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EVALUATION OF FENOXAPROP-P-ETHYL RESISTANT LITTLESEED CANARYGRASS (*Phalaris minor*) IN PAKISTAN[1]

*Avaliação de Erva-Cabecinha (**Phalaris minor**) Resistente a Fenoxaprop-P-Ethyl no Paquistão*

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ABSTRACT - To test resistance status of *Phalaris minor* (littleseed canary grass) to fenoxaprop-P-ethyl in Pakistan, a field survey was conducted during 2014. Uncontrolled *P. minor* plants were selected for seed collection from wheat fields where fenoxaprop-P-ethyl was used to control *P. minor*. Seeds were collected from eight different locations near Fasialabad, Pakistan. Susceptible plants were also selected near Faisalabad having no history of fenoxaprop-P-ethyluse for comparison. These seeds were grown in pots for resistance confirmation using completely randomized design with factorial arrangement having four replicates. Four doses of fenoxaprop-P-ethylcontrol (0X), 46.9 (0.5X), 93.7 (1X) and 187 (2X) g a.i. ha^{-1} were sprayed at 3 to 4 leaf stage of *P. minor*. Three weeks after fenoxaprop-P-ethyl spray, percent mortality and biomass of different biotypes were recorded. Dose killing 50% plants (LD_{50}) and resistance index (RI) were calculated on the basis of percent mortality. Results revealed that out of eight biotypes (PM-FS-1, PM-FS-2, PM-FS-3, PM-FS-4, PM-FS-5, PM-FS-6, PM-FS-7 and PM-FS-8) four biotypes (PM-FS-1, PM-FS-2, PM-FS-6 and PM-FS-7) showed resistance to fenoxaprop-P-ethyl. Percent mortality for the resistant biotypes was 51 to 71% even at 2X. Resistance index of the resistant biotypes was 2.13-6.00. Biomass reductions were also significantly lesser in resistant biotypes. Evolution of *P. minor* resistance to fenoxaprop-P-ethyl is first case of herbicide resistance in Pakistan. Research is needed to assess the infestation of herbicide resistant *P. minor* area in other locations and suggest control measures to evolve the effective management strategy to control the future spread of resistant biotypes.

Keywords: ACCase-inhibiter, LD_{50}, *Phalaris minor*, herbicide resistance, resistance index, survey.

*RESUMO - A fim de testar o status de resistência de **Phalaris minor** (erva-cabecinha) ao fenoxaprop-p-ethyl no Paquistão, uma pesquisa de campo foi realizada durante o ano de 2014. Plantas de **P. minor** sem controle foram selecionadas para coleta das sementes em campos de trigo em que o fenoxaprop-p-ethyl havia sido usado para controle de **P. minor**. As sementes foram coletadas a partir de oito locais diferentes próximos a Fasialabad, no Paquistão. Plantas suscetíveis também foram selecionadas próximo a Faisalabad, sem histórico de uso de fenoxaprop-p-ethyl, para fins de comparação. Essas sementes foram semeadas em vasos para confirmar sua resistência, usando um delineamento inteiramente casualizado com arranjo fatorial e quatro réplicas. Quatro doses de fenoxaprop-p-ethyl, controle (0X), 46,9 (0,5X), 93,7 (1X) e 187 (2X) g i.a. ha^{-1}, foram pulverizadas no estádio de 3 a 4 folhas de **P. minor**. Três semanas após a pulverização de fenoxaprop-p-ethyl, o percentual de mortalidade e biomassa dos diferentes biótipos foi registrado. A dose capaz de eliminar 50% das plantas (LD_{50}) e o índice de resistência (RI) foram calculados com base no percentual de mortalidade. Os resultados revelaram que, dos oito biótipos (PM-FS-1, PM-FS-2, PM-FS-3, PM-FS-4, PM-FS-5, PM-FS-6, PM-FS-7 e PM-FS-8), quatro (PM-FS-1, PM-FS-2, PM-FS-6 e PM-FS-7) mostraram resistência ao fenoxaprop-p-ethyl. O percentual de mortalidade para os biótipos resistentes foi de 51 a 71%, mesmo a 2X. O índice de resistência dos biótipos resistentes foi de 2,13-6,00. As reduções de biomassa também foram significativamente inferiores nos biótipos resistentes. A evolução da resistência de **P. minor** ao fenoxaprop-p-ethyl é o primeiro caso de resistência a herbicidas no Paquistão. Pesquisas adicionais são necessárias para avaliar a infestação da área de **P. minor** resistente ao herbicida em outros locais e sugerir medidas de controle para a evolução da estratégia de manejo eficaz no controle da infestação futura de biótipos resistentes.*

Palavras-chave: inibidor da ACCase, LD_{50}, *Phalaris minor*, resistência a herbicidas, índice de resistência, pesquisa.

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INTRODUCTION

Since the beginning of agriculture, weeds have been the most harmful biotic factor that reduces yield and quality of crops. Weeds cause up to 34% of losses in crop yield worldwide (Oerke, 2006), making uncontrolled arable weeds a dangerous factor for food security. Weed control was more labor-intensive and less efficient before the introduction of chemical herbicides. After the marketing of the first herbicide in the 1940's, weed control has become more effective (Oerke, 2006). The herbicides used may control on average >90% of weeds; this is the most efficient weed control method than all other tools ever developed to control weeds (Delye, 2013). Due to its greater efficacy, the chemical weed control method has rapidly extended all over the world and become one of the most used tools to control weeds (Oerke, 2006). Unfortunately, this golden era of herbicide use was challenged by herbicide resistance by the evolution of first herbicide resistance case in 1957 (Hilton, 1957). Herbicide resistance has been reported in 222 weed species against more than 150 herbicides, which cover all major modes of action of herbicides marketed worldwide (Duke, 2012; Heap, 2015).

Phalaris minor Retz. has been distributed throughout the world and described as a troublesome weed for wheat, barley, vegetables, rotational crops and several other winter crops (Singh et al., 1999; Jabran et al., 2010). *P. minor* can cause up to 95% of yield reduction in wheat (Chhokar and Sharma, 2008). Manual control of *P. minor* is difficult because of its mimicry with wheat plants until flowering. It produces from 300 to 475 seeds per plant and matures about 2 weeks before wheat (Rammoorthy and Subbain, 2006; Walia, 2006; Yasin and Iqbal, 2011).

Therefore, farmers are completely dependent on early post-emergence crop selective herbicides to control *P. minor* (Beckie et al., 2002). Continuous dependence on the same target-site-specific her-bicides against *P. minor* has increased the herbicide resistance risk. Heap (2015) reported that the herbicide selection pressure is continually increasing the resistant biotypes of different weeds. After the first case of *P. minor* resistance to isoproturon during 1995 in India, *P. minor* biotypes have developed resistance to ALS-inhibiting herbicide (sulfosulfuron), ACCase-inhibiting herbicides (clodinafop, fenoxaprop and tralkoxydim) and other herbicides with these modes of actions (Chhokar and Malik, 2002; Chhokar and Sharma, 2008; Gherekhloo et al., 2011). Many *P. minor* biotypes have evolved cross and multiple resistance to herbicides, having different modes of action in different parts of the world (Heap, 2015). The multiple resistance problem of *P. minor* at a few locations in India is so severe that it is causing huge yield reductions (Chhokar and Sharma, 2008). To prevent it from spreading to other fields and the further build-up of the herbicide resistance problem, early detection is essential. It also helps us to devise an effective integrated weed management system.

In Pakistan, *P. minor* is considered as one of the most troublesome grassy weed for wheat (Jabran et al., 2010). Fenoxaprop-P-ethyl has been used for more than fifteen years in Pakistan. Reports from farmers show that *P. minor* has become more difficult to control using fenoxaprop-P-ethyl in wheat fields of Faisalabad, Pakistan. Therefore, this study was conducted to identify *P. minor* resistance to fenoxaprop-P-ethyl. In Pakistan, no resistance case had been reported before this.

MATERIALS AND METHODS

Collection of seeds

For the resistance evaluation test, *P. minor* seeds were collected from eight different locations in Faisalabad District (31º252 - 31º362 N, 73º42 -73º72 E), Pakistan. After consulting the Department of Agricultural Extension, wheat fields were selected where farmers used fenoxaprop-P-ethyl during the then current year and uncontrolled *P. minor* plants were used for seed collection on April, 2014. Seeds collected from villages viz. Gojra, Jamiaabad, Sahianwala, Chak 73 GB, Chak 44 GB, Chak Jjhumra, Pathan Kot and Niddokay were named as PM-FS-1, PM-FS-2, PM-FS-3, PM-FS-4, PM-FS-5, PM-FS-6, PM-FS-7 and PM-FS-8, respectively. The field history of eight different locations where *P. minor* biotypes were selected for seed collection is shown on Table 3. The collected seeds were separated, sun-dried, and stored in paper bags at room temperature. Seeds of all biotypes were imbibed in distilled water for 24 h before sowing to promote germination (OM et al., 2004).

Pot experiments

Pot experiments were conducted twice in the screen house, at the Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan, starting on September 10 and November 3, 2014. Fifteen seeds of both susceptible and resistant suspected biotypes were sown in each pot separately (12x13x5 cm) and ten plants per pot were ultimately maintained. Soil was collected from the Agronomy Research Area having no history of herbicide application. Soil was mixed with farmyard manure (2:1, W/W). Seeds were spread on the soil surface and covered with an equal weight of soil in each pot to ensure uniform depth. Distilled water was regularly applied to keep the plants healthy. All pots were placed in the screen house on a completely randomized design with factorial arrangement, and replicated four times. The pots were randomized after each 4-5 days during the entire duration of the experiment to provide uniform conditions for the plants. Fenoxaprop-P-ethyl (Puma Super®750 EW, Bayer crop science, Pakistan) was sprayed during the 3 to 4-leaf stage as post emergence in four doses, control (0X), 46.9 (0.5X), 93.7 (1X) and 187(2X) g a.i. ha^{-1} both for susceptible plants and those with suspected resistance. Percent mortality and biomass data were recorded three weeks after the treatment with herbicide. The biomass data of weed biotypes obtained by oven drying above ground parts at 70 ^{o}C until the constant weight was obtained are stated as a percentage of the untreated control. Mortality data were subjected to probit analysis with the use of nonlinear sigmoid curves in JMP 11 to determine the lethal dose needed to kill 50% (LD_{50}) of each biotype. The resistance level for different biotypes was expressed in the form of resistance index (RI), which was calculated as the ratio of the LD_{50} of each resistant accession by the LD_{50} of the most susceptible (PM-FS-0) biotype (Travlos and Chachalis, 2010; Travlos et al., 2011).

The weed biomass data are presented in the form of dry weight per surviving plant (percentage of control). Plants that were not killed through the use of herbicide were considered surviving plants. Fisher's analysis of variance technique was used to analyze the data and the comparison of treatment means at each dose was conducted using Tukey's test at 5% probability level (Steel et al., 1997).

RESULTS AND DISCUSSION

The experiment was repeated twice. The results of the second experiment have been described in the text since repeated experiments showed similar results. Results showed that out of eight *Phalaris minor* collected biotypes, four biotypes (PM-S-1, PM-S-2, PM-S-6 and PM-S-7) showed resistance to wheat selective ACCase-inhibiting herbicide fenoxaprop-P-ethyl. The four remaining biotypes (PM-FS-3, PM-FS-4, PM-FS-5 and PM-FS-8) showed slight tolerance to fenoxaprop-P-ethyl, but they were not considered resistant due to their higher mortality and biomass reduction. Visual observation showed that three weeks after the herbicide application, there was a clear difference in the percent mortality among different biotypes and at different herbicide doses. All biotypes showed a difference in the percent mortality in relation to control, however, it is important to note that for PM-FS-1, PM-FS-2, PM-FS-6 and PM-FS-7, the percent mortality was very low: 71, 56, 54 and 51 respectively, even at 2X. For PM-FS-3, PM-FS-4, PM-FS-5 and PM-FS-8, the percent mortality was significantly higher than other resistant biotypes, where up to 95% and 99% of mortality occurred at 1X and 2X respectively, but they were statistically similar to each other (Table 1). By increasing the dose of the herbicide, the percent mortality significantly increased even in resistant biotypes.

The data regarding biomass, LD_{50} and resistance index (RI) showed that four selected biotypes of *P. minor* showed resistance to fenoxaprop-P-ethyl; the level of resistance was different for each accession. At 0.5X, the biomass reduction for resistant biotypes was about 13–23%, but in susceptible accession (PM-FS-0), the biomass reduction was up to 66%. The minimum biomass reduction occurred in PM-FS-7 (13%), which was followed by PM-FS-2 (16%) and PM-FS-6 (20%). It is important to notice that even at 1X (recommended fenoxaprop-P-ethyl dose), the biomass reduction of resistant biotypes was less than 36%, while the biomass reduction for the susceptible accession (PM-FS-0) was 100%. Furthermore, even at 2X, all resistant biotypes showed biomass reduction of less than 57% (Table 2).

The LD_{50} values of resistant biotypes ranged from 67.93 to 182.83 g a.i. ha^{-1}. The resistant index of different biotypes revealed that they showed different levels of resistance against fenoxaprop-P-ethyl. The resistance index of the PM-FS-3, PM-FS-4, PM-FS-5 and PM-FS-8 biotypes was equal to or less than 1, therefore, these biotypes were considered as non-resistant biotypes. The maximum RI of 6.00 was shown by PM-FS-7 and PM-FS-2 (Table 3). Our results revealed a wide spread resistance in *P. minor* to wheat selective herbicide fenoxaprop-P-ethyl. Different biotypes that were collected from eight locations showed resistance to fenoxaprop-P-ethyl. However, the level of resistance was different for each biotype. The prolonged use of the same herbicide imposes the selection pressure on weeds to increase the widespread of resistant biotypes (Owen et al., 2007). *Phlaris minor* resistance to fenoxaprop-P-ethyl and other ACCase inhibitors have been reported in different countries worldwide including India, Iran, Mexico, South Africa, United States and Australia (Heap, 2015). The first herbicide resistance case against fenoxaprop-P-ethyl in relation to other herbicides in Pakistan may be due to its earlier registration, the long use of a single herbicide and its good control efficacy against *P. minor*. It is important to notice that the biotypes showed a higher level of resistance when collected from a rice-wheat cropping system, where fenoxaprop-P-ethyl had been used from many years continually. Wheat fields with a more prolonged use of fenoxaprop-P-ethyl (PM-S-1, PM-S-2, PM-S-6 and PM-S-7) showed a higher level of resistance than fields with a short history of fenoxaprop-P-ethyl use (PM-FS-3, PM-FS-4, PM-FS-5 and PM-FS-8). The lack of crop rotation and the use of the same herbicide for many years are the main causes of evolution of herbicide resistance (Tal et al., 1996; Travlos and Chachalis, 2010). The higher seed producing ability of *P. minor* and the earlier seed maturity in relation to wheat are also very important factors to increase the resistant seed ratio in soil seed banks to increase the dominance of resistant *P. minor* plants in the existing weed population (WALIA, 2006; YASIN; IQBAL, 2011).

Variable *P. minor* resistance levels against fenoxaprop-P-ethyl have also been reported in other countries (Travlos, 2012). The high level of resistance in *P. minor* is due to the presence of altered ACCase enzyme. Mutations in the gene encoding the ACCase enzyme are responsible for the insensitivity of *P. minor* to fenoxaprop-P-ethyl (Gherekhloo et al., 2012). A difference in the resistance level of collected biotypes might be due to their unique

Table 1 - Percent mortality in selected *P. minor* biotypes three weeks after the application of fenoxaprop-P-ethyl

P. minor biotypes	Percent dry weight			
	0X	0.5X	X	2X
PM-FS-1	0.0 ± 0.0 a	41 ± 3.1 d	59 ± 0.68 d	71 ± 0.86c
PM-FS-2	0.0 ± 0.0 a	8.0 ± 1.3 ef	22 ± 1.0 f	56 ± 0.73 d
PM-FS-3	0.0 ± 0.0 a	77 ± 1.6 c	85 ± 1.2 c	99 ± 0.0 a
PM-FS-4	0.0 ± 0.0 a	84 ± 2.2 b	92 ± 0.94 bc	99 ± 0.32 a
PM-FS-5	0.0 ± 0.0 a	91 ± 1.5 ab	97 ± 1.4 ab	99 ± 0.0 a
PM-FS-6	0.0 ± 0.0 a	13 ± 0.8 e	37 ± 0.94 e	54 ± 0.94 d
PM-FS-7	0.0 ± 0.0 a	5.0 ± 1.5 f	21 ± 1.6 f	51 ± 1.1 de
PM-FS-8	0.0 ± 0.0 a	72 ± 2.0 c	88 ± 0.68 c	95 ± 1.3 b
PM-FS-0	0.0 ± 0.0 a	94 ± 1.6 a	99 ± 0.77 a	99 ± 0.0 a

X- Is the recommended dose of fenoxaprop-P-ethyl. Only the means in the same column were compared. The means marked with the same letter are not significantly different at the 5% reliability level. The data are the means ± standard error.

Table 2 - Biomass (control percentage) of selected *P. minor* biotypes three weeks after the application of fenoxaprop-P-ethyl

P. minor biotypes	Percent dry weight			
	0X	0.5X	X	2X
PM-FS-1	99 ± 0.0 a	77 ± 7.3 b	64 ± 1.7 c	43 ± 2.3fd
PM-FS-2	99 ± 0.0 a	84 ± 1.6 ab	81 ± 2.6 a	61 ± 2.4 c
PM-FS-3	99 ± 0.0 a	41 ± 2.3 fd	12 ± 1.4 d	0.0 ± 0.0 e
PM-FS-4	99 ± 0.0 a	38 ± 1.7 d	8.0 ± 0.72 e	0.0 ± 0.0 e
PM-FS-5	99 ± 0.0 a	34 ± 1.9 d	0.0 ± 0.0 f	0.0 ± 0.0 e
PM-FS-6	99 ± 0.0 a	81 ± 2.3 ab	75 ± 2.9 b	49 ± 2.1 b
PM-FS-7	99 ± 0.0 a	87 ± 2.4 a	78 ± 1.4 a	67 ± 2.6 da
PM-FS-8	99 ± 0.0 a	48 ± 1.6 c	15 ± 0.81 h	0.0 ± 0.0 e
PM-FS-0	99 ± 0.0 a	34 ± 1.8 d	0.0 ± 0.0 f	0.0 ± 0.0 e

X- is the recommended dose of fenoxaprop-P-ethyl. Only the means in the same column were compared. The means marked with the same letter are not significantly different at the 5% probability level. The data are the means ± standard error.

Table 3 - Field history, fenoxaprop-P-ethyl dose required to kill 50% of plants (LD_{50}) and resistance index (RI) of different biotypes of *P. minor*. See the note in MM

P. minor biotypes	Field history of wheat and herbicide use (years)		LD_{50} (g a.i ha^{-1})[1/]	Resistance index (RI)[2/]
	Wheat	fenoxaprop-P-ethyl		
PM-FS-1	>20.0	5.00-6.00	104.37	2.13
PM-FS-2	>20.0	>8.00	268.52	5.48
PM-FS-3	>20.0	3.00	<46.90	--
PM-FS-4	>20.0	5.00	<46.90	--
PM-FS-5	10.0	4.00	<46.90	--
PM-FS-6	>20.0	>10.0	242.55	4.95
PM-FS-7	>20.0	6.00	294.00	6.00
PM-FS-8	1.00	1.00	<46.90	--
PM-FS-0	0.00	0.00	49.00	--

[1/] LD_{50} was determined by conducting probit analysis in JMP 11. [2/] RI was calculated by dividing the LD_{50} dose (g a.i. ha^{-1}) of the resistant biotype by the LD_{50} dose of the susceptible biotype.

evolutionary herbicide selection pressure, since different biotypes of *P. minor* were selected from different locations with different wheat and herbicide crop histories. Fields from different locations received different herbicidal and non-herbicidal weed control approaches (Travlos et al., 2011). A difference in resistance level could also be due to different resistance mechanisms (altered target site, enhanced metabolism, existence of modified ACCase, compartmentalization or over-expression of the target protein) present in *P. minor* biotypes and other weed species (Maneechote et al., 1994; Gherekhloo et al., 2011; Travlos et al., 2011).

Conclusively, our research revealed that four biotypes out of eight collected from eight different locations have developed resistance to fenoxaprop-P-ethyl. The resistance is likely due to the lack of crop rotation and herbicide rotation. Due to the lack of herbicides with new modes of action, it is very important to know about the resistance status of different weeds against herbicides and to introduce alternate methods of weed control for the sustainability of the cropping system. Crop rotations, herbicide rotation, herbicide mixture, non-herbicidal weed control and other agronomic practices including cultural, mechanical and biological weed control methods need to be integrated in the weed management program to reduce the weed resistance problem (Cavan et al., 2000; om et al., 2004; Travlos et al., 2011).

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