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RESEARCH ARTICLE

Management of bispyribac-sodium-resistant populations of small-flowered umbrella sedge (*Cyperus difformis*) using alternative herbicides

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ABSTRACT

The over-reliance on the acetolactate synthase-inhibiting herbicide bispyribac-sodium for broad-spectrum weed control in the rice-rice cropping system has led to an increase in the population of bispyribac-sodium resistant weeds. *Cyperus difformis* L. is one of the problematic and difficult-to-control weeds in the rice ecosystem with steady rise in the occurrence of bispyribac-sodium resistant populations. Therefore, to assist the rice farmers containing bispyribac-sodium resistant *C. difformis*, alternate options were tested. A pot study was carried out during the rainy season of 2019 and 2020 at the ICAR-Directorate of Weed Research, Jabalpur to evaluate herbicides with different sites of action against susceptible and resistant populations of *C. difformis*. Fluorpyrauxifen-benzyl at 31.25 g/ha at 3-5 leaves stage of *C. difformis* caused 100% reduction, over untreated check, in *C. difformis* density, plant height, fresh and dry shoot biomass, and visible mortality at 21 days after treatment. Next best herbicide was bentazone at 960 g/ha (>90%). Similarly, chlorimuron + metsulfuron at 4 g/ha and 2,4-D amine salt at 500 g/ha also resulted in a substantial reduction in the growth of susceptible and resistant populations. It can be concluded that fluorpyrauxifen-benzyl at 31.25 g/ha could be a potential herbicide against *C. difformis* and to be evaluated under field situations to verify and confirm its efficacy.

Keywords: Bispyribac-sodium, Herbicide resistance, *Cyperus difformis*, Resistant populations, Resistance management, Rice

INTRODUCTION

In the recent past, scarcity of labour and higher wages has forced the adoption of herbicide-based weed management in India (Rao *et al*. 2007). Although herbicide provides cost-effective weed control and saves labour, the sole dependence on herbicides for weed control with repeated use of the same mode of action can lead to the rapid development of herbicide resistance in weeds (Bhullar *et al.* 2017; Singh 2016; Chhokar *et al.* 2017; Zakaria *et al.* 2018 and Soni *et al.* 2021).

Bispyribac-sodium is an excellent herbicide that gives broad-spectrum weed control in rice. In the recent past, the lesser efficacy of herbicides or escape after herbicide applications (possibly herbicide resistance) has emerged as the major concern of contemporary agriculture. For weed management in rice, acetolactate synthase (ALS)-inhibiting herbicides have been extensively used for more than a decade mainly due to their broad-spectrum weed control, availability, and affordability by the rice growers (Choudhary and Dixit 2018; Mascazoni *et al.* 2018). However, these herbicides are more prone to the development of resistance in weeds (Heap 2021). The continuous use of bispyribac-sodium over a decade in rice-rice cropping systems along with inappropriate application techniques [*i.e.* lesser spray volume, faulty nozzle (hollow cone nozzle), late application, swing pattern of spraying, impure water, etc.] (personal observations by VK Choudhary) are the main cause of the development of resistance. In rice-rice system, the bispyribac-sodium resistant small-flowered umbrella sedge (*Cyperus difformis*) populations have been gradually increasing. This problem may further intensify mainly due to sharing of rice seeds among farmers, some of which may be contaminated with herbicide-resistant seeds. Thus, the main weed management tool is at a risk; thus, the sustainability of rice production is a serious problem as insufficient weed control also leads to low yield and grain quality losses and higher production costs (Marchesi and Saldain 2019).

Cyperus difformis is the most predominant *Cyperus* species in wetland/lowland rice cultivation systems. It forms dense mats of vegetation in young rice and reduces the rice yield by 12-50% (https://www.cabi.org/isc/datasheet/17495). Due to the intensification of rice cultivation and the repeated use of herbicides for weed management, *C. difformis* has evolved resistance to ALS-inhibiting herbicides in many rice areas worldwide (Merotto Jr *et al.* 2009).

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Some of the *C. difformis* populations from Chhattisgarh and Kerala states of India have developed resistance against bispyribac-sodium (Choudhary *et al.* 2021a). Herbicide resistance in weeds has become a major threat to the sustainability of wheat production in rice-wheat cropping system of Indo-Gangetic Plains (Kaur and Singh 2019). Early control of resistant populations using alternate herbicides with a different mode of action is important for sustainable rice production (Marchesi and Saldain 2019). Herbicide rotation with a diverse mode of action may reduce selection pressure and delays herbicide resistance evolution (Burgos 2015; Choudhary *et al.* 2021b). Thus, there is an urgent need to develop potential management strategies to control resistant populations of *C. difformis* to further overstate the problem in other areas. Hence, the present study was conducted to evaluate the efficacy of different alternate herbicides against bispyribac-sodium resistant populations of *C. difformis*.

MATERIALS AND METHODS

A pot study was conducted during the rainy season of 2019 and 2020 at ICAR-Directorate of Weed Research, Jabalpur (23°132'N and 79°592'E with an elevation of 388 m above mean sea level), Madhya Pradesh, India in a completely randomized design (CRD) with three replications. Three resistant populations (CGDCD-11, CGDCD-12, and CGRCD-20) with a resistance index of 10 - >20 based on ED_{50} and one population (CGDCD-1) of susceptible biotype of *C. difformis* were chosen for the study. These populations were collected from the previously conducted resistance study of bispyribac-sodium applied at 25 g/ha (field dose). The basic information about the populations was published earlier (Choudhary *et al.* 2021a). Five herbicides were evaluated at recommended dose along with untreated control (**Table 1)**.

Soil samples from 2-15 cm soil depth was taken from the field where rice-wheat system has been in practice for >10 years. The soil was clay-loam (Typic chromusterts) in texture with neutral in reaction with 7.1 pH and 0.21 dS/m of electrical conductivity. The organic carbon was 0.65% with available nitrogen, phosphorus and potassium were 254.0, 16.8 and 365.0 kg/ha, respectively. The soils were dried and autoclaved to kill the existing weeds and then filled in the pots with 17 cm x 17 cm dimensions.

Fresh seeds of *C. difformis* possess dormancy and do not germinate immediately after sowing (Derakhshan and Gherekhloo 2013). Therefore, to break the seed dormancy, seeds were first scarified in cotton cloth by rubbing and then mixed with autoclaved sand. The seed mixture (seeds+sand) was uniformly broadcasted on the saturated pots filled with autoclaved soils. Later, the seed mixture was smeared with soil. After 5 days, there was an excellent emergence. At 10 days after sowing, pots were thinned and 25 seedlings of *C. difformis* retained in the single pot and the rest were uprooted. The post-emergence application (PoE) of herbicides treatments was done at 20 days after sowing (DAS), as per the schedule using solar-cum-battery operated knapsack sprayer fitted with a flat-fan nozzle delivering 375 L/ha of spray volume at 350 kPa pressure. An untreated control was maintained for comparison.

Observations on the survived plants/pot, plant height, fresh and dry shoot biomass, and visible mortality were recorded 3 weeks after the treatments. All these observations were compared with untreated control and presented as % reductions. The plants that remained green at 21 days after treatment were considered as surviving plants. The dead plants were considered as a reduction in plant population and expressed in percentage. Plant height was recorded for green plants from the base to tip of the plant and expressed in cm. The reduction in plant height of survived treated plants was estimated by comparing with untreated plants and expressed in percentage. Fresh weight of green plants was recorded from the base of survived plants and compared with untreated control of the same population. Likewise, dry shoot biomass was recorded from survived plants and compared with untreated control of the same populations (samples were dried in a hot air oven at 65±2 ^{0}C till constant weight was achieved) and expressed in percentage. Visible mortality was recorded by three experienced persons giving a score of 0-100 (0 means no control and 100 means complete control), and the mean was used to compare against untreated populations. Statistical analysis of all the parameters was done using OPSTAT software.

RESULTS AND DISCUSSION

Reduction in *C. difformis* growth

Fluorpyrauxifen-benzyl 31.25 g/ha post-emergence (PoE) recorded an absolute reduction in population, plant height, effective suppression in fresh shoot weight with 100% control of susceptible and resistant populations of *C. difformis* followed by bentazone 960 g/ha, chlorimuron + metsulfuron 4 g/ha and 2,4-D 500 g/ha over untreated control (**Table**

2). Bispyribac-sodium 25 g/ha (field dose) completely controlled the susceptible biotype (CGDCD-1), whereas CGDCD-11 (biotype resistant up to 2X) had shown only 9% of reduction in plant population but CGDCD-12 and CGRCD-20 biotypes (resistant > 4X) had no reduction in populations, lesser reduction in plant height (4-8%), fresh shoot weight (1-3%), and lesser control/suppression (only 4-8%) (**Table 3**).

Visible plant mortality

Fluorpyrauxifen-benzyl 31.25 g/ha PoE has caused complete mortality (visual) of the resistant and susceptible populations of *C. difformis*. Bentazone 960 g/ha PoE recorded 87-95% mortality while chlorimuron+metsulfuron at 4 g/ha and 2,4-D at 500 g/ha caused 72-78% and 60-67% mortality, respectively over untreated control. Bispyribac-sodium recorded complete mortality of CGDCD-1, but it was ineffective in managing resistant populations (CGDCD-11, CGDCD-12, and CGRCD-20) (**Table 4**). Among different tested herbicides, fluorpyrauxifen-benzyl was found to be an effective treatment that provided excellent control of *C. difformis* followed by bentazone suggesting that these herbicides may serve as alternative options that may help rice growers to control bispyribac-sodium resistant populations. Their usage may also delay or stops the evolution of herbicide resistance when used as a component of integrated weed management

Table 1. Alternate herbicides tested to manage bispyribac-sodium resistant populations of *Cyperus difformis*

Herbicide	Dose (g/ha)	Site of action	WSSA	HRAC
Florpyrauxifen-benzyl	31.25	Synthetic auxins	4	O
Bentazone	960	Photosystem II inhibitors	6	C3
Chlorimuron + metsulfuron	4	Acetolactate synthase inhibitors	2	B
2,4-D amine salt	500	Synthetic auxins	4	O
Bispyribac-sodium	25	Acetolactate synthase inhibitors	2	B

WSSA: Weed Science Society of America; HRAC: Herbicide Resistance Action Committee

Table 2. Bispyribac-sodium resistant and susceptible *C. difformis* populations' control as measured by plant population and plant height reduction (%) at 21 days after treatments (mean of two years)

Treatment	Dose (g/ha)	Reduction in plant populations				Reduction in plant height			
		Susceptible	Resistant			Susceptible	Resistant		
		CGDCD -1	CGDCD -11	CGDCD -12	CGRCD -20	CGDCD -1	CGDCD -11	CGDCD -12	CGRCD -20
Untreated control		Plant population (#/pot)				Plant height (cm)			
		25	25	25	25	14.3	14.2	14.7	15.0
		% control over untreated control							
Florpyrauxifen-benzyl	31.25	100aA	100aA	100aA	100aA	100	100	100	100
Bentazone	960	93aB	95aB	91bB	91bB	49	50	49	50
Chlorimuron + metsulfuron	4	83aC	83aC	79bC	77bC	48	40	42	42
2,4-D	500	79aD	73bD	69cD	68cD	47	32	30	33
Bispyribac-sodium	25	100aA	9bE	0cE	0cE	100	8	5	4
Untreated control	-	0	0	0	0	0	0	0	0

Means for each population within a column followed by the same uppercase letters and means for each herbicide within a row followed by the same lowercase letters are not significantly different according to Fisher's protected LSD test (p = 0.05).

Table 3. Reduction (%) in fresh and dry shoot biomass of bispyribac-sodium resistant and susceptible *C. difformis* populations at 21 days after treatment (mean of two years)

Treatment	Dose (g/ha)	Reduction (%) in fresh shoot biomass				Reduction (%) in dry shoot biomass			
		Susceptible	Resistant			Susceptible	Resistant		
		CGDCD -1	CGDCD -11	CGDCD -12	CGRCD -20	CGDCD -1	CGDCD -11	CGDCD -12	CGRCD -20
Untreated control		Fresh shoot weight (mg/plant)				Dry shoot biomass (mg/plant)			
		330	332	327	334	46.8	46.6	45.7	46.4
		% control over untreated control							
Florpyrauxifen-benzyl	31.25	100aA*	100aA	100aA	100aA	100aA	100aA	100aA	100aA
Bentazone	960	93aB	90bB	88cB	89bB	93aB	92aB	91bB	90bB
Chlorimuron + metsulfuron	4	77aC	75bC	74cC	74aC	80aC	78bC	76cC	77cC
2,4-D	500	71aD	68bD	64cD	65cD	71aD	67bD	64dD	65cD
Bispyribac-sodium	25	100aA	3bE	1cE	1cE	100aA	8bE	4dE	5cE
Untreated control	-	0	0	0	0	0	0	0	0

*as suggested in Table 2

Table 4. Visible mortality (%) of bispyribac-sodium resistant and susceptible *C. difformis* populations at 21 days after treatments (mean of two years)

Treatment	Dose (g /ha)	Susceptible CGDCD-1	Resistant CGDCD-11	CGDCD-12	CGRCD-20
			% control over untreated control		
Florpyrauxifen-benzyl	31.25	100aA*	100aA	100aA	100aA
Bentazone	960	95aB	94aB	87bB	87aB
Chlorimuron + metsulfuron	4	78aC	76bC	72cC	72aC
2,4-D	500	67aD	65bD	62cD	60aD
Bispyribac-sodium	25	100aA	0bE	0bE	0aA
Untreated control	-	0	0	0	0

*as suggested in Table 2

including crop rotation, herbicides rotation with different sites of action and use of herbicide mixtures at field rates (Choudhary and Dixit 2021) with other agronomic manipulations (competitive cultivars, optimum sowing window, seeding rate, etc.) (Choudhary *et al.* 2021b). It is also suggested to remove weeds surviving the herbicide treatments before the seed is set to reduce weed seed bank and avoid seed dispersal. However, the price of the fluorpyrauxifen-benzyl has not been announced by manufacturers, thus based on the field trials and price of the product, the economics analysis need to be done and communicated to the rice farmers.

Based on the experiment, it can be concluded that fluorpyrauxifen-benzyl 31.25 g/ha PoE appeared to be a potential herbicide to control bispyribac-sodium resistant populations of *C. difformis* followed by bentazone 960 g/ha PoE . However, in the absence of resistant biotypes, bispyribac-sodium 25 g/ha PoE continues to provide excellent control of *C. difformis*. Therefore, it is suggested to rotate these herbicides in rice-growing areas to control >90% of *C. difformis*.

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