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Weed dominance and status of herbicide-resistance in *Ischaemum rugosum* in direct-seeded rice in low country dry zone

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Abstract

Weed species-dominance significantly contributes to yield losses in Direct-Seeded Rice (DSR) production systems. This study aimed at determining the dominance nature of weeds and development of herbicides resistance in *I. rugosum* populations found in paddy fields. Studies were carried out in 40 farmer fields at the Hambantota district in Sri Lanka during 2017/2018 *Maha* (wet season; October to February) and 2018 *Yala* (dry season; March-September) seasons using 5 m^2 sampling size per field. Data were collected from each site at 8 weeks after crop establishment. The contribution of each weed species to the weed community in paddy fields was determined using the relative weed density and relative dry weight. Dose responses experiments were conducted using *I. rugosum* against the pre-mix herbicide formulations; Bispyribac sodium 40 g/1 + Metamifop 100 g/l SC, Clomazone 200 g + Propanil 400 g/l and Thiobencarb 400 g/l +Propanil 200 g/l EC. Results showed that the C4 grasses dominated the paddy fields in both seasons, and they were more productive in the relatively dry *Yala* season compared to that of wet-*Maha* season. The C4 grasses *E. crus-galli* and *I. rugosum* were the dominant weeds in 2017/18 *Maha* and 2018 *Yala* Season, respectively. Three *I. rugosum* populations exhibited possible multiple resistance development to the pre-mix herbicide formulation Bispyribac sodium 40 g/1 + Metamifop 100 g/1 SC, containing herbicides with two different modes of action (ALS-

inhibitor and ACCase inhibitor, respectively), showing a resistance index (RI) of 1.8, 2.8 and 1.68. Continuous use of herbicide influences the level of dominance of weed species in DSR and hence, weed management practices should be implemented based on the nature of weed dominance.

Key words: Herbicide resistance, *Ischaemum rugosum*, Weed dynamics

Introduction

Weeds are the major biological constraint in rice production systems worldwide. However, the impact of weed infestations depends on the crop establishment method, climate and management practices (Singh *et al*., 2005). Weeds contribute to crop losses depending on the cultivation system, the dominant weed community, and methods of weed control adapted by farmers (Labrada, 2003). Yield losses in paddy due to weed pressure have been estimated in different countries, i.e. 30-73% in Colombia (Cobb and Reade, 2010), 71% in India (Singh *et al*., 2005) and 20-30% in Sri Lanka (Amarasinghe and Marambe, 1998). A density of 50 weeds/m^2 could account for 38% yield loss of rice (Herath *et al*., 2018). Weed management is therefore, crucial in achieving the potential yield of rice.

Ischaemum rugosum Salisb is one of the major weeds found in rice fields in Asia, India, Thailand and Sri Lanka (Chauhan and Johonson, 2010). It is an annual grass and exhibit C_4 photosynthesis path way and native to tropical Asia, and considered as a highly competitive weed in rice. About 48% rice yield loss has been reported in Malaysia (Itoh, 1991) and 25% in India (Tarlok *et al*., 1991) owing to *I. rugosum*. The weed has been reported in the recent past to be dominant among grass weeds in paddy fields in Sri Lanka (Herath *et al*., 2017, 2018).

With increasing cost of production and labour scarcity, herbicides remain the most cost effective weed control techniques among the majority of rice farmers in Sri Lanka. However, over-reliance of herbicides and incorrect usage have become common issues in the country (Herath *et al*., 2017). Repetitive and incorrect use of herbicides causes many negative impacts to rice-growing ecosystem such as development of herbicide-resistant weed species, poor efficacy of herbicides and changes in weed populations.

Weed dynamics, which is the change in composition of weed community considering the relative dominance of each species in the system (Jakelaitis *et al*., 2003), is an important parameter to be considered in adopting effective weed control methods. Dominance of the weed flora depends on the climatic conditions (Andreasen and Streibig, 2010), soil management systems (Soares *et al*., 2012), agronomic practices, and herbicide application (Jakelaitis *et al*., 2003; Booth *et al*., 2003).

Continuous use of herbicides with similar mode of action has resulted in evolution of herbicide resistance in weeds. Different resistance mechanisms have been reported, *i.e*. cross resistance where plants possess one mechanism providing the ability to withstand herbicides from different chemical families, and multiple resistances with more than one resistance mechanism (HRAC, 2009). Resistance development in *I. rugosum* against Bispyribac sodium has been reported in Sri Lanka (Sangakkara *et al*., 2004), Malaysia (Heap, 2013) and Colombia (Plaza and Hernández, 2014). Further, Baltazar and Johnson (2013) reported of multiple resistances in *I. rugosuma* against herbicides that inhibit ACCase (Fenoxaprop, profoxydim) and ALS (Bispyribac sodium) activities.

Herbicide application is a key tool that determines the dominance weed species in rice-growing ecosystems. Therefore, identification of dominant and herbicide-resistant weed species in a crop production system is a must to develop effective weed management approaches. This study aimed at determining the dominance nature of weeds in paddy fields as influenced by seasonal variation and exploration of herbicides resistance in *I. rugosum* in direct-seeded rice in low county dry zone ($DL1_b$ and DL5).

Materials and methods

Study on weed dominance

The study was carried out in farmer fields in the Ambalantota Agrarian Division (covering agro-ecological regions $DL1_b$ and DL5) of Hambantota District, Sri Lanka, during the 2017/2018 *Maha* season (October-February) and 2018 *Yala* season (March to September). Forty rice-growing locations (paddy fields) managed by the farmers were randomly selected and the same fields were used in both seasons for the experiment. Five sampling points were randomly marked in each field. Weed data were collected from each site using a $1m^2$ quadrat at 8 weeks from crop establishment. Individual weeds were identified at species level using herbarium samples and dry weights (oven dried at 80 °C until constant weight) were recorded. The relative weed density (RD; Equation 1) and relative dry weight (RDW; Equation 2) was calculated.

$$RD\ \% = \frac{\text{Density of Individual weed species in the community}}{\text{Total density of all weed species in the community}} \times 100 \ldots\ldots \text{Equation 1}$$

$$RDW\ \% = \frac{\text{Dry weight of Individual weed species}}{\text{Dry weight of all weed species}} \times 100 \ldots\ldots\ldots\ldots \text{Equation 2}$$

Herbicide usage pattern of rice-growing farmers

The study was conducted in Hambantota district in both 2017/18 *Maha* and 2018 *Yala* seasons. A survey using a pre-tested structured questionnaire through face-to-face interviews was conducted with the involvement of 200 randomly selected rice-growing farmers in each season to collect data on herbicide use in rice cultivation. Data were subjected to analysis by using STAR for Windows version 2.1 (IRRI, 2014). Descriptive statistics were used for quantitative data analysis.

Dose-response of *I. rugosum* to herbicides

The herbicide dose-responses of *I. rugosum* to three commonly used herbicides with different modes of action, selected from the questionnaire survey, were determined in a pot experiment under greenhouse condition. Mature seeds of *I. rugosum* from suspected herbicide-resistant (R- biotype) populations were collected from the lowland rice fields in the Hambantota district and seeds of herbicide-susceptible (S-biotype) were collected from rice fields of the Rice Research Station, Ambalantota located in the DL1b agro ecological zone and an abandoned rice field, where no herbicides had been applied for the past five years. The site for collection of R-biotypes were selected based on the results of the farmer field survey carried out in the Hambantota district during 2016 and 2017 (four consecutive cultivating seasons), reporting a poor control of *I. rugosum*. Seeds of R-biotypes were collected from those sites with a past record of continuous use of the three different herbicides namely, Bispyribac sodium 40 g/1 + Metamifop 100 g/1 SC (eight samples: S1 to S8) for more than 4 years, Clomazone 200 g + Propanil 400 g/l (four samples: S9 to S12), and Thiobencarb 400 g/l +Propanil 200 g/l EC (four samples: S13-S16) for 3 years. Seeds collected were stored for six months in a freezer (-18 °C) before conducting the experiment. The seeds were sown in plastic trays (45 cm length x 35 cm width x 15 cm height) filled with top soil (2 kg/tray) at 5 cm row spacing, having a density of 100 seeds per tray. Soil used was Reddish Brown Earth with pH ranging from 6.4 to 6.8, CEC from 33.0 to 39.9 meq (100 g)-1, total N=0.0036%, total P=11.58 ppm and total K=26.68 ppm. Soil in trays was maintained at saturated water condition throughout the experiment in the green house.

The three herbicide formulations were selected to apply as six doses (Table 1) when *I. rugosum* seedlings were at 2-3 leaf stage, using a Knapsack sprayer (spray volume 400 L/ ha) equipped with a flat-fan nozzle (delivery rate 620 ml/min). Both R- and S-biotypes, grown on separate trays, received the same set of treatments. Further, any popular tank mix with the selected pre-mix formulations were also incorporated to the study based on the survey results. All seedling trays, allocated for a respective treatment were randomly placed in a 20 m^2 area in the greenhouse and the spraying was done from low to higher dosages separately, covering the whole area using a calibrated sprayer. Tap water was used for the control. Sprayer was washed thoroughly using tap water between different herbicide treatments. After imposing herbicide treatment, the trays were moved to their original position in the experimental layout.

Table 1. Dosages of different pre-mixed herbicide formulations used

Treatment number	Herbicide formulations and different dosages applied (L of product/ ha)		
	Bispyribac sodium 40 g/1 + Metamifop 100 g/1 SC	***Clomazone 200 g/l + Propanil 400 g/l***	***Thiobencarb 400 g/l +Propanil 200 g/l EC***
1	0.156	0.625	0.875
2	0.3125	1.25	1.75
3	0.625 (recommended dosage)	2.5 (recommended dosage)	3.5 (recommended dosage)
4	1.25	5.0	7.0
5	2.5	10.0	14.0
6	5.0	20.0	28.0

The treated trays were impounded with water 2 days after spraying. The experiment was conducted in a complete randomize design with nine replicates. Seedling mortality and remaining stand counts were taken at 7 and 14 days after spraying of the herbicide. A log logistic model (Seefedt *et al*., 1995) was fitted to estimate the ED_{50} and to compare the susceptibility of the biotypes to herbicides. Resistance Index (RI), which is the ratio between ED_{50} (R-biotype) and ED_{50} (S-biotype) was calculated as described by Sibony *et al*. (2001). Data were subjected to analysis using statistical package, STAR for Windows version 2.1 (IRRI, 2014). Rainfall data during the experimental period were collected from the weather station at the Rice Research Station, Ambalantota.

Results and discussion

Weed dominance

Relative density of weeds

Similar weed species were observed in the surveyed paddy fields during both 2017/18 *Maha* and 2018 *Yala* seasons, however, variation in weeds relative dominance (RD) was observed between the two seasons. Grasses dominated during the *Yala* season (RD>70%, *I.rugosum* (27%), *E. crus-galli* (31%) and *I. globosa* (12%) while sedges were dominant in the *Maha* season (RD>37%; *I. rugosum* >7%, *E. crus-galli* >10 and *I. globosa*>20). In the *Yala* season, the *I. rugosum* was the most dominant weed species with a RD>30% followed by *E. crus-galli* (Figure 1A). *C. difformis* was the most dominant in the *Maha* season (Figure 1B) followed by *I. globosa*. Among the broadleaves, *A. philoxeroides* was the dominant weed species in both seasons.

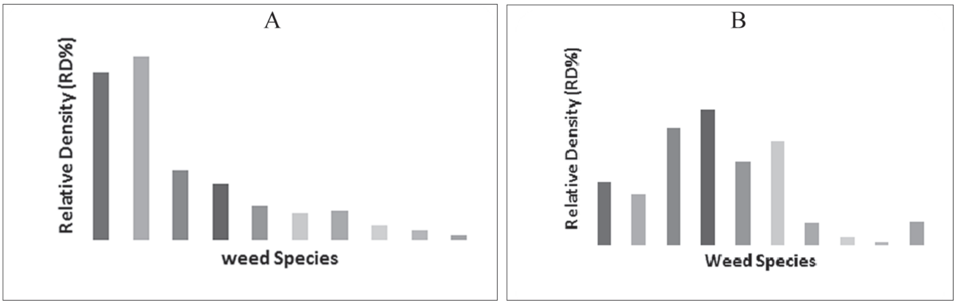


Figure 1. A: Relative density of weeds in 2018 *Yala* and B: 2017/2018 *Maha* season

*(Grasses:*ECR=*Echinochloa crusgalli,* IR=*Ischaemum rugosum,* IG=*Isachne globosa; Sedges:* CD=*Cyperus difformis,* CI=*Cyperus iria,* CR=*Cyperus rotundus; Broadleaves:* AP=*Alternanthera philoxeroides,* LO=*Ludwigia octovalvis,* AE=*Aeschynomene indica,* MO=*Monochoria vaginalis).*

The results clearly indicated the differences in changes in population structure of weed species between cultivating seasons. The *Yala* season is the relative dry period due to low rainfall received in the study region (Table 2). All dominant grass weeds recorded in this study are C_4 that has been originated in warmer climates compared to C_3 plants (Sage and Monson, 1999) and survive better in drier seasons compared to wet seasons as shown in the present study. The worlds' most troublesome weeds has the C_4 photosynthetic pathway (Lara and Andreo, 2011). The behavior of *I. globosa* was different to that of other two grasses showing a higher abundance during *Maha* season compared to that *Yala* season, probably due to the different propagating mechanisms of the weed (e.g. seeds and stolons) making it survive better in moist environments.

Table 2. Rainfall figure during 2017/2018 *Maha* and 2018 *Yala* cultivation period*

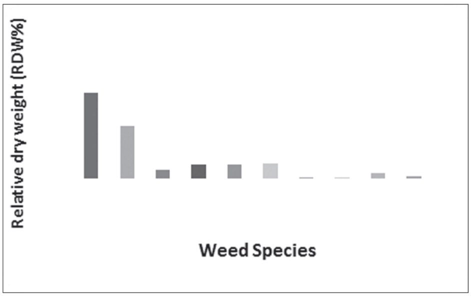
Details of rainfall	2017/2018 *Maha*	2018 *Yala*
Total number of rainy days	48	46
Total rain fall for the experimental period	773.39 mm	305.76 mm
Average rainfall of the period	4.20 mm/day	1.60 mm/day
Highest recorded rainfall	90.89 mm/day	49.0 mm/day
Lowest recorded rainfall	11.50 mm/day	8.00 mm/day
Rainfall intensity	4.27 mm	1.66 mm

**Information from the weather station reports of Rice Research Station, Ambalantota*

Relative dry weight (RDW %) of weeds

Dry weight of the weed species indicates the pressure imposed on crop species and other species due to weed competition. Figure 2 illustrates that grasses had the highest RDW in both *Yala* and *Maha* seasons (94.7% and 73.6%, respectively) in the paddy fields, despite sedges having a higher population density during the latter (Figure 2A). *I. rugosum* showed the highest RDW (70.9%) amongst the recorded weed species in the 2018 *Yala* season followed by *E. crus-galli*, where the latter recorded the highest RDW in 2017/18 *Maha* Season (42.6%; Figure 2B).

A



B

Figure 2. Relative dry weight (RDW%) of weeds in 2018 *Yala* (A) and 2017/2018 *Maha* season (B)

(Grasses: ECR=*Echinochloa crus-galli,* IR=*Ischaemum rugosum,* IG=*Isachne globosa; Sedges:* CD=*Cyperus difformis,* CI=*Cyperus iria,* CR= *Cyperus rotundus; Broadleaves:* AP=*Alternanthera philoxeroides,* LO=*Ludwigia octovalvis,* AE=*Aeschynomene indica,* MO=*Monochoria vaginalis).*

The C_4 grasses with higher RDW in both seasons shown in the present study have clearly indicated their ability to be more productive under resource limited situations, especially under low water availability during the *Yala* season. The C_4 plants are well known to exhibit higher photosynthetic and growth rates due to the gain from water, carbon and nitrogen use efficiency rates (Lara and Andreo, 2011). Thus, the C_4 weeds such as *I. rugosum* and *E. crus-galli* could be more productive in *Yala* season irrespective of their population densities (Figures 1 and 2) and emerge as better competitors reducing the yield of rice (C_3 plant) more in the *Yala* season compared to that of the *Maha* season. In a field situation, the more prostrate growth habit of *I. globosa* may get affected by mutual shading of other vertically-growing C_4 grasses thus affecting its photosynthetic process and productivity.

Herbicide usage in rice fields

Herbicides have become the key tool to manage weeds in rice cultivation in Sri Lanka. However, misuse of herbicides is becoming a major drawback to the system (Herath *et al*., 2017). Results of the present study showed that 80% of the farmers used tank mixtures of herbicide formulations, a practice not recommended by the Department of Agriculture of Sri Lanka (Table 3). In addition, majority of the farmers were applying higher rates of the herbicides than the recommended rate (Unpublished data). The tank mix of the pre-mix formulation Thiobencarb 400 g/l + Propanil 200 g/l EC with Carfentrazone-ethyl 240 g/1EC was the most popular in both seasons with good weed control as perceived by the selected farmers. The higher usage of this tank mixture may be due to its efficacy on the most dominant grass weed species found in the study area namely, *I. rugosum* and *E. crus-galli.* Moreover, results showed that the three Propanil-based pre-mixtures are more popular among the farming community, which also could be due to the higher efficacy of those herbicides in controlling a wide array of weeds, including C_4 grasses. Farmers may also decide on herbicides based on the nature of weed dominance. Pretilachlor 30% EC is also a popular herbicide formulation among the majority of the farmers due to its good weed controlling ability at early emergence stages. Pretilachlor 30% EC was reported to be used at higher volumes in the *Maha* season than in *Yala* season, indicating that the herbicide is effective during wet (*Maha*) season than the dry (*Yala*) season. The mode of action of the herbicide requires a moist environment, which is well supported by the rainfall during the *Maha* season than in the *Yala* season.

Table 3. Use of herbicides and their tank mixtures by farmers in the Hambantota district in 2017/18 *Maha* and 2018 *Yala* seasons

Herbicides and Herbicides combinations	% Farmers	
	2017/18 *Maha*	**2018 *Yala***
(Thiobencarb 400g/l +Propanil 200 g/l EC)[a] with Carfentrazone-ethyl 240g/1EC[b]	24	26
Pretilachlor 30% EC	16	9
(Clomazone 200g/l + Propanil 400 g/l)[a] with Carfentrazone ethyl 240g/1EC[b]	15	19
(Pretilachlor 170 g/l+ Propanil330 g/l EC)[a] with Carfentrazone-ethyl 240g/1EC[b]	13	8
(Bispyribac sodium 40g/1 + Metamifop 100g/1 SE)[a] with Carfentrazone-ethyl 240g/1EC[b]	11	15
Metamifop 10% EC with Carfentrazone-ethyl 240g/1EC[b]	9	11
(Pretilachlor 300g + Pyribenzoxim20g/1 EC)[a] with Carfentrazone-ethyl 240g/1EC[b]	6	6
Pyribenzoxim 5% EC with Carfentrazone-ethyl 240g/1EC[b]	3	4
Others	3	2

a= Pre-Mixture, b= tank mixture. (Source: Survey results)

Dose-response relationship of herbicides

The weed dynamic studies showed that *I. rugosum* has become most predominant weed species in Hambantota district during the *Yala* season and this was the most competitive weed species in rice production system in the study area. In agreement with the findings of Herath *et al*. (2017,2018), the farmer-field surveys also revealed that *I. rugosum* has become a troublesome weed in the recent past, and was effectively controlled by many herbicides in the past using the herbicides recommended by the DOA. Results revealed that the recommended dosage of the pre-mix formulation of Bispyribac sodium 40 g/1 + Metamifop100 g/1 SC controlled 98% of the S-biotype of *I. rugosum*, but did not have a significant impact (P>0.05) on the three selected R-populations (S-1,S-2 and S-3) of the weed. The dose–response curves (Figures 3 A, B and 4A) showed that the three suspected R-biotypes of *I. rugosum* are 1.68, 1.8 and 2.8 times resistant to Bispyribac sodium 40 g/1 + Metamifop 100 g/1 SC, respectively. Similar results were reported previously in experiments conducted using Bispyribac sodium (Sangakkara *et al*., 2004). Apóstolo (2009) also reported the development of resistance in *I. rugosum* to bispyribac-sodium (an ALS-inhibitor) in Venezuela. Moreover, the fourth suspected R-biotype of *I. rugosum* (S-4) collected from farmer fields did not show a resistance-buildup against the pre-mix herbicide formulation Bispyribac sodium 40 g/1 + Metamifop100 g/1 SC (Figure 4B). *I. rugosum* samples S5 to S8 showed a RI<0.5, indicating that those populations have not developed resistance to the pre-mix herbicide formulation Bispyribac sodium 40 g/1 + Metamifop 100 g/1 SC. Further, *I. rugosum* populations S9 to S16, when evaluated against the said herbicides, showed a RI<0.5 indicating that there is no significant resistance development to the herbicide formulations (data not shown). This could be the reason that a higher % of farmers in the study area use the two pre-mix formulations Clomazone 200 g + Propanil 400 g/l and Thiobencarb 400 g/l +Propanil 200 g/l EC for weed control in paddy fields.

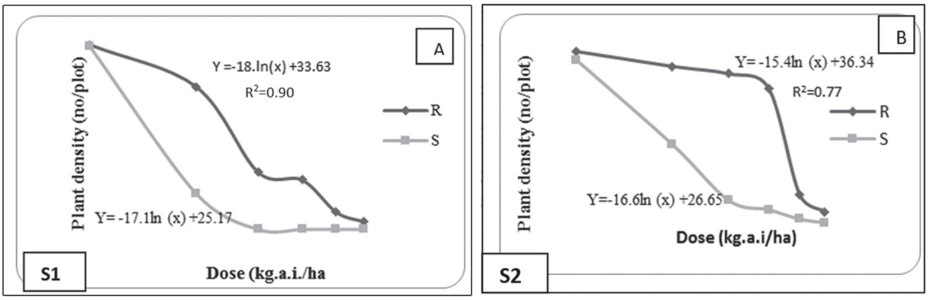


Figure 3. Dose response of Bispyribac sodium 40 g/l + Metamifop100 g/l SE in susceptible (S) and resistant (R) biotypes of *I. rugosum* grown at green house condition, using a log -logistic model (Samples S-1 and S-2)

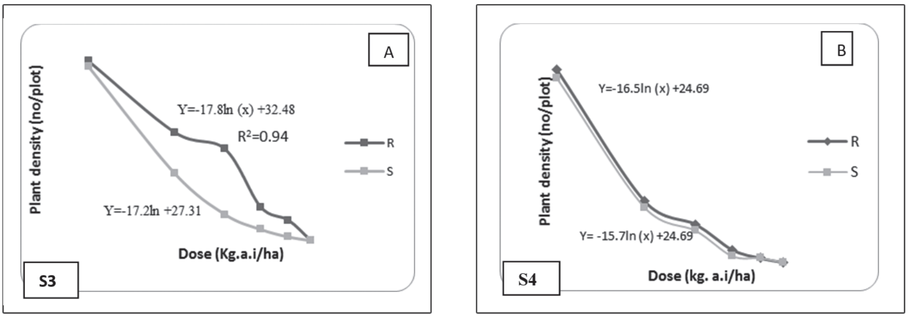


Figure 4. Dose response of Bispyribac sodium 40 g/1 + Metamifop100 g/1 SE –susceptible (S) and resistant (R) biotypes of *I. rugosum* grown at green house condition using a log logistic model (Samples S-3 and S-4)

The results revealed that *I. rugosum* has become the most dominant weed species probably due to the development of multiple resistances for pre-mix herbicide formulations used by farmers. Evolution of herbicide resistance in weed could be due to prolong use of the herbicides with same mode of action (Gressel and Segel, 1990). Therefore, continuous use of the pre-mix formulation containing Bispyribac sodium (ALS-inhibitor) and Metamifop (ACCase inhibitor) could have contributed to the evolution of multiple resistances in this weed. Herath *et al.* (2017) also reported that the ALS-inhibitor herbicides are the most popular among the rice-growing farmers in the Hambantota district of Sri Lanka, which could have aggravated the multiple resistance development to pre-mix formulation where ALS-inhibitor herbicides is a component. Propanil-resistant *E. crus-galli* has been reported in Sri Lanka (Marambe and Amarasinghe, 2002; Marambe and Samitha, 2002). However, the present study showed that *I. rugosum* has not developed ay detectable resistance to the propanil-based pre-mix formulations Clomazone 200 g/l + Propanil 400 g/l and Thiobencarb 400 g/l +Propanil 200 g/l EC having herbicide combinations with different modes of action.

Conclusion

Dominance of weed species in paddy fields has shown seasonal variation. Paddy farmers in Sri Lanka use tank mixtures of herbicides without any official recommendation from the Department of Agriculture, Sri Lanka. The C4 grass weeds were dominant in both *Yala* and *Maha* seasons where *I. rugosum* was the most dominant weed in the relatively dry, *Yala* season. *I. rugosum* populations in selected farmer fields have probably developed multiple resistances against the pre-mix formulation of Bispyribac sodium 40 g/1 + Metamifop 100 g/1 SE, having two modes of action namely, ALS-inhibitor and ACCase inhibitor, respectively. Continuous use of the same herbicide or herbicide mixtures should be avoided to minimize the occurrence of herbicide-resistant weeds. Weed dominance and dynamics would determine the type of herbicides to be used for weed control in paddy fields. These findings can be used effectively for the development of weed management strategies for lowland rice cultivation in Sri Lanka.

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