ORIGINAL RESEARCH

Rice flatsedge (*Cyperus iria* L.) is Resistant to the Auxinic Herbicide 2-methyl-4-chlorophenoxyacetic acid (MCPA)

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Abstract

This study assessed the potential resistance build-up in rice flatsedge (*Cyperus iria* L.), a dominant sedge weed in paddy fields of Sri Lanka, to MCPA (2-methyl-4-chlorophenoxyacetic acid; WSSA/HRAC Group 4). Mature seeds of *C. iria* were collected from susceptible (S) and suspected-resistant (R) biotypes in five districts of the Northern Province of Sri Lanka. Experiments were conducted in a completely randomized design with five replicates. A knapsack sprayer fitted with a flat-fan nozzle was used to spray MCPA on *C. iria* seedlings at the three-leaf stage at ten dosages ranging from 25% (0.54 kg a.i. ha-1) to 300% (3.24 kg a.i. ha-1) of the recommended dose (1.08 kg a.i. ha-1), with a water-only control. Seedling survival % was estimated at ten days after treatment.

The seed germination of MCPA-S and MCPA-R biotypes of *C. iria* did not significantly differ across districts (P>0.05). Probit analysis and ED50 values calculated using log-logistic model-fitting showed that *C. iria* has developed resistance against MCPA (Resistance Index ranging from 1.59 to 1.62), with no significant difference among districts (P>0.05). Auxin-mimicking Florpyrauxifen-benzyl effectively controlled the MCPA-R biotype with no indication of cross-resistance.

The other four herbicides, namely, Bispyribac sodium, Carfentrazone ethyl, Pretilachlor + Pyribenzoxim and Fenoxaprop-p-ethyl + Ethoxysulfuron, did not effectively control MCPA-R and MCPA-S biotypes. However, multiple resistance in *C. iria* cannot be ruled out. Interestingly, MCPA at the recommended dosage also showed relatively poor control of MCPA-S biotypes, indicating the need to revisit the herbicide recommendation against the sedge weed in paddy fields.

Keywords: *Cyperus iria*, herbicide resistance, MCPA, paddy fields, Sri Lanka

Introduction

The input-intensification in agriculture, including herbicides, has progressively increased herbicide-resistant weeds (Heap and Duke, 2018; Hulme, 2023), thus limiting crop productivity and production worldwide. It has become one of the significant challenges in the sustainable development of agronomic practices. Rice is the main staple for Asians, and the annual crop yield loss due to weed infestation is about 15–21% (Karim *et al*., 2004). In the Sri Lankan context, a 20-40% loss in rice yields was reported due to weed infestation (Herath Banda *et al.,* 1998; Amarasinghe and Marambe, 1998). The weed diversity is the highest in the family Poaceae, with at least 70 species, followed by Cyperaceae, with more than 55 species (Rao *et al*., 2017).

Herbicide use has become the most effective practice in weed management (De Prado *et al.*, 2004; Travlos et al., 2017). However, the shift in weed flora (Marambe, 2002) development of herbicide resistance (De Prado *et al*., 2000; Marambe and Amarasinghe, 2002; Preston and Powles, 2002; Travlos and Chachalis, 2010; Travlos *et al*., 2018) and other environmental concerns (Balderrama-Carmona, 2020) are some of the adverse effects of continuous and misuse of herbicides.

The number of resistant biotypes of weeds is increasing alarmingly in many cropping situations (Duary, 2008; Travlos *et al.,* 2020). The evolution of herbicide resistance in weeds has increased the cost of chemical control measures (Kniss *et al*., 2022), thus affecting the cost of production of crops in addition to various other challenges.

Rice flatsedge (*Cyperus iria* L.), an annual herbaceous sedge (Family Cyperaceae), is among the most troublesome weeds in rice fields in Sri Lanka, India, Pakistan and the Philippines (Awan *et al*., 2022). The weed *is listed* as one of the weeds of national significance in direct-seeded rice cultivation in Sri Lanka (Rao *et al*., 2017).

The extensive use of the direct seeding method of rice (DSR) has resulted in *C. iria* becoming a notorious weed in rice fields (Azmi and Baki, 2002), with a 64% reduction in paddy yield when the weed infests the whole crop duration (Dhammu and Sandhu, 2002). In Sri Lanka, about 7-10% yield loss was recorded in paddy in the Northern Province due to *C. iria* infestation (PDOA, 2020). Sedge weeds, especially the smaller-statured ones, are known to be susceptible to the herbicide MCPA (2-methyl-4-chlorophenoxyacetic acid; WSSA/HRAC 4), which is an auxinic, selective herbicide. MCPA is among the most extensively used herbicides in paddy in Sri Lanka and has been used since the 1960s (Bandara *et al*., 2017; Dissanayake *et al*., 2019; Piyasiri *et al*., 2022).

Based on the field observations, Abeysekera *et al*. (2017) speculated a potential resistance build-up in *C. iria* and Dirty Dora (*C. difformis* L.) in paddy fields against MCPA. Our discussions with experienced paddy farmers, research officers, and agricultural instructors of the Provincial Department of Agriculture and field surveys conducted in 2018-2021, especially in the Northern Province of Sri Lanka, also supported the hypothesis that *C. iria* might have developed resistance to the continuous use of MCPA.

The preliminary surveys also revealed that the farmers in different districts in the Northern Province have been using MCPA at variable dosages, not following the recommendation made by the Department of Agriculture (DOA, 2019).

Further investigations on the regional differences in potential resistance development in *C. iria* in paddy fields to MCPA are warranted as no such scientific evidence is available. Hence, we conducted the study reported herein to detect whether *C. iria* biotypes have already developed resistance to MCPA, quantify the resistance level, and suggest alternate treatments to control such resistant biotypes in rice agriculture in Sri Lanka.

Materials and Methods

The experimental sites were in the five districts of the Northern Province of Sri Lanka, namely Jaffna, Kilinochchi, Mannar, Mullaitivu and Vavuniya. Paddy fields infested with weeds were selected from five districts (Figure 1) based on farmer surveys at those locations where high densities of the *C. iria* had been previously reported (i.e. ≥ 20 *C. iria* plants /m^2).

The main criterion for paddy field selection was the continuous use of the popular sedge-control herbicide MCPA (CAS number 94-74-6] for more than five years in the paddy fields (two cultivating seasons per year) for weed control. The *C. iria* plants in paddy fields, where the herbicide MCPA of 600 g L^{-1} SL (Soluble Concentrate) formulation was applied at the recommended rate (1.08 kg of a.i. ha^{-1}; DOA, 2019) but could not successfully control the weed in the past two years (including the current season) were considered as the suspected resistant (MCPA-R) populations (biotypes).

The *C. iria* populations in paddy fields in each district, where MCPA has continuously controlled the weed, including the current season, were considered susceptible (MCPA-S) biotypes.

Seed Collection

Mature seeds from suspected MCPA-S and MCPA-R biotypes were collected separately from at least five paddy fields per district belonging to several farmers. **Figure 2** illustrates the status of paddy fields where heavy infestation of *C. iria* was observed. Twenty-five mature inflorescences from MCPA-R biotypes of *C. iria* were collected randomly from selected paddy fields that the researchers suspected to have developed resistance to MCPA based on continuous field surveys.

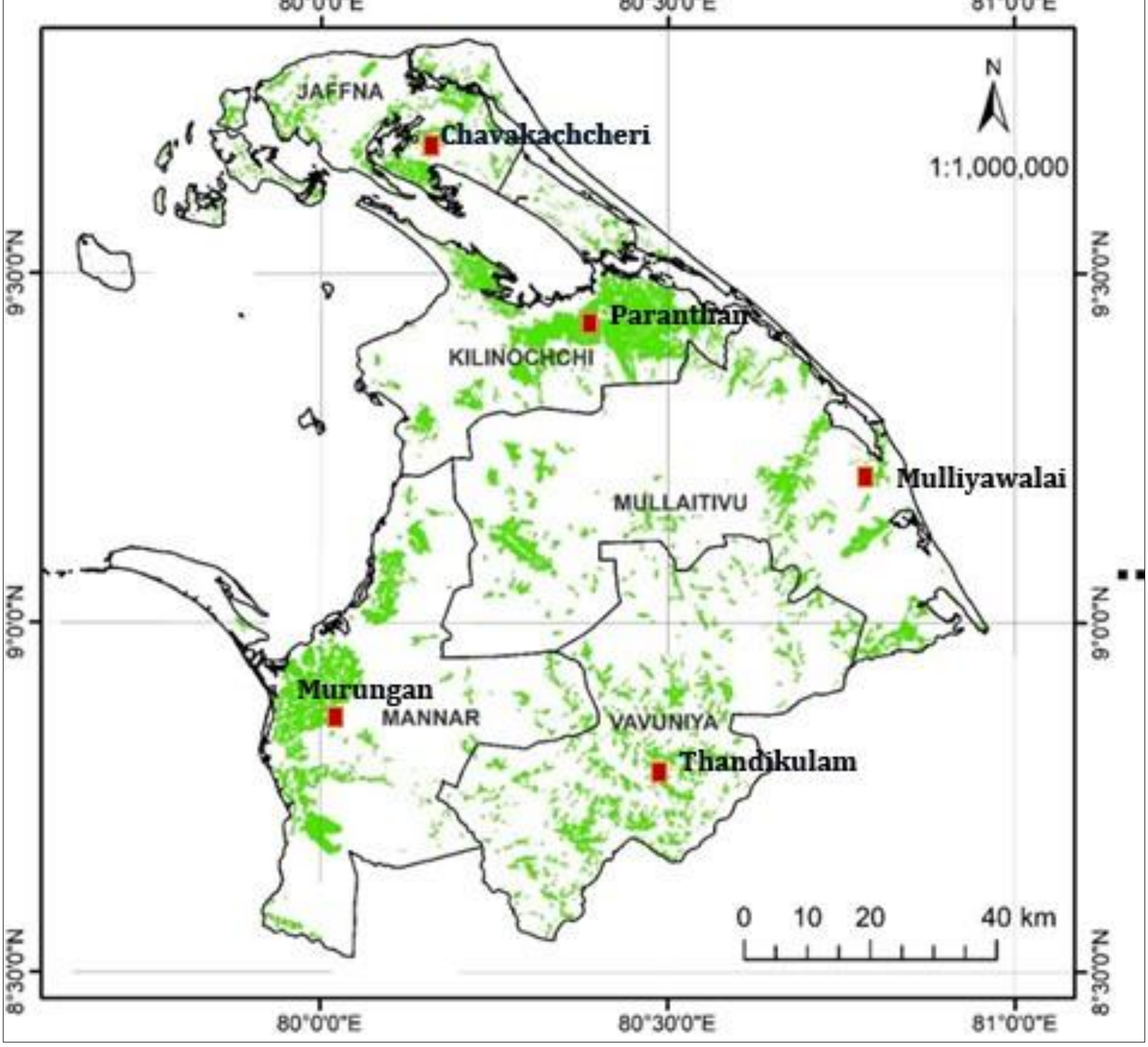

Figure 1. Locations of the paddy fields in five districts in the Northern Province of Sri Lanka from where seeds of the suspected MCPA-resistant *C. iria* biotypes were collected

Figure 2. (A) Paddy fields infested with *Cyperus iria* in the Northern Province of Sri Lanka; (B) Mature inflorescence of *C. iria*

More than 50 inflorescences were collected from several locations in each district, and composite samples were prepared for each district separately as MCPA-S biotypes. The samples were then transported to the laboratory; mature seeds were manually extracted separately and preserved in plastic containers unde

r refrigerated conditions (4 °C) until further use. The study comprised two experiments. The first experiment investigated the potential development of resistance to the commonly used herbicide MCPA. The second experiment explored the potential of alternate herbicides to control the resistant biotypes of *C. iria* in paddy fields.

Seed Germination

Fifty seeds of MCPA-S and suspected MCPA-R biotypes of *C. iria* were placed on Petri dishes laid with Whatman No. 1 filter paper at 27/16 °C day/night room temperature. The Petri dishes with seeds were covered with Aluminium foil to simulate seed burial under natural conditions. The germination rate was recorded for ten days. The Petri dishes were moistened once every 48 hours until the end of the experiment.

Experimental Conditions

Plastic containers (25 cm length x 22.5 cm width x 19 cm height) were used for the experiment conducted in a plant house at the Faculty of Agriculture, University of Jaffna, Kilinochchi, Sri Lanka. The representative paddy soil samples collected from each district were used to grow MCPA-R and MCPA-S biotypes of *C. iria* collected from the respective district. The containers were filled with paddy soil to a height of 15 cm.

Fifty mature seeds harvested from MCPA-S and MCPA-R biotypes from each district were planted in rows in separate containers. The soil was moistened continuously using a hand sprayer. All weeds emerging, excluding the planted *C. iria*, were carefully removed from the pots with minimum disturbance to the soil.

Treatment Regimes

The experiment comprised 11 treatments based on the different rates of application of MCPA (600 g L^{-1} SL formulation); T1: No herbicide application (water-only control), T2: 25% of the recommended dosage (0.27 kg a.i. ha^{-1}), T3: 50% of the recommended dosage (0.54 kg a.i ha^{-1}), T4: 75% of the recommended dosage (0.81 kg a.i ha^{-1}), T5: Recommended dosage (1.08 kg a.i ha^{-1}), T6: 125% of the recommended dosage (1.35 kg a.i ha^{-1}), T7: 150% of the recommended dosage (1.62 kg a.i ha^{-1}), T8: 175% of the recommended dosage (1.89 kg a.i ha^{-1}), T9: 200% of the recommended dosage (2.16 kg a.i ha^{-1}), T10: 250% of the recommended dosage (2.7 kg a.i ha^{-1}), and T11: 300% of the recommended dosage (3.24 kg a.i ha^{-1}).

Each treatment was replicated five times and laid down in a complete randomized design. Herbicide treatments were applied at the 3-leaf stage *of C. iria*. Plastic pots were moved into an open space and placed evenly before spraying the herbicide.

The treatments were imposed separately for S and R biotypes, ensuring no cross-contamination. A lever-operated 16 L Knapsack sprayer equipped with a flat-fan nozzle was used, with a nozzle deliverer rate of 576 mL min^{-1} to cover the whole area of the open space. The spray volume was 320 L ha^{-1}.

Alternative Herbicides to Control *C. iria*

Tray experiments were conducted under the same plant house conditions using weed seeds collected from the five districts to identify alternative control measures for the MCPA-R weeds. The trays used in the first experiment were also used, and soil was filled to 15 cm height using the same paddy to grow the MCPA-S and MCPA-R biotypes. Fifty seeds of each of the MCPA-S and MCPA-R biotypes were planted in each container separately. Five alternate herbicides, which are also recommended and used by the farmers in the province to control sedge and broadleaf weeds, were evaluated for their efficiency in controlling *C. iria* (Table 1).

Of the five herbicides, one was an auxin-mimic, as in the case of MCPA. The herbicides were applied using the same method adopted in the previous experiment. The herbicide rates were derived from recommendations of DOA (2019). The experiment was conducted in a completely randomized design with five replicates. The application method and equipment used were the same as previously reported in this paper.

Table 1 Alternate herbicides used in the experiment

No.	Herbicide and Formulation	Mode of Action	Recommended dose of commercial product
H1	Bispyribac sodium (100 g a.i. L-1) EC	Acetolactate synthase (ALS) inhibitor	225 L ha-1
H2	Carfentrazone ethyl (240 g a.i. L-1) EC	Protoporphyrin oxidase (PPO) inhibitor	90 L ha-1
H3	Pretilachlor (300 g a.i. L-1) + Pyribenzoxim (20 g a.i. L-1) EC	Very long chain fatty acid and ALS inhibitor	1.25 L ha-1
H4	Fenoxaprop-p-ethyl (69 g a.i. L-1) + Ethoxysulfuron (20 g a.i. L-1) OD	Acetyl-CoA Carboxylase (ACCase) + ALS inhibitor	0.5 Lha-1
H5	Florpyrauxifen-benzyl (25 g a.i. L-1) EC	Auxin-mimic	1.2 L ha-1

Measurements and Statistical Analysis

The number of seedlings killed and survived ten days after each treatment was counted. Data are presented as % of seedlings that survived in each treatment. The Chi-Square test (p=0.05) was conducted to test the association between the resistance build-up and seed germination.

The resistance development was assessed using the dose-response curves from probit analysis (NCSS Statistical Software, Chapter 575). A log-logistic model was fitted to estimate the ED50 (effective dosage to kill 50% of the population of a given biotype). The Resistance Index (RI) was calculated using Equation 1 as given in Pilho *et al.* (2009).

$$\text{Resistance Index (RI)} = \frac{ED_{50} \text{ of the Resistant Population}}{ED_{50} \text{ of Susceptible Population}} \quad \ldots\ldots\ldots$$

Results

Seed Germination (%) of MCPA-S and MCPA-R *C. iria* biotypes

A similar germination % (Figure 2) among the districts was observed in MCPA-S and MCPA-R within each district. Among the districts, MCPA-S ($\chi^2_{df=4}$ = 1.034; p=0.90) and MCPA-R ($\chi^2_{df=4}$ = 2.19; p=0.69) showed no significant differences in the germination pattern of the *C. iria* seeds collected.

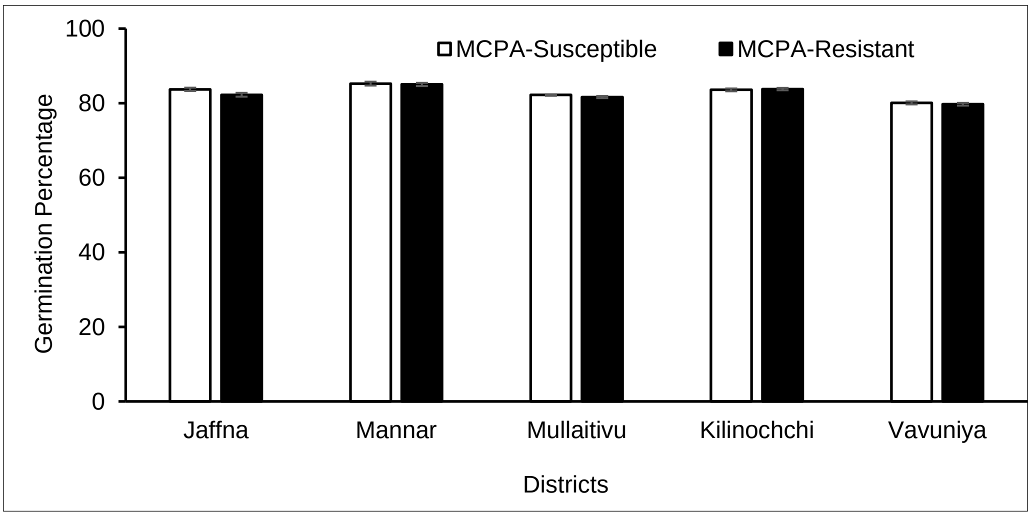

Figure 2. Seed germination % of MCPA-S and MCPA-R biotypes collected from different districts in Sri Lanka. Vertical lines are the Standard Errors of Means (N=5).

Resistance Build-Up in *C. iria* populations to MCPA

Based on the parallel and non-parallel model fitting, the non-parallel dose-response curves were selected to explain the results, as the responses showed a genetic (G) x environment (E) interaction. Inverse Sigmoidal dose-response curves of *C. iria* seedlings exposed to different concentrations of MCPA indicated a strong relationship between increased MCPA concentrations and mortality rates (Figure 3). The herbicide controlled the weeds effectively (100% control) at a 25% higher dosage than the recommended (Figure 3). According to the ED50 values, the MCPA-R biotype had an RI of 1.59-1.62 in all five districts, indicating resistance build-up compared to the MCPA-S biotype (Table 2).

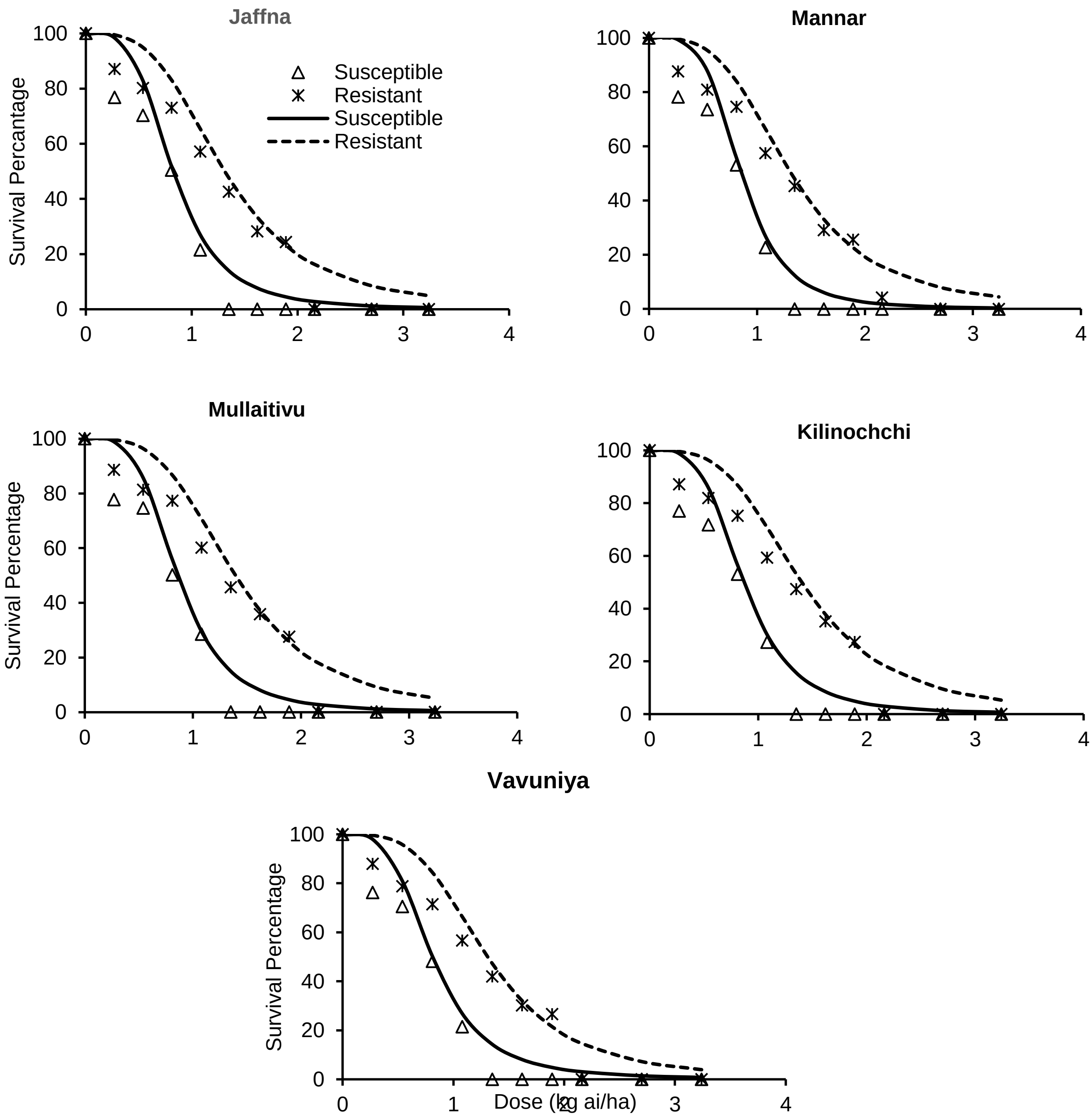

Figure 3. Non-parallel dose-response curves of susceptible and resistant biotypes of *C. iria* from five districts in the Northern Province to MCPA, using the log-logistic model. Lines are response curves predicted from non-linear regression. Symbols represent the mean survival rate of five replicates.

Table 2. ED_{50} values of MCPA-S and MCPA-R biotypes and Resistance Index (RI) of *C. iria* in five districts of Sri Lanka

Districts	ED50 Values for MCPA-S Biotype	ED50 Values for MCPA-R Biotype	RI Index
Jaffna	0.82	1.3	1.59
Mannar	0.84	1.34	1.62
Mullaitivu	0.9	1.44	1.6
Kilinochchi	0.9	1.44	1.6
Vavuniya	0.85	1.35	1.59

ED_{50} = herbicide dosage required to kill 50% of the weed population, R = resistant biotype; S = susceptible biotype, RI = Resistance Index

The ED50 values for MCPA-S biotype ranged from 0.82 to 0.9 kg a.i. ha^{-1} and that for MCPA-R biotype raged from 1.59 to 1.62 kg a.i. ha^{-1}. The results of the present study also showed that even the MCPA-S biotypes identified by the farming community were not effectively controlled by the recommended dosage of MCPA (1.08 kg a.i. ha^{-1}).

The Effectiveness of Alternative Herbicides to Control MCPA-Resistant *C. iria*

None of the five alternative herbicides tested provided satisfactory control of *C. Iria*, irrespective of whether the biotypes were MCPA-S or MCPA-R (Figure 4). Notably, all MCPA-R biotypes were collected from the five districts of the Northern Province of Sri Lanka. Although the differences were statistically insignificant, the resistant biotypes showed a marginally better survival rate than the susceptible biotypes ($P>0.05$).

Discussion and Conclusions

Differences in seed germination rates have been observed among the herbicide-resistant and susceptible weed biotypes (Alcocer-Ruthling et al., 1992; Torres-Cgarcia et al., 2015). However, similar to the results observed in our study, Shaeffer et al. (2021) also reported identical germination patterns in the two biotypes, suggesting that the germination behaviour of herbicide-susceptible and resistant weed biotypes would vary depending on conditions encountered in the ecosystems.

The results clearly showed G x E interactions among the biotypes of *C. iria* biotypes collected from the five districts in the Northern Province for both MCPA-S and MCPA-R biotypes, even when the herbicide was used at the recommended dosage.

Our preliminary analysis has confirmed that, though the extent of farmer fields varied significantly, there was only a marginal variability in the use of other agricultural inputs, such as fertilizer in the study sites and that all paddy farmers used herbicides for weed control in paddy fields (data not shown). Though it was difficult to estimate the actual dosages of MCPA applied to the paddy fields by individual farmers in five different districts, it is still reasonable to conclude that several biotypes of *C. iria* in the study sites are resistant to the herbicide MCPA.

The number of weed species resistant to synthetic auxin herbicides mimicking indole-3-acetic acid (IAA) is relatively low, considering their long-term use globally (Busi *et al.,* 2018). Of the 570 cases reported as herbicide resistant (Heap, 2024), 44 were related to synthetic auxin-type herbicides, while 14 weeds were reported as resistant to MCPA with no records on *Cyperus* spp.

We did not study the resistance mechanism to MCPA in *C. iria* biotypes. However, the differences in the rate of translocation and metabolism of the MCPA among plant populations are considered as a possible mechanism of evolution of resistance to the herbicide in weeds (Singh *et al.* 2023), where the resistant biotypes might have translocated lesser amounts of MCPA while they metabolized the herbicide more rapidly.

Weinberg *et* al. (2006) reported the involvement of more than one genetic locus with additive effects in the absorption, translocation and metabolism of MCPA in the roots of the resistant populations. The MCPA resistance in wild radishes is controlled by a single gene, where resistant plants rapidly translocated more 14C-MCPA to roots than the susceptible plants, resulting in its exudation from the plant (Jugulam *et al.,* 2013).

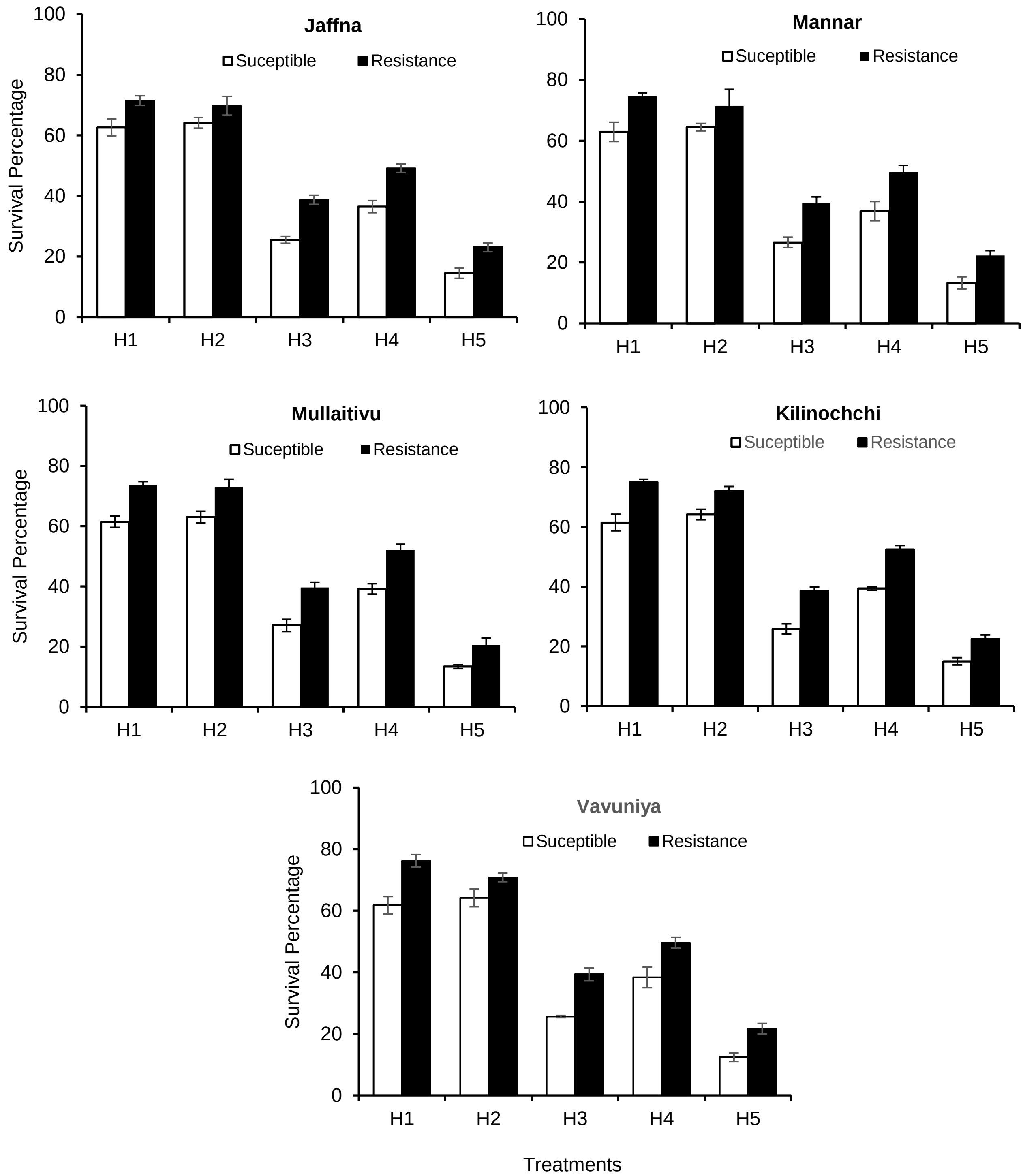

Figure 4. The survival rate of MCPA- Susceptible (S) and Resistant (R) populations of *C. iria* when treated with alternate herbicides (H1 = Bispyribac sodium, H2: Carfentrazone ethyl, H3 = Pretilachlor + Pyribenzoxim, H4 = Fenoxaprop-p-ethyl + Ethoxysulfuron, and H5 = Florpyrauxifen-benzyl. The vertical line on each bar represents the standard error (SE; n=5). Refer to Table 1 for the recommended dosages.

Moreover, Jasieniuk *et al.* (1996) reported that herbicide-resistant populations could be the source of resistant alleles for the nearby susceptible populations, thus aggravating the problem of herbicide-resistant weeds in crop cultivation.

The phytotoxic effects of MCPA on nursery tea plants (Sayanthan *et al.*, 2021) and rice (Bandara *et al*., 2017) are well established. The recommended dosage of MCPA to control sedges and broad leaf weeds applied 20-21 days after planting paddy has affected the paddy plant itself (Shibayama, 1980; Bandara *et al.,* 2017). However, it is essential to note that early application or higher dosages of MCPA would be detrimental to paddy plants despite the sedge weed control observed in this study.

Further, Jayasiri *et al.* (2022) reported a high environmental impact score of 7.4 and a high variability of EIQ-FUR for MCPA. Hence, the study reveals the importance of revisiting the recommendations to control sedge weeds such as *C. iria* in paddy cultivation in Sri Lanka.

The results of the present study also revealed the poor control of *C. iria* by the alternate herbicides with different modes of action, with a marginal increase of survival by the MCPA-R biotypes compared to the MCPA-S biotypes of the weed.

Multiple resistance could occur in weeds owing to the co-evolution of multiple mechanisms in either target site, non-target site resistance, or a combination (Torra *et al.*, 2019).

This study did not investigate the resistance mechanisms or assess the development of multiple resistance in MCPA-R biotypes of *C. iria*. However, these aspects warrant further studies. Tarvlos (2012) reported that the degree of selection pressure will determine the levels and patterns of herbicide resistance and the development of cross-resistance to multiple herbicides.

Cyperus iria, a major sedge weed species in the lowland paddy fields in the Northern Province of Sri Lanka, has developed resistance to MCPA, most likely due to the herbicide’s long application history.

To prevent the dominance of MCPA-resistant *C. iria* in paddy fields and avoid the development of cross- or multiple resistance to herbicides, rotational use of alternate herbicides or non-herbicide control measures should be encouraged. Continuous programmes aimed at farmer education on the impending threat of herbicide-resistant weeds in paddy fields are recommended.

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