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Efficacy of sequential application of herbicide mixture for management of multiple herbicide resistant in *Phalaris minor* in wheat (*Triticum aestivum*)

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ABSTRACT

The emergence of multiple herbicide resistance in *Phalaris minor* is a major threat to wheat (*Triticum aestivum* L.) production in north-western Indo-Gangetic Plains of India. The present study was carried out during winter (*rabi*) seasons of 2020–21 and 2021–22 at research farm of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana for management of herbicide resistance in *Phalaris minor* Retz. in wheat using pre and post-emergence herbicide mixtures with different mode of action. The experiment was laid out in randomized block design (RBD) with 15 treatments, replicated thrice. Sequential application of flumioxazin 125 g/ha with pendimethalin (tank mix) 1500 g/ha *fb* clodinafop + metribuzin 174 g/ha resulted in significantly lowest weed density, dry matter of *P. minor* and broad-leaf weeds, and it was statistically comparable to application of flumioxazin alone or as mixture with pendimethalin as pre-emergence; pyraxosulfone + pendimethalin 127.5 + 1500 g/ha *fb* PoE (post-emergence) application of either mesosulfuron + iodosulfuron 14.4 g/ha; sulfosulfuron + metsulfuron, pinoxaden + metsulfuron 50 + 4 g/ha, clodinafop + metribuzin 174 g/ha, respectively. Phytotoxicity of flumioxazin was observed on wheat after 2–3 days of 1st irrigation i.e. 25 DAS (days after sowing). Application of pyroxasulfone + pendimethalin pre *fb* PoE mesosulfuron + iodosulfuron resulted in significantly higher grain yield (5847 and 5369 kg/ha, during 2021 and 2022, respectively) as compared to other herbicides.

Keywords: Flumioxazin, Multiple herbicide resistance, Pendimethalin, Pyraxosulfone, *Phalaris minor*, Wheat

The rice (*Oryza sativa* L.)-wheat (*Triticum aestivum* L.) cropping system plays a crucial role towards food security for the entire world's population. Asia has 13.5 million hectares dedicated to its vast cultivation, with 57% of that land located in south Asia. More than 85% of the land area is under rice-wheat cropping system and is located in south Asia's Indo-Gangetic Plains (Upadhaya *et al*. 2022). In India, rice-wheat cropping system occupies an area of about 10 Mha. The western Indo-Gangetic Plain has the highest mean levels of wheat productivity in the area, which encompasses the Indian states of Punjab and Haryana (McDonald *et al*. 2022). Wheat is India's second most important food grain crop after rice. Worldwide, wheat grown on an area of 226.5 million hectares with a production of 781.1 Mt in the year 2021–22 (Anonymous 2022a). India produces 109 Mt of wheat from a land area of 31.1 Mha with average national productivity of 35.7 q/ha (Anonymous 2022b). *Phalaris minor* Retz. is the common herbicide resistant grassy weed in wheat under rice-wheat system of India as well as the world, viz. United States, Australia, Iran, Israel, Mexico, Pakistan and South America (Heap 2023) affecting the sustainability of rice-wheat cropping system and possess a major challenge to food security (Raj *et al*. 2020, Chaudhary *et al*. 2022, Ahlawat *et al*. 2023). Because of the extremely aggressive capacity, *P. minor* develops alongside the germinating wheat crop and significantly reduces crop yield (Malik and Singh 1995). *P. minor* seeds can withstand an anaerobic environment in rice fields by entering secondary dormancy and avoiding anaerobic deterioration, which may be one of the reasons for the high prevalence of *P. minor* in rice-wheat cropping system. It is extremely prevalent in the rice-wheat cropping system due to morphological similarities, concurrent and asynchronous early development, and perpetual seed shattering before crop harvest. The evolution of resistance was aided by monocropping, increased nitrogen fertilizer uses and inadequate or excessive herbicide application (Singh *et al*. 2021). *P. minor* appears with the germinating wheat crop and its infestation can reduce grain yield to an extent of 25–50% (Chhokar and Sharma 2008). The rice-wheat system is seriously threatened by *P. minor*'s rising multi-herbicide

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resistance, underscoring the importance of diversifying weed control tactics for its efficient management. Due to cost and time-effectiveness, herbicides have been the primary tool for the control of *P. minor* and are commonly employed by wheat producers in northern India (Rasool *et al*. 2019). Using herbicides that have different modes of action provides a way to reduce selection pressure from any one group and improve the sustainability of wheat output (Abbas *et al*. 2018). Hence, this study was carried out to manage the multiple herbicide resistance of *P. minor* in wheat through pre- and post-emergence herbicides and their combination having different mode of actions.

MATERIALS AND METHODS

A field experiment was conducted during the winter (*rabi*) seasons of 2020–21 and 2021–22 at research farm of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana. The experiment was laid out in randomized block design (RBD) with 3 replications. The size of each plot was 6 m × 6 m. The experiment was performed in the field where multiple herbicide resistant of *P. minor* to ACCase (clodinafop and pinoxaden) and ALS inhibitors herbicides (sulfosulfuron and iodosulfuron + mesosulfuron (RM) was already reported in previous years (AICRP-Weed Management 2019–20). Wheat (cultivar WH 1105) was sown in 20 cm rows on 25th November 2020 and 2th November 2021 using a seed-cum-fertilizer seed drill at a seeding depth of 4 cm with a seed rate of 100 kg/ha. During both the years, wheat seed was treated with Vitavax (carboxin) at 2 g/kg seed before sowing. The crop was fertilised with 150 kg nitrogen and 60 kg P_2O_5/ha. First irrigation was scheduled at 22 days after sowing (DAS). Direct seeded rice was grown in *kharif* season followed by wheat in *rabi*. The experimental soil was sandy loam (*p*H of 7.7 and 7.9), having low organic carbon (0.35 and 0.38%) and nitrogen (190 and 194 kg/ha), medium available phosphorus (16 and 15 kg/ha) and high available potash (296 and 295 kg/ha) during 1st and 2nd year of experimentation, respectively. To control herbicide resistant of *P. minor*, a variety of pre- and post- herbicide combinations were included in the herbicide programme. A weed-free plot with manual weeding at regular intervals and non-treated plot (weedy check) where no herbicide was applied, was kept as control for comparison (Table 1). Herbicides were sprayed as per treatments, pre-emergence herbicides applied immediately after sowing and post-emergence herbicides were applied at 35 DAS (except metribuzin which was applied at 25 DAS as mix with urea) using a knapsack sprayer fitted with flat fan nozzle with a 500 litre/ha spray volume for pre-emergence herbicide application and a 375 litre/ha spray volume for post-emergence herbicide application.

The weather data were collected from the Department of Agricultural Meteorology, CCS Haryana Agricultural University, Hisar, Haryana. During the cropping season 2020–21, the mean weekly maximum and minimum temperatures ranged from 16.4–37.2°C and 2.6–17.9°C, respectively, whereas the mean weekly maximum and minimum temperatures during the cropping season 2021–22 ranged from 14.0–41.1°C and 3.3–21.2°C, respectively. A maximum air temperature of 37.2°C and 41.1°C was recorded in the 15th standard meteorological week of 2020–21 and 2021–22, respectively. Minimum air temperatures of 2.6°C and 3.3°C was recorded during the 52nd and 51st standard meteorological weeks of 2020–21 and 2021–22, respectively.

Data collection: The density of *P. minor* and broad-leaf

Table 1 List of herbicides, application rate and time of application used for control multiple herbicide resistance in *Phalaris minor* in wheat

	Herbicide treatment	Rate (g/ha)	Time of application
$T_{1,}$	Pendimethalin	1500	PRE
$T_{2,}$	Flumioxazin	125	PRE
$T_{3,}$	Flumioxazin + pendimethalin (TM)	125 + 1500	PRE
$T_{4,}$	Pyroxasulfone + pendimethalin (TM)	127.5 + 1500	PRE
$T_{5,}$	Pendimethalin *fb* mesosulfuron + iodosulfuron (RM)	1500 *fb* 14.4	PRE/35 DAS
$T_{6,}$	Pyroxasulfone + pendimethalin (TM) *fb* mesosulfuron + iodosulfuron (RM)	127.5 + 1500 *fb* 14.4	PRE/35 DAS
$T_{7,}$	Pendimethalin *fb* sulfosulfuron + metsulfuron (RM)	1500 *fb* 32	PRE/35 DAS
$T_{8,}$	Pendimethalin *fb* metribuzin*	1500 *fb* 315	PRE/25 DAS
$T_{9,}$	Pyroxasulfone + pendimethalin (TM) *fb* sulfosulfuron + metsulfuron (RM)	127.5 + 1500 *fb* 32	PRE/35 DAS
$T_{10,}$	Pendimethalin *fb* pinoxaden + metsulfuron (TM)	1500 *fb* 50 +4	PRE/35 DAS
$T_{11,}$	Pyroxasulfone + pendimethalin (TM) *fb* pinoxaden + metsulfuron (TM)	127.5 + 1500 *fb* 50 + 4	PRE/35 DAS
$T_{12,}$	Pyroxasulfone + pendimethalin (TM) *fb* clodinafop propargyl + metribuzin (RM)	127.5 + 1500 *fb* 174	PRE/35 DAS
$T_{13,}$	Flumioxazin + pendimethalin (TM) *fb* clodinafop propargyl + metribuzin (RM)	125 + 1500 *fb* 174	PRE/35 DAS
$T_{14,}$	Weed free	-	-
$T_{15,}$	Weedy check	-	-

*PRE, Pre-emergence; TM, Tank mix; RM, Ready mix; DAS, Days after sowing.

weeds was recorded at 30 and 50 DAS by using a quadrat of 0.5 m^2 placed (twice) randomly in wheat plots and finally expressed as per m^2. Weed plants that were counted during density ratings were cut/clipped at soil surface, placed in paper bags and dried in shade for 1–2 days and than dried in oven at 65°C until a constant weight was reached. Aboveground biomass of weeds was transformed into a per cent biomass reduction by comparing with the untreated control. Relative density of weeds in weedy check treatment was calculated by comparing number of weeds of particular species with total number of weeds.

Phytotoxicity symptoms of herbicides on wheat crop were also observed visually at 10, 20 and 30 DAT if any found they were rated on a scale range from 0 (no phytotoxicity to wheat) to 100% (fully phytotoxic or complete death of plant) (Kaur *et al*. 2017). The crop was harvested manually on the 20^{th} and 18^{th} of April, 2021 and 2022, when the straw became dry and brittle, and the grains became hard. The harvested crop was tied in labelled bundles, sun-dried, and weighted to determine biological yield before being threshed with a power thresher. The grain yield was obtained from each plot after threshing, which was then converted and expressed as grain yield kg/ha.

Data analysis: Data were subjected to ANOVA using R software (4.2.1) package Agricolae version 1.3-5. Prior to analysis, the data on *P. minor* density and per cent dry weight reduction were transformed using the square root and arcsine square root, respectively; however, the back-transformed data were shown with the mean separation based on the transformed data. The F-test was used to determine the significant treatment impact at a 5% level of significance. The graph was made using Origin v.8.5 software (Originlab Corporation, Northampton, USA).

RESULTS AND DISCUSSION

Weed studies: Major weeds infesting the experimental field were *P. minor*, *Convolvulus arvensis* and *Medicago denticulata.* In weedy check treatment an average density of *P. minor, Convolvulus arvensis* and *Medicago denticulata* was 55.7 and 54.9%; 18.6 and 21.8%; 25.9 and 23.3% at 30 and 50 DAS, respectively, during 2021 and 2022.

At 30 DAS, minimum density and maximum reduction in density of *P. minor* and broad-leaf weeds was observed with application of Flumioxazin-PPO inhibitor as PRE (pre-emergence), alone or in combination with pendimethalin - microtubule assembly inhibitor (Table 2 and Table 3). It was statistically comparable to weed free and PRE pendimethalin in tank mix with pyraxosulfone. A reduction of 63.7–67.9 and 68.7– 71.7% in biomass of *P. minor*, during 2021 and 2022, respectively was observed when pendimethalin was applied as PRE-alone in comparison to weedy check. Pre-emergence application of pendimethalin alone does not reduce the *P. minor* count significantly when compared to its tank mix with flumioxazin or pyraxosulfone. At 50 DAS, among different herbicides, sequential application of flumioxazin in tank mix with pendimethalin *fb* clodinafop + metribuzin (RM) resulted in significantly least weed count and maximum dry matter reduction of *P. minor* and it was statistically comparable to application of flumioxazin alone or in mix with pendimethalin as PRE; pyraxosulfone + pendimethalin as PRE *fb* PoE application of either mesosulfuron + iodosulfuron (RM), sulfosulfuron + metsulfuron (RM), pinoxaden + metsulfuron (TM), clodinafop + metribuzin (RM) at 35 DAS. Flumioxazin alone or in tank mix with pendimethalin as PRE reported 95–95.6% and 96.7–98.7% reduction in biomass of *P. minor* during 2021 and 2022, respectively. Tank mix application of pendimethalin + pyraxosulfone as PRE reported better control of broad-leaf weeds up to 30 DAS but its efficiency decreased significantly in the absence of post emergence herbicides.

Pre-emergence herbicides, especially in combination with pendimethalin, effectively target emerging weeds, including resistant *P. minor* and broad-leaf weeds. Combining pendimethalin with pyraxosulfone or flumioxazin significantly improved its weed control performance. Flumioxazin, while potent in weed control, showed phytotoxicity to wheat, impacting its growth and yield. Additionally, post-emergence herbicides like mesosulfuron + iodosulfuron (RM), pinoxaden + metsulfuron (TM), and clodinafop propargyl + metribuzin (RM) efficiently managed later-emerging weeds. Similar results were reported by Chand and Puniya. (2017), Chokkar *et al*. (2019), Kaur *et al*. (2019), Raseed *et al*. (2020) and Parita *et al*. (2021).

Phytotoxicity symptoms on wheat: No phytotoxicity of any herbicide was observed at 10 and 20 DAT. However, phytotoxicity symptoms in treatments where flumioxazin was used alone or in combination with pendimethalin were appeared after 2 days of first irrigation (25 DAS). Severe yellowing of whole plants i.e. leaves and stem was visualized (Fig. 1). Phytotoxicity range from 38–41% and 30–35% during 2020–21 and 2021–22, respectively.

Grain yield: Sequential application of pyroxasulfone + pendimethalin (TM) *fb* POE mesosulfuron + iodosulfuron (RM) resulted in significantly higher grain yield (5847 and 5369 kg/ha) when compared to other treatments and it was statistically at par with weed free (6051 and 5514 kg/ha); PRE pyroxasulfone + pendimethalin (TM) *fb* PoE pinoxaden + metsulfuron (TM) (5818 and 5342 kg/ha) (59.1 and 68.1% higher than weedy check) or clodinafop propargyl + metribuzin (RM) (5661 and 5220 kg/ha) (54.9 and 64.7% higher than weedy check) (Fig. 2).

Early-stage weed control by pre-emergence herbicides, followed by post-emergence herbicides for later-emerging weeds, minimized resource competition between crops and weeds. Wheat benefited from reduced weed competition, resulting in enhanced growth and higher grain yield. However, the use of flumioxazin, especially in combination with pendimethalin, negatively impacted yield due to phytotoxicity under sandy loam soils. Additionally, variations in seasonal weather, notably higher temperatures in March 2022, resulted in reduced grain yield during second year of study. These findings are in line with Dhillon (2018), Kaur and Singh (2019) and Kumar *et al*. (2022).

Based on the 2-years experiment it can be Hundal and

Table 2 Effect of application of pre- and post-emergence herbicides on density and per cent reduction in dry weight of *P. minor*

Herbicide treatment	Density (no./m^2) of *P. minor* at 30 DAS		Percentage reduction in dry weight of *P. minor* at 30 DAS		Density (no./m^2) of *P. minor* at 50 DAS		Percentage reduction in dry weight of *P. minor* at 50 DAS		Density (no./m^2) of *C. arvensis* at 30 DAS		Density (no./m^2) of *C. arvensis* at 50 DAS	
	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22	202–21	2021–22	2020–21	2021–22
T_1	7.0b	8.0bc	66.3b	71.7b	10.3b	11.56b	61.0c	66.6c	7.3b	6.0bc	9.0b	7.8b
T_2	0.0c	0.0d	100.0a	100.0a	1.3de	0.51fg	95.0a	96.7a	1.0de	0.7de	2.2e	1.0fgh
T_3	0.0c	0.0d	100.0a	100.0a	1.31de	0.34fg	95.6a	98.7a	1.7cd	0.0e	2.3e	0.3hi
T_4	1.0c	0.7d	91.8a	97.5a	5.15c	3.74de	81.1b	83.5b	3.0c	1.7d	5.0d	3.5e
T_5	7.7b	9.7bc	65.9b	73.4b	6.31c	6.97c	82.4b	83.2b	7.7b	6.3bc	6.5cd	4.7de
T_6	0.3c	0.3d	97.1a	98.5a	0.98de	0.51fg	96.4a	97.9a	2.7c	1.0de	2.7e	0.8gh
T_7	7.3b	10.7b	67.5b	70.9b	6.30c	8.16bc	78.5b	78.4b	7.3b	5.7c	6.5cd	4.8cd
T_8	6.3b	6.3c	67.1b	68.7b	6.14c	5.1cd	78.4b	77.6b	9.0b	8.3ab	7.3bc	6.3bc
T_9	0.7c	0.3d	94.3a	98.6a	2.30d	1.7ef	93.7a	95.5a	3.0c	1.7d	2.5e	1.5fg
T_{10}	8.0b	7.3bc	63.7b	71.1b	6.64c	6.63c	78.4b	79.3b	7.3b	6.0bc	6.5cd	5.5cd
T_{11}	0.7c	0.3d	94.1a	98.0a	1.30de	0.68fg	95.8a	98.0a	2.3c	1.3d	2.7e	1.7f
T_{12}	1.0c	0.7d	91.9a	97.3a	1.64d	1.02fg	92.4a	94.5a	2.7c	1.7d	2.3e	1.3fg
T_{13}	0.0c	0.0d	100.0a	100.0a	0.64de	0.17g	98.2a	98.9a	1.0de	0.0e	2.0e	0.8fgh
T_{14}	0.0c	0.0d	100.0a	100.0a	0.00e	0g	100.0a	100.0a	0.0e	0.0e	0.0f	0.0i
T_{15}	38.0a	35.3a	0.0c	0.0c	41.64a	40.29a	0.0d	0.0d	13.7a	10.7a	17.0a	15.5a

Treatment details are given under Table 1. DAS, Days after sowing.

Table 3 Effect of application of pre- and post-emergence herbicides on density of *Convolvulus arvensis* and *Medicago denticulata*

Herbicide treatment	Density (no./m^2) of *M. denticulata* at 30 DAS		Density (no. /m^2) of *M. denticulata* at 50 DAS		Percent reduction in dry weight of BLWs at 30 DAS		Percent reduction in dry weight of BLWs at 50 DAS	
	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22	2020–21	2021–22
T_1	7.7b	10.3b	9.0b	11.3b	43.9f	38.7g	43.9f	38.7g
T_2	1.3fg	1.0efg	1.8g	1.3efg	92.4b	91.5b	92.4b	91.5b
T_3	0.7gh	0.7fgh	1.0h	1.0fg	94.1b	93.3b	94.1b	93.3b
T_4	2.3def	1.7def	4.0e	4.7d	64.9c	62.2cd	64.9c	62.2cd
T_5	6.0bc	7.3c	5.0d	5.7cd	60.4d	56.8ef	60.4d	56.8ef
T_6	1.7ef	1.3def	1.7g	1.2fg	94.6b	94.3b	94.6b	94.3b
T_7	6.3bc	6.3c	5.5cd	5.0cd	59.5d	60.6de	59.5d	60.6de
T_8	7.3b	8.0c	6.2c	6.3c	67.9c	66.0c	67.9c	66.0c
T_9	2.7de	2.0de	2.7f	1.8ef	92.5b	92.8b	92.5b	92.8b
T_{10}	5.0c	6.3c	8.5b	5.0cd	49.9e	53.9f	49.9e	53.9f
T_{11}	2.0def	2.3d	2.7f	2.2e	90.2b	90.8b	90.2b	90.8b
T_{12}	3.0d	1.0efg	2.8f	1.0fg	93.6b	92.4b	93.6b	92.4b
T_{13}	0.7gh	0.3gh	1.3gh	0.7gh	93.3b	93.8b	93.3b	93.8b
T_{14}	0.0h	0.0h	0.0i	0.0h	100.0a	100.0a	100.0a	100.0a
T_{15}	17.0a	17.0a	17.2a	17.7a	0.0g	0.0h	0.0g	0.0h

Treatment details are given under Table 1. DAS, Days after sowing.

Fig. 1 Phytotoxicity of flumioxazin on wheat at 25 days after sowing.

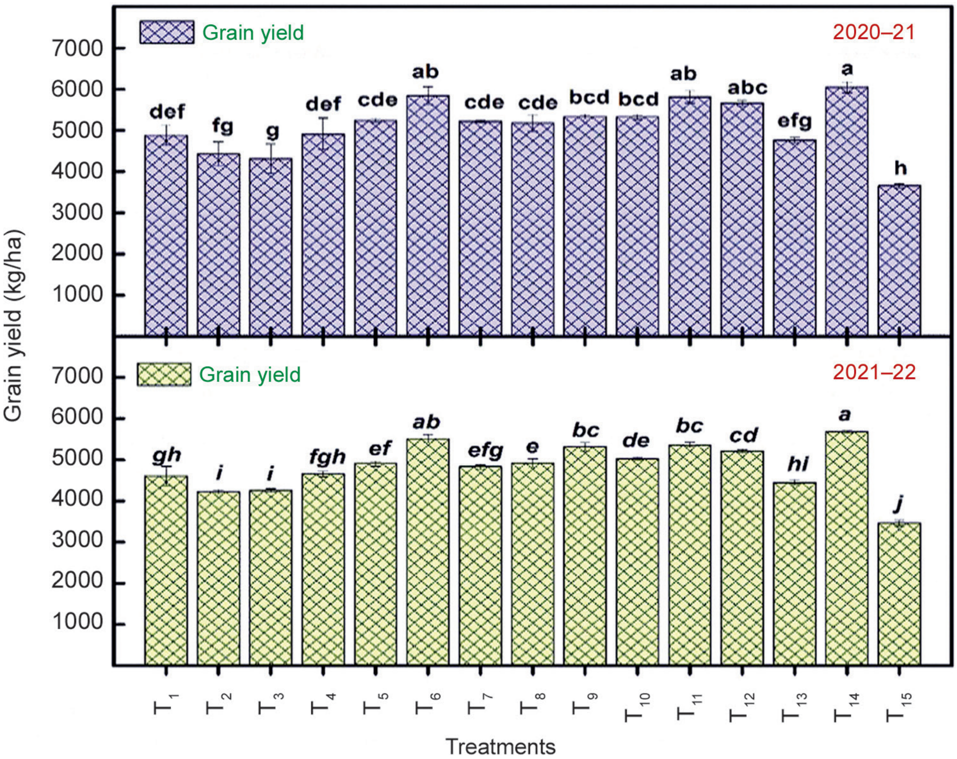

Fig. 2 Effect of different herbicidal treatments on grain yield of wheat.
*Error bars denotes the SEM (standard error of mean). Treatment details are given under Table 1.

concluded that pre-emergence flumioxazin alone 125 g/ha or with pendimethalin 1500 g/ha effectively controlled weeds but caused significant phytotoxicity to wheat, leading to reduced grain yield. Sequential applications involving specific herbicides demonstrated the best control of resistant *Phalaris minor* along with broad-leaf weeds and increased grain yield, particularly pyroxasulfone + pendimethalin 127.5 + 1500 g/ha (PRE) followed by post emergence mesosulfuron + iodosulfuron 14.4 g/ha; or pinoxaden + metsulfuron 50 + 4 g/ha; or clodinafop propargyl + metribuzin (RM) 174 g/ha; at 35 DAS.

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