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RESEARCH ARTICLE

Assessment of herbicide resistance in *Phalaris minor* and managing clodinafop-resistance with alternative herbicides

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ABSTRACT

Studies were conducted to assess and quantify the level of herbicide resistance in *P. minor* towards major used herbicides through a field survey in 2015-16 followed by dose-response assays in 2016-17. A total of 16 *P. minor* populations were collected from farmer's fields and screened against four majorly used herbicides in Haryana, *viz.* clodinafop, sulfosulfuron, mesosulfuron + iodosulfuron {Ready mix (RM)} and pinoxaden with its four graded doses (0X, ½X, X and 2X times of recommended dose). It was found that even at double of recommended dose, <80% mortality was observed in seven different populations under clodinafop; three under sulfosulfuron; one each under mesosulfuron + iodosulfuron (RM) and pinoxaden. These tested populations are generally categorized as resistant to highly resistant levels. Out of 16 populations, one showed multiple resistance towards ALS and ACCase inhibitor herbicides. Hence, for effective management of resistant *P. minor* and minimizing the probability of resistance development, field experiment with 16 different herbicide combinations was conducted during 2016-17 followed by 2019-20 to identify effective herbicide combinations for better management of clodinafop resistant *P. minor.* Field experiment advocated that sequential application of tank-mixed pre-emergence herbicides (pendimethalin with pyroxasulfone or metribuzin) followed by post-emergence (mesosulfuron + iodosulfuron (RM) or pinoxaden) along with their rotational application was effective against clodinafop-resistant *P. minor* and possibly a potent tool to minimize chances of resistance development.

Keywords: *Phalaris minor*, herbicide resistance, dose-response assay, sequential application of herbicide, wheat

INTRODUCTION

North-western Indo-Gangetic plains (IGPs) of India comprising states of Haryana, Punjab, and western Uttar Pradesh contributing more than 50% of national wheat production. Haryana producing 11.87 mt from an area of 2.53 mha with a productivity of 4.7 t/ha (Anonymous 2021), is one of the major wheat-growing states of India. However, weeds are the major biotic constraint in wheat production. They emerge concurrently along with crop seedling and if not managed till critical crop - weed competition period may cause significant reduction in crop yield (≥15-40% or more) and quality (Punia and Yadav 2009), having substantial economic impact on overall wheat production (Mamta and Sharma 2019).

Wheat is generally infested by diverse weed flora encompassing both grassy and broad-leaved weeds (BLWs). Among them, *Phalaris minor* Retz. (little seed canary grass) is major problematic and mimic weed of wheat. It predominates in the irrigated rice-wheat cropping system and severely infests wheat fields in the north-western IGPs of India including Haryana (Singh *et al.* 1999). Cultivation of semi-dwarf wheat varieties provides favourable and conducive micro-climate for the growth and development of *P. minor* (Singh *et al.* 1995). Additionally, rice straw burning before sowing of wheat tends to boost *P. minor* germination (Chhokar *et al.* 2009). Due to its morphological similarity to wheat, it frequently eludes manual and mechanical control methods. Thus to control this weed, application of selective herbicides is the most appropriate tool along with cost- and time effectiveness. However, use of same herbicide repeatedly develops selection pressure resulting resistant weed population. Simultaneously, the sole dependence on herbicides with a single mode of action has the greatest risk for herbicide-resistance evolution (Beckie 2006). During 1991-92, the first case of herbicide resistance was testified in *P. minor* against isoproturon in India (Malik and Singh 1995). The sole dependence on herbicides led to the evolution of multiple resistance in *P. minor* in due course of time. Also, some of the biotypes developed

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resistance to some new herbicides, *viz*. pinoxaden and mesosulfuron + iodosulfuron (RM). As of now, multiple herbicide-resistant *P. minor* is endemic causing significant yield reductions in rice-wheat cropping system of IGPs; and it is estimated that *P. minor* invades about 50% (15 mha) of the wheat growing areas in India. Of this area, the multiple herbicide-resistant *P. minor* affects about 3.0 mha (20% of *P. minor* infected area) of wheat (Chhokar *et al*. 2019, Soni *et al*. 2023). However, the exact information on herbicide resistance level in *P. minor* under different herbicides is still not known. Therefore, information on the level of herbicide resistance in *P. minor* is of prime importance. This can be managed within a field by using different herbicides with different mode of actions (MOAs). Herbicide-resistant *P. minor* in wheat was found susceptible to pre-emergence (PE) herbicides such as pendimethalin, metribuzin and pyroxasulfone (Dhawan *et al*. 2012). The PE herbicides offer an alternate mode of action to many post-emergence (PoE) herbicides, reduce selection pressure on subsequent PoE herbicide applications along with a reduction in early season weed competition for crop. Moreover, only PE herbicide application is not enough to control *P. minor* and its cohorts. The PE herbicides require a mixing partner for improved and broad-spectrum control. Mixing partners, such as herbicide combinations or compatible mixtures, provide various advantages including broad-spectrum action, increased efficacy through synergistic or additive effects, reduced application quantities, cost-effective weed management, prevention of weed shifts and resistance mitigation (Powles and Shaner 2001). Cavan *et al*. (2000), through a simulation model, showed that alternate herbicides with a different mechanism of action used in rotation resulted in delaying of development of resistance up to 45 years. In light of this background, an experiment was undertaken to determine the level of herbicide resistance by screening graded doses of herbicides and at the same time searching the effective combination of herbicides and their sequential application to manage this problem.

MATERIALS AND METHODS

Survey and collection of *P. minor* seeds

Based on the problem lodged by farmers towards poor efficacy of herbicides against *P. minor* at recommended dose (RD), a survey was conducted during *Rabi* (winter) 2015-16 from different locations in Haryana state, India. To represent one population, 30 matured ear heads of survived *P. minor* were randomly collected from a particular locality (Burgo 2015). Similarly, a total of 16 locations were surveyed and sampled that represented 16 populations (**Table 1**). The collected ear heads were shade dried, seeds were removed from each ear head and stored in craft paper bags at room temperature.

Dose-response bioassay for confirmation of resistance level

A dose-response bioassay for determination of herbicide resistance level in 16 *P. minor* populations was conducted in the earthen pots (20 cm diameter and 20 cm height) at the screen house of CCS HAU, Hisar (29°8’41.50"N, 75°42’15.72"E). The soil was taken from HAU Research Farm that was not subjected to any herbicide application for the last two years and was free from *P. minor* infestation. The soil was air-dried, crushed, well-grounded and passed through a 2 mm sieve. Earthen pots were filled with sieved soil: vermicompost mixture of 4:1 ratio. Seeds of each *P. minor* population were sown during *Rabi* (winter) 2016-17 and thinning was done at 15 days of germination to keep 20 plants per pot. The experimental units consisting of pots were arranged in factorial completely randomized block design under 16 populations screened with post-emergence application of 0.0, 0.5, 1.0 and 2.0 time of recommended dose (RD) of herbicides, *viz*. clodinafop (RD: 60 g/ha), sulfosulfuron (RD: 25 g/ha), mesosulfuron + iodosulfuron (RD: 14.4 g/ha) and pinoxaden (RD: 50 g/ha) with four replications. The herbicides were sprayed at 3-4 leaf stage {30 days after sowing (DAS)}. Plots were arranged outside the screen house and marked area was used for calculation of required quantity of herbicide corresponding to its dose. Herbicides were applied using knapsack sprayer fitted with a flat fan nozzle in 375 L water volume/ha.

Per cent control of *P. minor* was recorded at 30 days after treatment (DAT) from 0 (zero) to 100 scale (0 indicated no control, 100 = complete control of *P. minor*). GR_{50} value of different *P. minor* populations sprayed with various herbicides was calculated on basis of per cent visual control of *P. minor* under different herbicides in graded doses. Data on the per cent inhibition of the *P. minor* populations were subjected to linear regression using the probit analysis (Das *et al*. 2014) by OPSTAT software (Sheoran *et al*. 1998).

Field experiment

In order to manage resistance in *P. minor* through alternate herbicides, a field experiment was conducted at Agronomy Research Farm, CCS HAU, Hisar (Haryana) in *Rabi* (winter) 2016-17 and 2019-20. Sixteen treatments were: pendimethalin 1500 g/ha PE; metribuzin 210 g/ha PE; pendimethalin + metribuzin tank mix (TM) 1500 + 175 PE; pendimethalin + metribuzin (TM) *fb* pinoxaden 1000 +175 *fb* 60 g/ha PE *fb* PoE; pendimethalin + metribuzin (TM) *fb* mesosulfuron + iodosulfuron (RM) 1000 + 175 *fb* 14.4 g/ha PE *fb* PoE; pendimethalin + pyroxasulfone (TM) 1500+102 g/ha; pendimethalin + pyroxasulfone (TM) *fb* pinoxaden 1500+102 *fb* 60 g/ha PE *fb* PoE; pendimethalin + pyroxasulfone (TM) *fb* mesosulfuron + iodosulfuron (RM) 1500+102 *fb*14.4 g/ha PE *fb* PoE; pendimethalin + metribuzin (TM) *fb* pinoxaden 1500 + 175 *fb* 60 g/ha before sowing *fb* PoE; sulfosulfuron *fb* pinoxaden 25 *fb* 60 g/ha BI *fb* PoE; pinoxaden 60 g/ha PoE; pinoxaden + metribuzin (TM) 50+120 g/ha PoE; pinoxaden + metribuzin (TM) 50+150 g/ha PoE; mesosulfuron + iodosulfuron (RM) 14.4 g/ha PoE; weed-free check; weedy check. Evaluated in a randomized block design (RBD) replicated thrice with plot size of 6m × 6m. Wheat cv HD 2967 was sown on 20 November 2016 and 5 December 2019 and harvested on 16 April 2017 and 27 April 2020 during two seasons. The recommended dose of fertilizer (RDF), *viz.* 150 kg N/ha and 60 kg P/ha was applied in both the crop seasons. Herbicides were applied as per treatment either as pre-emergence (PE), post-emergence (PoE) at 35 days after sowing (DAS) of wheat, or PE followed by (*fb*) PoE, before sowing of wheat seeds *fb* PoE or before first irrigation (BI) at 18 DAS *fb* PoE. In weeds-free plots, weeds were removed manually as and when appeared; and no weeding was done in weedy check.

Plant dry matter, yield attributing parameters, grain and biological yield of wheat were recorded as per the standard observation and computing methods. Data on *P. minor* dry weight, total weeds dry weight and weed control efficiency (WCE) were recorded at 60 and 120 DAS, respectively. All the weeds taken with quadrate from four places selected at random from each plot for dry matter accumulation at 60 and 120 DAS. Individual weeds were separated, sun-dried and then kept in an oven at 65±5 ^{0}C till a constant weight was achieved. The dried samples of individual weeds were weighed and the final dry weight of total weeds was expressed as g/m^2. Whereas, WCE was calculated using following formula:

$$\mathrm{WCE} = \frac{(\mathrm{Wc} - \mathrm{Wt})}{\mathrm{Wc}} \times 100$$

Where, Wc = dry weight of weeds in weedy plot (g); Wt = dry weight of weeds in treated plot (g). While weed index (WI) of different treatments was calculated using formula given below and expressed in %.

$$\mathrm{WI} = \frac{(\mathrm{Yc_0} - \mathrm{Yt})}{\mathrm{Yc_0}} \times 100$$

Where, Yc_0 = Yield obtained from weed-free plot (control plot) and Yt = Yield obtained from treatment for which WI is to be worked out

Statistical analysis

The data underwent statistical analysis using Analysis of Variance (ANOVA) *via* OPSTAT software (Sheoran *et al.* 1998). Weed dry weight data were subjected to square-root $(\sqrt{x+1})$. The responses of various treatments remained consistent across both years and passed the homogeneity test; thus, the data

Table 1. Details of *P. minor* populations collected from different locations in Haryana state under rice-wheat cropping system

P. minor Population	Village name	District	Latitude	Longitude
P_1	Nangla-1	Fatehabad	29°37'14.39"N	75°53'38.65"E
P_2	Pipaltha-1	Jind	29°45'28.38"N	76° 5'41.14"E
P_3	Nangla-2	Fatehabad	29°36'39.41"N	75°53'33.35"E
P_4	Laloda-1	Fatehabad	29°38'30.92"N	75°52'39.07"E
P_5	Pipaltha-2	Jind	29°45'42.07"N	76° 5'33.18"E
P_6	Barwala	Hisar	29°21'4.57"N	75°53'44.69"E
P_7	Pipaltha-3	Jind	29°45'51.98"N	76° 6'7.27"E
P_8	Khedi	Kaithal	29°46'49.98"N	76°29'39.13"E
P_9	Ludas	Hisar	29° 9'28.35"N	75°38'42.83"E
P_{10}	Danora	Ambala	30°29'38.66"N	77° 7'42.37"E
P_{11}	Ujhana	Jind	29°43'18.59"N	76° 7'41.49"E
P_{12}	Dhos	Kaithal	29°49'30.36"N	76°32'11.80"E
P_{13}	Danoda	Jind	29°31'7.32"N	76° 3'1.17"E
P_{14}	Samain	Fatehabad	29°37'19.98"N	75°55'25.30"E
P_{15}	Rasidan	Jind	29°43'30.55"N	76° 1'44.74"E
P_{16}	Kalwan	Jind	29°42'50.16"N	75°58'3.05"E

were statistical examination, accordingly. The significance of treatments was determined using the 'F' test with a least significant difference (LSD) of 5%.

RESULTS AND DISCUSSION

Dose-response bioassay for confirmation of resistance level

Sixteen populations of *P. minor* collected from different locations in Haryana state were subjected to graded doses of herbicides at 30 DAS (**Table 2**). At 30 days after treatment (DAT), averaged doses of clodinafop showed significantly least mortality in P_9 (19.9%) followed by P_2 (26.6%). At half of RD, only 5 (P_3, P_6, P_{10}, P_{13} and P_{15}) populations depicted with >80% mortality while at RD, six populations displayed >80% mortality and five (P_1, P_2, P_7, P_9 and P_{16}) showed <50% mortality. Whereas, at double of RD of clodinafop, significantly least mortality was recorded in P_9 (33.3%) followed by P_2 (43.3%). Similarly, application of sulfosulfuron resulted in significant variation in per cent mortality of *P. minor* population (**Table 2**), mean data of sulfosulfuron doses exhibited that significantly lower mortality was observed in P_9 (25.6%) followed by P_8 (41.1%). At half of RD, 8 populations depicted with >80% mortality and at RD, four populations (P_1, P_8, P_9 and P_{15}) exhibited <80% mortality. At double of RD, significantly least was recorded in P_9 (35.0%) followed by P_{15} (59.8%). Most of the tested populations were found sensitive to mesosulfuron + iodosulfuron (RM). Significantly least mortality was depicted by P_{15} (38.1%) followed by P_{16} (41.7%) under its averaged doses (**Table 2**). At half of RD, 11 populations depicted with >80% mortality. While at RD, P_{15} and P_{16} showed <50% mortality. At double of RD, significantly least mortality was recorded in P_{15} (53.3%). Similarly, under pinoxaden, significantly least mortality per cent was observed in P_{14} (38.0%) followed by P_1 (66.6%) under the averaged doses. At half of RD, 7 populations depicted with >80% mortality. While, at RD, only P_{14} witnessed least visual mortality (31.8%). At double of RD of pinoxaden, significantly least mortality was recorded in P_{14} (78.3%). Percentage mortality is an index of sensitivity of *P. minor* population over the years towards different herbicides. The resistance to herbicides can be attributed to the use of wheat monoculture in the given areas along with the repeated use of the same herbicide for a long period of time levied a persistent selection pressure resulting in resistance development (Chhokar *et al.* 2012). Clodinafop is an aryloxyphenoxypropionate type herbicide inhibiting acetyl-CoA carboxylase (ACCase) enzyme (Golmohammadzadeh *et al.* 2019). Resistance towards clodinafop is most common phenomenon of insensitivity of ACCase target site. This target site resistance in *P. minor* can appear within 10 years of its continuous application (Beckie 2006). Clodinafop is being used for more than 15 years under monocropping in the region/state (Haryana) under study. This might be the reason that nearly 62% of screened populations were observed with <80% mortality under recommended dose.

Table 2. Per cent visual mortality of different *P. minor* populations under graded doses of herbicides at 30 DAT

Population	Percent mortality (%)															
	Clodinafop				Sulfosulfuron				Mesosulfuron + Iodosulfuron				Pinoxaden			
	30 g/ha	60 g/ha	120 g/ha	Mean	12.5 g/ha	25 g/ha	50 g/ha	Mean	7.2 g/ha	14.4 g/ha	28.8 g/ha	Mean	25 g/ha	50 g/ha	100 g/ha	Mean
P_1: Nangla1	42.3	48.3	51.7	47.4	30.0	65.0	85.0	60.0	58.3	83.3	93.3	78.3	43.3	73.3	83.3	66.6
P_2: Pipaltha1	16.7	19.7	43.3	26.6	78.3	91.7	98.3	89.4	85.0	88.3	100.0	91.1	63.3	88.3	96.8	82.8
P_3: Nangla2	83.0	90.0	100.0	91.0	85.0	91.7	96.7	91.1	83.3	91.7	98.3	91.1	86.8	91.8	100.0	92.9
P_4: Laloda1	23.3	65.0	96.7	61.7	68.3	95.0	100.0	87.8	93.3	100.0	100.0	97.8	46.8	86.8	96.8	76.8
P_5: Pipaltha2	41.7	55.0	90.0	62.2	61.7	88.3	100.0	83.3	93.3	100.0	100.0	97.8	35.0	93.3	100.0	76.1
P_6: Barwala	90.0	95.0	100.0	95.0	85.0	93.3	100.0	92.8	93.3	100.0	100.0	97.8	90.0	100.0	100.0	96.7
P_7: Pipaltha3	32.7	36.7	58.3	42.6	88.3	98.3	100.0	95.6	95.0	98.0	100.0	97.7	18.3	93.3	100.0	70.5
P_8: Khedi	51.7	85.0	93.3	76.7	15.0	38.3	70.0	41.1	68.3	93.3	100.0	87.2	86.0	100.0	100.0	95.3
P_9: Ludas	4.7	21.7	33.3	19.9	16.7	25.0	35.0	25.6	50.0	81.7	98.3	76.7	88.0	100.0	100.0	96.0
P_{10}: Danora	90.0	95.0	100.0	95.0	82.3	95.0	96.7	91.3	86.7	93.3	98.0	92.7	93.0	100.0	100.0	97.7
P_{11}: Ujhana	28.3	50.0	53.8	44.0	85.0	96.7	98.3	93.3	80.0	90.1	96.7	88.9	15.0	93.3	100.0	69.4
P_{12}: Dhos	11.7	56.7	65.0	44.5	90.0	95.0	100.0	95.0	90.0	98.0	100.0	96.0	68.3	98.3	100.0	88.9
P_{13}: Danoda	85.0	100.0	100.0	95.0	90.0	95.0	100.0	95.0	88.3	96.7	100.0	95.0	96.8	100.0	100.0	98.9
P_{14}: Samain	41.7	78.3	95.0	71.7	80.5	97.5	100.0	92.7	90.0	100.0	100.0	96.7	3.8	31.8	78.3	38.0
P_{15}: Rasidan	90.0	95.0	100.0	95.0	33.3	38.3	59.8	43.8	21.7	39.3	53.3	38.1	92.0	99.0	100.0	97.0
P_{16}: Kalwan	33.3	38.3	55.0	42.2	56.7	88.3	98.3	81.1	0.0	31.8	93.3	41.7	31.8	86.8	98.3	72.3
Mean	47.9	64.4	77.2		65.4	80.8	89.9		73.5	86.6	95.7		59.9	89.7	97.1	
LSD (p=0.05)																
Population(P)	3.0				3.3				3.0				2.8			
Herbicide(H)	1.3				1.4				1.3				1.2			
PXH	5.1				5.6				5.2				4.8			

Herbicide sulfosulfuron and mesosulfuron + iodosulfuron (RM) are acetolactate synthase (ALS) enzyme inhibitors that inhibit ALS enzyme. Target site mutation in amino acid sequence of ALS is the possible cause of resistance. The earlier finding indicates that herbicides inhibiting ALS enzyme are more efficient than ACCase inhibitor herbicides for control of herbicide resistant population of *P. minor* (Kaur *et al.* 2016). However, continuous use of ALS herbicide leads to decreased efficacy towards *P. minor* population forcing farmers to apply higher doses. While, pinoxaden is ACCase inhibitors herbicide of the most recently introduced chemistry phenylpyrazolin (Linda *et al.* 2010). It was very effective against resistant populations of *P. minor*. However, being one of the costlier herbicides, though, it was used in farmers' fields at lower scale, yet, its continuous use over years under monoculture has also resulted in the raising the resistance cases towards it.

The GR_{50} values of clodinafop for seven populations (P_1, P_2, P_8, P_9, P_{11}, P_{12} and P_{16}) were more than RD (60 g/ha). Similarly, GR_{50} values of sulfosulfuron for three populations (P_8, P_9 and P_{15}) were estimated more than RD (25 g/ha) with highest in P_9 (131.8 g/ha). Whereas, GR_{50} values of mesosulfuron + iodosulfuron (RM) for two populations (P_{15} and P_{16}) were found more than RD (14.4 g/ha) with the highest value in P_{15} (24.5 g/ha). The GR_{50} value of pinoxaden for P_{14} (61.7 g/ha) was more than RD (50 g/ha). The higher dose of clodinafop to control resistant *P. minor* populations as evident from findings of study that GR_{50} value of 62% of populations estimated between 34.7-193.6 g/ha, which were 2.8 to 14.3 times higher than their most susceptible population (**Table 3**). Similarly, out of 311 populations of *P. minor* collected from Haryana and Punjab, 71 showed RF value between 2-41 for clodinafop (Das *et al.* 2014). Likewise, Chhokar and Sharma (2008) found some of the *P. minor* populations recorded with >10 times higher GR_{50} value of clodinafop than that of most susceptible one. The GR_{50} values of sulfosulfuron for *P. minor* populations were 5 g/ha before 2005 in Haryana (Yadav and Malik, 2005). Over a period of time, GR_{50} values rose up to 10-fold against sulfosulfuron (Dhawan *et al.* 2009). Similarly reduced efficacy of mesosulfuron + iodosulfuron (RM) against some populations of *P. minor* has also been reported by Kaur *et al.* (2016). Earlier researchers also documented that continuous use of pinoxaden on clodinafop resistant *P. minor* populations brought reduced sensitivity towards pinoxaden resulting in higher dose requirement for controlling *P. minor* (Chokkar *et al.* 2008). Some of the tested *P. minor* populations recorded GR_{50} value >120 g/ha towards pinoxaden and indicated progressive development of resistance in *P. minor* towards pinoxaden (Dhawan *et al.* 2010).

Present study also confirmed that population (P_1 and P_{14}) have developed cross-resistance against clodinafop (GR_{50}: 34.7-86.9 g/ha) and pinoxaden (GR_{50}: 28.2-61.7 g/ha). Also, neither clodinafop nor sulfosulfuron or pinoxaden could control P_1 population, that indicats development of multiple resistance towards ALS and ACCase inhibitor herbicides (Pieterse and Kellerman, 2002). Earlier findings also indicate that ALS and ACCase inhibitors herbicides are highly suspectable towards resistance evolution if being used continuously under monocropping (Das *et al.* 2014). However, variable resistance among the *P. minor* populations might be due to varied selection pressure across fields, crop rotation and cropping pattern; cultural practices,

Table 3. GR_{50} value (g/ha) of different herbicides against *P. minor* populations at 30 DAT

Population	Clodinafop (g/ha)	Sulfosulfuron (g/ha)	Mesosulfuron + iodosulfuron (RM) (g/ha)	Pinoxaden (g/ha)
P_1: Nangla1	86.9	19.1	5.6	28.2
P_2: Pipaltha1	193.6	5.5	4.8	18.2
P_3: Nangla2	19.0	2.3	2.2	13.2
P_4: Laloda1	44.7	10.0	2.1	25.1
P_5: Pipaltha2	41.7	11.5	2.1	28.2
P_6: Barwala	12.6	7.1	1.9	7.4
P_7: Pipaltha3	50.6	5.4	1.7	32.4
P_8: Khedi	91.2	31.6	5.9	13.1
P_9: Ludas	179.5	131.8	7.2	8.5
P_{10}: Danora	12.9	3.0	1.4	5.6
P_{11}: Ujhana	62.7	2.6	2.4	33.1
P_{12}: Dhos	72.4	5.4	2.8	18.2
P_{13}: Danoda	14.8	5.4	3.2	9.8
P_{14}: Samain	34.7	7.3	2.8	61.7
P_{15}: Rasidan	12.9	34.1	24.5	7.7
P_{16}: Kalwan	102.3	10.7	14.8	30.2

intensity and extent of herbicide usage; and the way farmers use the herbicide (Abbas *et al*. 2017).

Field experiment

Weed studies: Field experiment was carried out in a field infested with clodinafop resistant *P. minor*. The results indicated that maximum reduction in dry weight of *P. minor* at 60 and 120 DAS was recorded in sequential application of PE tank-mix pendimethalin + pyroxasulfone (1500 + 102 g/ha) *fb* PoE application of mesosulfuron + iodosulfuron (RM; 14.4 g/ha) or pinoxaden (60 g/ha) that was statistically at par with PE tank-mix pendimethalin + metribuzin (1000 + 175 g/ha) *fb* PoE mesosulfuron + iodosulfuron (RM; 14.4 g/ha) in both years. Significantly lowest total weeds dry weight and highest total WCE was observed under sequential application PE tank-mixed pendimethalin + pyroxasulfone (1500 + 102 g/ha) or pendimethalin + metribuzin (1000 + 175 g/ha) *fb* PoE mesosulfuron + iodosulfuron (RM; 14.4 g/ha) (**Table 4 & 5**). Similarly, weed index (WI) was significantly influenced by different weed control treatments. Apart from weed-free, lowest yield reduction (0.6-2.1% over weed-free) was recorded in PE pendimethalin + pyroxasulfone *fb* PoE mesosulfuron + iodosulfuron (RM) and it was statistically at par with PE pendimethalin + metribuzin *fb* PoE mesosulfuron + iodosulfuron (RM; 2.7-3.5%) and PoE mesosulfuron + iodosulfuron (RM). In contrast, significantly higher yield reduction was recorded in weedy check (29.9-34.6%) (**Table 5**). The variation in weed dry weight under different herbicides could be due to variable resistance patterns towards *P. minor*, cohorts of weeds and its composition in a different time interval. Alone pre-emergence or post-emergence herbicides recorded higher weed dry weight and least WCE compared to tank-mixed sequential application of herbicides. Among the solely applied PE herbicides, pendimethalin belongs to dinitroaniline group inhibiting cell division; and metribuzin possesses protoporphyrinogen oxidase (PPO) inhibitor activity that inhibits PSII. These two herbicides have the potential to manage herbicide resistance in *P. minor* and possess a lower risk for selection pressure (Dhawan *et al.* 2012, Kaur *et al.* 2016). Mixing PE herbicides with its compatible mixture with different alternate modes of action provided effective control of susceptible and resistant *P. minor* and other weeds (Evans *et al.* 2016). This mixture eliminates early-season weed competition pressure on the crop. PE tank mixture of pendimethalin with pyroxasulfone provides WCE of resistant *P. minor* up to 87%. This was highest among all the PE tank mix herbicides application, while pendimethalin applied alone gave only 54% control. Pyroxasulfone is a new class of chemistry known as isoxazoline that inhibits the biosynthesis of very-long-chain fatty acids in *P. minor* and other narrow-leaved weeds (Tanetani *et al.* 2011). It's mixing with pendimethalin offers a compatible mixture with alternate modes of action, reduced selection pressure, controlling weed cohorts and

Table 4. Effect of different weed control treatments on weed dry weight

Treatment	Dry weight (g/m^2)							
	P. minor				Total weeds			
	60 DAS		120 DAS		60 DAS		120 DAS	
	2016-17	2019-20	2016-17	2019-20	2016-17	2019-20	2016-17	2019-20
Pendimethalin 1500 g/ha PE	3.5(11.1)	3.5(11.2)	6.3(38.9)	7.4(53.6)	3.9(14.1)	3.8(13.6)	7.2(50.8)	8.0(62.9)
Metribuzin 210 g/ha PE	3.6(12.0)	4.1(15.8)	7.3(52.2)	8.7(74.7)	4.4(18.5)	4.6(19.7)	8.8(77.3)	9.5(89.4)
Pendimethalin + metribuzin (TM) 1500 + 175 PE	2.6(5.6)	2.9(7.6)	4.8(21.9)	5.7(31.0)	3.0(8.1)	3.3(9.8)	5.7(31.8)	6.1(35.9)
Pendimethalin + metribuzin (TM) *fb* pinoxaden 1000 +175 *fb* 60 g/ha PE *fb* PoE	1.4(1.1)	1.5(1.4)	2.6(6.0)	2.0(2.9)	2.2(4.0)	2.3(4.3)	4.5(19.1)	3.5(11.3)
Pendimethalin + metribuzin (TM) *fb* mesosulfuron + iodosulfuron (RM) 1000 + 175 *fb* 14.4 g/ha PE *fb* PoE	1.0(0.0)	1.3(0.6)	1.4(1.0)	1.7(1.8)	1.2(0.5)	1.5(1.1)	2.2(3.6)	1.9(2.5)
Pendimethalin + pyroxasulfone (TM) 1500+102 g/ha	1.8(2.2)	1.9(2.6)	3.5(10.9)	3.5(11.3)	2.6(5.7)	2.6(5.8)	5.0(23.9)	5.1(25.0)
Pendimethalin + pyroxasulfone (TM) *fb* pinoxaden 1500+102 *fb* 60 g/ha PE *fb* PoE	1.0(0.0)	1.3(0.6)	1.0(0.0)	1.2(0.5)	2.0(3.2)	2.1(3.4)	3.6(11.9)	3.4(10.7)
Pendimethalin + pyroxasulfone (TM) *fb* mesosulfuron + iodosulfuron (RM) 1500+102 *fb*14.4 g/ha PE *fb* PoE	1.0(0.0)	1.0(0.1)	1.0(0.0)	1.0(0.0)	1.1(0.3)	1.1(0.2)	1.6(1.6)	1.1(0.3)
Pendimethalin + metribuzin (TM) *fb* pinoxaden 1500 + 175 *fb* 60 g/ha before sowing *fb* PoE	1.3(0.7)	1.5(1.2)	2.0(3.1)	2.0(3.1)	2.3(4.3)	2.2(4.0)	4.2(16.3)	3.9(14.0)
Sulfosulfuron *fb* pinoxaden 25 *fb* 60 g/ha BI *fb* PoE	1.1(0.3)	1.4(0.9)	1.4(1.0)	1.9(2.6)	2.0(2.9)	2.0(3.1)	4.0(14.9)	3.3(9.9)
Pinoxaden 60 g/ha PoE	1.5(1.2)	1.6(1.4)	2.4(4.9)	2.6(6.0)	3.1(8.6)	3.1(8.3)	5.7(31.1)	5.4(28.6)
Pinoxaden + metribuzin (TM) 50+120 g/ha PoE	1.5(1.3)	1.5(1.4)	2.8(7.1)	2.5(5.3)	2.2(3.9)	2.1(3.2)	4.2(16.2)	3.4(10.8)
Pinoxaden + metribuzin (TM) 50+150 g/ha PoE	1.4(0.8)	1.5(1.3)	2.4(4.8)	2.5(5.2)	1.9(2.7)	1.8(2.4)	3.3(10.2)	3.0(8.3)
Mesosulfuron + iodosulfuron (RM) 14.4 g/ha PoE	1.2(0.6)	1.4(0.9)	2.2(3.8)	2.2(4.0)	1.6(1.5)	1.7(1.7)	3.0(8.2)	2.9(7.3)
Weed-free check	1.0(0.0)	1.0(0.0)	1.0(0.0)	1.0(0.0)	1.0(0.0)	1.0(0.0)	1.0(0.0)	1.0(0.0)
Weedy check	4.4(18.4)	4.8(22.3)	8.8(76.3)	10.5(108.9)	5.6(30.4)	5.6(30.6)	10.9(117.2)	11.7(135.6)
LSD (p=0.05)	0.3	0.3	0.6	0.6	0.3	0.2	0.2	0.5

Data given in parenthesis are original values, and outside are square-root transformed value ($\sqrt{x+1}$), *fb*: followed by, PE: pre-emergence, PoE: post-emergence, and BI: before irrigation, TM: tank mix, RM: ready mix elimination of early-season weeds competitive with crop. Whereas, solely applied PoE herbicides pinoxaden and mesosulfuron + iodosulfuron (RM) control resistant *P. minor* more efficiently than PE solely applied or its tank mixture. Nonetheless, continuous application with the same herbicide or other herbicide with the same mode of action exerts more selection pressure that would be highly susceptible towards early development of resistance in *P. minor* (Das *et al.* 2014). Therefore, sequential application of tank-mixed PE herbicides followed by PoE herbicide leads to higher WCE for a longer period. Present findings showed that tank mix application of PE pendimethalin with metribuzin or pyroxasulfone followed by PoE pinoxaden or mesosulfuron + iodosulfuron (RM) provided the most efficient control of clodinafop resistant *P. minor*. However, if a field is dominated with both resistant *P. minor* and other weeds, then application of PE pendimethalin with metribuzin or pyroxasulfone followed by PoE mesosulfuron + iodosulfuron (RM) provides most efficient weed control. This was due to the fact that pendimethalin, metribuzin, pyroxasulfone and mesosulfuron + iodosulfuron (RM) provides control of broad-spectrum (narrow and broad-leaved) weeds, while, pinoxaden is very effective against narrow-leaved weeds only (Punia *et al*. 2020, Soni *et al*. 2021). Therefore, in the long run, rotational use of herbicides is recommended to minimize selection pressure. Effective control of resistant *P. minor* and other weeds with longer weed-free window provides congenial micro-climate for the crop to utilize nutrients, moisture, light and space available to crop which led to healthier crop and consequently higher yield brought least weed index (Chandana *et al*. 2019).

Crop studies: Crop growth and yield attributing parameters varied significantly with treatments (**Table 6**). Apart from weed-free plot, sequential application of pre-emergence pendimethalin + pyroxasulfone (or) pendimethalin + metribuzin followed by post-emergence application of mesosulfuron + iodosulfuron (RM) (or) pinoxaden resulted in maximum plant dry weight and yield attributes, *viz*. number of effective tillers per m^2 and grains per spike; while, least values of these parameters were obtained in weedy check followed by PE metribuzin. Similarly, the highest grain yield (5.45-6.28 t/ha) was observed under PE pendimethalin + pyroxasulfone *fb* PoE mesosulfuron + iodosulfuron (RM) with higher harvest index (48.0%) which was statistically at par with PE pendimethalin + metribuzin *fb* PoE mesosulfuron + iodosulfuron (5.37-6.15 t/ha and 47.9-48.0%). The lowest grain yield and HI were obtained in weedy check (3.91-4.14 t/ha with HI 44.6-44.7%). The improvement in crop growth, yield attributes, yield

Table 5. Effect of different weed control treatments on WCE

Treatment	WCE (%)								Weed index (%)	
	P. minor				Total weeds					
	60 DAS		120 DAS		60 DAS		120 DAS			
	2016-17	2019-20	2016-17	2019-20	2016-17	2019-20	2016-17	2019-20	2016-17	2019-20
Pendimethalin 1500 g/ha PE	36.4	49.6	47.8	50.5	52.6	55.6	55.6	53.5	21.3	19.7
Metribuzin 210 g/ha PE	31.1	29.0	30.8	30.9	38.0	35.3	33.0	33.9	27.5	23.4
Pendimethalin + metribuzin (TM) 1500 + 175 PE	68.1	66.0	71.0	71.1	73.1	68.3	72.6	73.3	16.5	14.9
Pendimethalin + metribuzin (TM) *fb* pinoxaden 1000 +175 *fb* 60 g/ha PE *fb* PoE	93.7	93.6	92.3	97.3	86.7	85.7	83.7	91.7	12.0	11.4
Pendimethalin + metribuzin (TM) *fb* mesosulfuron + iodosulfuron (RM) 1000 + 175 *fb* 14.4 g/ha PE *fb* PoE	100.0	97.3	98.7	98.3	98.3	96.3	96.9	98.2	2.7	3.5
Pendimethalin + pyroxasulfone (TM) 1500+102 g/ha	86.5	88.4	85.5	89.4	80.8	81.0	79.4	81.5	19.5	17.7
Pendimethalin + pyroxasulfone (TM) *fb* pinoxaden 1500+102 *fb* 60 g/ha PE *fb* PoE	100.0	97.4	100.0	99.5	89.5	88.9	89.8	92.1	8.5	8.0
Pendimethalin + pyroxasulfone (TM) *fb* mesosulfuron + iodosulfuron (RM) 1500+102 *fb*14.4 g/ha PE *fb* PoE	100.0	99.6	100.0	100.0	99.1	99.2	98.7	99.8	0.6	2.1
Pendimethalin + metribuzin (TM) *fb* pinoxaden 1500 + 175 *fb* 60 g/ha before sowing *fb* PoE	95.8	94.5	96.1	97.2	86.0	86.8	86.3	89.6	14.1	10.6
Sulfosulfuron *fb* pinoxaden 25 *fb* 60 g/ha BI *fb* PoE	98.2	95.9	98.6	97.6	90.3	90.0	87.3	92.7	8.8	10.0
Pinoxaden 60 g/ha PoE	93.4	93.5	93.5	94.5	70.9	72.6	73.0	78.9	16.0	16.7
Pinoxaden + metribuzin (TM) 50+120 g/ha PoE	93.2	93.8	90.6	95.1	87.1	89.5	86.1	92.1	10.9	11.8
Pinoxaden + metribuzin (TM) 50+150 g/ha PoE	95.8	93.9	93.6	95.2	91.4	92.2	91.4	94.0	9.6	7.3
Mesosulfuron + iodosulfuron (RM) 14.4 g/ha PoE	96.9	95.7	95.1	96.3	95.2	94.3	93.1	94.7	6.3	5.5
Weed-free check	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	0.0
Weedy check	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	34.6	29.9
LSD (p=0.05)	11.0	4.1	8.7	7.0	5.8	3.5	7.3	4.4	7.1	7.5

fb: followed by, PE: pre-emergence, PoE: post-emergence, and BI: before irrigation, TM: tank mix, RM: ready mix

Table 6. Effect of different weed control treatments on crop growth and yield parameters; and grain yield of wheat

Treatment	Plant dry weight (g/plant) 120 DAS		Effective tillers (no./m^2)		Grains per spike (no.)		Grain yield (t/ha)		Harvest index (%)	
	2016-17	2019-20	2016-17	2019-20	2016-17	2019-20	2016-17	2019-20	2016-17	2019-20
Pendimethalin 1500 g/ha PE	14.5	13.2	386	374	47	45	4.98	4.47	47.0	46.2
Metribuzin 210 g/ha PE	14.2	12.9	378	354	46	43	4.58	4.27	45.2	45.4
Pendimethalin + metribuzin (TM) 1500 + 175 PE	15.6	13.6	384	369	48	46	5.28	4.74	46.7	46.1
Pendimethalin + metribuzin (TM) *fb* pinoxaden 1000 +175 *fb* 60 g/ha PE *fb* PoE	16.1	13.8	402	396	52	50	5.56	4.93	46.8	46.6
Pendimethalin + metribuzin (TM) *fb* mesosulfuron + iodosulfuron (RM) 1000 + 175 *fb* 14.4 g/ha PE *fb* PoE	16.7	14.3	431	415	54	52	6.15	5.37	48.0	47.9
Pendimethalin + pyroxasulfone (TM) 1500+102 g/ha	15.2	13.9	394	390	49	49	5.09	4.58	47.2	47.2
Pendimethalin + pyroxasulfone (TM) *fb* pinoxaden 1500+102 *fb* 60 g/ha PE *fb* PoE	16.4	14.2	410	402	53	51	5.78	5.13	47.6	47.2
Pendimethalin + pyroxasulfone (TM) *fb* mesosulfuron + iodosulfuron (RM) 1500+102 *fb*14.4 g/ha PE *fb* PoE	17.9	15.4	442	421	57	53	6.28	5.45	48.0	48.0
Pendimethalin + metribuzin (TM) *fb* pinoxaden 1500 + 175 *fb* 60 g/ha before sowing *fb* PoE	15.8	13.6	402	385	51	47	5.43	4.98	47.5	47.3
Sulfosulfuron *fb* pinoxaden 25 *fb* 60 g/ha BI *fb* PoE	16.0	14.1	405	404	51	48	5.76	5.01	47.7	47.3
Pinoxaden 60 g/ha PoE	15.2	13.3	380	368	48	45	5.31	4.64	47.0	46.5
Pinoxaden + metribuzin (TM) 50+120 g/ha PoE	15.0	13.4	394	386	50	46	5.63	4.91	46.4	46.7
Pinoxaden + metribuzin (TM) 50+150 g/ha PoE	15.2	13.5	412	398	52	47	5.71	5.16	46.6	46.7
Mesosulfuron + iodosulfuron (RM) 14.4 g/ha PoE	15.5	13.6	418	409	52	49	5.93	5.26	47.0	47.1
Weed-free check	17.9	15.6	445	427	57	54	6.32	5.57	48.1	48.1
Weedy check	13.2	12.2	354	341	43	40	4.14	3.91	44.7	44.6
LSD (p=0.05)	0.8	0.5	33	30	5	4	0.45	0.41	1.2	1.1

fb: followed by, PE: pre-emergence, PoE: post-emergence, and BI: before irrigation, TM: tank mix, RM: ready mix

and harvest index could be due to overall improvement in crop vigour and growth, reflected by improved dry weight/plant resulting in higher translocation of photosynthates from source to sink. This could be due to sequential application of pre-followed by post-emergence herbicides with more than one mode of action, provided efficient control of resistant *P. minor* and all other weeds in wheat field which resulted in less crop-weed competition for resources (water, nutrients, space, sunlight) and their efficient utilization for growth and development leading to higher production efficiency/productivity (Yadav *et al*. 2016, Kaur *et al*. 2019).

Conclusion

The continuous application of clodinafop, sulfosulfuron, mesosulfuron + iodosulfuron (RM) and pinoxaden herbicides year after year for the control of *P. minor* lead to the development of herbicide resistance. It was observed that out of 16 populations (collected from farmers' fields of different locations in Haryana, India); 7, 3, 2 and 1 populations recorded GR_{50} values higher than recommended for clodinafop (60 g/ha), sulfosulfuron (25 g/ha), mesosulfuron + iodosulfuron (14.4 g/ha) and pinoxaden (50 g/ha), respectively. These populations fall under resistant to highly resistant category to respective herbicides. To manage clodinafop resistance under field conditions, sequential application of tank-mixed pre-emergence pendimethalin + pyroxasulfone (1500 + 102 g/ha) (or) pendimethalin + metribuzin (1000 +175 g/ha) *fb* post-emergence pinoxaden (60 g/ha) (or) mesosulfuron + iodosulfuron (RM; 14.4 g/ha) brought 95-100% reduction in dry weight of resistant *P. minor* and total weed dry weight with higher WCE at different crop growth stages. This resulted in higher crop height, dry matter production, yield attributes, and 43-46% higher grain yield as compared to weedy check (4.0 t/ha). Application of single herbicide either as pre- or post-emergence proved ineffective for control of clodinafop resistant *P. minor* and recorded lower grain yield.

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