



Multiple herbicide resistance in *Phalaris minor* Retz. in Haryana, India

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ABSTRACT

A screen house study was conducted at CCS Haryana Agricultural University, Hisar during (winter) Rabi 2018-19 and 2019-20 to evaluate the resistance in various populations of *Phalaris minor* Retz. in Haryana against clodinafop and also to evaluate the efficacy of alternate herbicides against the herbicide resistant weed populations. The alternate herbicides were sulfosulfuron, pinoxaden and mesosulfuron + iodosulfuron (ready-mix). The seeds of *P. minor* were sown in pots and 20 plants per pot were maintained. All the herbicides were applied at 2-3 leaf stage of *P. minor* i.e. about 25-27 days after sowing. Herbicides were applied with graded doses viz. 1/2X, X (recommended dose), 2X and 4X dose. The variation in the percentage decrease in the biomass was observed amongst *P. minor* populations with the application of different herbicides under their doses. Clodinafop (60 g/ha) and mesosulfuron + iodosulfuron (14.4 g/ha) provides <30% decrease in the biomass of Sitamai, Karnal population at the recommended dose. The poor efficacy of clodinafop at recommended dose was observed in most of the *P. minor* populations except those populations of Hindwan, Hisar (susceptible population) and Laloda, Fatehabad. An increase in the reduction of biomass was observed with an increase in the dose to 2X and 4X but at 2X dose of clodinafop, Kalwan, Jind population showed minimum decrease in the biomass during both years of the study. There was decrease in the efficacy of sulfosulfuron during 2nd year of study particularly in Rasidan, Jind population. The reduced efficacy (<70%) against clodinafop, sulfosulfuron, mesosulfuron + iodosulfuron and pinoxaden was observed in Sitamai, Kalwan, Ramba and Ramba populations of *P. minor*, respectively.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second most important crop in India, grown in 29.57 million ha (Anonymous 2019). In wheat, weeds are the major concern which can cause up to 40% reduction in the yield (Das 2008). Among the weeds in wheat, *Phalaris minor* Retz. is major weed (Singh *et al.* 1992). Wheat yield can be reduced from 30% by 150 plants/m² (Balyan and Malik 1989) to complete crop loss by a density of 2000-3000 plants/m² of *Phalaris minor* (Das *et al.* 2014).

Manual weeding is labor intensive, expensive, tedious and ineffective method, as *P. minor* can escape during manual weeding due to its phenotypic mimicry with wheat, even though experts can easily differentiate it because of pink coloration stem near the base at early stages. Hence, farmers prefer to rely on the herbicides, which is comparatively a cheaper method to control weeds. Isoproturon had been recommended for the effective control of *P. minor* in

wheat since 1977 (Gill *et al.* 1978). But the continuous reliance on the same herbicide isoproturon to control the weeds in wheat, has led to the development of resistance in *P. minor*, which is a major issue since it was reported in early 1990's. It was observed that *Phalaris minor* has developed resistance against isoproturon due to enhanced degradation via N-dealkylation and ring alkyl oxidation by reduced nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P-450 monooxygenase (Singh *et al.* 1998). A similar P-450 monooxygenase system operates in wheat, degrading isoproturon. This type of resistance can lead to the evolution of cross-resistance or multiple resistance against herbicides of different modes of action (Singh 2007, Chhokar and Sharma 2008). The alternate herbicides, viz. clodinafop-propargyl, fenoxaprop-p-ethyl, sulfosulfuron and tralkoxydim were recommended in 1997-98 to control the resistant populations of *P. minor* (Das 2008). Later on, resistance in *Phalaris minor* against alternate

herbicides was also reported, with fenoxaprop-p-ethyl being the first herbicide (Abbas *et al.* 2016). Other herbicides have also shown poor efficacy and instances of multiple herbicide resistance in *P. minor* have been noticed.

The herbicide resistant *Phalaris minor* populations have spread in all rice-wheat growing areas of Haryana (Punia *et al.* 2020), which is a serious concern for the sustainability of rice-wheat cropping system. Herbicides applied in the mixtures can provide acceptable control of *P. minor*, wild oat and some broad-leaved weeds also. Tank mixture of clodinafop + sulfosulfuron (3: 1) at 60 g/ha and fenoxaprop + sulfosulfuron (4: 1 and 5: 1) at 120 g/ha provided 85–90% control of *Avena ludoviciana* and *Phalaris minor* and 60% control of broad-leaved weeds like *Chenopodium album*, *Melilotus indica* and *Rumex retroflexus* (Punia *et al.* 2005). Selection pressure can be reduced by use of alternate herbicides, use of herbicide mixtures, herbicide rotation and other practices. However, the continuous monitoring of extent of herbicide resistance amongst *P. minor* populations is essential for effectively managing them. Hence, a study was conducted to assess the efficacy of different herbicides in managing *P. minor* populations vis-a-vis herbicide resistance.

MATERIALS AND METHODS

The experiment was conducted during (winter) *rabi* 2018-19 and *rabi* 2019-20 in the screen houses of Department of Agronomy, CCS Haryana Agricultural University, Hisar. The seeds of 15 *P. minor* populations (14 populations with poor control history and one susceptible population for comparison) were collected during April 2018 and April 2019 from wheat fields of farmers on the basis of problem reported by the farmers. All the populations were taken from rice-wheat cropping system except susceptible population (Hindwan, Hisar) which was taken from cotton-wheat cropping system. Of these 15 populations; four were from Karnal (Kachwa, Ramba, Sitamai, Uchana), four from Jind (Rasidan, Kalwan, Danoda, Ujhana), two from Kaithal (Kheri raiwali and Teek), two from Hisar (Hindwan and CCSHAU Farm), one each from Yamuna Nagar, Fatehabad and Kurukshetra districts of Haryana (Table 1). The population collected from Hindwan, Hisar was taken as susceptible population to clodinafop.

The soil for the pot experiment was taken from CCSHAU farm where there was no herbicide application during last two years, in order to attain the

Table 1. *Phalaris minor* populations collected for study from various districts of Haryana

District	Village	Population code	Latitude and Longitude
Karnal	Kachwa	1	29.7274° N, 76.8872° E
	Ramba	5	29.7935° N, 76.9837° E
	Sitamai	11	29.7837° N, 76.7629° E
	Uchana	13	29.7403° N, 76.9704° E
Jind	Rasidan	7	29.7256° N, 76.0319° E
	Kalwan	10	29.7063° N, 75.9709° E
	Danoda	12	29.5218° N, 76.0508° E
	Ujhana	14	29.7153° N, 76.1349° E
Kaithal	Kheri raiwali	4	29.8643° N, 76.5546° E
	Teek	8	30.0379° N, 76.7853° E
Hisar	Hindwan	2	29.1191° N, 75.6121° E
	CCSHAU Farm	6	29.1504° N, 75.7057° E
Yamuna Nagar	Khijrabad	3	30.2919° N, 77.4974° E
	Raiyawala		
Fatehabad	Laloda	9	29.6407° N, 75.8752° E
Kurukshetra	Chanarathal	15	30.0701° N, 76.8671° E

proper effect of the tested herbicides. Soil was sieved before filling the pots. Soil: Vermicompost – 4:1 mixture was used to fill 1020 pots of 8 inch diameter in which *P. minor* seeds were surface seeded with seeds just covered with soil, followed by watering the pots to facilitate germination. After germination, the *P. minor* populations were thinned out to 20 plants per pot. Pots were watered regularly as per the requirement.

All the herbicides were applied at 25-27 DAS as post-emergence application at 2-3 leaf stage of *P. minor*. Clodinafop was applied at 30 g/ha (1/2X), 60 g/ha (X: recommended rate), 120 g/ha (2X), 240 g/ha (4X); sulfosulfuron at 12.5 g/ha (1/2X), 25 g/ha (X), 50 g/ha (2X), 100 g/ha (4X); mesosulfuron + iodosulfuron at 7.2 g/ha (1/2X), 14.4 g/ha (X), 28.8 g/ha (2X), 57.6 g/ha (4X); pinoxaden at 25 g/ha (1/2X), 50 g/ha (X), 100 g/ha (2X), 200 g/ha (4X). The pots were arranged in completely randomized design in the screen house. A control without herbicide application was maintained for all the *P. minor* populations for comparison. Total number of pots used were 1020 for the 15 populations with 4 replications. The pots were arranged outside the screen house for herbicide application. These pots were arranged in marked area and the required quantities of herbicides, corresponding to a dose, were applied with 300 L/ha of water (calibrated earlier) with a manually operated knapsack sprayer. Flat-fan nozzle was used for the application. After 30 days of the application, mean dry weight of per plant was recorded and compared with the control pots (where there was no application of herbicide). Percent decrease in the dry weight with respect to increase in the dose of different herbicides was calculated using by using the following formula. Data was statistically analyzed by using OP Stat online statistical tool (Sheoran *et al.* 1998).

$$\text{Decrease in dry weight (\%)} = \frac{\text{DMC} - \text{DMT}}{\text{DMC}} \times 100$$

DMC= Dry matter of weeds in control (untreated) pots

DMT= Dry matter of weeds in treated pots

RESULTS AND DISCUSSION

P. minor populations showed variable response to the recommended dose of tested post-emergence herbicides, viz. clodinafop-propargyl 60 g/ha, sulfosulfuron 25 g/ha, mesosulfuron + iodosulfuron (RM) 14.4 g/ha and pinoxaden 50 g/ha. Among the tested populations; Ramba, Karnal showed very poor control with clodinafop 60 g/ha (Figure 1). Laloda, Fatehabad and Hindwan, Hisar showed maximum decrease in the dry matter with the application of recommended dose of clodinafop (Figure 1). The Sitamai, Karnal population's percentage decrease in dry weight was less with the application of clodinafop, sulfosulfuron and mesosulfuron + iodosulfuron (RM) (Figure 1, 2 and 3). Most of the farmers relied on the single herbicide for more than four years for the control of *P. minor* in the problematic areas. During second year of the study, higher dry weight of some populations was observed

as compared to the previous year, which indicated a decrease in the herbicide efficacy with the repeated use of single herbicide in long run. Decrease in efficacy of mesosulfuron + iodosulfuron (RM) was recorded in Kheri Raiwali, Kaithal population during second year of study and in Sitamai, Karnal population during both the years. A decrease in efficacy of mesosulfuron + iodosulfuron (RM) against *P. minor* populations was also observed in this study which might be due to continuous reliance on sulfonylureas (sulfosulfuron and mesosulfuron + iodosulfuron) (Figure 2 and 3). Abundant evidence is available on loss of sensitivity in majority of the *P. minor* populations against clodinafop with its long-term use (Chhokar and Sharma 2008, Dhawan *et al.* 2009, Smit and Cairns 2000, Gherekhloo *et al.* 2011, Das *et al.* 2014). The repeated use of herbicides with similar modes of action for weed control in wheat leads to evolution of multiple herbicide resistance in *P. minor* (Bhullar *et al.* 2017).

In 2018-19, populations from Kalwan, Jind followed by Ramba, Karnal showed minimum decrease in the dry matter with the 2X dose (double of the recommended dose) application of clodinafop. The Sitamai, Karnal followed by Kheri Raiwali,

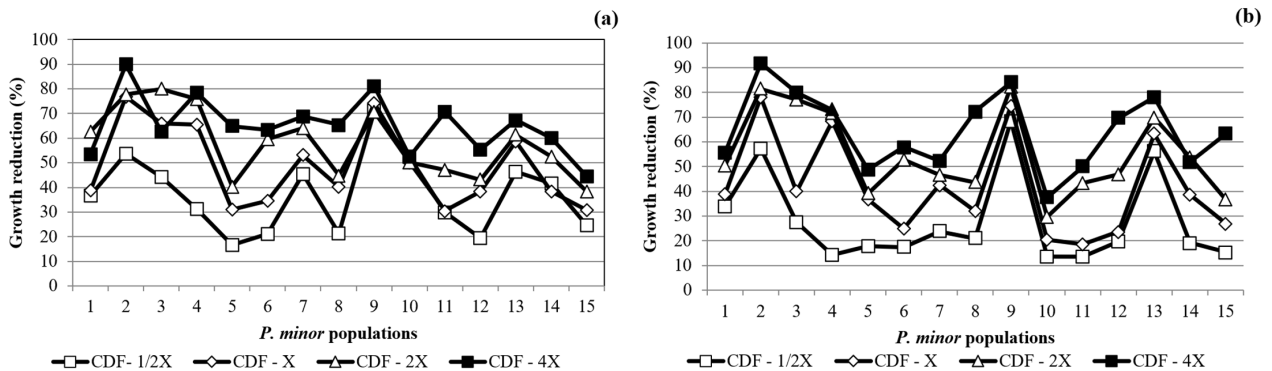


Figure 1. Growth reduction (%) of *P. minor* population with graded dose of clodinafop during 2018-19 (a) and 2019-20 (b); 1/2X – 30 g/ha, X – 60 g/ha, 2X – 120 g/ha, 4X- 240 g/ha

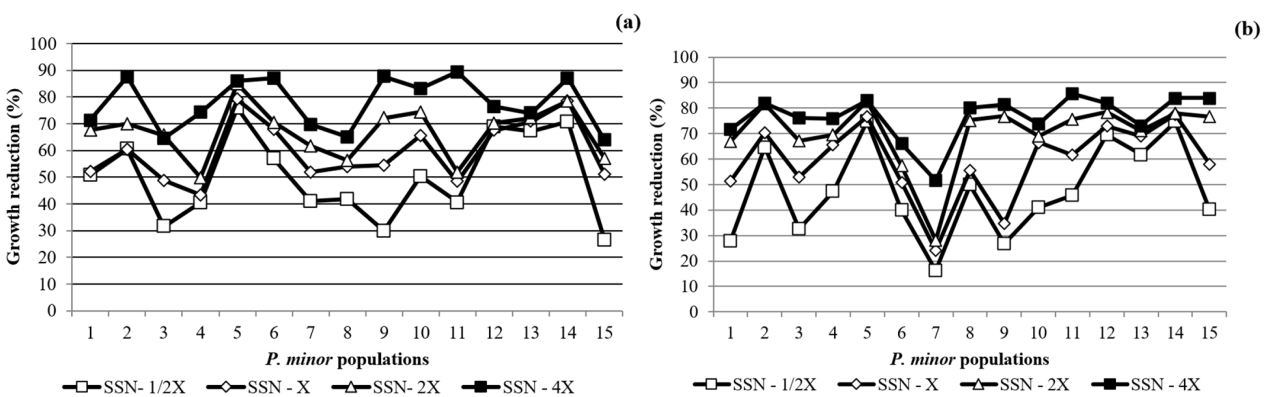


Figure 2. Growth reduction (%) of *P. minor* populations with graded doses of sulfosulfuron during 2018-19 (a) and 2019-20 (b); 1/2X – 12.5 g/ha, X – 25 g/ha, 2X – 50 g/ha, 4X- 100 g/ha

Kaithal and Rasidan, Jind populations showed minimum decrease in the dry matter with the application of sulfosulfuron (Figure 2). Sitamai, Karnal population showed lowest decrease in the dry matter among the tested populations with the 2X dose of mesosulfuron + iodosulfuron (ready-mix) (Figure 3). A decrease in efficacy of this ready-mix herbicide on the *P. minor* population was observed.

In 2019-20, Kalwan, Jind followed by Ramba, Karnal population showed minimum decrease in the dry matter with the application of clodinafop at 2X dose; whereas Laloda, Fatehabad and Hindwan, Hisar (susceptible population) showed the maximum reduction in the dry matter with the application of clodinafop at similar dose (Figure 1). Rasidan, Jind showed minimum reduction in the dry matter with the application of sulfosulfuron at 2X dose (Figure 2). Among the tested populations, Sitamai, Karnal followed by Kheri Raiwali, Kaithal population showed lower efficacy of mesosulfuron + iodosulfuron (RM) even at 2X dose (Figure 3). But when compared with the first year of study, there was decline in the efficacy of herbicides with less dry matter reduction observed during the second year (Table 2). The need for 10-fold increase in dose for fenoxaprop and sulfosulfuron and 2-3-fold dose increase of clodinafop for 50% growth reduction was observed earlier also (Dhawan *et al.* 2005). Punia *et al.* (2012) reported decrease in the efficacy of ready-mix formulation of sulfonylurea herbicides viz. mesosulfuron + iodosulfuron.

Pinoxaden application at the rate of 2X of the recommended dose resulted in more than 80% decrease in the dry matter over the control in most of the populations except Kalwan, Jind and Kachwa, Karnal populations indicating higher efficacy of pinoxaden as compared to the other herbicides tested

(Figure 4). This indicated suitability of this herbicide in tackling the problem of resistance in *P. minor* in wheat.

To check the level of resistance among the tested populations, herbicides were applied even up to 4X dose. During 2018-19, clodinafop 4X application resulted in minimum decrease in the *P. minor* populations' dry matter followed by sulfosulfuron at 4X (Figure 1 and 2). Resistance in *P. minor* to clodinafop and sulfosulfuron was also reported by Bhullar *et al.* (2014). Less decrease in the dry matter with the application of 4X of mesosulfuron + iodosulfuron (ready-mix) was recorded in Sitamai, Karnal populations followed by Rasidan, Jind. Rasidan, Jind recorded minimum decrease in the dry matter with pinoxaden 4X (Figure 3). During 2019-20, Kalwan, Jind showed lowest decrease in the dry matter (37%) followed by Ramba, Karnal (<50%) with 4X dose of clodinafop (Figure 1). Amongst the tested *P. minor* populations, Rasidan, Jind population showed less decrease in the dry matter with the 4X dose of the sulfosulfuron (Figure 2). Sitamai, Karnal populations showed lowest decrease in the dry matter production (50%) among all the tested populations. Khijrabad Raiyawala, Yamuna Nagar populations showed reduction in the efficacy of mesosulfuron + iodosulfuron (ready-mix) with minimum decrease in the dry matter with its application (Figure 3). A decrease in the efficacy of mesosulfuron + iodosulfuron (ready-mix) was observed in this study (Table 2). Only Kalwan and Jind populations showed less than 80% decrease in the dry matter with the application of 4X dose of pinoxaden, while rest of the populations showed more than 84% decrease in the dry matter (Figure 4). *P. minor* has developed multiple resistance across three modes of action: photosynthesis at the PS- II site, acetyl CoA carboxylase (ACCase) and acetolactate synthase

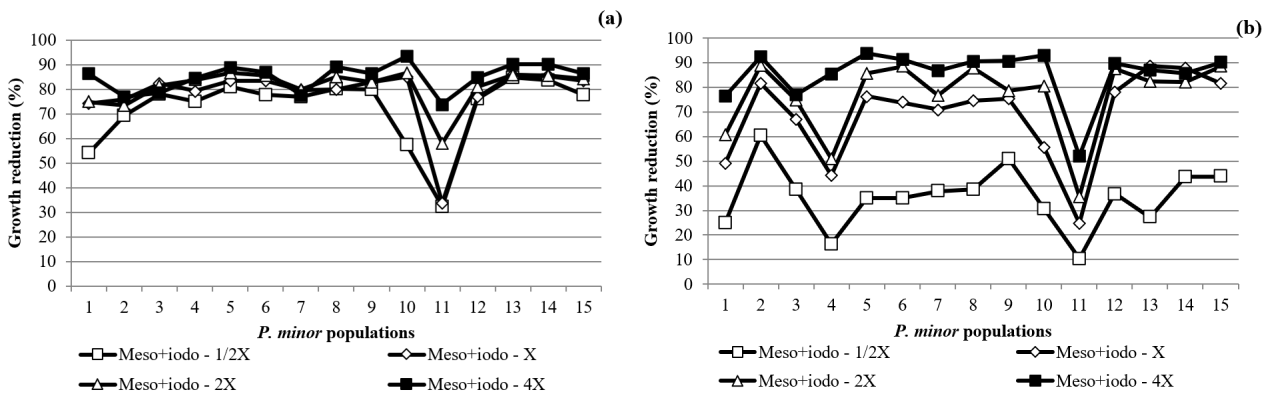


Figure 3. Growth reduction (%) of *P. minor* populations with graded doses of mesosulfuron+ iodosulfuron (ready-mix) during 2018-19 (a) and 2019-20 (b); 1/2X – 7.2 g/ha, X – 14.4 g/ha, 2X – 28.8 g/ha, 4X- 57.6 g/ha

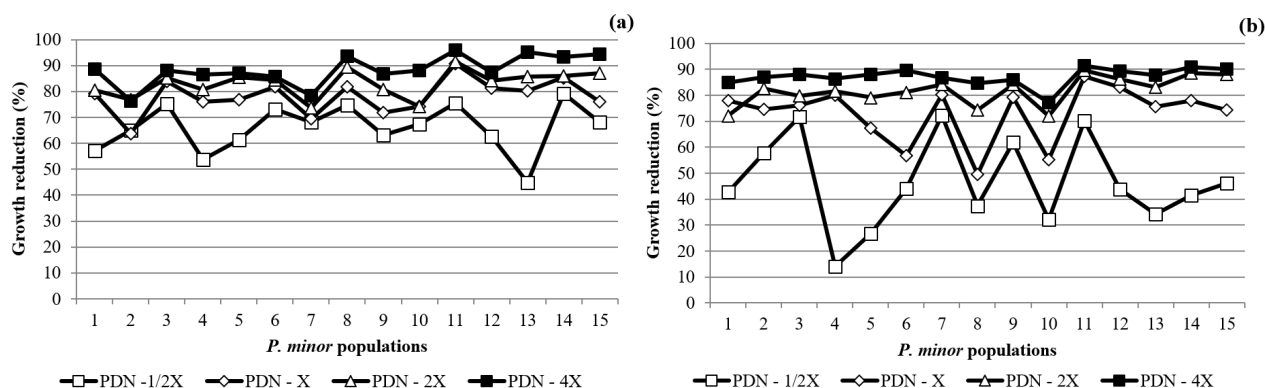


Figure 4. Growth reduction (%) of *P. minor* populations with graded doses of pinoxaden during 2018-19 (a) and 2019-20 (b); 1/2X – 25 g/ha, X–50 g/ha, 2X– 100 g/ha, 4X- 200 g/ha

Table 2. Mean percent decrease in the dry weight of *P. minor* populations against graded doses (1/2X to 4X) of herbicides

Populations	Clodinafop		Sulfosulfuron		Mesosulfuron + iodosulfuron (ready-mix)		Pinoxaden	
	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20	2018-19	2019-20
Kachwa	47.87	44.73	60.53	54.43	72.44	52.82	76.33	69.40
Hindwan	74.55	77.16	69.59	74.67	73.83	80.83	70.46	75.44
Khijrabad Raiyawala	63.18	56.09	52.66	57.20	80.11	64.29	83.15	78.86
Kheri Raiwali	62.79	56.86	51.97	64.53	80.64	49.24	74.29	65.50
Ramba	38.22	35.66	81.51	79.22	84.98	72.65	77.70	65.29
HAU Farm	44.64	38.30	70.62	53.63	83.42	72.12	81.32	67.84
Rasidan	57.85	41.35	56.07	29.97	78.51	68.02	72.43	80.84
Teek	42.98	42.28	54.26	65.17	83.58	72.86	84.95	61.46
Laloda	74.20	77.27	61.03	54.89	83.05	73.89	75.72	77.85
Kalwan	51.34	25.34	68.41	62.59	80.63	64.96	75.96	59.10
Sitamai	44.56	31.44	57.56	67.16	49.48	30.72	88.54	84.59
Danoda	39.04	40.05	70.76	75.74	79.70	73.02	78.87	75.60
Uchana	58.41	66.82	71.04	68.74	86.69	71.29	76.51	70.19
Ujhana	48.19	40.89	78.75	78.52	86.21	74.81	85.99	74.70
Chanarathal	34.59	35.61	49.68	64.74	82.94	75.97	81.39	74.59
LSD (p=0.05)	13.53	8.25	11.40	6.83	8.08	6.01	9.73	5.21

(ALS) inhibitors (Heap 2021). The multiple herbicide-resistant populations showed a low level of sulfosulfuron resistance, moderate level of resistance to pinoxaden and a high level of resistance to clodinafop and fenoxaprop (Chhokar and Sharma 2008).

Based on the current study, it may be concluded that efficacy of all the tested herbicides against *P. minor* in Haryana has reduced to a significant extent. The clodinafop has the least efficacy against *P. minor* populations, which might be due to continuous reliance on a single herbicide. The efficacy of the sulfonylureas (sulfosulfuron and mesosulfuron + iodosulfuron) has been also reduced due to use of different herbicides but with same mode of action. The better control of most of *P. minor* populations by pinoxaden indicated towards its cautious use in management of resistant *P. minor* populations. To control the resistant weeds, integrated weed management approach with use of

herbicides with different modes of action may be the most sustainable approach.

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