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Little seed canary grass resistance to sulfonyl–urea herbicides and its possible management with pendimethalin

Rupa S. Dhawan*, Neha Singh and Samunder Singh[1]
Department of Botany and Plant Physiology, CCS HAU Hisar, Haryana 125 004

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ABSTRACT

In this study, response of 20 *Phalaris minor* Retz. (little seed canary grass) populations against sulfosulfuron and its ready–mix formulation sulfosulfuron + metsulfuron and mesosulfuron + iodosulfuron was studied. Out of 20 populations, 12 showed high resistance, 8 showed medium resitance and none were susceptible to sulfosulfuron (25 g/ha). GR_{50} value was in the range of 30-110 g/ha in *P. minor* populations tested. In wheat, it was more than 200 g/ha. Eleven populations showed resistance to ready mix formulation of sulfosulfuron + metsulfuron, 8 populations were medium R/medium S and one population was susceptible. Similarly, 11 showed resistance to ready mix formulation of mesosulfuron + iodosulfuron, 7 populations were medium R/medium S and one biotype was susceptible. GR_{50} values tested were in the range of 30-110 g/ha in contrast to 5 g/ha at the time of recommendation.

Key words: Pendimethalin, Resistance, *Phalaris minor*, Sulfosulfuron, Sulfosulfuron + metsulfurn, mesosulfuron + iodosulfuron

Phalaris minor Retz (little seed canary grass) is a major weed of wheat crop in northern part of India. The crop suffers a yield loss of 25-30% due to infestation of this weed (Malik and Singh 1995) and it is very difficult to distinguish it from wheat plant in its early growth stages. The weed evolved insensitivity to isoproturon–a urea herbicide after its continuous use for over 15 years (Malik and Singh 1995, Walia *et al*. 1997. Alternative herbicides belonging to group I [(acetyl co-A carboxylase (ACCase) inhibitors] and group II [acetolactate synthase (ALS) inhibitors] were recommended for its management in 1997-98 (Yadav *et al.* 1995, 1997; Brar *et al.* 1999). While the impact of ACCase inhibiting herbicides has been evaluated in an earlier investigation (Dhawan *et al*. 2010), this study was carried out with the objective to evaluate the effect of sulfosulfuron singly, as a ready–mix formulation with another herbicide metsulfuron (Total), and a ready–mix formulation of sulfonylurea herbicides, *viz.* mesosulfuron + iodosulfuron–methyl sodium (Atlantis). Efficacy of pendimethalin belonging to group III herbicides with different mode of action (microtubule assembly inhibitors) was evaluated with a view to develop management options for ALS resistant populations of *P. minor*.

MATERIALS AND METHODS

Seeds of 20 populations of *P. minor* were collected randomly from cropped fields at different locations of Haryana (with and without history of herbicide resistance) in April 2010. Seeds were sown by November end during both the years in sandy loam soil in earthen pots (9" dia). Five plants were maintained in four replications for each population. Sulfosulfuron (25 g/ha), ready–mix formulation of mesosulfuron + iodosulfuron (32 g/ha) and ready mix formulation of mesosulfuron + iodosulfuron (14.4 and 28.8 g/ha) were sprayed at 2-3 leaf stage *i.e*. 30-35 days after sowing (DAS) with a knapsack sprayer using flat fan nozzle with a spray volume of 500 ml for spray based on 5 x 2 m area in a randomized block design. The plants that remained unsprayed with herbicide served as control. Observations were recorded on 30 DAS on weed mortality. Percentage mortality was calculated by the formula; no. of plants that survived after 30 days/ no. of plants at the time of spray x 100). The populations that showed mortality in the range of 70-100% were classified as susceptible (S), those that showed mortality in the range of 35-70% were classified as medium susceptible/medium resistant (MS/MR) and those in the range of 0-35% as resistant (R) populations .

In another experiment, six selected populations (Table 2) of *P. minor* along with wheat variety '*WH 711*' were tested for GR_{50} values against sulfosulfuron and ready mix formulation of mesosulfuron + iodosulfuron. Seedlings were raised in the same manner as described above. The dose range of sulfosulfuron sprayed was 0, 12.5, 25, 50 and 100 g/ha and dose range for mesosulfuron +

***Corresponding author:** rupadhawan@hotmail.com
[1]*Department of Agronomy*

iodosulfuron was 0, 7.2, 14.4 and 28.8 g/ha. Observations were recorded at 30 DAS on weed mortality and biomass accumulation. For plotting dose response curves and calculation of GR_{50} values were made as per methodology reported earlier (Dhawan *et al.* 2009).

In yet another experiment, effect of ready mix formulation of mesosulfuron + iodosulfuron (14.4 and 28.8 g/ha) was studied on physiological indicators like photosynthetic pigments, membrane integrity, lipid peroxidation, proline content and activity of antioxidant enzymes in six selected populations and wheat variety '*WH 711*'.Seedlings were raised in the same manner as in earlier two experiments. Leaf samples(youngest 2-3 leaves) were collected 10 days after spray of the herbicide. Chlorophyll was extracted by the method of Arnon (1949) using 80% acetone. Membrane stability was assessed by the method of vanStove and Stobbe (1977) as described earlier (Dhawan *et al.* 2010). Free proline was assessed by the method of Bates *et al.* (1973). Acid ninhydrin was prepared by dissolving 1.25 g ninhydrin in 30 ml acetic acid and 20 ml phosophoric acid with continuous stirring until dissolved and stored at room temperature before use. 200 mg of fresh tissue was homogenized in 2 ml of aqueous sulfosalicylic acid in a mortar and centrifuged at 4000 rpm for 20 minutes. The residue was re-extracted with 2 ml of 3% sulfosalicylic acid and centrifuged. The supernatants were combined and volume was made to 10 ml. One ml of this aliquot was transferred in a test tube of 1 ml each of acid- ninhydrin and acetic acid was added. The mixture was heated on a boiling water bath at $100^{O}C$ for one hour after which the reaction was terminated by placing the tubes in ice bath. The reaction mixture was shaken with 2 ml toluene and kept for several hours at room temp. Chromatograph was extracted in toluene phase and O.D. was measured at 520 nm using toluene as blank. Standard curve was prepared with graded doses of DL-proline.

For extraction of enzymes, 500 g leaf tissue was homogenized in 3 ml phosphate buffer 0.8 M pH 7.0) in a pre-chilled glass pestle mortar at $4^{O}C$. This was centrifuged at 10,000 rpm for 30 M. Pellet was discarded and supernatant was used for enzyme assay. Peroxidase was assayed by the method of Plewa *et al.* (1991). Guaiacol oxidation was monitored by reading the absorbance at 470 nm at the moment of enzyme extract addition and 1 minute later. The difference in absorbance (470 nm) was divided by the tetraguaiacol molar extinction co-efficient (25.5 m/M/cm) and the enzyme activity was expressed as mol of H_2O_2 used/min/mg protein. Peroxidase unit was calculated for the formation of 1mM tetraguaiacol for 1 M. The enzyme units were calculated by formulae given by Kokkinakis and Brooks (1999). Catalase was measured by the reduction of potassium dichromate to chromic acid by hydrogen peroxide (Sinha 1972). The reaction mixture containing 0.5 ml of 0.2 M H_2O_2, 0.8 ml of enzyme extract and 0.7 ml of 0.1M phosphate, buffer pH 7.0 was incubated at $37^{O}C$ for 30 M. After that 4.0 ml of dichromate acetic acid reagent (5% potassium dichromate + glacial acetic acid in a ratio of 1:3) was added and mixture heated in a boiling water bath for 10 M and cooled. Green colour of chromic acetate thus formed was measured at 570 nm. One unit of enzyme has been defined as amount of enzyme required to utilize 1 millimole of hydrogen peroxide under assay conditions. The specific activity has been expressed as units/min/mg protein.

To study the effect of pendimethalin, earthen pots (9" dia) filled with soil were sprayed with 0.25, 0.50 and 1.0 kg/ha pendimethalin prior to sowing. The herbicide was mixed thouroughly in the upper soil layer manually to ensure uniformity of appication. Twenty seeds of six *P. minor* populations as indicated in the Table 3 and wheat were sown 24 h after spray in four replicates. Per cent emergence was recorded 30 days after spray. In another experiment Petri-plate assay was conducted. Twenty seeds of *P. minor* populations and wheat were sown in Petri-plates lined with filter paper and soaked in different concentrations of pendimethalin (2.5, 5.0 and 25 g/ha). Number of seeds germinated, hypocotyl length and radical length was recorded after 7 days.

RESULTS AND DISCUSSION

After sulfosulfuron (25 g/ha) spray, mortality was less than 30% in 12 populations which were identified as resistant populations. These were Ambala – Jansui Head, Jind- Majra, Hisar-Nangla, Karnal-Uchana, Karnal-Sagga, Fatehbad-Badi Birthal, Jind-Raseedan, Kurukshetra-Chanarthal, Kurukshetra- Munak, Hisar- HAU farm, Kaithal-Gumthala and Hisar-Lalodha. Eight populations showed mortality in the range of 30-70% and were categorized as medium susceptible/medium resistant (MR/MS) populations. After spray with 32 g/ha sulfosulfuron + metsulfuron, 11 populations were found to be resistant, 8 populations MR/MS and one population from Hisar – Nangla was found to be susceptible. Similarly, after spray with 14.4 g/ha mesosulfuron + iodosulfuron, 12 populations were found to be resistant, 7 MS/MR and one population from Jind-Pipaltha was found to be susceptible to Atlantis (Table 1). GR_{50} values were higher than the rec-

ommended dose of sulfosulfuron *i.e.* 25 g/ha in all populations. These were in the range of 30-50 g/ha in populations from Karnal-Uchana, Kurukshetra-Neemwali, Kurukshetra-Chanarthal, Jind-Raseedan and Jind-Pipaltha. Populations from Karnal-Sagga showed GR_{50} value of 110 g/ha. Wheat showed GR_{50} value more than 200 g/ha. After spray with mesosulfuron + iodosulfuron (28.8 g/ha) per cent mortality increased in all populations and up to 75% in many populations. GR_{50} values for mesosulfuron + iodosulfuron varied between 7.2 to >84. Populations from Uchana and Sagga showed GR_{50} values higher than 84. Populations from Kurukshetra –Neemwali showed a value of 16.2 and those from Jind-Raseedan, Kurukshetra-Chanarthal and Jind-Pipaltha showed GR_{50} values of 7.8. Wheat showed a GR_{50} value of 28 (Table 2).

Table 1. Effect of sulfosulfuron, sulfosulfuron + metsulfuron and mesosulfuron + iodosulfuron on mortality in different *P. minor* populations

Population	Mortality (%) of *P. minor*			
	S	SM	M	MI
Ambala–Jansui Head	15	40	45	60
Ambala–Adumajra	35	30	30	60
Jind–Majra	20	40	45	55
Hisar–Nangla	20	70	25	60
Karnal–Uchana	15	5	40	40
Karnal–Sagga	15	15	25	10
Fatehbad–Badi Birthal	15	45	5	75
Jind–Raseedan	25	15	45	75
Kurukshetra–Chanarthal	25	50	15	75
Jind–Pipaltha	30	50	75	75
Katihal–Thana	30	15	25	55
Kurukshetra–Neemwali	30	0	15	25
Kurukshetra–Munak	25	35	20	75
Rohtak	45	25	60	75
Hisar, HAU–Farm	25	25	30	75
Hisar-Bass	45	20	15	75
Kaithal–Gumthala	15	5	20	75
Hisar–Lalodha	25	20	20	75
Hisar–Vaibhalpur	35	20	20	75
Sirsa, University Farm	30	50	5	75
LSD (P=0.05)	6.9	7.1	10.3	20.5

S - Sulfosulfuron (25 g/ha); SM - Sulfosulfuron + metsulfuron (32 g/ha); M - Mesosulfuron + iodosulfuron (14.4 g/ha); MI - Mesosulfuron + iodosulfuron (28.8 g/ha)

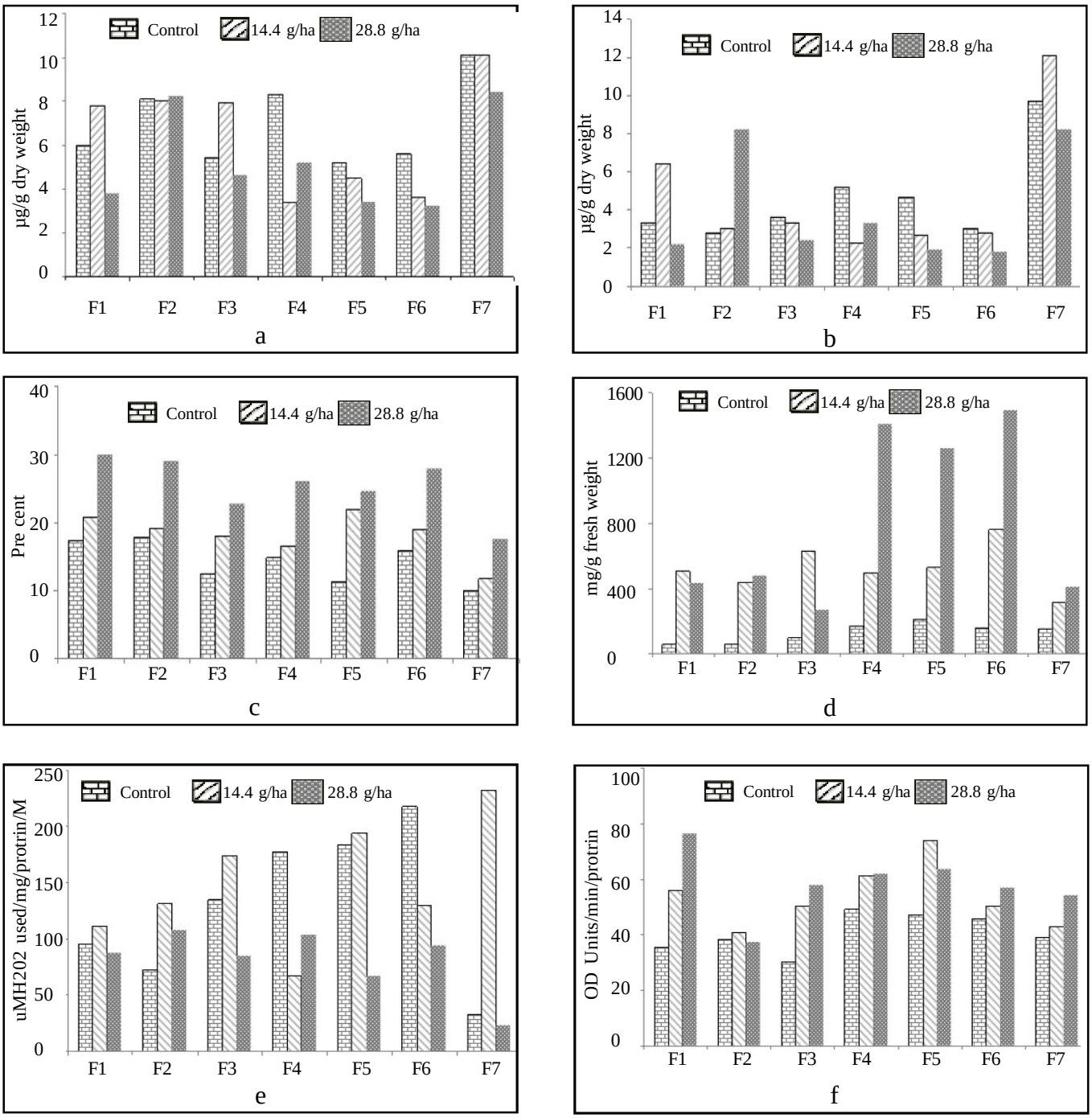
A decline in total chlorophyll content was observed 20 days after spray with 14.4 g/ha mesosulfuron + iodosulfuron in populations from Jind-Raseedan, Jind-Pipaltha and Kurukshetra-Chanarthal. An increase in chlorophyll content was observed in populations from Karnal-Uchana and Kurukshetra-Neemwali of *P. minor*. No decline was observed in populations from Karnal-Sagga and wheat. At 28.8 g/ha further decline was observed in all populations except Karnal-Sagga and Jind Raseedan (Fig. 1a). A decline in carotenoid content was also observed 20 DAS with mesosulfuron + iodosulfuron (14.4 g/ha) in populations from Jind-Raseedan and Kurukshetra-Chanarthal. A less or no decline was observed in populations from Karnal-Sagga, Kurukshetra-Neemwali and Jind-Pipaltha of *P. minor*. An increase in carotenoid content was observed with mesosulfuron + iodosulfuron (14.4g/ha) in *P. minor* population from Karnal-Uchana and wheat. After spray with 28.8 g/ha mesosulfuron + iodosulfuron carotenoid content declined in all populations except Karnal-Sagga where it showed an increase at this dose (Fig. 1b).

The ion efflux increased in all populations after spray with 14.4 g/ha of mesosulfuron + iodosulfuron. The increase being highest in Kurukshetra-Chanarthal and least

Table 2. Effect of sulfosulfuron and mesosulfuron + iodosulfuron on GR_{50} values of different *P. minor* populations

Population	GR_{50} value (g/ha)	Regression equation $Y= (a+bx+cx^2)$	R^2
Sulfosulfuron			
F_1	100	$Y= 87 + 32x-11x^2$	0.69
F_2	100	$Y= 83 + 24x-8.2x^2$	0.99
F_3	55	$Y= 127 - 17x-2.5x^2$	0.82
F_4	80	$Y= 42.2 + 105x-27.5x^2$	0.49
F_5	80	$Y= 75 + 64x-18.4x^2$	0.35
F_6	75	$Y= 104 + 8x-6.3x^2$	0.74
F_7	>200	$Y= 121 - 37x+6.9x^2$	0.13
Mesosulfuron + iodosulfuron			
F_1	>84	$Y= 134 - 40x + 5.9x^2$	1
F_2	>84	$Y= 159 -77x +17.8x^2$	1
F_3	16.2	$Y= 161 - 70x + 9.3x^2$	1
F_4	7.2	$Y= 260 - 199x + 39.8x^2$	1
F_5	7.2	$Y= 282 - 227x + 47.5x^2$	1
F_6	7.2	$Y= 271 -217x - 47x^2$	1
F_7	28.8	$Y= 54 +150x -43.6x^2$	1

F_1 - Karnal-Uchana; F_2 - Karnal-Sagga; F_3 - Kurukshetra-Neemwali; F_4 - Jind -Raseedan; F_5 - Kurukshetra-Chanarthal; F_6 - Jind-Pipaltha; F_7 - Wheat



F1 = Karnal-Uchana, F2 = Karnal-Sagga, F3 = Kurukshetra-Neemwali, F4 = Jind -Raseedan, F5 = Kurukshetra-Chanarthal, F6 = Jind-Pipaltha, F7 = Wheat

LSD (P=0.05) - Population x herbicide - total chlorophyll =1.105; carotenoid content =1.035; ion efflux=5.8; proline=123; catalase 4.4; peroxidase=8.4

Fig. 1. Effect of mesosulfuron + iodosulfuron on total chlorophyll content (a), carotenoid content (b), ion efflux(c), proline content (d) ,catalase(e) and peroxidase(f) activities

in Karnal-Sagga and wheat. This increased further after spray with 28.8 g/ha mesosulfuron + iodosulfuron (Fig. 1c). An increase in proline content was observed in all populations of *P. minor* and wheat after spray with 14.4 g/ha mesosulfuron + iodosulfuron. At 28.8 g/ha, the increase was more in populations from Jind-Raseedan, Kurukshetra-Chanarthal and Jind-Pipaltha(Fig. 1d). Catalase activity increased after spray with 14.4 g/ha mesosulfuron + iodosulfuron in populations from Karnal-Uchana, Karnal-Sagga, Kurukshetra-Neemwali. In wheat it increased to much higher levels than the *P. minor* populations. A decline in catalase activity was observed in populations from Jind Raseedan and Jind-Pipaltha. At 28.8 g/ha a further decline in catalase activity was observed in all populations (Fig. 1e). Peroxidase activity increased in all populations of *P. minor* and wheat after spray with 14.4 g/ha mesosulfuron + iodosulfuron. The enzyme activity increased with increasing dose to 28.8 g/ha except for Karnal-Sagga where it did not show this change and in Kurukshetra-Chanarthal where it declined at this dose (Fig. 1f).

None of the populations was susceptible to sulfosulfuron. All the populations tested were either resistant or medium resistant to the herbicide. GR_{50} values (herbicide dose required for 50% reduction of the growth) were in the range of 30-110 g/ha, well above the recommended dose of the herbicide. The population from Karnal-Sagga showed highest value of 110 g/ha. It is pertinent to note here that GR_{50} values of sulfosulfuron against *P. minor* populations at the time of recommendation of the herbicide was 5 g/ha (Yadav and Malik 2005). A test of other sulfonyl urea herbicides, *viz.* (sulfosulfuron + metsulfuron) and (mesosulfuron + iodosulfuron) showed similar response with most of the populations being either resistant or medium resistant to these herbicides. Brar and Walia (2009) reported significant decline in *P. minor* density after spray with mesosulfuron + iodosulfuron. Singh *et al.* (2010a) however, reported lower efficacy of mesosulfuron + iodosulfuron on some *P. minor* populations. Data on chlorophyll content after spray with 14.4 g/ha mesosulfuron + iodosulfuron indicated a decline in the 3 medium resistant populations, *viz.* Jind–Raseedan, Jind-Pipaltha and Kurukshetra-Chanarthal but an increase in the resistant populations, *viz.* Karnal-chana, Karnal-Sagga and Kurukshetra-Neemwali. No change was observed in wheat. A decline in chlorophyll content after spray with isoproturon has earlier been reported in *P.minor* in susceptible populations. A further decline was observed with a higher dose of mesosulfuron + iodosulfuron in all populations except Karnal-Sagga which is highly resistant. Carotenoid content increased in resistant population from Karnal-Uchana and wheat after spray with 14.4 g/ha with mesosulfuron + iodosulfuron. In resistant population from Karnal–Sagga it increased after spray with 28.8 g/ha mesosulfuron + iodosulfuron. Wheat showed the highest carotenoid content at 14.4 g/ha and same as *P. minor* population from Karnal-Sagga at 28.8 g/ha. The content of photosynthetic pigments provides clues for their impact on biomass accumulation (Singh *et al.* 2010b). Carotenoids in addition to their role as accessory pigments for photosynthesis are also known to act as photoprotective agents against reactive enzyme species (ROS) produced as a result of stress.

As a result of herbicide spray due to stress effects, free radicals and other active derivatives of oxygen are produced. These reduced oxygen species such as hydrogen peroxide (H_2O_2), O_2 and OH radicals inactivate enzymes and damage cellular components. Singlet oxygen produced through the triplet state of chlorophyll is highly destructive. This oxygen species initiates lipid peroxidation which results in membrane destruction and increase in membrane permeability. Data on ion efflux after spray with mesosulfuron + iodosulfuron indicates a progressive increase in permeability with increase in dose of the herbicide. The ion efflux in wheat was relatively lesser at all doses as compared to *P. minor* populations. This indicates lesser damage in wheat as compared to the *P. minor* populations. Accumulation of proline in response to various stresses has been reported in a number of plant species under water and salt stress (Hsiao 1973, Waldren and Tuare 1974) , in cold treated plants (Tantau and Dorfling 1991), as part of plant's defense reaction against abiotic and biotic stresses (Reddy and Veeragnjaneygulu 1991). It is also seen to accumulate in response to stresses caused by herbicides (Toteva *et al.* 2004). In the present investigation proline levels increased in all the populations after spray with mesosulfuron + iodosulfuron. The level remained higher in medium resistant populations as compared to the resistant ones at both the lower and higher doses. The high concentration of proline corresponds to its osmotic role but other functions including radical detoxification and regulation of cellular redox status have also been suggested (Hare and Cress 1997). Enzymes like peroxidase and catalase have antioxidant functions in crops as well as weeds after spray with herbicides (Pan *et al.* 2008). In the present investigation catalase activity increased in wheat as also in populations from Karnal-Uchana, Karnal-Sagga, Kurukshetra-Neemwali and Kurukshetra-Chanarthal. Peroxidase activity increased in all populations except one from Karnal-Sagga. Studies on

Table 3. Effect of pre-emergence application of pendimethalin in pot-culture on per cent emergence and plant height of different *P. minor* populations and wheat at 30 days after seeding

Population	Pendimethalin (kg/ha)			
	0	0.25	0.50	1.0
Emergence (%)				
F_1	52.5	7.5	0	0
F_2	50	12.5	2.5	0
F_3	52.5	15	0	0
F_4	62.5	5	0	0
F_5	55	17.5	0	0
F_6	50	10	0	0
F_7	65	67.5	57.5	22.5
Plant height (cm)				
F_1	31.2	3.7	0	0
F_2	34.2	23	7.5	0
F_3	34.5	21	0	0
F_4	26.5	3.5	0	0
F_5	23.5	17.5	0	0
F_6	28.2	8.25	0	0
F_7	42.2	29.2	29	19.5

Population details are given in Table 2

chlorosulfuron transformed plants have indicated a need for a balanced interaction of protective enzymes (Toteva *et al.* 2004).

The study on evaluation of pendimethalin in pot culture assay indicated that 50-60% emergence was observed in all *P. minor* populations and wheat in control. In pots sprayed with 0.25 kg/ha pendimethalin, emergence declined to 5-17% in *P. minor* populations but not in wheat. At 0.50 and 1.0 kg/ha^{-} pendimethalin, emergence declined further to zero except in case of population from Karnal-Sagga where it was 2.5% at 0.5 kg/ha pendimethalin. Plant height also decreased after a pre-emergence spray of 0.25 kg/ha in *P. minor* population as well as wheat. Decline was more in *P. minor* populations (Table 3). In Petri-plate assay germination percentage declined with increase in dose of pendimethalin. At a dose equivalent to 25 g/ha it was more in Kurukshetra-Chanarthal and Kurukshetra-Neemwali. Hypocotyl and radical length also declined significantly with pendimethalin (2.5 to 25 g/ha) in all *P. minor* populations.

Table 4. Germination, hypocotyl length and radical length of different *P. minor* populations and wheat at 7 days after application of different concentrations of pendimethalin in petri-plates

Population	Pendimethalin (g/ha)			
	0	2.5	5.0	25
Germination (%)				
F_1	93	93	76	76
F_2	93	96	73	80
F_3	90	93	95	63
F_4	90	96	85	80
F_5	96	75	85	45
F_6	91	88	85	83
F_7	100	100	96	93
Hypocotyl length (cm)				
F_1	2.8	0.15	0.15	0.15
F_2	2.6	0.16	0.15	0.16
F_3	2.6	0.15	0.5	0.16
F_4	2.8	0.15	0.15	0.15
F_5	2.7	0.15	0.15	0.15
F_6	3	0.15	0.15	0.15
F_7	5	4	1.5	1.5
Radical length(cm)				
F_1	2.3	0.3	0.1	0.07
F_2	2.2	0.3	0.1	0.06
F_3	1.5	0.3	0.1	0.1
F_4	2.9	0.3	0.1	0.1
F_5	2.7	0.3	0.1	0.08
F_6	2.2	0.3	0.1	0.1
F_7	6.6	6.0	2.0	1.5

Population details are given in Table 2

In wheat, however the suppression was lesser at 2.5 gha as compared to 5.0 and and 25 g/ha (Table 4).

For management of the weed species resistant or cross resistant to one or two categories of herbicides, herbicides with different mode of action are advocated to be employed. Pendimethalin belonging to Group III herbicides could completely arrest the growth of all *P. minor* populations tested in pot culture as well Petri-plate assay. Management of isoproturon resistant *P. minor* by trifluralin derivatives had been advocated earlier (Yaduraju and Ahuja 1995). With limited herbicides options available pendimethalin appears to be the best option for management of *P. minor*.

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