained by the difference in the water temperature.

### (2) Aigamo-robot experiment

Aigamo-robot controlled annual paddy weeds (including *M. vaginalis*), if it was operated three times per week. However, the most of the uncontrolled weeds remained at the space near the hills (intra-row spacing).

## CONCLUSION

It is expected that Aigamo-robot can control paddy weeds more effectively if the paddling time is delayed.



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## *Herbicide and Herbicide Resistant Weeds*

# MECHANISMS OF GLYPHOSATE RESISTANCE IN ITALIAN RYEGRASS (*LOLIUM MULTIFLORUM*) FOUND IN LEVEES OF RICE PADDY FIELDS IN JAPAN

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**ABSTRACT** The rapid range expansion of naturalized Italian ryegrass (*Lolium multiflorum* Lam.) in farmland is a serious problem in Fukuroi City, Shizuoka, Japan. Glyphosate has been used to control Italian ryegrass in the levees of rice paddy and wheat fields for about 20 years in this area. Some wild populations in the levees show high survival rates after the application of glyphosate at 2.3 kg ai $ha^{-1}$, the recommended dose. This suggests that resistance to glyphosate has evolved in the wild populations of Italian ryegrass. We are investigating the mechanisms conferring glyphosate resistance on these populations. The mechanisms of glyphosate resistance have been determined for several weed species: a mutation in 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) gene; amplification of the *EPSPS* gene; and reduced glyphosate translocation. Our experiments showed that any mutation in the *EPSPS* gene and amplification of the *EPSPS* gene were not found on these Italian ryegrass populations. Studies on the translocation of glyphosate in resistant Italian ryegrass biotypes are undergoing.

***Keywords:*** Herbicide resistance, EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase, mutation, gene amplification, translocation.

## INTRODUCTION

Glyphosate (*N*-[phosphonometyl]glycine) is the world's most important herbicide and is used widely in agricultural and non-agricultural areas, including roadsides and recreational areas, to control a broad spectrum of weed species (Dill *et al*. 2008). In the late 1990s, glyphosate resistance in weeds was first reported for rigid ryegrass (*Lolium rigidum* Gaudin) populations from Australia (Powles *et al*. 1998; Pratley *et al*. 1999). Since then, an increasing number of glyphosate-resistant weed species has been reported.

Italian ryegrass (*Lolium multiflorum* Lam.) is a widely distributed winter annual grass that is cultivated as a forage crop and is a noxious weed in agricultural fields in the temperate zone. Glyphosate-resistant Italian ryegrass was first reported in the orchards of Chile in 2003 (Perez & Kogan 2003) and now is reported in the orchards and cereal crop fields of five countries: Argentina, Brazil, Spain, the USA and Japan (Heap 2013; Niinomi *et al*. 2013).

The rapid range expansion of naturalized Italian ryegrass in farmland is a serious problem in Fukuroi City, in the western part of Shizuoka Prefecture, Japan, where rice, wheat and soybean are cultivated systematically. Moreover, naturalized Italian ryegrass in paddy levees harbors insect pests such as mirid bugs that damage the commoditized rice grain quality, thereby causing severe economic losses (Higuchi 2010).

Since the early 1990s, glyphosate has been used to control the weeds on the levees of rice paddy and wheat fields in Fukuroi City for about 20 years. However, some wild populations in the levees show high survival rates after the application of glyphosate at 2.3 kg ai $ha^{-1}$, the recommended dose. This suggests that resistance to glyphosate has evolved in the wild populations of Italian ryegrass.



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The mechanisms of glyphosate resistance have been determined for several weed species: a mutation in the target enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) gene (Baerson *et al*. 2002; Ng *et al*. 2004); amplification of the *EPSPS* gene (Gaines *et al*. 2010; Salas *et al*. 2012); and reduced glyphosate translocation (Lorraine-Colwill *et al*. 2003; Wakelin *et al*. 2004). The mechanisms conferring glyphosate resistance on these populations are investigated by molecular and biochemical approaches.

## MATERIALS AND METHODS

### Plant materials

The fresh leaves of Italian ryegrass were collected from six naturalized Italian ryegrass populations in Fukuroi City, the western part of Shizuoka Prefecture, Japan, in May 2012. Four glyphosate-resistant populations were located on large-scale levees of paddy field and wheat field, containing a part of area where glyphosate has been applied two times per year for about 20 years. Two susceptible populations were located on a terrace paddy levee and a river bank (Niinomi *et al.* 2013). These collected leaves were stored at -80˚C until RNA or DNA extraction.

### Mechanisms of resistance to glyphosate

Complementary DNA (cDNA) sequencing analysis of the *EPSPS* gene was conducted to elucidate a mutation in the *EPSPS* gene. Total RNA was extracted from the leaves using RNeasy Mini Kit (QIAGEN). Synthesis of cDNA from mRNA was performed using a reverse transcriptase (SuperScript III First-Strand Synthesis System for RT-PCR, Life Technologies). PCR was conducted by using a pair of primers designed based on the *EPSPS* gene sequence of Italian ryegrass to amplify a fragment of the *EPSPS* gene containing codon 106 (Perez-Jones *et al*. 2007). PCR products were directly sequenced.

Amplification of the *EPSPS* gene was investigated by quantifying genomic copy number of the *EPSPS* gene. DNA was extracted from the leaves using a slightly modified CTAB method. Quantitative real-time PCR was conducted by using two pair of primers, *EPSPS* primer and ciinamoyl-CoA reductase (*CCR*) primer (Salas *et al*. 2012). *CCR* gene is constitutively expressed and is present as a single copy gene in perennial ryegrass. *EPSPS* copy number relative to *CCR* was calculated.

Studies on the translocation of glyphosate in resistant Italian ryegrass biotypes are undergoing.

## RESULTS AND DISSCUSSION

Complementary DNA sequencing analysis of the *EPSPS* gene indicated that there were no mutations conferring amino acid substitution at codon 106 in the *EPSPS* gene.

The genomic copy number of the *EPSPS* gene was estimated at 0.5 to 1.5 relative to the copy number of the *CCR* gene. This result suggested that amplification of the *EPSPS* gene did not occur in glyphosate-resistant biotype.

Our experiments showed that any mutation in the *EPSPS* gene and amplification of the *EPSPS* gene were not found in these Italian ryegrass populations. Researches on the translocation of glyphosate in resistant Italian ryegrass biotypes are in progress.

## ACKNOWLEDGEMENTS

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# IDENTIFICATIONS OF HERBICIDE-RESISTANT BARNYARDGRASS (*ECHINOCHLOA CRUS-GALLI*) BIOTYPES IN KOREA

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**ABSTRACT T**he continuous use of acetolactate synthase (ALS) and acetyl-CoA carboxylase (ACCase) inhibiting herbicides in the direct-seeded rice fields in Korea has lead to the selection of herbicide resistant barnyardgrass populations since 2009. This study was conducted to investigate the cross- and multiple-herbicide resistance of suspected barnyardgrass biotypes. We collected barnyardgrass seeds from 155 uncontrolled individual plants at 15 sites in five locations in 2012. ALS inhibitors (sulfonylurea and imidazolinone herbicides) and ACCase inhibitors (cyclohexanedione and aryloxyphenoxypropionate herbicides) were used to determine cross- and multiple-resistance. 24% of populations collected from Taeahn-Gun were partially resistant to ACCase inhibitors and 22% of populations collected from Kimjae-Si were partially resistant to ALS inhibitors. However, 8.2% of the populations from both sites were resistant to both ALS and ACCase inhibitors. The continuous use of these herbicides in the rice fields will increase populations of herbicide-resistant barnyardgrass. Alternative control methods should be applied to control herbicide-resistant barnyardgrass in the infested fields.

***Keywords:*** herbicide-resistance, barnyardgrass, ALS, ACCase

## INTRODUCTION

The continuous use of the same herbicide or herbicides acting on the same target site leads to the selection of herbicide resistant weed populations (Maxwell & Mortimer, 1994). In Korea, the first resistance selected with acetolactate synthase(ALS) inhibitors was reported in *Monochroria korsakowii* in 1998 (Park et al. 1999). Since then, 12 weed species have developed resistance to ALS inhibitors.

Barnyardgrass (*Echinochloa curs-galli* L.) is one of the most important weed species in rice cultivation in Korea. Herbicides inhibiting ALS or Acetyl-CoA carboxylase (ACCase) have been used successfully to control barnyardgrass in rice fields. However, ALS or ACCase inhibiting herbicide resistant barnyardgrass was reported in rice fields in 2009.

The objectives of this research were to identify barnyardgrass populations resistant to ALS and/or ACCase inhibitors in the direct-seeded rice fields in Korea.

## MATERIALS AND METHODS

### Sampling of barnyardgrass seeds

Monitoring of established barnyardgrass plants in the direct-seeded rice fields was conducted at 15 sites in five locations in Oct. 2012. Seeds from 155 uncontrolled individual plants were collected and immersed in 4C water for 30 days to remove germination inhibitors of seeds. Seedlings were grown in a 10 cm diameter pot containing potting mix. Plants were grown in a growth chamber with 30/25 C and 16/8 h day/night temperature.



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**Resistance test**

Two ALS inhibitors (sulfonylurea (SU) and imidazolinone (IMI)) and two ACCase inhibitors (cyclohexanedione and aryloxyphenoxypropanoate) were used to determine cross- and multiple-resistance patterns of barnyardgrass populations. Pre- and post-emergence applications were used for flazasulfuron (SU) and imazaquin (IMI), and sethoxydim (DIM) and fluazifop-p-butyl (FOP), respectively. Resistance of seedlings was rated visually 14 days after treatment.

## RESULTS AND DISCUSSION

We collected 155 barnyardgrass seeds from 15 sites in five locations in 2012. It was found that 42 plants out of 155 plants were resistant to at least one of ALS or ACCase inhibiting herbicides. 24% of populations collected from Taeahn-Gun was partially resistant to ACCase inhibitors and 22% of populations collected from Kimjae-Si wase partially resistant to ALS inhibitors. However, 8.2% of the populations from both sites were resistant to both ALS and ACCase inhibitors. The continuous use of these herbicides in the rice fields will increase populations of herbicide-resistant barnyardgrass. Alternative control methods should be applied to control herbicide-resistant barnyardgrass in the infested fields.

**Table 1**. Number of barnyardgrass plants resistant to ALS and ACCase inhibitors.

| Sites | no. of samples | ALS inhibitors | | | ACCase inhibitors | | | ALS/ ACCase |
|---|---|---|---|---|---|---|---|---|
| | | SU | IMI | SU/IMI | DIM | FOP | DIM/FOP | |
| Taeahn-Gun | 63 | 6 | 1 | 1 | 14 | 3 | 2 | 4 |
| Kimjae-Si | 59 | 12 | 6 | 5 | 13 | 3 | 1 | 6 |
| Others | 33 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 155 | 21 | 7 | 6 | 27 | 6 | 3 | 10 |

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# DETECTION OF RESISTANCE POPULATIONS OF JAPANESE FOXTAIL (*ALOPECURUS JAPONICUS*) IN CHINA TO ACCASE-INHIBITORS AND ALS-INHIBITOR

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**ABSTRACT** Fenoxaprop-p-ethyl and chlorsulfuron were applied over 20 years in the winter wheat in south of China, and local farmers complained the reduced efficacy of the fenoxaprop to the Japanese foxtail. It is very necessary to detect the resistant level of the ACCase inhibitors and ALS inhibitors which are newly registered and used in China, and find some good solutions to manage resistant weeds. Fifty Japanese foxtail populations collected from Anhui, Jiangsu and Hubei Provinces of China's wheat fields were used to detect the resistance to ACCase and ALS-inhibitors from 2012 to 2013 in Beijing. Fifty Japanese foxtail seeds of the populations were planted into 12-cm-diam pots containing moist loam soil. Pots were placed in the greenhouse, watered and fertilized as required. The seedlings were thinned to 30 evenly-sized plants per pot before herbicide application. The herbicides representing different chemical classes were applied respectively to the Japanese foxtails at the three-leaf growth stage. Clodinafop-propargyl belongs to APP chemical class of ACCase inhibitor, and pinoxaden belongs to PPZ chemical class of ACCase inhibitor, mesosulfuron-methyl was belonging to ALS inhibitor. The 15% Clodinafop-propargyl, wettable powder, supplied by the Syngenta (China) Company, was applied at 67.5 g ai $ha^{-1}$ while 50g/L Pinoxaden, emulsifiable concentration, supplied by the Syngenta (China) Company at 75.0 g ai $ha^{-1}$, and 30g/L mesosulfuron-methyl, emulsifiable concentration, supplied by the Bayer Company, was similarly applied at 15.7 g ai $ha^{-1}$. Every treatment was accorded with ten replicates. The experiment was conducted twice to check its reproducibility. The weeds were checked if they were killed or otherwise to determine the resistance after treatment 21 days. The results showed that out of 50 populations of Japanese foxtail, 43 displayed high level resistance to clodinafop-propargyl, and 39 populations of these 43 resistant populations expressed cross-resistance to pinoxaden, and 6 populations of these 43 resistant populations expressed multiple-resistance to mesosulfuron-methyl. It is very necessary to study adapt technology to manage the cross-resistance and multiple-resistance issues.

***Keywords:*** Japanese foxtail (*Alopecurus japonicus*), clodinafop-propargyl; pinoxaden; mesosulfuron-methyl, ACCase inhibiting herbicides, ALS inhibiting herbicide, herbicide resistance.

## INTRODUCTION

The increasing use of ACCase-inhibiting and ALS-inhibiting herbicides have resulted in evolved resistance in key grass weeds infesting cereal cropping systems, and Japanese foxtail (alopecurus japonicas) is one o fthe most important grass weeds species in wheat and canola in China (Fig. 1.). The fenoxaprop-p-ethyl being one of most common ALS-inhibiting herbicide were applied over 20 years in the winter wheat in south of China, and local farmers complainded the efficacy of them to the Japanes Foxtail. It's very necessary to detect the



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resistant level of the ACCase inhibitors and ALS inhibitors, and find some good solutions to manage the resistant weeds.

**Fig. 1.** Infestation of Japanese foxtail in the wheat field.

## MATERIALS AND METHODS

### The resistant detection

The seeds of fifty Japanese foxtail populations were collected in 2011 from wheat fields in Anhmi province, Hubei province and Jiangsu province, etc.

Two ACCase inhibitors representing two differen chemical classes (APP and PPZ) and one ALS-inhibitor representing SU chemical class were applied respectively by whole plant experimental method.

The populations were grown in the greenhouse and treated with clodinafop-propargyl at 67.5 g/ha. With pinoxaden at 75 g/ha and with mesosulfuron-methyl at 15.7 g/ha at the three-leaf growth stage, respectively. There were at least 100 plants of one population for resistant detection, and the spraying volume was 367.5L/ha.

The results showed that the 43 poupulations of them expressed resistance o clodinafop-propargyl, and 39 populations of these 43 resistant populations expressed cross-resistance to pinoxaden, and 6 populations of these 43 resistant populations expressed multiple-resistance to mesosulfuron-methyl. The distributions of them were showed in Figure 2.

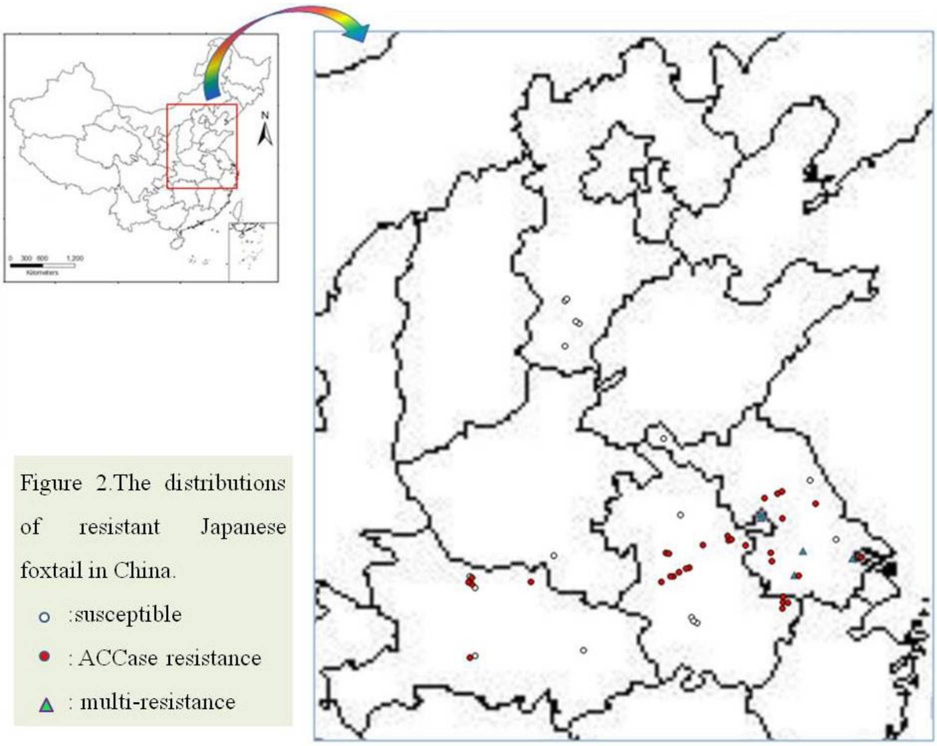


Figure 2.The distributions of resistant Japanese foxtail in China.



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## RESULTS AND DISCUSSION

### Resistant Mechanism

The two resistant and one susceptible populations were also selected to elucidate the resistant mechanism. The susceptible ACCase gene fragment sequence of the Japanese foxtail have very high homology (99.61%) with the black grass (Alopecurus myosuroides). Comparison of the ACCase gene sequences of the susceptible and the resistant populations with black-grass revealed that tryptophan at position 2027 of the ACCase gene was substituted by leu

tcine in populations Aloja-JS10-R2. The study firsly confirmed Japanese foxtail resistance to ACCase inhibitor fenoxafop, cross-resistance to other ACCase inhibitors, and the resitance mechanism being conferred by specific ACCase point myutation at amino acid position 1781 and 20127 (Table 1).

**Table 1.** DNA and derived amino acid sequences of ACCase gene from susceptible population S and resistant populations R1 and R2 of Japanese foxtail. Amino acid positions were correspond to the full-length plastidic ACCase in A.myosuroides.

| Population | Ile-1781 | Trp-2027 |
|---|---|---|
| Aloja-JS10-S | - - - I- - - | - - -W- - - |
| | ATA | TGG |
| Aloja-JS10-R1 | - - - I- - - | - - -C- - - |
| | ATA | TGT |
| Aloja-JS10-R2 | - - - L- - - | - - -W- - - |
| | CTA | TGG |

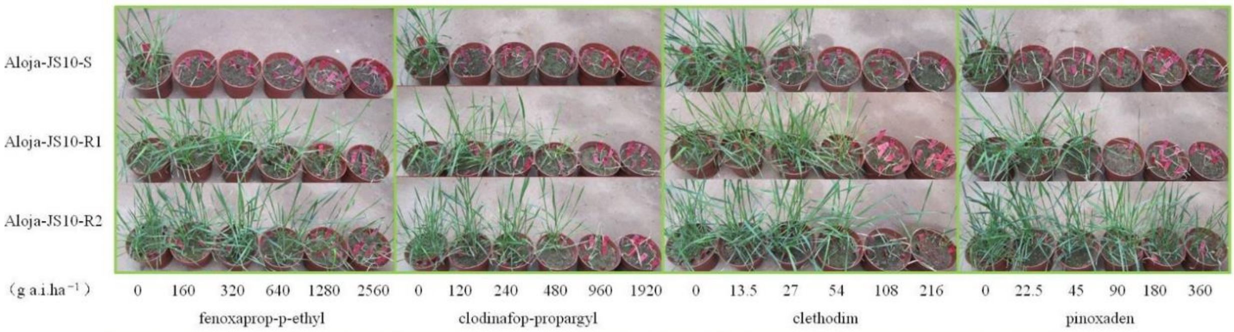


**Fig. 3.** The cross-resistance of the populations of Japanese foxtail to the four ACCase inhibitors by whole –plant experiment method.

The above two populations being resistan to clodinafop were selected to conduct the cross-resistance with four ACCase inhibitors. The response of the populations to differen herbicides at some dosages were showed in figure 3, and cross-resistance could be determined. The two populations Aloja-JS10-R1 and Aloja –JS10-R2 expressed high resitance to fenoxaprop-p-ethyl with resistance index (R1) being 61.5 and 82.5. They also expressed high cross resistance to clodinafop-propargyl with R1 being 18.3 and 20.7 and moderate cross-resistance to clothedium and pinoxaden with R1 ranging from 2.9 to 15.0. It's also first time to find that the populations Aloja-JS10-R2 with lle-1781-Leu showed much higher resistance to the APPs, CHDs and PPZs, comparing to the Aloja-JS10-R1 with Tro-2027-Cys.

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# OPTIMUM APPLICATION TIMING OF BENTAZON FOR BROADLEAVED WEED CONTROL IN SOYBEAN CROP IN MIYAGI PREFECTURE, JAPAN

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**ABSTRACT** In order to identify the optimum application time of bentazon, we conducted field experiments to determine the relationship between application time of bentazon and weed age in soybean crop of Miyagi. We applied bentazon at the third trifoliolate stage (V3), sixth trifoliolate stage (V6), seventh trifoliolate stage (V7), and the ninth trifoliolate stage before the bloom (V9). In this study we identified spraying made at V3 was more effective than those made at V9. Our results indicated that optimum bentazon application time for controlling broadleaved weeds was around soybean at V3 stage (30 - 35 days after sowing and before first inter-row tillage), which is early stage as recommend on the label for herbicide.

***Keywords:*** Soybean, bentazon, application timing, weed.

## INTRODUCTION

The area planted with soybean in Miyagi Prefecture is about 10,000 ha, representing the second biggest production district in Japan. Following high demand of the soybeans produced in Miyagi, improvement of seed yield and quality is desired. Weed problem is also an important issue in soybean production. As of 2013, bentazon [3-isopropyl-1H-2,1,3-benzothiadiazin-(4)3H-one 2,2-dioxide] is the only the post-emergence herbicide for efficacious broadleaved weed control in soybean registered as over the top application, and has been used widely in Miyagi. Devil's beggarticks (*Bidens frondosa*) and pale persicaria (*Polygonum lapathifolium*) are major species, susceptible to bentazon (Shibuya et al. 2006; Taira and Yoshida 2004), to be controlled. However, such species still remained problematic in many soybean fields in Miyagi. The registered application time of the bentazon formulation is soybean second trifoliated stage (V2) to before the bloom stage in Japan. In order to identify the optimum application time of bentazon, we conducted field experiments to determine the relationship between application time of bentazon and weed age in soybean crop of Miyagi.

## MATERIALS AND METHODS

Experiments were conducted in 2011 and 2012 at Miyagi Prefectural Furukawa Agricultural Experiment Station in Japan. In experiments, soybeans were planted at 20 cm intervals in rows at 75 cm apart on June 4, 2011 and May 31, 2012. After planting soybean in 2011 dimethenamid [2-chloro-*N*-(2,4-dimethyl-3-thienyl)-*N*-(2-methoxy-1-methylethyl)acetamide], and linuron [3-(3, 4-dichlorophenyl)-1-methoxy-1-methylurea], premix formulation was applied in 2011 and dimethenamid was applied in 2012 as PRE. Sethoxydim [2-[1-ethoxyimino-butyl]-5-[2-(ethylthio)-propyl]-3-hydroxy-2-cyclohexen-1-one] was applied as POST treatment to the entire experimental area for grass weeds control.

In both 2011 and 2012, 0.6 kg a.i./ha bentazon (40% a.i.) was applied with a boom sprayer at the third trifoliolate stage (V3), sixth trifoliolate stage (V6), seventh trifoliolate stage (V7), and the ninth trifoliolate stage before the bloom (V9), respectively. In addition to these treatments, non-application plots were served as control. Three replications of each treatment were made. Bentazon were applied over the canopy as broadcast treatments. Management calendars for two years, such as bentazon application, are shown in Table 1. The maximum weeds age (2011) and weeds age distribution of 30 individuals (2012) were measured at each application. Additionally, in 2012, in each application, spray exposure area



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ratio was also measured using water sensitive papers placed on the soil surface within row. Weeds were harvested before soybean harvest, oven-dried, and the shoot dry weight recorded.

**Table 1**. Soybean growth stage at bentazon application

| | | | The Application time of bentazon | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Soybean grouth stage | | | V3 | - | V6 | - | V7 | V9 | The bloom |
| The date | 2011 | | July, 6 | July, 7 | July,11 | July, 13 | July, 15 | July, 22 | July, 26 |
| | 2012 | | July, 3 | July, 4 | July, 10 | July, 13 | July, 17 | July, 25 | July, 31 |
| Inter-row tillage | | | | First | | Second | | | |
| Sethoxydim | 2011 | june, 30 | | | | | | | |
| | 2012 | june, 27 | | | | | | | |

V3-V9 indicate the soybean growth stage at each application. V3: the third trifoliolate stage, V6: sixth trifoliolate stage, V7: seventh trifoliolate stage, V9: the ninth trifoliolate stage

## RESULTS AND DISCUSSION

In our weeds age study in 2011, after V6, both of lambsquarters (*Chenopodium album*) and pale persicaria were over the six leaf stage, and in 2012, at V3, 83% of common lambsquarters and 63% of pale persicaria were over the six leaf stage (Fig. 1). The application time of bentazon formulation is registered till the weed sixth leaf stage in Japan. But at V9, all of the common lambsquarters and pale persicaria exceeded the sixth leaf stage.In 2012, water sensitive paper tests revealed that exposure area ratio within-row was largest at V3. Late application time led to smaller exposed area. Applied at V9, exposed area was decreased to 34% compared with application made at V3.

Weed shoot dry weight at soybean harvest in 2012 was greater than 2011. Major residual weed species was lambsquaters in both years. Pale persicaria and other species were effectively suppressed. Spraying made at V3 was more effective than those made at V9, reducing weed dry weight at soybean full maturity by 86% and 56%, in 2011 and 2012, respectively (Fig. 2). Our results indicated that optimum bentazon application time for controlling broadleaved weeds was around soybean at V3 stage (30 - 35 days after sowing and before first inter-row tillage), which is early stage as recommend on the label for herbicide. Furthermore, controlling common lambsquarters with bentazon was erratic, and became more tolerant at later stage. Thus, spraying in earlier growth stage is needed to reducing weeds infection.

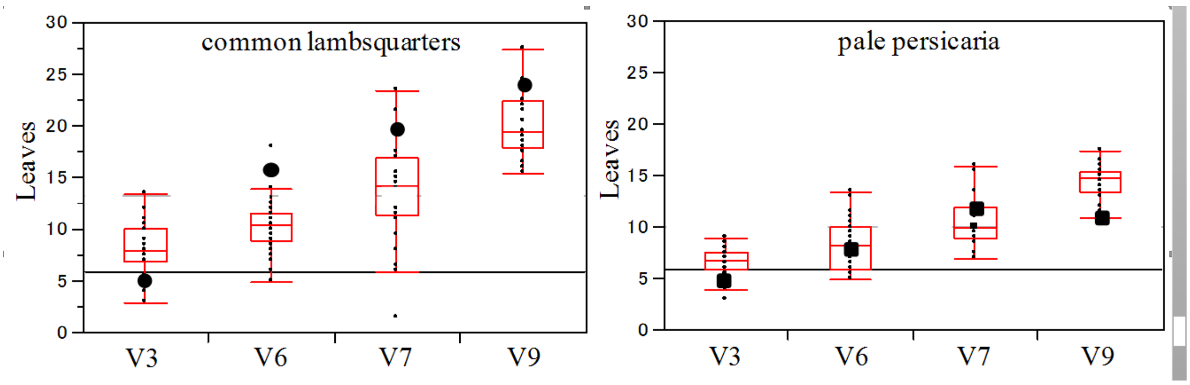


**Fig.1.** Weeds age at different application time of bentazon Marker (● and ■) indicate maximum weeds age of 2011.Box-and-whisker plot indicate weeds age distribution of 30 individuals of 2012. The horizontal line indicates 6 leaves of weeds.



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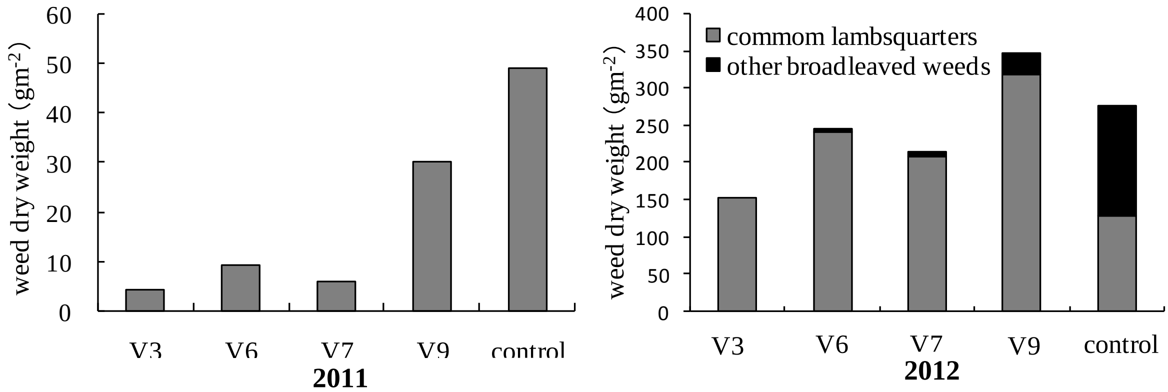


**Fig. 2.** Effect of times of bentazon application on reduction of broadleaf weeds biomass at soybean full maturity.

Dimethenamid and linuron in 2011, and dimethenamid in 2012 applied as PRE after planting soybean. Sethoxydim was applied as POST for grasses in both 2011 and 2012.

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## *Allelopathy and Allelochemicals*

# A POTENT GROWTH INHIBITORY SUBSTANCE OCCURRED IN DECOMPOSITION PROCESS OF JAPANESE RED PINE LITTER

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**ABSTRACT** Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) was considered to have strong allelopathy. Based on the literatures, allelopathic substances are probably released and accumulated in the soil by leaching from needles and degradation of the litter. Therefore, allelopathic substances in the soil are important for the elucidation of allelopathy. Although several growth inhibitory substances were isolated from the pine needles and litter, the active compounds in soil are limited. In this study, potent inhibitory substance in the soil under the pine forests has been isolated and identified. The soil extract showed the concentration-dependent inhibition on the growth of cress and barnyard grass, which suggest that the soil may contain growth inhibitory substances. A main growth inhibitory substance in the soil extract was isolated and characterized by spectral data as 7-oxodehydroabietic acid. This compound is one of abietic acids which are main components of resin produced in Pinaceae plant families. Another oxidized abietic acid, 9,13-Epidioxyabiet-8(14)-en-18-oic acid had been isolated from red pine needles and litter as growth inhibitory substance. Considering to the same carbon structure of these two active substances, it is possible that 9,13-epidioxyabiet-8(14)-en-18-oic acid in pine needles may be delivered into soil as litter and may change the structure to 7-oxodehydroabietic acid in the soil during litter decomposition. These results suggest that 7-oxodehydroabietic acid may play an important role in the allelopathy of Japanese red pine.

***Keywords:*** Allelopathy, forest floor, growth inhibitor, *Pinus denshiflora*

## INTRODUCTION

The vegetation under Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) forests is sparse although sunlight intensity under the forests is sufficient for herbaceous plants to grow. The sparse vegetation of the red pine forest floors could be due to their allelopathic properties. Based on the literatures, allelopathic substances are probably released into the soil either by leaching from the red pine needles or degradation of their litters (Lee and Monsi 1963). To date several putative allelopathic substances have been isolated from aqueous methanol extracts of pine needles and litter (Kato-Noguchi et al. 2009; 2011; 2012). These active substances may be accumulated in the soil, causing the growth inhibition of neighboring plants and thereby regulate the structure of the plant community. Although allelopathic substances in the soil are imperative for the elucidation of red pine allelopathy, there have been very few studies so far. The present research work was conducted to isolate and identify the potent allelopathic substances from the soil under the pine forests.

## MATERIALS AND METHODS

### Extraction

Soils under red pine (Pinus densiflora Sieb.et Zucc.) forest were randomly collected below 20 trees (young and old trees) in area of 10 × 10 $m^2$, from mountainous terrain of Takamatsu, Japan in June, 2011. The collected soils (400 g dry weight) were then extracted with 2 L of



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80% (v/v) aqueous methanol for 2 days. After filtration using filter paper (No. 2; Toyo Ltd., Tokyo), the residue was extract again with 2 L of cold methanol for 2 days and filtered. The two filtrates were combined and evaporated with a rotary evaporator at 40°C to produce an aqueous residue.

**Bioassay**

Sample for bioassay was evaporated to dryness and dissolved in methanol, added to the filter paper in 2.8 cm Petri dishes, and the methanol was evaporated. Then the filter paper was moistened with 0.6 mL of 0.05% (v/v) Tween 20. Ten seeds of cress or 10 germinated seeds of barnyard grass were arranged on the filter paper in Petri dishes. Barnyard grass was germinated in dark at 25°C for 48 h. The hypocotyl/coleoptile and root lengths of cress and barnyard grass were measured at 48 h after incubation in dark at 25°C. Control seeds were also arranged on the filter paper moistened with the aqueous solution of Tween 20 without the extracts.

**Isolation and identification of an allelopathic substance**

The aqueous residue was then adjusted to pH 7.0 with 1 M phosphate buffer, partitioned three times against an equal volume of ethyl acetate. The ethyl acetate fraction was evaporated to dryness and chromatographed on a column of silica gel, Sephadex LH-20, reverse phase $C_{18}$ cartridges and HPLC. In every step of purification, biological activity was detected by cress bioassay according to the aforementioned procedure. Finally, an active substance was characterized by $^{1}H$- and $^{13}C$-NMR and mass spectra.

## RESULTS AND DISCUSSION

Aqueous methanol extracts of soil under red pine forest inhibited hypocotyl/coleoptile and root growth of cress and barnyard grass, and increasing the extract concentration increased the inhibition. The results suggest that soil may contain growth inhibitory substances and may possess allelopathic potential.

A main growth inhibitory substance in the soil extract was isolated and characterized by spectral data as 7-oxodehydroabietic acid. This compound is one of abietic acids which are main components of resin produced in Pinaceae plant families. The concentrations required for 50% inhibition of 7-oxodehydroabietic acid on the hypocotyl and root of cress were 0.18 and 0.17 mM, respectively. The growth of cress was completely inhibited at the concentrations greater than 1 mM.

On the other hand, 9,13-epidioxyabiet-8(14)-en-18-oic acid had been isolated from red pine needles and litter as a growth inhibitory substance (Kato-Noguchi et al. 2009). 9,13-Epidioxyabiet-8(14)-en-18-oic acid and 7-oxodehydroabietic acid have same abietic acid structure. Thus, it is possible that 9,13-epidioxyabiet-8(14)-en-18-oic acid in pine needles may be delivered into soil as litter and may change the structure to 7-oxodehydroabietic acid in the soil during litter decomposition. As accumulation of allelopathic substances in the soil is very crucial for regulating the neighboring plant germination and growth, 7-oxodehydroabietic acid may be one of the main factors responsible for the sparse vegetation under Japanese red pine.

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# HERBICIDAL POTENTIALITY OF FIVE LAMIACEAE PLANT SPECIES ON *ECHINOCHLOA CRUS-GALLI*

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**ABSTRACT** Uninterrupted use of synthetic herbicides to control *Echinochloa crus-galli* L. not only makes them resistance to certain herbicides on their sites of action but also creates environmental pollution. To overcome those problems, researchers are currently interested in searching new novel compounds in allelopathic medicinal plants to develop natural herbicides. Plants belongs to the Lamiaceae family attracted the attention of many researchers, mainly for their myriad of pharmacological and toxicological properties. To date, very limited information is available in the literature about the allelopathy of this family. Hence, to explore the allelopathy of five Lamiaceae plant species: *Leucas aspera* L., *Leonurus sibiricus* L., *Ocimum tenuiflorum* L., *Mentha sylvestris* L. and *Hyptis suaveolens* L., the aqueous methanol extracts of those plants were tested against *E. crus-galli* at four different concentrations. The root growth was more sensitive to the plant extracts than the coleoptile growth. The inhibitory activities were concentration and plant extract dependent. At 100 mg DW equivalent extract/mL, *L. aspera* and *H. suaveolens* plant extracts strongly inhibited the seedling growth of *E. crus-galli*. However, at the same concentration the lowest inhibition was observed in case of *O. tenuiflorum* plant extract. Considering $I_{50}$ values, the sensitivity of E. crus-galli was in the order of *L. aspera*>*H. suaveolens*>*L. sibiricus*>*M. sylvestris*>*O. tenuiflorum*. These results suggest that *L. aspera* and *H. suaveolens* possess strong allelopathic potential and therefore, could be used as the good candidates for isolation and identification of allelochemicals to develop environment friendly, bio-degradable natural herbicides to control *E. crus-galli*.

***Keywords:*** Allelopathy, allelochemicals; natural herbicides; paddy weed

## INTRODUCTION

*Echinochloa crus-galli* (L.) P. Beauv. is considered as the most deleterious paddy weeds throughout the world (Holm et al., 1977). The $C_4$ photosynthetic pathway system of *E. crus-galli* makes it more competitive against $C_3$ plants like rice (Vidotto et al., 2007; Ampong-Nyarko and De Datta, 1991). Additionally, the special characteristic, ‘mimicry’ with rice seedlings helps it to escape manual weeding (Barrett, 1983). Stephenson (2000) reported that each year on an average three million tonnes of synthetic herbicide is used by the farmers in agriculture to control weeds. Despite some benefits of synthetic herbicides, over-reliance on it may cause a number of environmental hazards as well as develops herbicide resistant weed biotypes (Vyvyan, 2002). These harmful effects of synthetic herbicide shifted the attention of the researchers towards the development new eco-friendly bio-degradable natural herbicides. Researchers around the world showed their keen interest on medicinal plants for searching new active allelochemicals, perhaps due to their existed certain metabolic compounds which was used for curing many diseases.

The plants belongs to Lamiaceae family attracted the attention of many researchers mainly due to their myriad of pharmacological and toxicological properties (Lovett and



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Weerakoon, 1983). But to date very few are known about their allelopathic properties. Hence, the current research was conducted to examine the allelopathic properties of five Lamiaceae plant species against *E. crus-galli*. The research findings could be helpful to find out the good candidates for isolation and identification of active allelochemicals for new natural herbicide development, which will effectively control the deleterious paddy weed *E. crus-galli*.

## MATERIALS AND METHODS

Five medicinal plant species from Lamiaceae family; *Leucas aspera* L., *Leonurus sibiricus* L., *Ocimum tenuiflorum* L., *Mentha sylvestris* L. and *Hyptis suaveolens* L. were collected from Bangladesh and extracted separately as per Islam and Kato-Noguchi (2013) with slight modifications. *E. crus-galli* was selected as a test plant species due to its worldwide acceptance as a major paddy weed**.** The bioassay was conducted with four extract concentrations of 3, 10, 30 and 100 mg DW equivalent extract/mL of each plant species. The bioassays were also performed according to Islam and Kato-Noguchi (2013). Experimental data were analyzed using statistical software SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA) and GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, California, USA).

## RESULTS AND DISCUSSION

The inhibitory activity of the aqueous methanol extracts of *L. aspera*, *L. sibiricus, O. tenuiflorum, M. sylvestris* and *H. suaveolens* on the coleoptile and root growth of *E. crus-galli* was determined. The results showed that the root growth was more sensitive to the plant extracts than the coleoptile growth. The inhibitory activities were concentration and plant extract dependent. At 100 mg DW equivalent extract/mL, both *L. aspera* and *H. suaveolens* shown more than 55% coleoptile and 95% root growth inhibition. Whereas, *L. sibiricus, O. Tenuiflorum* and *M. sylvestris* shown less than 25% coleoptile and more than 60% root growth inhibition. Considering total average inhibition, both *L. aspera* and *H. suaveolens* shown more than 20 and 50% coleoptile and root growth inhibition, respectively. In contrast, the other three plant extracts stimulated the coleoptile growth of *E. crus-galli* and shown less than 30% root growth inhibition. The sensitivity of *E. crus-galli* was followed the trend of *L. aspera*>*H. suaveolens*>*L. sibiricus*>*M. sylvestris*>*O. tenuiflorum*, respectively depending on the concentration required for 50% inhibition ($I_{50}$).

Our results indicate that all the five Lamiaceae plants may have allelopathic properties, even though their effectiveness on *E. crus-galli* was different from others. Among the five plant extracts *L. aspera* and *H. suaveolens* have the highest potential to inhibit the seedling growth of *E. crus-galli*. Therefore, this two plant species could be the good candidates for isolation and identification of their active allelochemicals, which might lead the chemical basis to develop natural herbicides to control *E. crus-galli* in more sustainable way. Nevertheless, their crude extract and/or residue could also be recommended to apply directly as bio-herbicide.

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# EFFECT OF AQUEOUS EXTRACT FROM DURIAN LEAVES AND PARTIALLY SEPARATION OF ACTIVE COMPOUNDS

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**ABSTRACT** Comparison of the aqueous extracts from five *Durio zibethinus* Murr. varieties. (Cha-nee, Kra-dum , Mawn-tawng, Gahn-yao and Poung-manee) and three leaf growth stages (terminal leaflet, young leaflet and mature leaflet) were assayed at concentrations of 50 mg/mL on seed germination and seedling growth of barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) and wild pea (*Phaseolus lathyroides* L.). The results showed that the degrees of their inhibitory effects were varieties dependent and varied among leaf growth stages. Among five varieties, Kra-dum variety was the most inhibition effects on seed germination and seedling growth of *P. lathyroides* and seed germination and seedling growth can be classified in order of decreasing inhibition as young leaf > terminate leaflet > mature leaflet. Thus, Kra-dum variety was selected for further partially solvent separation of inhibitory allelochemicals. The ethanol crude extract (OR) of Kra-dum varieties young leaflets was partitioned into ethyl acetate soluble acidic (AE), neutral fraction (NE), and aqueous fraction (AQ). The inhibitory effects of AQ, NE, and AE fractions were compared with the OR fraction. The AE fraction showed the greatest inhibitory effect fraction with complete inhibition of seed germination and seedling growth of both bioassay species at the concentration of 10,000 ppm, followed by NE fraction, AQ fraction and OR fraction, respectively.

***Keywords:*** *Durio zibethinus* Murr., acid-base solvent partitioning, *Echinochloa crus-galli* (L.) Beauv., *Phaseolus lathyroides* L.

## INTRODUCTION

Durian (*Durio zibethinus* Murr.) is the most famous and popular seasonal fruit in South East Asia, especially in ASEAN countries. It is considered exceedingly delicious and quality fruits are most expensive. Durian is a tropical fruit belonging to the Malvales order, in the Bombacaceae family and genus Durio (Roy and Joshi 1995). Thailand is a country with the potential to produce the best fruit, tropical country and exporter of durians, followed by Malaysia and Indonesia (Nanthachai 1994). There are 30 kinds of fruit with at least 9 species of edible *Durio zibethinus* but only one type and only Five varieties to commercial of durian(Cha-nee, Mawn-tawng, Kra-dum, Poung-manee and Gahn-yao). In 2012, durian products exported from Thailand totally 365,914 metric tons and export earnings from durian sales amounted to 7,167 million baht (The Office of Agricultural Economics 2012). However, there are no reported on allelopathic potential of durian. Therefore, this research was to study on the allelopathic potential of 5 durian varieties.

## MATERIALS AND METHODS

Five durian varieties (Cha-nee, Kra-dum , Mawn-tawng, Gahn-yao and Poung-manee) were collected from Horticultural Recearch Center, Chantaburi province, Thailand. Three leaf growth stages (terminal leaflet, young leaflet and mature leaflet) of each durian variety were dried in a hot-air oven at 45°C for 3 days. They were then extracted by distilled water and diluted with distilled water to give final concentrations of 25, 50, 75 and 100 $gL^{-1}$. Each extract concentration was bioassay on germination and seedling growth of barnyardgrass (*Echinochloa crus-galli* Beauv.) and wild pea (*Phaseolus lathyroides* L.). Treatments with distilled water were used as the negative controls. Germination and seedling growth were



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determined at 7 days after treatments. Seedling growth was measured as the root and shoot lengths at 7 days after treatments. Data were subjected to an analysis of variance, and means were compared using the Tukey's Studentized Range Test at a 5% level of significance. The most effective treatment was selected for solvent partitioning of active compounds experiment**.** The crude extracts were prepared by extracted of the most effective plant material with ethanol and evaporated leaving a sticky residue (OR fraction). The sticky residue was diluted with 500 mL of distilled water and partially separated in to gross allelochemical groups by acid-base partitioning methods (Laosinwattana et al. 2007). The inhibitory activities of each fractions (OR, AQ, NE and AE fractions), were prepared as same as previously described. Each fraction of OR, AQ, NE and AE fractions was prepared to contain four concentrations of each fraction from 1000 to 8000 ppm. Bioassays on seed germination and seedling growth were tested as previously described.

## RESULTS AND DISCUSSION

The results showed that the degrees of their inhibitory effects were varieties dependent and varied among leaf growth stages. Among five varieties, Kra-dum variety was the most inhibition effects on seed germination and seedling growth of *P. lathyroides* and seed germination and seedling growth can be classified in order of decreasing inhibition as young leaf > terminate leaflet > mature leaflet. Thus, Kra-dum variety was selected for further partially solvent separation A ethanol crude extract (OR) from Kra-dum variety was partitioned into aqueous (AQ) fraction, neutral (NE) and acidic (AE) fractions. The growth inhibition activities of AQ, NE and AE fractions were compared with the OR at the concentrations for bioassay varied from 0.125 – 1 % were evaluated on seed germination and seedling growth of *E. crus-galli* and *P. lathyroides* (Fig. 1). After solvent partitioning, the inhibition increased as compared to the OR fraction. The AE fraction showed the greatest activity fraction with complete inhibition of germination and seedling growth of both bioassay species at concentration of 1%, followed by NE fraction, AQ fraction and OR fraction, respectively. These results indicated that most allelochemical compounds produced by Kra-dum variety could be presented in AE fraction. It was similar results to Teerarak et al. (2010), who reported that a secoiridoid glucoside named oleuropine which identified as an allelopathic compound from AE fraction of a related *Jasminum officinale* var. *grandiforum*. It is suggested that the mixture compound in AE fractions gave significantly inhibited the tested weed species.

## CONCLUSION

The results indicated that young leaflets from Kra-dum variety was the strongest allelopathic potential and after acid-base solvent partition of active compounds, acidic fraction (AE) was the most effective with complete inhibition of germination, shoot length and root length of both bioassay species.



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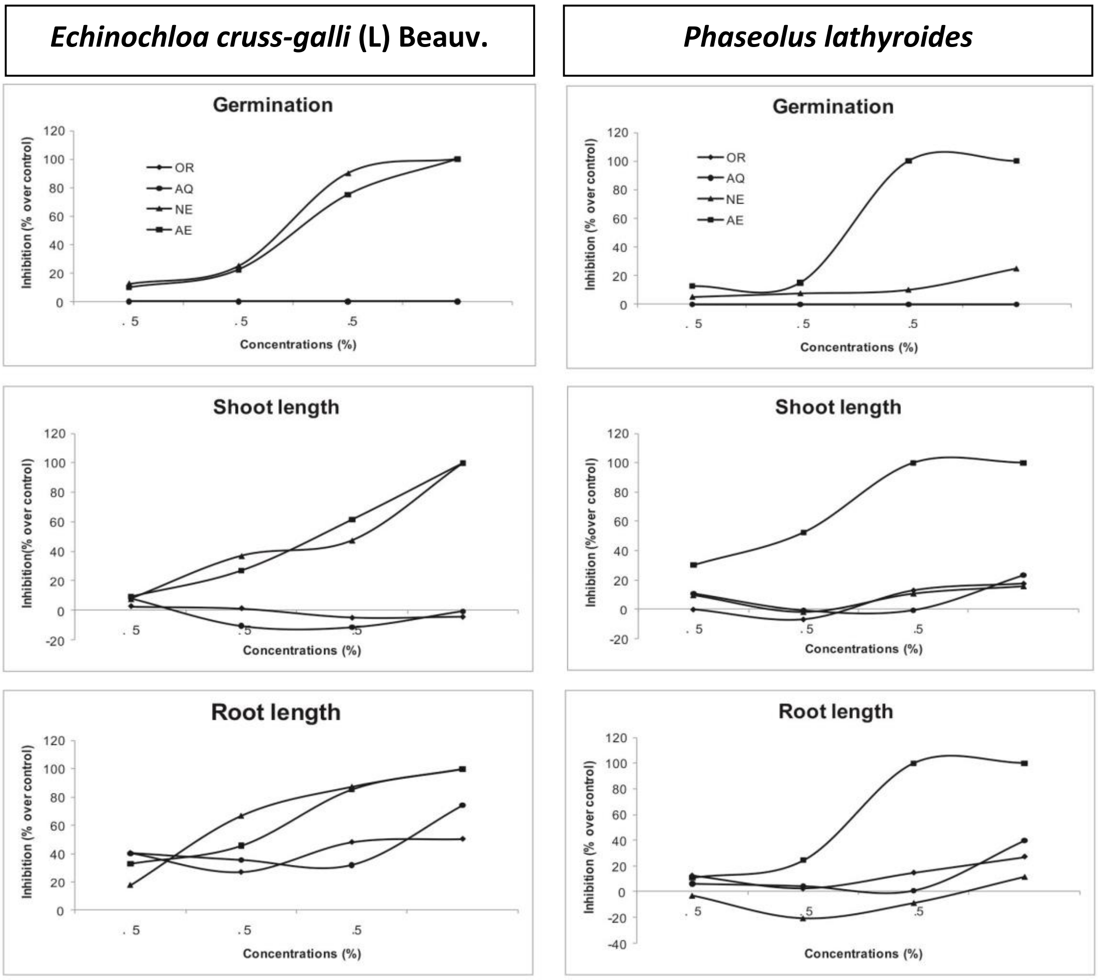


**Fig. 1.** Effect of ethanol (OR), aqueous (AQ), neutral (NE) and acidic (AE) fractions at the concentrations of 0.125 to 1 % on germination, shoot length and root length of *E. crus-galli* and *P.lathyroides* L.

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# MOMILACTONE B RESPONSIVE PROTEINS IN ARABIDOPSIS

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**ABSTRACT** Momilactone B is a potent allelopathic substance of rice (*Oryza sativa* L.) and inhibits the growth of some weeds on paddy field. However, information about the inhibitory mechanism of momilactone B is limited. As proteins are known to have very important roles in plant growth directly or indirectly, it is necessary to study the effects of momilactone B on the expression of proteins. The present research was conducted to identify momilactone B responsive proteins in Arabidopsis. Arabidopsis seedlings were treated with momilactone B and incubated, and control seedlings were also set under same condition without momilactone B. The protein extracts were separated by two-dimensional electrophoresis. Then, the momilactone B responsive proteins, which increased or decreased by momilactone B, were identified by MALDI TOF-MS and mascot. Four proteins, subtilisin-like protease, amyrin synthase LUP2, beta-glucosidase and malate synthase were decreased significantly and four proteins, cruciferin 2, translationally-controlled tumor protein-like protein, glutathione S-transferase GST6 and 1-Cys peroxiredoxin 1 were increased significantly by momilactone B. Therefore, just 1 h after application, momilactone B increased four proteins and decreased other four proteins in Arabidopsis seedlings. The present research suggests that momilactone B affected the protein expression in Arabidopsis and some of the proteins may be involved in the growth inhibition.

***Keywords:*** allelopathy, momilactone B, protein expression, *Arabidopsis thaliana*

## INTRODUCTION

Weeds are the common constraint of rice production and cause yield losses in all rice production system and in all seasons (Zoschke, 1990). Weed management using allelopathy may effect a yield improvement without environmental cost, which is one of the most important considerations for scientists working to secure the world's food supply for future generations (Rice, 1974; Khanh et al., 2007).

Momilactone B is released from rice, inhibits the growth of typical rice weeds such as barnyard grass and *Echinochloa colonum*, and may have an important role in the rice allelopathy (Kato-Noguchi et al., 2005; Kato-Noguchi, 2011). However, information about the inhibitory mechanism of momilactone B is limited. Proteins have important functions in plant development, including germination and growth, and many proteins undergo post-translational modifications and glycosylation, which are extremely important for protein activities and subcellular localization (Shunping et al., 2005). Proteomics is a powerful tool to reveal the protein complement of cell organelles and to obtain new insights into intracellular protein sorting and biochemical pathways (Kleffmann et al., 2004). Therefore this research focused on momilactone B responsive proteins in Arabidopsis by two-dimensional electrophoresis.

## MATERIALS AND METHODS

Seeds of *Arabidopsis thaliana* (L.) were incubated in light for 24 h and darkness for 16 h after imbibitions to grow seedlings. Then the seedlings were treated with momilactone B and incubated in darkness for 1 h. Control seedlings were also grown under same condition without momilactone B. After sampling, these seedlings were homogenized with five volumes of ice-cold solution containing 10mM Tris-HCl (pH 8.1), 0.5% (w/v) sodium dodecyl



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sulphate-polyacrylamide (SDS), 0.3% (v/v) Triton X-100, Protease inhibitor cocktail (EDTA free), 6.3 units/mL DNase and 0.5 units/mL RNase A. After homogenation, these crude extracts were centrifuged for 20 min at 12000 rpm. These protein extracts were then separated by two-dimensional electrophoresis. After electrophoresis, the gels were stained by silver (Yan et al. 2001). Then, the momilactone B responsive proteins were identified by MALDI TOF-MS and mascot.

## RESULTS AND DISCUSSION

The gels which were stained by silver showed that several proteins were decreased or increased by momilactone B. Decreased proteins were subtilisin-like protease, amyrin synthase LUP2, beta-glucosidase and malate synthase. On the other hand, increased proteins were cruciferin 2, translationally-controlled tumor protein-like protein, glutathione S-transferase GST6 and 1-Cys peroxiredoxin 1. Momilactone B increased glutathione S-transferase GST6 and 1-Cys peroxiredoxin on the growth of Arabidopsis. These proteins protect plant cell from ROS damage (Radhika et al., 1998; Camilla et al., 2003), which are important regulators of plant development and signalling molecules to control various processes. Moreover, they can cause extensive cell injury or death (Apel et al., 2004; Davletova et al., 2005; Gapper et al., 2006). Therefore, momilactone B may disturb ROS regulation to inhibit the growth of plants. The present research indicates that momilactone B affected the protein expression in Arabidopsis, which are directly or indirectly involved in the growth inhibition of Arabidopsis. Plants have evolved intricate mechanism to perceive external signals to survive under various conditions (Fujita et al., 2006).

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# ALLELOPATHY AND NOVEL ALLELOPATHIC SUBSTANCE IN JAVA TEA (*ORTHOSIPHON STAMINEUS*)

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**ABSTRACT** Java tea (*Orthosiphon stamineus*) has been widely used as traditional herb, and there are many commercial herbal tea products, known as Java tea, derived from "Kumis Kucing (Misai Kucing)", which is the name of *O. stamineus* in Indonesia. Several bioactive compounds against animal cells have been isolated from *O. stamineus.* However, no bioactive compound against plants has been reported so far. Therefore, we investigated possible allelopathic properties and allelopathic substances in *O. stamineus*. Aqueous methanol extracts of *O. stamineus* inhibited root and hypocotyl growth of cress (*Lepidium sativum*) and lettuce (*Lactuca sativa*) seedlings. Increasing the extract concentration increased the inhibition, which suggests that *O. stamineus* may have allelopathic properties. The extract was further purified mornitaring the inhibitory activity, and a main allelopathic substance was isolated and identified as 13-*epi*-orthosiphol N, a novel compound, by spectral data. 13-*epi*-Orthosiphol N inhibited root and hypocotyl growth of cress and lettuce at concentrations greater than 10 μM. The concentrations required for 50% inhibition ranged from 41 to 102 μM. These results suggest that 13-*epi*-orthosiphol N may be an allelochemical and main contributor to the growth inhibitory effect of *O. stamineus* and may have potential as a template for the development of new plant control substances.

***Keywords:*** Allelopathy, biological activity, growth inhibitor, Lamiaceae

## INTRODUCTION

Plants produce numerous secondary metabolites, and some of these compounds show allelopathic activity, such as growth inhibitory effects on other plants. Some plant species provide excellent weed control in intercropping or as soil additives. Allelopathic substances have potential as either herbicides or templates for new synthetic herbicide classes, and are more environmentally benign than most synthetic herbicides.

Java tea (*Orthosiphon stamineus*) has been widely used as traditional herb to treat diabetes, kidney and urinary disorders, high blood pressure and bone or muscular pain. In addition, there are many commercial herbal tea products, known as Java tea, derived from "Kumis Kucing (Misai Kucing)", which is the name of *O. stamineus* in Indonesia. The tea of *O. stamineus* is known to be effective in treatments of kidney stones, depression and gall bladder problems.

Several biologically active compounds, including terpenes, have already been isolated from *O. stamineus*. These compounds exhibited anti-inflammatory and anti-proliferate activities, cytotoxicity and nitric-oxide inhibition in animal cells. However, no bioactive compound against plants has been reported so far.

## MATERIALS AND METHODS

Therefore, we investigated possible allelopathic properties and allelopathic substances in *O. stamineus*.

## RESULTS AND DICUSSION

The aqueous methanol extract of *O. stamineus* inhibited root and hypocotyl growth of cress and lettuce seedlings. Increasing the extract concentration resulted in an increase in the



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inhibition. The extract obtained from 30 mg of *O. stamineus* dry shoots inhibited cress root and hypocotyl growth to 21 and 34% of the control, respectively, and in lettuce to 32 and 41. These results suggest that the extract of *O. stamineus* may have allelopathic properties and, thus, contain allelopathic substances.

The ethyl acetate and aqueous fractions of the aqueous methanol extract, both suppressed root and hypocotyl growth of cress seedlings. The inhibitory activity of the ethyl acetate fraction was greater than that of the aqueous fraction. The ethyl acetate fraction was then separated on a silica gel column and the biological activity of the fractions was determined. The most active fraction was further purified by Sephadex LH-20, reverse-phase $C_{18}$ Sep-Pak cartridges and HPLC with monitoring the inhibitory activity by cress bioassay and a main active compound was isolated. The active compound was identified as 13-epi-orthosiphol N, a novel compound, by spectral data.

13-epi-Orthosiphol N inhibited root and hypocotyl growth of cress and lettuce at concentrations greater than 10 μM. The inhibition increased with increasing concentrations of 13-epi-orthosiphol N. The concentrations required for 50% growth inhibition ($I_{50}$) of cress roots and hypocotyls, as determined by a logistic regression analysis, were 39 and 83 μM, respectively, and the $I_{50}$ for lettuce roots and hypocotyls was 47 and 102 μM.

The aqueous methanol extract of *O. stamineus* showed growth inhibitory activity. The extract was purified and a main active substance isolated. The chemical structure of the substance was determined as a novel compound, 13-*epi*-orthosiphol N. The present results suggest that *O. stamineus* has allelopathic properties, and that 13-*epi*-orthosiphol N may also have potential as a template for the development of new growth inhibitory agents.



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# STUDIES ON ALLELOPATHY OF *PHYLA NODIFLORA* ON SEVERAL CROPS

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**ABSTRACT** This paper aims at the investigation on the effect of allelopathy of native and domesticated *Phyla nodiflora* introduced from Japan on wheat, radish and rape. The results indicated that the inhibitory effect of the germination rates of the three crops did not reach significantly higher levels in six aqueous extract treatments from six breeds of *P. nodiflora*; there were inhibitory effect on the height, root length, fresh weight of root and aerial part of the three crops' seedlings in some cases, but were not significantly. The domesticated species did not have obviously stronger allelopathy on the three crops than those native species as well. Therefore, the preliminary conclusion is that the introduced domesticated *P. nodiflora*, according to the allelopathy effect, will not harm the growth of domestic wheat, radish and rape.

***Keywords:*** *Phyla nodiflora*, allelopathy.

## INTRODUCTION

*Phyla nodiflora* (L.) Greene (Verbenaceae), common names are YanChuiCao, ShiXian, YaSheHuang, YaSheCao, and GuoJiangTeng, originated from Jiangsu, Fujian, Hunan, Hubei, Guangdong, and Guangxi Provinces, China (Anon 1982). *Phyla nodiflora*, is a perennial creeping herb with growing rapidly and high stress resistance. It plays an active role on reforesting barren mountains, harnessing waste lands and steep hills, conserving earth and water, guarding against gale and fixating sand. The aims of this study were to determine whether *P. nodiflora* performs allelopathy effect on crops and to evaluate the risk in introducing *P. nodiflora* by studying the allelopathy of *P. nodiflora* on several crops.

## MATERIALS AND METHODS

### *Phyla nodiflora* Collection

Native species was collected from Guangzhou Province of China and five domesticated species being introduced from Japan were collected in Beijing, Tianjin, Shanghai, Jiangxi and Jiangyan, and cultivated in the greenhouse at CAAS of Beijing.

### Preparation of Aqueous Extracts of Fresh P. nodiflora

With tissue disruption, fresh plant material was extracted in distilled water at room temperature (about 26℃). The aqueous extract was decanted, vacuum filtered through 4 layer filter paper, the filtrate was identified as 4% (ratio of tissue to water), and further diluted at the ratio of 1%, 1‰. Finally the aqueous extracts was filtered with microporous membrane and stored at 4℃ until use.

### Growth Test (Bioassays)

Referred to research methods of Shen *et al.* (2005), the mature, uniform seeds of wheat, radish, and rape were selected, surface-sterilized with 1% sodium hypochlorite, washed three times with distilled water, drained to dry with filter paper, then immersed in distilled water for 8 h, and promoted germination in a dark incubator. Each experimental unit consisted of 10 germinating seeds, placed on 2 layer filter paper in a Petri dish and each treatment has three duplications. Two militers of each extract types which were prepared above was added to the Petri dish, and 2 ml distilled water was added to the contrast. All Petri dishes were placed in



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an incubator set at 28-18℃, on a 12h day-light cycle and 75% relatively humidity maintained. The root length, seedling height, fresh weight of root and fresh weight of aerial part of the seedlings were measured after 72 hours.

**Data Analysis**

The calculation of allelopathy response index (RI) was based on the methods of Jiang et.al. (2007). RI = 1- C/T (T≥C) or RI = T/C – 1 (T＜C). Where C is for the data of contrast； T is for the data of treatment. RI＞0 is for promoted function, RI＜0 for inhibitory function. The absolute value is consistent to intensity of effect. The statistical analyses of all data were performed using SPSS 13.0.

## RESULTS AND DISCUSSION

**Effect of *P. nodiflora* Aqueous Extracts on Several Crops Seed Germination Rate**

The analysis results indicated that the effect of *P. nodiflora* breeds, aqueous extract concentration, and interaction of breed and concentration on seed germination rate of the three crops were not significantly.

**Effect of Aqueous Extracts from *P. nodiflora* on Wheat Seedling Growth**

The breeds of *P. nodiflora* had no significant effect on the wheat' seedling root length, seedling height, fresh weight of root and fresh weight of aerial part of seedlings; but the concentration of aqueous extracts had significant effect on wheat' root length and fresh weight, the effect on wheat' seeding height and fresh weight of aerial part was not significant;and the interaction of breed and aqueous extracts concentration had no significant effect on four physiological indicators of wheat.

**Effect of Aqueous Extracts from *P. nodiflora* on Radish Seedlings Growth**

Different breeds of *P.nodiflora* had significant effect on the radish' seedling root length and seedling height, but not on fresh weight of root and fresh weight of aerial part; similarly, the concentration of aqueous extracts had significant effect on radish' seedling root length and seedling height, but not on fresh weight of root and fresh weight of aerial part; and the interaction of breeds and aqueous extracts concentration had significant effect on the four physiological indicators of radish.

**Effect of Aqueous Extracts from *P. nodiflora* on Rape Seedlings Growth**

The analysis results indicated that the breeds of *P. nodiflora* had no significant effect on the rape seedling growth; the concentration of aqueous extracts had significant effect on the seedling height and fresh weight of aerial part, but not on root length and fresh weight of roots; and the interaction of breeds and aqueous extracts concentration had no significant effect on the four physiological indicators of rape. The analysis results indicated that the effect of *P. nodiflora* aqueous extracts on seeds germination rate of the three crops were not significantly, but there were certain inhibitory effect on seedling growth of the three crops. This inhibitory effect did not increase with an increasing extract concentration; therefore, there was no rule to follow. We speculated that crops physiological indicators by treatment with three concentrations of aqueous extracts decided by their own vitality, the allelopathy effect was not the role. Meanwhile, this inhibitory effect was more pronounced with the five domesticated species than that of the native wild species, since the native wild species did not become weed and harm the agricultural production, nor did the introduced species. As for the allelopathy effect of the introduced species on other crops as well as on China's agricultural production potential hazards still requires further study.



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# ALLELOPATHIC POTENTIAL OF *TAGETES ERECTA* LINN; OPTIMAL EXTRACTION SOLVENT AND ITS PARTIAL SEPARATION OF ACTIVE COMPOUNDS

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**ABSTRACT** Recently, the allelopathy has been introduced as a viable option for alternative weed management under sustainable agriculture. Marigold (*Tagetes erecta* L.) plant growing around the experiment field at the King Mongkut's Institute of Technology Ladkrabng, Bangkok, Thailand was used for all experiments. Allelopathic effects of stem, leaf, flower and root aqueous extract of *T. erecta* were assayed on seed germination and seedling growth of bioassay plants. The results showed that the degrees of growth inhibition can be classified in order of decreasing inhibition as leaf > root > flower > stem extract. To study the optimization of solvent extraction of crude extract from *T. erecta* leaf, 5 different solvent mixtures of ethanol containing of water (100, 75, 50, 25 and 0%) were tested. The quantity of crude extracted materials was found to bee dependent on solvent proportion. The recovery of crude extraction increased with increase in the water concntrations. A mixture of ethanol with water at 75:25 (v/v) was the most effective and gave the greatest quantity of crude extract from *T. erecta*. In the second phase, extraction residue of each solvent mixture was repeated using the same ethnaol containing different volumes of water for 3 times. The result showed that recovery of crude extract was 45-60% in the first number of extraction. These results indicated that extraction of crude extract from *T. erecta* with ethanol:water mixtures must be repeated at least two times of extraction. Crude ethanol:water (75:25 (v/v)) was separted by acid-base solvent partitioning into acidic fraction (AE), neutral fraction (NE), hydrolyze fraction and aqueous fraction (AQ). AE fraction showed the greatest inhibitory effect on bioassay plant, followed by crude ethanol, NE fraction and AQ fraction, respectively.

***Keywords:*** *Tagetes erecta*, marigold, allelopathy, solvent separation

## INTRODUCTION

Marigold is in the family Asteracaea Genus Tagetes, a widespread ornamental plant and is commonly known as marigold which bears bright yellow and orange flowers. It is available in many parts of the world. Its is annual aromatic plant widely grown in Thailand and South East Asia for ornamental plant. The species *Tagetes erecta* (Rhama and Madhavan 2011), *T. minuta* (Tereschuk et al. 1997), *T. patula* (Szarka et al. 2010) are most common, other species referred to are often specific to a region *T. lucida* (Capunzo et al. 2003), *T. mendocina* (Lima et al. 2009). This plant exhibits nematocidal, fungicidal, and insecticidal activity (Vasudevan et al. 1997; Xu et al. 2011). It has been used as cover crop to protect plant-parasite nematodes, inhibition of growth of microorganism and pesticide an attractive crop to natural enemies for long times (Silveira et al. 2009; Hooks et al. 2010). Allelopathy is a plant-plant or plant-microorganism biochemical interaction (Rice 1984). Allelopathic compounds have received considerable attention as potential sources of novel natural herbicides. *Tagetes* species had been vastly reported. Aqueous extracts from *T. minuta* significantly inhibited seed germination and seedling growth of *Lotus corniculata* var. japonicus and *Lactuca sativa* (Kil et al. 2002). Aqueous extract and essential oil of *T. minuta* inhibited the induction of callus and growth of *Oryza sativa*, *Brassica campestris*, *Raphanus sativus* and *Sesamum indicum* (Lee et al. 2002). *T. minuta* leaf powder at 1 and 2 t ha$^{-1}$ significantly reduced weed emergence and dry weight in rice field, and also significantly increased rice yield (Batish et



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al. 2007). It not only possesses excellent pesticidal properties but also has strong antioxidant properties (Maity et al. 2011).

This research was studied on allelopathic and herbicidal potential of *T. erecta* on seed germination and seedling growth of wild pea (*Phaseolus lathyroides* L.).

## MATERIALS AND METHODS

### Plant materials

Whole plants (leaves, stem, flower and root) of *Tagetes erecta* L. plants growing at the experimental field at the King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand were collected at 45 days after planting. The plants were cleaned from soil immediately with running tap water, dried-up in a hot-air oven at 45°C for 3 days and ground to powder (100 mesh) in an electrical blender. Seed of wild pea (*Phaseolus lathyroides* L.) collected from paddy field in the Ladkrabang district, Thailand. Hard seed coats of *P. lathyroides* were scrubbed with No.0 sandpaper to break their dormancy. These species were selected for the experiment due to big seeds and tolerate allelochemicals.

### Aqueous extracts bioassay

Aqueous extracts were prepared from leaf, stem, flower and root materials from each *T. erecta* L. by dissolving 10 grams of each powered material in 100 mL of distilled water at 8°C for 72 h, followed by filtration though three layer of cheesecloth to remove any debris. The supernatant was then filtered through Whatman No.1 filter paper (Whatman Inc. Clifton, NI, USA) to a concentration of 100 mg/mL of dried plant material and stored in a refriferator at 4°C until bioassay. Dilutions of each *T. erecta* L. extract of 12.5, 25, 50 and 100 mg/mL were prepard in distilled water. Five milliliters was added in each 9 cm diameter Petri dish, lined with filter paper and twenty healthy seeds of *P. lathyroides* were placed in each Petri dish. Four replicates were maintained per treatment in a completely randomized manner in a growth chamber with a temperature of 25–32°C, a 12/12 hour dark/light photoperiod, with light intensity (cool White 840) of 100 µmol $m^{-2}$ $s^{-1}$ and relative humidity of around 80%. Treatment with distilled water was used as the control. Germination was deemed to have occurred only after the radicle had protruded beyond the seed coat by at least the dimension of the seed at seven days after treatment. Seedling growth was measured as the root and shoot lengths at seven days after treatment.

### Effect of solvent extraction on the crude extracts yield and bioassay

Ten grams of 100 mesh of *T. erecta* L. leaf power were extracted (ratio 20g: 200 mL) with a different solvent system at room temperature for 48 hours. Solvent systems used were absolute ethanol containing different volumes of distilled water (75%, 50%, 25% and 0%) and distilled water. After 48 hours of extraction, the brown supernatants were filtered through three layers of cheeseclothes and re-filtered through Whatman No.1 filter paper. Following filtration, the brown supernaytants were dried by evaporation of the solvent using a rotary evaporator (BUCHI Rotavapor R255), BUCHI, Lausanne, Switzerland) under a partial vacuum at 45°C until constant crude extract weight was reached. After that each residue was re-extracted 3 times with the same extraction solvent as the same condotion of the first extraction procedure, and then crude extract number 1, 2 and 3 were combined. Stock solution of each crude extract generated by ethanol in water at 100%, 75%, 50%, 25% and 0% (v/v) was prepared by dissolvoing each sticky crude extract with acetone in a motar jar and wettable power (bentonite:anionic surfactant; 95:5 (w/v)), was added in to motar jar in a 3:7 ratio (crude fraction: wettable power). The mixture was slowly pulverized until completely dried; acetone was added three times and kept in the dark at a low temperature until used. Each concentration of crude extracts (100%, 75%, 50%, 25% and 0% ethnaol in water) was performed in wettable power by dissolved in distilled water to contain four concentrations



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ranging from 1250 to 10000 ppm. Bioassays on seed germination and seedling growth were tested as previously described.

**Solvent partitioning of active compounds and bioassay**

The crude extract was prepared from *T. erecta* L. leaf power by extraction with 75% ethanol in distilled water. After filtration using Whatman No.1 filter paper, the residue was repeatedly extracted 3 times with 75% ethanol for 72 hours at 25 °C and filtered. The filtrates were combined and evaporation in the rotary evaporator at 45°C, leaving a sticky residue (original crude fraction; OR fraction). This residue was then diluted with 500 mL of distilled water and stirred vigorously on a magnetic stirrer at 45°C for 20 min, resulting in an aqueous solution which was acidified to pH 3 by 6N HCl. The filtrate was extracted with ethyl acetate three times (0.5 L x 3). After adjusting the pH to neutral, the aqueous phase was dried up by reducing presure at 45°C, and an aqueous residue was obtained (AQ fraction). The ethyl acetate solutions were combined and then treated with anhydrous magnesium sulfate. This solution were divied to 2 parts, the first was evaporated to dryness obtained EtOAc fraction, another was concentrated to about 0.5 L., and then extracted three times with saturated aqueous $NaHCO_3$ (0.5 L x 3). The ethyl acetate phase was dried over with anhydrous magnesium sulfate and concentrated by reducing pressure, and an ethyl acetate-soluble neutral fraction was obtained (NE fraction). The combined sodium bicarbonate phase was evaporated to about 1 L, adjusted to pH 7 by 6 HCl, and then extracted with ethyl acetate (0.5 L x 3). The ethyl acetate solutions were combined, dried over $MgSO_4$, and then evaporated to obtain the ethyl acetate-soluble acidic fraction (AE fraction), and the remains of the aqueous phase were discarded. The inhibitory activities from each fractions (OR, AQ, NE and AE fraction), were prepared as same as previously described (Fig. 1). Each fraction of OR, AQ, NE and AE fraction was prepared to contain four concentrations from 2000 to 16000 ppm. Bioassays on seed germination and seedling growth were tested as previously described.

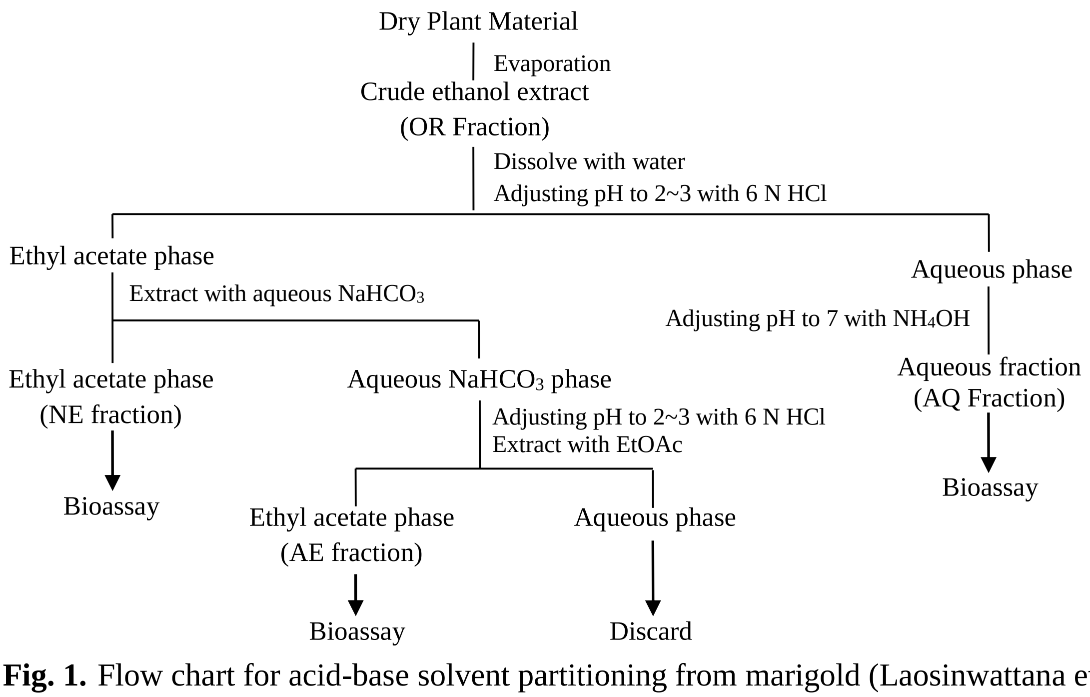


**Fig. 1.** Flow chart for acid-base solvent partitioning from marigold (Laosinwattana et al. 2007)

**Statistical analysis**

Data are analyzed using analysis of variant (ANOVA). Whenever ANOVA indicated significant effects ($p < 0.05$), a pairwise comparison of means by Tukey's studentized range test is carried



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## RESULTS AND DISCUSSION

### Aqueous extracts bioassay

Inhibition effects of stem, leaf, flower and root aqueous extract of *T. erecta* were assayed at concentrations of 12.5, 25, 50 and 100 mg/mL for their effect on seed germination and seedling growth of *P. lathyroides* compared with that of distilled water control. The results indicated that degree of inhibitory was significantly different depending on difference concentration and plant parts extract being tested. The degree of inhibition increased with increasing concentration. Among plant parts, leaf aqueous extracts was the most inhibition effect on germination, shoot length and root length of *P. lathyroides*. The leaf showed the greatest inhibitory effect on bioassay plant, followed by root, flower, and stem extract, respectively (Fig. 2). It was similar results to Laosinwattana et al. (2009), who reported that leaf and branches aqueous extracts of *Aglaia odorata* inhibitory effects of barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) and wild pea (*Phaseolus lathyroides* L.) Lin et al. (2006), who reported that the inhibitory effects of Saururaceae (*Houttuynia cordata* Thunb.) varied with the weed species.

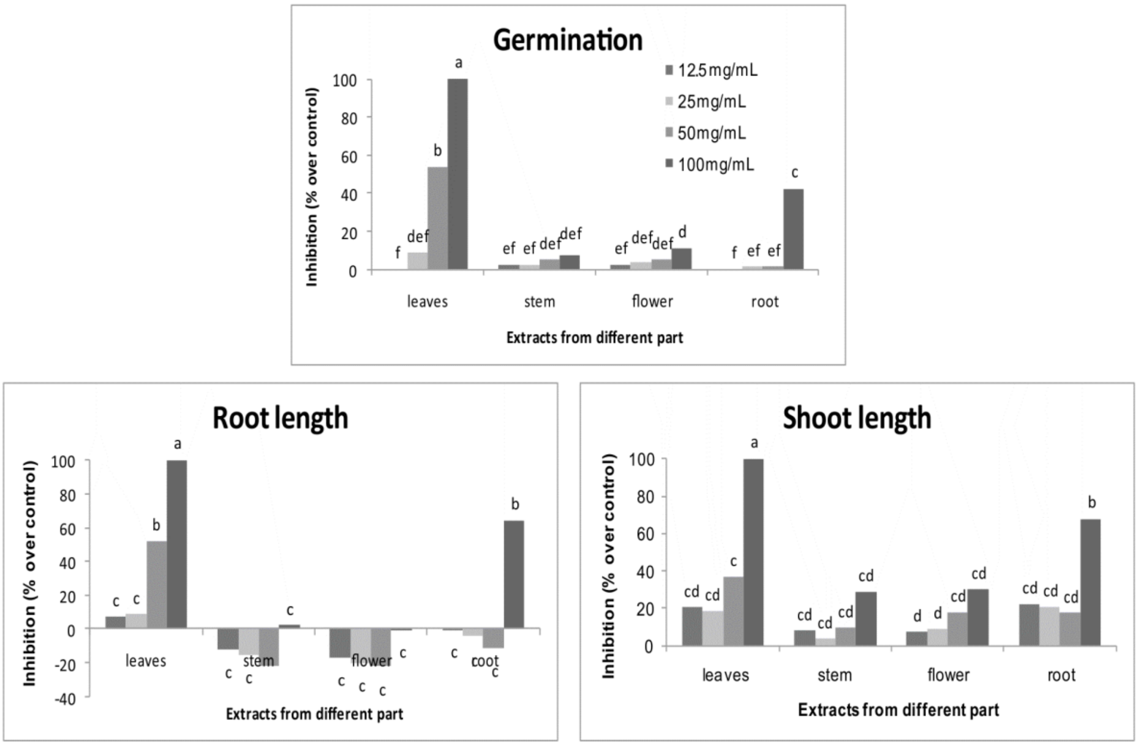


**Fig. 2.** Effects of aqueous extracts of from different *Tagetes erecta* L. plant parts on germination of *P. lathyroides* seeds at 7 days after treatment. Means followed by the same letter(s) are not significantly different by Tukey's (p=0.05).

### Effect of solvent extraction on the crude extracts yield.

The results in Fig. 3 show that the dried leaf of *T. erecta* L. extracted by various solvent systems showed different inhibitory influence on *P. lathyroides* seed germination and seedling growth in various ways. The degree of inhibitory was different depending on source of crude extract and concentration being tested. At 10000 ppm concentration, *P. lathyroides* seed germination was reduced by 7%, 12%, 5%, 5%, and 5% over control (distilled water) when treated with crude extract obtained from 100%, 75%, 50%, 25% and 0% ethanol in water, respectively. Thus, it can be seen that extracts prepared by different solvents carried varying degrees of inhibitory activities. These results indicate that selective extraction from natural sources by appropriate solvent systems is important for obtaining fractions with high allelopathic potential and high crude extraction yield. It was significant difference results to Poonpaiboonpipat et al. (2011), who reported that 50% ethanol in water demonstrated the



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highest potential extraction activity produced by *Jasminum sambac*. This finding is supported by Li et al. (2006); Luthria et al. (2007); Garcia et al. (2010), who reported that different solvent systems have been used for the extraction of secondary metabolites from plant materials because their extraction efficacy depends on their chemical nature.

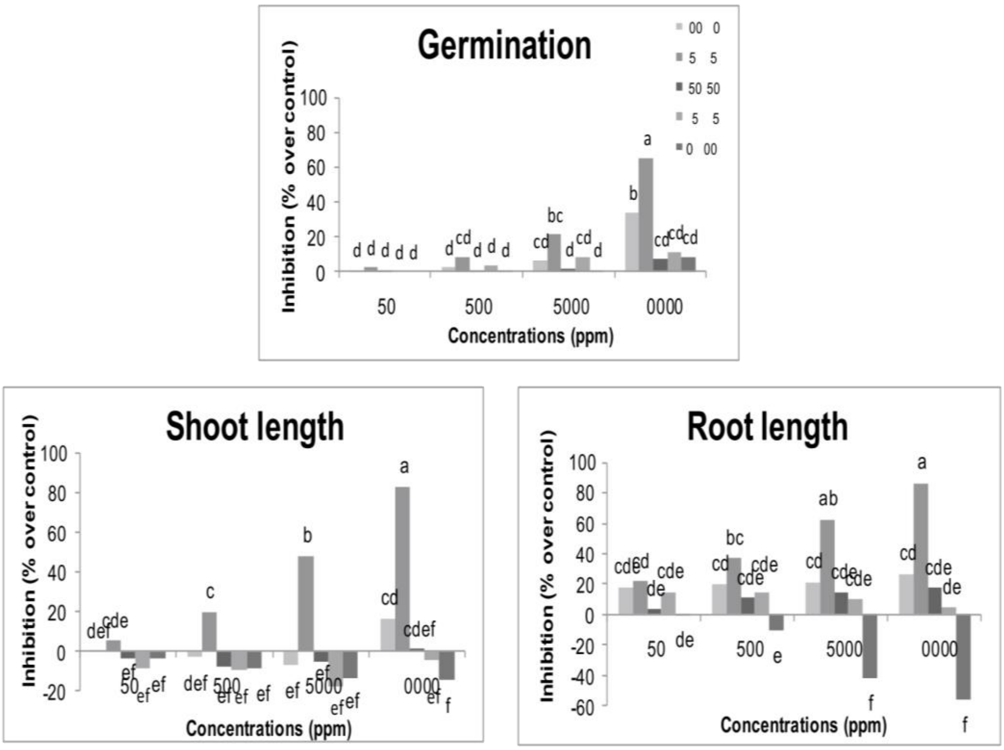


**Fig. 3.** The inhibitory effect of crude extraction obtained by different ethanol percentages in water from from *T. erecta* on germination, shoot length and root length of *P. lathyroides* seeds at 7 days after treatment. Means followed by the same letter(s) are not significantly different by Tukey's (p=0.05)

**Solvent partitioning of active compounds**

An aqueous-ethanol crude extract fraction (OR) from *T. erecta* was separated into three fractions : the aqueous (AQ) fraction, neutral compound (NE) fraction, and acidic compound (AE) fraction. The growth inhibitory activities of AQ, NE, AE fractions and OR at the concentration of 2000 to 16000 ppm were evaluated on seed germination and seedling growth of *P. lathyroides*. The results showed that (Fig. 4) OR fraction showed strong activity, giving 88.75%, 100% and 100% inhibition of *P. lathyroides* germination, shoot length and root length, respectively at the concentration of 16000 ppm. After solvent partitioning, at this step, relative inhibition increased, as compared to the OR fraction. The AE fraction showed the greatest activity fraction with complete inhibition of germination, shoot length and root length of *P. lathyroides* at the concentration of 16000 ppm. The NE and AQ fractions showed a weaker inhibition effect on *P. lathyroides* germination shoot length and root length when compared with the OR fraction. These results indicated that most of phytotoxic compounds produced by *T. erecta* could be presented in AE fraction. It was similar results to Poonpaiboonpipat et al. (2011), who reported that AE fraction was the most allelochemical compounds produced by *Jasminum sambac* and Teerarak et al. (2010), reported that a secoiridoid glucoside named oleuropine which identified as an allelopathic compound from AE fraction of a related *Jasminum officinale* var. *grandiforum.* The importance of allelochemicals mixtures is recognized both in herbicide research and exploring plant allelochemicals (Inderjit *et al*., 2002). It is suggested that the mixture compound in AE fractions gave significantly inhibited the tested weed species.



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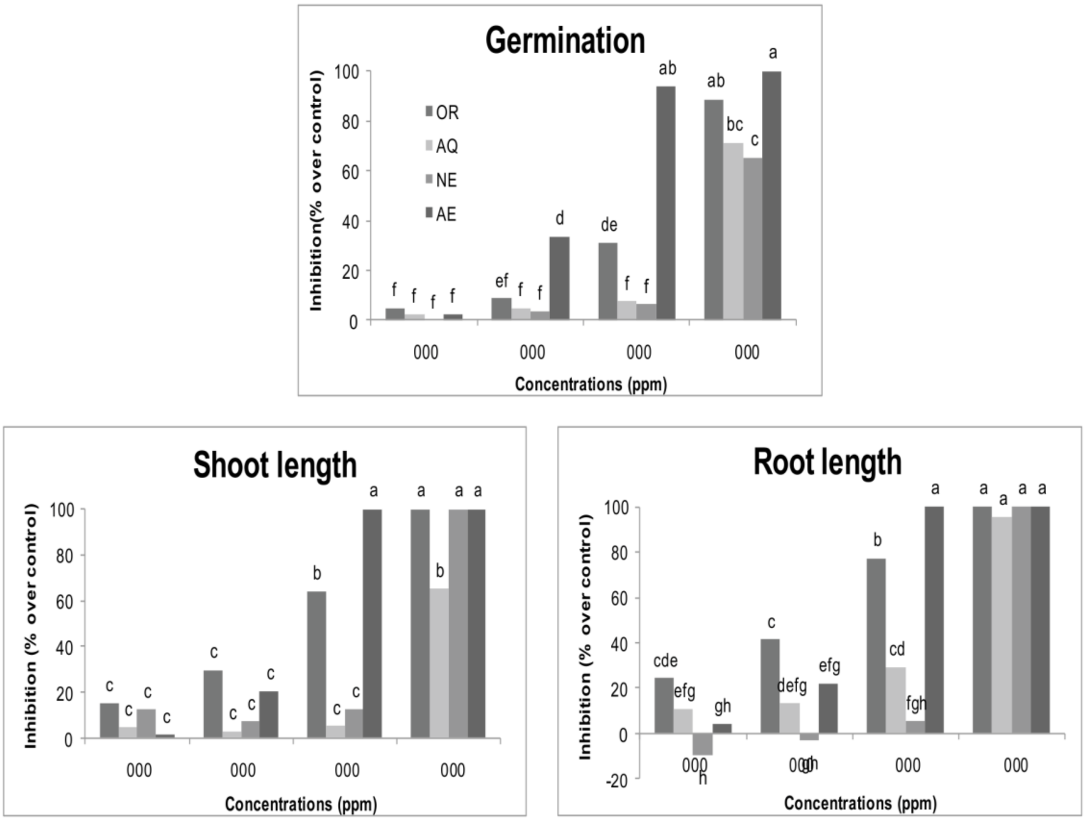


**Fig. 4.** Effect of aqueous-ethanol (OR), aqueous (AQ), neutral (NE) and acidic (AE) fractions at the concentrations of 2,000 to 16,000 ppm on germination, shoot length and root length of *P. lathyroides* seed.

## CONCLUSIONS

Allelopathic effects of stem, leaf, flower and root aqueous extract of *T. erecta* were assayed at concentrations of 12.5, 25, 50 and 100 mg/mL for their effects on seed germination and seedling growth of *P. lathyroides*. Aqueous extracts from leaf extract had a greater inhibitory effect on seed germination and seedling growth of *P. lathyroides* more than root, flower, and stem extract, respectively. The crude ethanol:water (75:25 (v/v)) fraction (OR) was separated by solvent partitioning into aqueous fraction (AQ), neutral compound fraction (NE), and acidic compound fraction (AE). AE fraction showed the greatest inhibitory effect on P. lathyroides, followed by NE fraction, AQ fraction and OR fraction, respectively.

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# RESIDUAL EFFECTS OF SYNTHETIC ALACHLOR HERBICIDE AND ITS CYTOGENETIC ON ROOT TIP CELL OF *ALLIUM CEPA* L.

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**ABSTRACT** The issue of environmental contamination of pesticide has increased in recent years, due to the heavy use of toxic chemicals in modern agriculture. The herbicide alachlor (2-cloro-N-(methoxymethyl)-N-(2,6-diethylphenyl)-acetamide) is an organochlorine (belonging to the α-haloacetanilide series) commonly employed for use for controlling annual grasses and broadleaf weeds in cultivation of brasicas, soybean and corn. The purpose of this study was to investigate the residue in soil and effects of alachlor on mitotic index (MI), mitotic phases and chromosomal aberrations. Alachlor residue in the soil was determined by applied it on soil surface at the rates of 0.625, 1.25 and 2.5 kg ai.ha$^{-1}$. The bioassay result indicated that Alachlor residue in the soil was longer than 3 months. The effects of alachlor herbicide on cytotoxic effects were examined on the root meristem cells of *Allium cepa* L. Six concentrations of alachlor herbicide (1.25 to 20 ppm) were applied for 12 h. The mitotic index in treated *A. cepa* root tips decreased with increasing concentrations of alachlor. In addition, the mitotic phase index was altered in *A. cepa* incubated with alachlor. The results showed that the increase in the percentage of the prophase phase in contrast to the percentage of remaining phases was found to be decreased. Alachlor produced the mitotic abnormalities resulting from its action on chromatin organization and mitotic spindle. In addition, the size of the nucleolus area decreased with increasing concentrations when compared with the control. For recovering treatment, *A. cepa* roots that have undergone the 12 hour treatment were transferred and grown in distilled water for 24 hours. The result obtained in a recovering treatment showed that alachlor exhibited a cellular damage and unrecovered cell to normal manner.

***Keywords:*** Synthetic herbicide, Alachlor, Chromosome aberration

## INTRODUCTION

The herbicide alachlor is an organochlorine (belonging to the α-haloacetanilide series) commonly employed for use for controlling annual grasses and broadleaf weeds in cultivation of brasicas, soybean and corn (Kiely et al. 2004; Hackett et al. 2005). Due to its extensive and occasionally inappropriate usage, alachlor can lead to environmental contamination of water, soil and air (Gabadón et al. 2002). Several studies have been published dealing with the mutagenic, genotoxic and carcinogenic potentials of alachlor (Osano et al. 2002; Siddiqui et al. 2012). Nucleolar organizer regions (NOR) are segments of DNA containing ribosomal genes, which can be historically detected by a silver staining technique as so-called AgNORs (Pederson, 1998). Specificity of changes in nucleolar characteristics in fish cell or plant cell and the use of nucleolar parameters in assessment of cytogenetic toxicity have also been shown (Teerarak et al. 2010).

The purpose of this study was to investigate the effects of alachlor on mitotic index (MI), mitotic phases, chromosomal aberrations and interphase AgNOR characteristics.

## MATERIALS AND METHODS

The herbicide LASSO (Monsanto S.A.) was used for all experiment. *A. cepa* bulbs with roots (1-2 cm long) were exposed to different concentrations of Alachlor at 1.25, 2.5, 5, 10 and 20



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ppm for 12 h. and cultured in water for 24 h. to study the recovery effects. Bulbs kept in distilled water served as control. For cytogenetic analysis, the *A. cepa* roots were cut and fixed immediately in a freshly prepared mixture of absolute ethyl alcohol and glacial acetic acid for 24 h. The fixed roots were macerated in hydrolytic enzyme mixture and then were stained with Giemsa. Some root tips squashed in absolute ethanol and acetic acid were stained with 50% silver nitrate and incubated in a moist chamber at 60 $^oC$ for 2 h to study AgNOR parameters (Teerarak, 2009). The statistical significance of the differences among values of mitotic index, mitotic phases, total abnormalities, and AgNOR parameters in the treated samples and the control was evaluated by means of Tukey's Studentized Range Test of significance at the $P \leq 0.05$ level.

## RESULTS AND DISCUSSION

The results of analysis of the mitotic index in the roots treated with alachlor at (1.25-20 ppm) for 12 h. revealed that all concentrations of alachlor could significantly inhibit cell division when compared to the control (Table 1). The percentages of total abnormalities increased as the concentrations of applied alachlor increased (Table 1). In the *A. cepa* root tips, several types of abnormalities were recorded at different mitotic stages with the treatments: spindle disturbance at prophase, c-metaphase, Sticky metaphase, Sticky anaphase, diagonal at anaphase, diagonal at telophase and delay anaphase. Furthermore, effects of recovery in water for 24 h. most frequently consisted of abnormal sticky anaphase, Spindle distribution at prophase and sticky metaphase (data not shown).

**Table 1.** Effect of different concentrations of alachlor on mitotic index and total cell abnormalities of *Allium cepa* root meristem cells for 12 h and recovery in distilled water for 24 h.

| Concentrations (ppm) | Mitotic index (mean+S.E.) | | (%) Total cell abnormalities (x±S.E.) | |
|---|---|---|---|---|
| | 12 h exposure | Recovery in distill water for 24 h | 12 h exposure | Recovery in distill water for 24 h |
| control | $9.28 \pm 0.93^{a}$ | $7.71 \pm 1.31^{a}$ | $0.00 \pm 0.00^{c}$ | $0.00 \pm 0.00^{c}$ |
| 1.25 | $6.99 \pm 0.58^{b}$ | $3.15 \pm 0.68^{b}$ | $3.72 \pm 1.31^{a}$ | $3.72 \pm 1.31^{a}$ |
| 2.50 | $4.30 \pm 0.93^{c}$ | $2.99 \pm 0.52^{b}$ | $2.57 \pm 1.31^{a}$ | $2.57 \pm 1.31^{a}$ |
| 5.00 | $2.73 \pm 0.26^{d}$ | $2.38 \pm 0.26^{bc}$ | $1.76 \pm 0.34^{b}$ | $1.76 \pm 0.34^{b}$ |
| 10.00 | $2.02 \pm 0.45^{d}$ | $1.80 \pm 0.66^{bc}$ | $1.17 \pm 0.40^{bc}$ | $1.17 \pm 0.40^{bc}$ |
| 20.00 | $0.60 \pm 0.23^{e}$ | $0.89 \pm 0.74^{c}$ | $0.17 \pm 0.14^{c}$ | $0.17 \pm 0.14^{c}$ |

Mean within a column for each Alachlor concentration followed by different letters (a–d) is significantly different according to Tukey's Studentized Range Test at $P < 0.05$ level.

For AgNOR area, cells were treated with all concentrations of alachlor for 12 h and the recovery in water for 24 h. With increasing Alachor concentrations, total area of AgNOR decreased, as illustrated in Table 2. Roots treated with alarchloa for 12 h and futher recovery treatment with distilled water 24 h also displayed smaller AgNOR size. The inhibitory effect of pesticide on root growth in *A.cepa* L. have been reported such as furadan and enodosulphan found both the pesticides showed genotoxic effects with variation with respect to dosage and exposure of time. Mitotic index of both Furadan and Enodosulphan treated root tip cells



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shows significant decrease in 50 and 100µg/ml concentration for 6 and 24 hrs. (Ananthakrishnan 2013).

**Table 2.** Change in total AgNOR area in *Allium cepa* L. tips treated with alachlor 12 hours and recovery in distilled water for 24 h.

| Conc. (ppm) | Total AgNOR area within different number of AgNOR per cell for 12 h. ($\mu m^2$) | | | | Total AgNOR area within different number of AgNOR per cell after recovery in water for 24 h. ($\mu m^2$) | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| control | 37.17$^{a}$ | 48.68$^{a}$ | 54.86$^{a}$ | 55.13$^{a}$ | 35.24$^{a}$ | 44.76$^{a}$ | 52.88$^{a}$ | 49.27$^{a}$ |
| 1.25 | 21.89$^{b}$ | 27.53$^{b}$ | 27.88$^{b}$ | 15.88$^{b}$ | 22.84$^{b}$ | 40.14$^{a}$ | 30.13$^{b}$ | 24.54$^{b}$ |
| 2.50 | 17.86$^{bc}$ | 24.05$^{b}$ | 22.12$^{c}$ | 12.48$^{bc}$ | 20.37$^{b}$ | 24.48$^{b}$ | 22.36$^{c}$ | 18.47$^{c}$ |
| 5.00 | 17.12$^{cd}$ | 17.96$^{c}$ | 17.54$^{c}$ | 12.70$^{bc}$ | 19.56$^{b}$ | 20.97$^{bc}$ | 20.95$^{c}$ | 15.84$^{c}$ |
| 10.00 | 13.24$^{d}$ | 15.93$^{c}$ | 16.28$^{cd}$ | 11.46$^{cd}$ | 13.24$^{c}$ | 17.43$^{c}$ | 19.58$^{cd}$ | 14.42$^{cd}$ |
| 20.00 | 6.56$^{e}$ | 8.65$^{d}$ | 9.59$^{d}$ | 7.77$^{d}$ | 7.34$^{d}$ | 10.72$^{d}$ | 12.38$^{d}$ | 10.93$^{d}$ |

Mean within a column for each alachlor concentration followed by different letters (a–d) is significantly different according to Tukey's Studentized Range Test at $P < 0.05$ level.

## CONCLUSSION

Alachor inhibited cell division, induced chromosome aberrations and decreased AgNOR size. Recovering treatment showed that alachlor exhibited a cellcaleular damage and unrecovered cell to normal manner.

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