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Herbicide resistance mechanism of *Phalaris minor* in Uttarakhand

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ABSTRACT

Isoproturon is a substituted urea herbicide effectively controlled Phalaris minor which was first recommended in 1977-78 and widely used since the early 1980s. However continuous use of this single herbicide for 10-15 years coupled with monocropping of rice-wheat led to evolution of resistance in this weed. Earlier first report of resistance was reported in 1993 in Haryana and Punjab. A pot culture experiment was conducted to evaluate the mechanism of resistance of P. minor by using piperonyl butoxide, an inhibitor of that enzyme along with three different doses viz. ISO 0.5 kg ha^{-1}, 1.0 kg ha^{-1} and 2.0 kg ha^{-1} of isoproturon. The bio efficacy of isoproturon could be enhanced by the addition of piperonyl butoxide (PBO) and a significant reduction of dry weight was recorded in different doses of Isoproturon. The mortality rate of P. minor was also increased by the addition of PBO. These results suggest that enhanced herbicide metabolism is the cause of isoproturon resistance in P. minor.

Keywords: Cytochrome P450 monooxygenase, herbicide resistance, isoproturon, Phalaris minor.

Phalaris minor is most troublesome an annual grass weed in the wheat crop mainly in the rice–wheat system of India. Most of wheat grown areas are heavily infested by *P. minor* which emerges with the germinating wheat crop, competes for water and nutrient requirement and reduces the grain yield (Bhan and Kumar, 1997). It has been estimated weeds cause yield losses to the extent of 33 per cent, which is more than losses caused by insects and pests (Kulshrestha and Parmar, 1992). Moreover, the morphological similarity of this weed with wheat makes it difficult to remove manually within crop rows (Cavan *et al.,* 2000). Isoproturon a postemergence herbicide was recommended for control of *P. minor* in wheat, and was largely accepted by the Indian farmers (Gill *et al.,* 1978). However, the continuous use of urea-based herbicides, particularly isoproturon, for more than a decade in wheat under a rice–wheat system has resulted in the evolution of herbicide resistant biotypes of *P. minor* (Malik and Singh, 1993, Yaduraju and Ahuja, 1995, Walia *et al.,* 1997) which have been found to tolerate 2 times and even higher dose than the recommended application than susceptible ones in Haryana (Malik and Singh, 1995) and more than 2 times in Punjab (Walia *et al*., 1997).

Two major mechanism of herbicide resistance *i.e.* target site modification and enhance metabolism. The target site modification is well understood in *P. minor,* the insensitive target enzyme based resistance mechanism is understood (Tal *et al.,* 1996). However, biochemical studies to characterize herbicide resistance due to detoxifying enzyme *i.e*. enhance metabolism resistance, particularly by the cytochrome P450 monooxygenase is still not much clear in *P. minor*. First report of herbicide resistance due to non-target site modification in *P. minor* was reported in 1998 (Singh *et al.,* 1998). Subsequently, in vivo studies on herbicide metabolism and cytochrome P450 inhibitors in resistant biotypes found that cytochrome P450 monooxygenase enhanced rates of metabolism of several herbicides (Powles, 2010). The evolution of non-target herbicide resistance due to enhanced rates of herbicide metabolism has been demonstrated in resistant biotypes of several weeds, such as *L. rigidum, A. myosuroides, Amaranthus hybridus, Bromus tectorum, Avena sterilis, Phalaris minor, Echinochloa phyllopogon, Stellaria media, Digitaria sanguinalis and Sinapis arvensis* (Preston, 2004 and Yun, *et al.,* 2005).

In this study, resistance due to non-targetsite modification in *P. minor* biotype to isoproturon is evaluated by the application of cytochrome P450 monooxygenase inhibitor. It has been hypothesized that the application of cytochrome P450 monooxygenase inhibitor (PBO) block the action of isoproturon metabolism and eventually the plant is expected to die. The effects of cytochrome P450 monooxygenase inhibitors on the dose response to isoproturon in *P. minor* were also investigated.

MATERIALS AND METHODS

A pot experiment was carried out in Department of Plant Physiology, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology Pantnagar. Seeds were sown in 11 cm diameter plastic pots filled with a 2:1 (wt/wt) mixture of sand and soil. Soil pH was 5.6 and the organic matter content was 1.4 per cent. Seeds collected from farmer's

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field were sown in pots. After emergence, the seedlings were thinned to 15 plants per pot. At the two to three-leaf stage, the plants were sprayed with different doses of Isoproturon using sprayer equipped with a flat-fan nozzle calibrated to deliver 480 L ha^{-1} at 230 kPa. It was applied at 0.5 kg ai/ha, 1.0 kg ai ha^{-1} (recommended dose, x) and 2.0 kg (2x dose) ai ha^{-1} at 2-3 leaf stage. The experiments were conducted by completely randomized design with three replications. Confirmation of resistance in *P. minor* was cross checked by the application of PBO, a mono-oxygenase inhibitor @ 2kg ha^{-1} sprayed along with all the three doses of Isoproturon. PBO was dissolved in 25 per cent acetone before mixing with Isoproturon and reference plants were treated with a mixture of 25 per cent acetone plus water. Observation on per cent mortality of *P. minor* plants was recorded at three weeks after spray. Three weeks after treatment, the above ground parts of the plants were harvested and the fresh weight was determined.

S1, S2, S3, S4 and S5 stands for the source of *P. minor* seeds which were collected from different farmers' wheat fields in winter season of Uttarakhand, where Isoproturon has been previously applied for various lengths of time. Some seeds were also collected from fallow land areas where Isoproturon had never been applied.

RESULTS AND DISCUSSION

Results of per cent mortality clearly indicate *P minor* biotypes collected from different locations were tolerant to Isoproturon. At recommended dose and also higher dose of Isoproturon is non toxic to wheat (Table 1).

Mortality percent

The effect of different doses of Isoproturon on percent mortality of *P. minor* is shown in table 1. An increasing trend of mortality per cent was observed with increasing doses of isoproturon in all the sources. However, among all the sources S1 exhibited highest percentage of mortality in all the doses while S3 showed least mortality percentage. Hence S1 considered as the most susceptible one and S3 as tolerant at the same time. Reduced potential of isoproturon to control *P. minor* was observed at recommended doses (ISO 2) due to evolution of resistance. At the recommended dose of Isoproturon, S2, S3 and S5 sources showed less mortality per cent in comparison to control and rest of the source *i.e*. S1 and S5 that indicate that these sources are able to successfully detoxifying the herbicide.

The per cent mortality was found to enhance with higher doses of isoproturon + PBO in all the sources. Noticeably the values were recorded higher under these combined doses in contrast to when isoproturon was used alone. Likewise at the recommended dose plus PBO, tolerant sources *i.e*. S2, S3 and S5 showed remarkable increase in mortality per cent in comparison to treated with isoproturon alone. It was also recorded that there were 16-55 per cent increase in mortality per centage. It should noticed that the values recorded here in S3 (considered as tolerant source) were also showed increased mortality percentage like other most susceptible source under isoproturon doses.

Dry weight

The effect of different doses of Isoproturon with and without PBO on dry weight of *P. minor* is presented in table 2. The dry weight values were observed to decrease with increasing doses of isoproturon in all the sources. S1 showed the minimum dry weight under ISO3 dose. The values of dry weight recorded in combined treatment were lower than the values when only isoproturon was used.

Science, Isoproturon is a urea herbicide which inhibits photosynthesis by either blocking or interfering with electron flow in the thylakoid membrane of chloroplasts. Photoaffinity studies with atrazine discovered that the binding site was a 32 kDa protein (Mordern, and Golden, 1989). The blocking of electron transport by PS II inhibiting herbicides generates oxidative stress resulting in the destruction of the reaction centre and the photooxidation of lipid and chlorophyll molecules. Modification of the amino acid residues in the QB binding site on the D1 protein conferred target site resistance to triazines in *P. minor* (Tripathi, 2003 and Tripathi *et al*, 2005) resulting in reduced herbicide affinity to displace plastoquinone and electron flow from QA to QB, thus conferring resistance to urea herbicides.

Evidence for cytochrome P450 monooxygenase involvement has been provided by pot experiments using the enzyme inhibitors. Several inhibitors of cytochrome P450 monooxygenase activity have been identified, including: 1-aminobenzo-triazole, tetcyclacis, piperonyl butoxide (PBO), carbon monoxide and tridiphane (McFadden *et al*,. 1989, Siminszky, 2006, and Zimmerlin *et al.*, 1990). These inhibitors have provided evidence for the involvement of cytochrome P450 monooxygenase in herbicide resistance and tolerance. Application of cytochrome P450 inhibitor PBO in vivo showed that PBO was synergistic with isoproturon. In this study it has been found that the combination of PBO and isoproturon was significantly more effective to control *P. minor* than isoproturon alone suggesting cytochrome P450 monooxygenase mediated metabolism was involved in *P. minor* resistance and link with its ability to withstand toxicity of Isoproturon.

Earlier it has also been reported that PBO has shown to be synergistic with alachlor, metolachlor, bispyribac-sodium, chlorofuluron and simazine action against several plants. Enhanced metabolism, mediated by cytochrome P450 monooxygenase, probably cause resistance to several herbicides, including chlorotoluron, simazine and chlorsulfuron in *L. rigidum* (Preston *et al.* 1996) , bispyribac sodium in *E. phyllopogon* (Fischer *et al.* 2000), mecoprop in *S. media* (Coupland, *et al*. 1990), fluazifop-P-butyl in *D. sanguinalis* (Hidayat and Preston, 2001), diclofop-methyl in *Avena sterills* (Maneechote *et al.* 1997), isoproturon in *P. minor* (Singh *et al.* 1998) and ethametsulfuron-methyl in *S. arvensis* (Veldhuis *et al.* 2000).

Biotypes of *P. minor i.e.* S2, S3, and S5 which are considered as resistant on the basis of above results, required even higher doses to show same effect as other biotype *i.e*. S1 and S4 showed. Treatments of Isoproturon along with cytochrome P-450 monooxygenase inhibitor, PBO significantly decreased the dry weight of the resistant biotypes of *P. minor* confer the mechanism of resistance could be due to enhance metabolism. Further, molecular study required to confirm resistance. In conclusion, cytochrome P450 monooxygenase played an important role in the Isoproturon herbicide resistance responses in *P. minor.*

Table 1 : Effect of different doses of Isoproturon alone and in combination with PBO on per cent mortality of *P. minor*

	Control	ISO I	ISO II	ISO III	PBO	ISO I +PBO	ISO II +PBO	ISO III +PBO
S1	0^{j}	28.89^{fgh}	57.78^{bc}	80.89^{a}	0^{h}	37.78^{g}	82.22^{bc}	93.33^{a}
S2	0^{j}	26.67^{ghi}	35.56^{efg}	51.11^{cd}	0^{h}	46.67^{efg}	80.00^{c}	91.11^{a}
S3	0^{j}	13.33^{ij}	48.89^{cde}	48.89^{cde}	0^{h}	51.11^{ef}	75.56^{d}	88.89^{ab}
S4	0^{j}	33.33^{fg}	68.89^{ab}	75.56^{a}	0^{h}	55.56^{e}	84.44^{bc}	91.11^{a}
S5	0^{j}	15.56^{hi}	42.22^{def}	57.78^{bc}	0^{h}	42.22^{fg}	82.22^{bc}	86.67^{ab}
SEM(±)	**-**	**4.765**	**3.718**	**4.66**	**0.21**	**3.58**	**2.43**	**3.29**
LSD (0.05)	**-**	**11.56**	**11.33**	**13.64**	**0.66**	**9.98**	**7.06**	**6.05**

Note: The mean values with same superscript are not significantly different in the post-hoc tests (P < 0.05).

Table 2. : Effect of different doses of Isoproturon alone and in combination with PBO on dry weight of *P. minor*

	Control	ISO1	ISO2	ISO3	PBO	ISO1+ PBO	ISO2+ PBO	ISO3+ PBO
S1	43.33^{a}	32.11^{cd}	22.50^{efg}	17.62^{g}	42.80^{a}	28.22^{efgh}	20.30^{ghij}	16.43^{j}
S2	42.20^{a}	37.78^{abc}	29.50^{de}	22.83^{efg}	36.13^{bcd}	32.21^{cde}	26.80^{efgh}	21.47^{ghij}
S3	40.60^{ab}	33.56^{bcd}	28.33^{de}	23.64^{efg}	37.20^{abc}	29.61^{def}	25.60^{efghi}	24.43^{fghi}
S4	42.73^{a}	27.44^{de}	23.54^{efg}	18.83^{fg}	42.20^{ab}	24.11^{fghi}	21.83^{ghij}	19.33^{ij}
S5	39.60	33.33^{bcd}	28.27^{de}	26.50^{def}	35.60^{cd}	31.38^{cde}	24.50^{fghi}	21.93^{ghij}
SEM(±)	**1.95**	**2.86**	**2.88**	**1.94**	**1.53**	**2.13**	**2.22**	**1.51**
LSD (0.05)	**6.17**	**6.03**	**7.08**	**6.12**	**5.12**	**4.71**	**5.20**	**4.77**

Note: The mean values with same superscript are not significantly different in the post-hoc tests (P < 0.05).

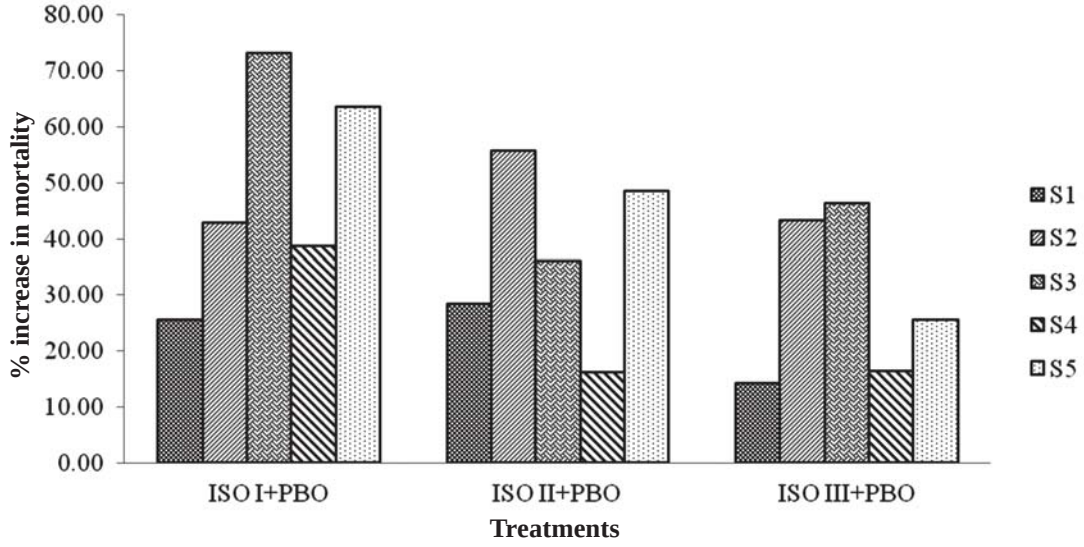

Fig. 1 : Per cent increase in mortality of *P. minor* after the treatment of isoproturon along with PBO.

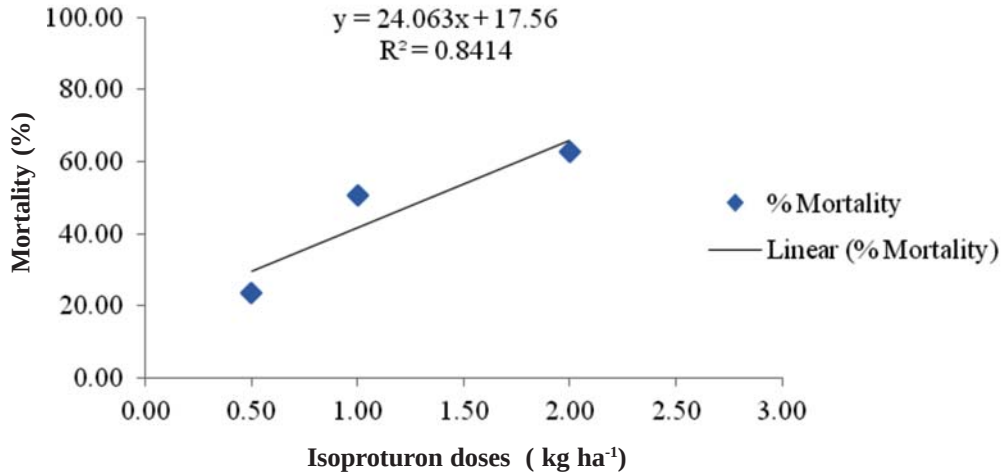

Fig. 2 : Linear regression of mortality % corresponded to Isoproturon doses of *P. minor.*

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