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RESEARCH NOTE

Susceptibility of long-term unexposed population of *Phalaris minor* to isoproturon

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ABSTRACT

Phalaris minor populations (95 No.) were collected from 14 districts of Punjab. All these populations were not exposed to isoproturon for more than 15 years. Plants of each population/pot were planted and treated with the graded doses of isoproturon 75WP (234.5, 469, 938, 1876, 3752 g/ha) 30 days after sowing as per treatment. The mortality data was recorded and converted to morality percentage in relation to untreated control populations. Nonlinear regression analysis was used to determine the mean dose that caused mortality by 50% (GR50). Out of 95 *Phalaris minor* populations, isoproturon (IPU) at 938 g/ha provided more than 90% mortality in 39 populations and 70-90% mortality in 17 populations, respectively.

Keywords: GR50, Herbicides, Isoproturon, *Phalaris minor*, Reverse resistance, Wheat

In North-West India, *Phalaris minor* became the dominant weed in wheat fields under the rice-wheat production system. The broad-spectrum herbicides, *viz*. isoproturon (IPU), metoxuron, chlortoluron, and methabenzthiazuron were recommended for its control in wheat in the mid-1970s. Isoproturon (IPU) was readily adopted for weed control in early years of its recommendation (Gill *et al.* 1978). Owing to its flexible application method and broader application window, broad-spectrum weed control, IPU became the first choice of farmers during the 1980s-1990s till the onset of resistance in *P. minor* against IPU in the early 1990s (Malik and Singh 1993). Its continuous use in rice-wheat rotation for a longer period coupled with monoculture led to the evolution of resistance in *P. minor* against IPU (Walia *et al.* 1997). The evolution of isoproturon (IPU) resistance in *P. minor* in Haryana was the first case of herbicide resistance reported in India (Malik and Singh 1995) and it was the first report of weed resistance to isoproturon (IPU) in the world (Bhullar *et al.* 2017).

Alternate herbicides, *viz.* clodinafop-propargyl, sulfosulfuron, and fenoxaprop were introduced to control isoproturon (IPU) resistant *P. minor.* These herbicides provided excellent control for 6-7 years but they also met with the same fate due to their continuous use for killing *P. minor* (Yadav and Malik 2005). Subsequently, the GR50 values (dose of herbicide required for 50% growth reduction) of these herbicides increased for better weed control. Bhullar *et al.* (2014) reported that alternate herbicides like clodinafop, sulfosulfuron, and fenoxaprop lost their efficacy to control *P. minor* after 10-15 years of their continuous usage by the farmers even at double doses than the recommended dose of respective herbicide for their field use. Multiple resistance in *P. minor* to various modes of action herbicides is now well-established and confirmed by various scientists in northern India (Punia *et al.* 2017, Yadav *et al.* 2016). High levels of resistance to fenoxaprop, clodinafop-propargyl, and pinoxaden in the multiple herbicide-resistant populations of *P. minor* have been reported from Punjab (Bhullar *et al.* 2002, Bhullar *et al.* 2014, Kaur *et al.* 2015). During the extensive weed survey conducted in Punjab, it was also noticed that some farmers are getting good weed control with isoproturon. Keeping this in view, an experiment was carried out to study the response of *P. minor* populations, collected from different areas in Punjab to graded doses of isoproturon application.

A field survey of wheat fields of the rice-wheat system was conducted in March-April 2018 following reports of poor weed control across fourteen districts of Punjab *viz.* Amritsar (A1-A3),

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Barnala (B1-B8), Ferozepur (F1-F8), Gurdaspur (G1-G5), Hoshiarpur (H1-H4), Jalandhar (J1-J8), Kapurthala (K1-K6), Ludhiana (L1-L15), Moga (M1-M7), Patiala (P1-P2), Ropar (R1-R7), Sangrur (S1-S16), Tarantaran (T1-T4), Fatehgarh Sahib (FS1-FS2). Seeds of *P. minor,* which escaped herbicides, were collected from more than 100 different locations (wheat fields) in the state of Punjab. At these farmers' fields, farmers applied isoproturon (IPU) in the early 1980s, for control of *P. minor* and other weeds, which continued till late 1990s. After the evolution of resistance in *P. minor* to IPU, farmers abandoned its use in the early 2000s and as per personal communication with the farmers they had not used IPU in their fields since then. The farmers shifted to alternate herbicides, *viz*. clodinafop, sulfosulfuron, and fenoxaprop which worked well for a decade and after that, their efficacy decreased due to the evolution of resistance in *P. minor* to alternate herbicides. These results were confirmed by a farmers' field survey conducted by Bhullar *et al.* (2014). Thereafter, pinoxaden and ready-mix of mesosulfuron + iodosulfuron were introduced which were followed by ready-mixes of fenoxaprop and clodinafop with metribuzin for management of resistant *P. minor*. Isoproturon has not been used at most of these farmers' fields since the early 2000s.

Apart from resistant populations, *P. minor* seeds to be used as susceptible control were collected from the population that had not previously been exposed to any herbicide. Germination test of seeds of all populations was conducted during November 2018, using the Petri plate method where 10 seeds per plate were sown on moist filtered paper and the number of seeds germinated was counted after 10 days of sowing. *Phalaris minor* populations having more than 80% germination were selected. The pots were filled with soil from the field where no rice-wheat cropping system was followed for more than ten years to avoid *P. minor* soil seed bank. The soil was sieved to remove unwanted material and then filled in pots. About 15 g well-prepared vermicompost was added to every pot to provide the desired nutrition to the plants. Out of the selected populations, 48 populations were used for sowing in the first year (2018-19) and 49 populations were used for the second year (2019-20). The filled pots were arranged as per treatments in 6 blocks and 75 cm distance was maintained among blocks. Every block had three rows of 50 pots for planting 50 populations with three replications. The commercial formulation of photosystem II inhibitor isoproturon (Isoguard® 75 WP, Gharda Chemicals Pvt Ltd) was used for testing herbicide resistance and one block was kept as control (no herbicide spray) except water spray. All populations of *P. minor* per block were planted and replicated thrice for each dose of herbicide. The tagging of pots was done as per the layout. The populations were planted at 40 seeds per population per pot for each replicate in the first week of December during 2018 and 2019. Seeds were thoroughly mixed with soil and water was given to the pots as per need to avoid moisture stress. Pots were covered with black polythene sheets for a few days to give them the desired temperature and to save the seeds from bird damage. Water was applied uniformly to all pots. The number of seedlings per pot was counted four weeks after sowing from each pot. Plants of each population/pot were treated with the doses of isoproturon 75WP, *viz*. 234.5, 469, 938, 1876, 3752 g/ha 30 days after sowing as per treatment. Isoproturon at 938 g/ha was the recommended dose by Punjab Agricultural University, Ludhiana for the control of *Phalaris minor.* It was applied using a knapsack sprayer fitted with a flat fan nozzle, calibrated to deliver 375 liters of water per hectare and the spray lance was kept at knee-high height while spraying. Every precautionary measure was taken to avoid the spray drift from one block to another. As a precautionary measure, adjacent blocks on both sides were kept covered while spraying a block. The mortality of plants of each population was recorded 28 days after spray.

The mortality data was converted to morality percentage compared to untreated control populations. Nonlinear regression analysis was used to determine the mean dose that caused mortality by 50% (GR50). 'R' software was used to simultaneously fit multiple dose-response curves (Ritz and Streibig 2005) and to graph the distribution of data and regression lines. The effective herbicide doses that inhibited plant population by 50% (GR50) concerning the untreated control were estimated for each population by using this model. The resistance factor (RF), which is the ratio of the GR50 of the resistant *P. minor* population to GR50 of the susceptible population, was calculated based on mortality percentage, to compare resistance levels of evaluated populations.

Susceptibility of *Phalaris minor* isoproturon

The data about the control of *P. minor* populations by isoproturon (IPU) has been presented in **Figure 1.** The data revealed that at the recommended dose of 938 g/ha, IPU recorded more than 90% control of 39 populations, 70-90% control

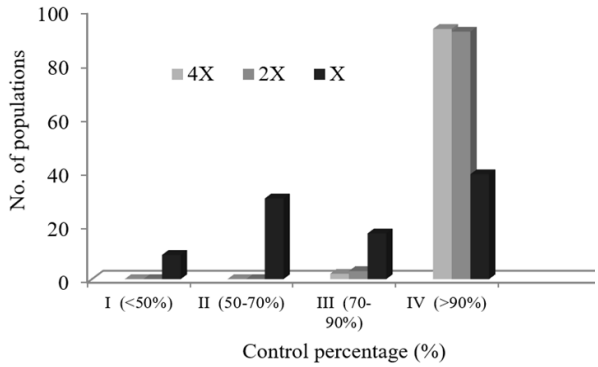

Figure 1. Percent control of *P. minor* populations in response to different doses of isoproturon (X represents the field dose of herbicide recommended by Punjab Agricultural University, Ludhiana)

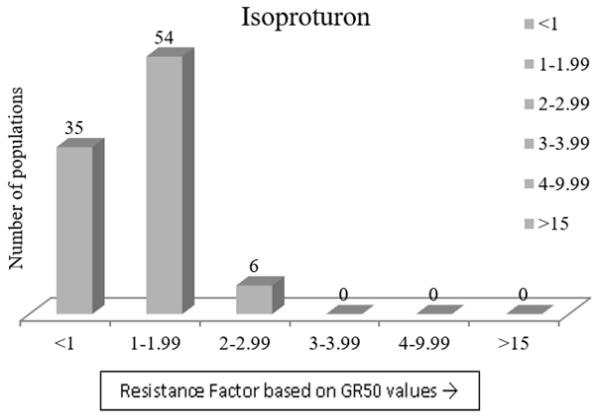

Figure 3. Categorization of *P. minor* populations from Punjab state based on resistance indices to isoproturon

of 17 populations, 50-70% control of 30 populations, and less than 50 % control of 9 populations of *P. minor*. At 1876 g/ha and 3752 g/ha, no population recorded less than 70% control. 92 populations showed more than 90% control whereas only 3 populations recorded 70-90 % control at 1876 g/ha. The respective figures were 93 and 2 at 3752 g/ha. Isoproturon (IPU) recorded remarkable control in most of the populations of *P. minor*.

GR50 and RF50 values for different *P. Minor* populations from 14 districts were calculated. A log-logistic model, with four parameters, was used using the dose-response curve graph the distribution of data, and regression lines, which has been presented in **Figure 2**. Out of 95 populations of *P. minor*, 37% had RF <1.0 at GR50, 57% had RF values between 1-2 and 6% of populations had RF >2.0 (**Figure 3**). Six populations of *P. minor, viz.* S4, R2, M6, T2, A2, and FS1 from districts Sangrur, Ropar, Moga, Tarntaran, Amritsar, and Fatehgarh Sahib, respectively, had RF between 2.0- 3.0. About 54 *P. minor* populations from Ferozepur, Gurdaspur, Kapurthala, Ludhiana, Hoshiarpur, Jalandhar, Patiala, and Bathinda districts were found susceptible to a low level (RF between 1 -2) of resistance to IPU. The effective control of *P. minor* populations with IPU indicates the evolution of reverse resistance in *P. minor* populations to IPU as these populations had not been exposed to IPU since the early 2000s.

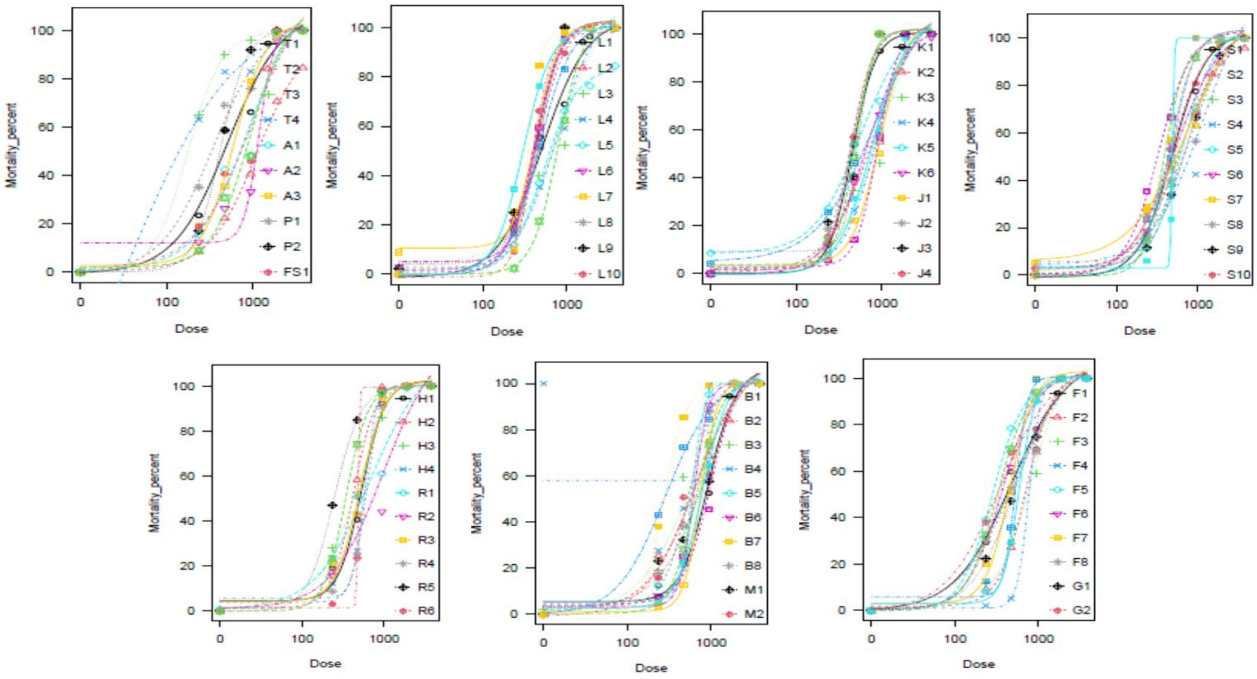

Figure 2. Estimated dose response curves for the *Phalaris minor* populations in response to different isoproturon concentrations

Conclusion

Out of 95 *Phalaris minor* populations, IPU at 938 g/ha provided more than 90% mortality in 39 populations and 70-90% mortality in 17 populations. The effective control of *P. minor* populations with isoproturon seems to be a fit case to investigate reverse resistance in *P. minor*.

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