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EVALUATION OF CLODINAFOP-PROPARGYL RESISTANT *AVENA FATUA* L. (WILD OAT) IN SARGODHA DIVISION OF PUNJAB-PAKISTAN

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Abstract

Avenafatua L. (Wild oat) is one of the world's worst agricultural weeds and is an ever-increasing threat in wheat growing areas of Pakistan. The injudicious application of herbicides for the control of *A. fatua* has resulted in the evolution of herbicide resistance and made it the second most herbicide resistance weed in the world. Studies were conducted to test the resistance status of *A. fatua*to clodinafop-propargyl during winter 2018-19 after conducting a field survey for the collection of suspected *A. fatua*seeds from various locations of Sargodha division of Punjab, Pakistan. For the resistance confirmation, dose-response assays were conducted under the laboratory and greenhouse conditions. Four doses of clodinafop-propargyl (0, 0.5X, 1X, and 2X) were applied at 3-4 leaf stages of *A. fatua.* Mortality percentage and dry biomass of different biotypes were recorded at three weeks after herbicide spray. Lethal dose to kill 50% of plants (LD_{50}), and resistance index (RI) were recorded based on mortality percentage. Results revealed that three biotypes (AF-SS-4, AF-SS-5 and AF-KSB-1) were resistant to clodinafop-propargyl. The mortality percentage for the resistant biotypes was 53%, 59% and 83%, respectively even at 2X. Resistance index was in the range of 5 to 7. Confirmation of *A. fatua*resistance to the selected herbicide was the second case for herbicide resistance in Pakistan. Further research is warranted to evaluate the spreading of herbicide-resistant *A. fatua* in other areas and to suggest alternate practices to control *A. fatua.*

Key words: ACCase-inhibiter; *Avena fatua*; Herbicide Resistance; LD_{50};Resistance index; Survey.

Introduction

Avena fatua L. (wild oat) is the most problematic annual weed plant, distributed in the temperate and subtropical areas of Asia, Canada, Europe, Australia and the USA (Holm *et al*., 1977; Beckie *et al*., 2012a; Ahmad-Hamdani *et al*., 2013; Harker *et al*., 2016). The distinct features like enormous seed production and the presence of varying levels of dormancy enable *A. fatua* to increase seed bank for several years (Qasem, 2007; Owen & Powles, 2009). The losses of crop yield and quality by the *A. fatua* interference and its control cost is an increasing threat throughout the world (Balyan *et al*., 1991, Jabran *et al*., 2010, Jack *et al*., 2017). Grassy areas, pastures, and uncultivated areas are also being infested by this weed (Beckie *et al*., 2012 a, b).

Avena fatua interference with cereal crops is causing severe yield losses up to 70% (Beckie *et al*., 2012b). It is a highly allelopathic weed and releases toxic phenolics in its rhizosphere (Ahmad *et al*., 2014), that inhibited growing crop seedling, soil microbes (Zhang *et al*., 2006; Jabran *et al*., 2010; Bajwa, 2014; Liu *et al*., 2016). Being extremely receptive to the applied nitrogen, *A. fatua*can utilize more resources (water and nutrients) than that of wheat (Balyan *et al*., 1991, SorkhyLalelo *et al*., 2008).

A lot of herbicides (barban, chlorfenprop, difenzoquat, linuron, metoxuron, metribuzin, monolinuron, and glyphosate) are being applied to control this weed (Terry, 1984; Beckie *et al*., 2002, 2006; Qasem, 2007; O'Donovan *et al*., 2013). It has been observed that the selected pressure for the application of herbicide is continuously increasing naturally arising resistant biotypes of various weeds including *A. fatua*. Herbicide resistance in this species was first reported from Western regions of the Australia in 1985, where this weed was resistant to diclofop-methyl in the wheat-growing fields (Heap, 2020). With the passage of time, this weed has shown resistance to the numerous marketed herbicides in different countries (Uludag *et al*., 2007, 2008; Adamczewski *et al*., 2013). This multiple resistance is evolving in *A. fatua* biotypes, particularly against ALS, ACCase inhibitor herbicides (Friesen *et al*., 2000; Tal *et al*., 2000; Beckie *et al*., 2008; Adamczewski *et al*., 2013).

Herbicides use is facing many challenges such as environmental and safety problems, and the evolution of resistance against different herbicides. In Pakistan, no study has been conducted to confirm the status of herbicide resistance of *A. fatua*. Further, there is a lack of accurate information on herbicide resistance in Pakistan besides Abbas *et al*., (2016) who reported the resistance level of *Phalaris minor* against fenoxaprop-P-ethyl (ACCase inhibitor). For sustainable wheat production, the evaluation and assessment of herbicide resistance levels in *A. fatua*and its subsequent control by using alternative herbicides and other methods are needed (Khan *et al*., 2019). Accordingly, this study was conducted to evaluate resistance and estimate the level of clodinafop-propargyl resistance in *A. fatua* and to determine alternative strategies for managing of *A. fatua* in wheat through chemical methods.

Materials and Methods

Field survey for the collection of *A. fatua* seeds: A field survey was conducted for the collection of *A. fatua* seeds from Sargodha, Khushab, and Mianwali districts of Sargodha division, Punjab, Pakistan. During the first phase of the survey, *A. fatua* seeds were collected from seven different locations of district Sargodha, for the evaluation of resistance. After the confirmation of herbicides resistance in *A. fatua* seeds of district Sargodha, two more districts (Khushab and Mianwali) were added in the second phase. Sites were selected for seeds collection of resistant plants which were suspected after discussing with the members of the department of agriculture extension.

Mostly those fields were selected where farmers were applying clodinafop-propargyl as a post-emergence herbicide for various years. Only, those plants were selected for seeds collection which survived after selected herbicides application during the current year. An initial trial was conducted for the evaluation of resistance status within different fields of the same location that showed similar results. Hence, these collected samples were representing each of the locations (Burgos *et al*., 2013). At maturity, seeds were collected by shaking the matured spikes. Seeds were placed under the shade for fully drying and stored at 25ºC (room temperature) in kraft paper bags. They were soaked for 24 hours in the distilled water to improve the germination before sowing (Om *et al*., 2004). Clodinafop-propargyl susceptible seeds of *A. fatua* were collected from a known site that has no herbicde use history.

Evaluation of clodinafop-propargyl resistance *Avena fatua* in Sargodha, Pakistan: Seeds of *A. fatua* biotypes were collected from seven different locations of Sargodha (31.41 N, 74.17 E), Pakistan. The collection of seeds from sites viz. Bhalwal, Bhera, KotMomin, Sahiwal, Sargodha, Shahpur and Sillanwali were named as AF-SS-1, AF-SS-2, AF-SS-3, AF-SS-4, AF-SS-5, AF-SS-6, and AF-SS-7, respectively. Historical data of different locations where seeds were collected are given in Table 3.

Confirmation of clodinafop-propargyl resistant *A. fatua* in two districts (Khushab and Mianwali) Punjab, Pakistan: After the evaluation of herbicides resistance in *A. fatua* seeds of district Sargodha, two more districts (Khushab and Mianwali) were added in the second phase. Seeds of *A. fatua* biotypes were collected from six different locations of districts Khushab and Mianwali. The collection of seeds from sites viz. NoorpurThal, Quaidabad, Khushab, Piplan, Isakhel and Mianwali were named as AF-KSB-1, AF-KSB-2, AF-KSB-3, AF-MNW-1, AF-MNW-2, and AF-MNW-3, respectively. Historical data of different locations where seeds were collected are given in Table 6.

Dose response bioassays for resistance confirmation: Repeated bioassays were conducted in the net house of College of Agriculture, University of Sargodha, Sargodha (31.41°N and 74.17°E), Punjab, Pakistan started during winter 2018. The soil was collected from the Agronomic Research field which has no history of herbicides application. Farmyard manure was mixed in the soil (1:2, w/w). Before putting into pots, the soil was dried, crushed and thoroughly mixed. Ten seeds were sown in the plastic pots (14×12×6 cm) separately from each susceptible and resistant collected samples. On the surface of soil, seeds were spread uniformly and hid with soil having the same weight in every pot to confirm uniform depth. Sprinkler irrigation with distilled water was applied as per need. The mean lowest and highest temperatures in the net house during the experiments were 20 ± 2 and 25 ± 2°C, respectively. The range of relative humidity was from 26-51%. The experiments were laid out in a completely randomized design (CRD) with a factorial arrangement having four replications.

Collected populations of *A. fatua* were sprayed at the 3-4 leaf stage (BBCH scale growth stage 13-14) at four rates (0, 0.5X, 1Xand 2X) of selected post-emergence herbicide clodinafop-propargyl. The recommended dose (X) for clodinafop-propargyl was 55g a.i. ha^{-1}. Distilled water was used for the preparation of different herbicide treatments and backpack sprayer with liquid CO_2 fixed pressure and TeeJet nozzle (8003VS) was used to spray. At 35 psi pressure, about 180 L ha^{-1} dose of herbicide was sprayed. After spraying of herbicide treatments, pots belonging to various populations were returned to the net house separately.

Parameter studied: Mortality percentage and biomass data were recorded three weeks after the application of herbicide. Percent mortality was the number of killed plants by the application of herbicide and the total number of plants before the spray of herbicide. The percentage of mortality (0% represented no plant injury while 100% represented the complete death of the plant) was calculated based on the average of ten randomly selected plants. Dry biomass of the plants was recorded by drying the above-ground parts of the already uprooted plants in the oven at 70°C until constant weight was obtained. The counted values of dry weight were presented as the control in percentage. The lethal dose needed to kill 50% (LD_{50}) of each biotype was determined by subjecting the calculated mortality data to probit the analysis with the application of nonlinear sigmoid curves in JMP 11.

$$f\big(x,(b,d,e)\big) = c + \frac{d-c}{1+\exp\{b\big(\log(x)-b\log(e)\big)\}}$$

where LD_{50} is represented by *e* while *d* and *c* represent the upper and lower limit, respectively. The parameter *b* indicates the relative slope around *e*.

The resistance level for each biotype was expressed in the form of resistance index (RI). It was obtained by dividing the LD_{50} value for resistant biotype with the LD_{50} value for susceptible biotype (Travlos & Chachalis, 2010; Travlos *et al*., 2011).

Statistical analysis

Collected data were statistically analyzed by using statistix 8.1® for ANOVA (Analysis of Variance) and at 5% probability level, Tukey's honestly significance difference (HSD) test was used for the testing of significance of treatment means (Steel *et al*., 1997).

Results

Evaluation of clodinafop-propargyl resistance *Avena fatua* in Sargodha, Pakistan: Two times repeated experiments were conducted and showed statistically same results. Therefore, only the second experiment's results have been explained in the text. Results revealed that out of seven *A. fatua* biotypes that were collected from different locations in Sargodha district, two biotypes (AF-SS-4 and AF-SS-5) were showed resistance against ACCase-inhibiting herbicide clodinafop-propargyl. There was highly reduction in mortality percentage and dry biomass of the plants three weeks after herbicide application. Visual observation of all the *A. fatua* biotypes showed a clear variance in the mortality percentage of various resistant and susceptible biotypes at different herbicide's doses as compared to the control. Data revealed that two biotypes AF-SS-4 and AF-SS-5 showed 59% and 53%, respectively, even at 2X of clodinafop-propargyl (Table 1, Fig. 1). While, in the other biotypes AF-SS-1, AF-SS-2, AF-SS-3, AF-SS-6, and AF-SS-7 mortality percentage was significantly more than resistant biotypes even at 2X. It is also observed that the mortality percentage was significantly increased by increasing herbicide dose, even in those biotypes which showed resistance against the herbicide.

The data regarding dry biomass, LD_{50} and resistance index (RI) represented that two selected biotypes of *A. fatua* showed resistance to clodinafop-propargyland the resistance level of each biotype was different at various rates of herbicide (Tables 2&3). At 0.5X of clodinafop-propargyl, the biomass reduction of resistant biotypes (AF-SS-4 and AF-SS-5) was 13-25%, while in the susceptible biotype (AF-SS-0) biomass reduction was about 72%. It is importantly noted that the biomass of resistant biotypes was reduced less than 37%, even at the 1X (recommended dose) of clodinafop-propargyl, while the biomass of susceptible biotype (AF-SS-0) was reduced up to 100% at the recommended dose of selective herbicide. It is also observed that the biomass of all the resistant biotypes was reduced even at the 2X (Table 2). Data analysis for LD_{50} and resistance index (RI) exposed that the LD_{50} values of resistant biotypes against clodinafop-propargyl ranged from 38.46 to 41.86 g a.i. ha^{-1}. Likewise, the resistant index of all biotypes explained that they indicated different resistance levels against the herbicide (Table 3). Data showed that the resistance index of *A. fatua* biotypes such as AF-SS-1, AF-SS-2, AF-SS-3, AF-SS-6, and AF-SS-7 was less than 5 against clodinafop-propargyl, so these nominated biotypes considered to be non-resistance biotypes.

Confirmation of clodinafop-propargyl resistant *A. fatua* in two districts (Khushab and Mianwali) Punjab, Pakistan: The symptoms from herbicidal injury on *A. fatua* were showing the yellow color of the leaves. These symptoms began from the earliest stages of the leaves and inhibited plant growth which is followed by the fully dead condition of the susceptible plants while there were minute injury symptoms in *A. fatua* resistant biotypes which were recovered over time. *Avena fatua* biotypes were collected from six different locations of district Khushab and district Mianwali and these biotypes showed different levels of resistance to clodinafop-propargyl. All the collected biotypes showed a different response to the herbicide for all the parameters such as mortality percentage, visual injury, dry biomass (% of the control), lethal dose to kill 50% (LD_{50}) (g a.i ha^{-1}) and resistance index (RI). Analysis of the data for mortality percentage showed that at 0.5X of clodinafop-propargyl only one biotype (AF-KSB-1) showed less than 40% mortality, while susceptible plants showed maximum mortality (100%). Similarly, at the recommended dose (1X), the resistant biotype (AF-KSB-1) showed less than 75% mortality (Table 4, Fig. 2).

Dry biomass, lethal dose to kill 50% (LD_{50}) and resistance index (RI) are considered as the most important parameters to assess resistance status in different weeds. Therefore, the data analysis regarding dry biomass indicated that the biomass of all the resistant and susceptible *A. fatua* biotypes was significantly influenced by different doses of clodinafop-propargyl. At 1X, two biotypes (AF-KSB-1 and AF-MNW-3) produced more than 50% biomass and four biotypes (AF-KSB-2, AF-KSB-3, AF- MNW-1 and AF-MNW-2) produced dry biomass more than 25% (Table 5). Similarly, data for LD_{50} and resistance index (RI) exposed that the LD_{50} value of resistant biotype against clodinafop-propargyl is 36.66 g a.i. ha^{-1}. While the resistant index of all biotypes explained that they indicated different resistance levels against selected herbicide. Data represented in table 6 showed that resistance index of *A. fatua* biotypes such as AF-KSB-2, AF-KSB-3, AF-MNW-1, AF-MNW-2, and AF-MNW-3 showed RI less than 5 against clodinafop-propargyl, so these nominated biotypes considered to be non-resistance biotypes against the herbicide.

Table 1. Mortality (%) of selected *A. fatua* biotypes three weeks after the application of clodinafop-propargyl.

A. fatua biotypes	Mortality (%)			
	0X	0.5X	X	2X
AF-SS-1	0.00 ± 0.00a	65 ± 2.7cd	79 ± 2.3c	91 ± 1.21b
AF-SS-2	0.00 ± 0.00a	76 ± 1.1c	83 ± 1.89c	99 ± 0.00a
AF-SS-3	0.00 ± 0.00a	59 ± 3.1d	77 ± 1.27c	85 ± 2.14bc
AF-SS-4	0.00 ± 0.00a	34 ± 0.89de	48 ± 2.88d	59 ± 0.99d
AF-SS-5	0.00 ± 0.00a	22 ± 0.2e	40 ± 0.39de	53 ± 0.35de
AF-SS-6	0.00 ± 0.00a	71 ± 3.5c	86 ±1.7bc	97 ± 0.5ab
AF-SS-7	0.00 ± 0.00a	84 ± 2.9b	90 ±2.54b	99 ± 0.41a
AF-SS-0	0.00 ± 0.00a	91 ± 2.7a	99 ± 1.04a	99 ± 0.00a

Here the required quantity of herbicide (clodinafop-propargyl-55 gram a.i. per hac) is denoted by "X". Means' comparison is represented in the similar column of table and the value of means having same letters which are statistically equal to each other at five percent probability level. The tabulated data is in the arrangement of means ± standard error

Table 2. Biomass (% of the control) of selected *A. fatua* biotypes three weeks after the application of clodinafop-propargyl.

*A. fatua*biotypes	Biomass (% of the control)			
	0X	0.5X	1X	2X
AF-SS-1	100 ± 0.00a	41 ± 3.67cd	24 ± 2.04d	0.00 ± 0.00e
AF-SS-2	100 ± 0.00a	33 ± 0.22d	9.0 ± 0.71e	0.00 ± 0.00e
AF-SS-3	100 ± 0.00a	49 ± 1.76c	28 ± 3.42d	0.00 ± 0.00e
AF-SS-4	100 ± 0.00a	75 ± 4.2b	63 ± 2.1bc	42 ± 5.11bc
AF-SS-5	100 ± 0.00a	87 ± 2.43a	79 ± 1.21a	60 ± 4.78da
AF-SS-6	100 ± 0.00a	39 ± 1.49cd	15 ± 0.96de	0.00 ± 0.00e
AF-SS-7	100 ± 0.00a	31 ± 0.94d	5.00 ± 1.03e	0.00 ± 0.00e
AF-SS-0	100 ± 0.00a	28 ± 0.31de	0.00 ± 0.00e	0.00 ± 0.00e

Here the required quantity of herbicide (clodinafop-propargyl-55 gram a.i. per hac) is denoted by "X". Means' comparison is represented in the similar column of table and the value of means having same letters which are statistically equal to each other at five percent probability level. The tabulated data is in the arrangement of means ± standard error

Table 3. Historical data of wheat growing fields, clodinafop-propargylrequired dose to kill 50% plants (LD_{50}) and resistance index (RI) of different biotypes of *A. fatua.*

A. fatua biotypes	Historical data of wheat fields and uses of herbicide (years)		LD_{50} (g a.i per hac)[a]	Resistance index (RI)[b]
	Wheat	Clodinafop-propargyl		
AF-SS-1	>18.0	4.00	26.46	4.35
AF-SS-2	15.0	3.00	22.54	3.70
AF-SS-3	>20.0	>2.00	27.68	4.55
AF-SS-4	13.0	6.00	38.46	6.32
AF-SS-5	>15.0	5.00-8.00	41.86	6.84
AF-SS-6	>20.0	>4.00	24.27	3.99
AF-SS-7	15.0	5.00	15.79	2.59
AF-SS-0	0.00	0.00	6.08	-

[a] LD_{50} was calculated through conducting probit analysis in JMP 11 statistical softewear

[b]RI was determined by dividing the value of LD_{50} dose (g a.i. ha^{-1}) for resistant biotype by the value of LD_{50} dose for susceptible biotype

Table 4. Mortality (%) of selected *A. fatua* biotypes three weeks after the application of clodinafop-propargyl.

A. fatua biotypes	Mortality (%)			
	0X	0.5X	1X	2X
AF-KSB-1	0.00 ± 0.00a	36 ± 1.34de	72 ± 1.74c	83 ± 2.39bc
AF-KSB-2	0.00 ± 0.00a	68 ± 1.49c	79 ± 3.56b	94 ± 2.77a
AF-KSB-3	0.00 ± 0.00a	76 ± 1.12bc	88 ± 2.61ab	100 ± 0.00a
AF-MNW-1	0.00 ± 0.00a	63 ± 2.59c	78 ± 1.26b	95 ± 3.45a
AF-MNW-2	0.00 ± 0.00a	82 ± 3.45b	97 ± 1.34a	100 ± 0.00a
AF-MNW-3	0.00 ± 0.00a	56 ± 3.10d	75 ± 0.44bc	90 ± 2.58ab
Susceptible biotype	0.00 ± 0.00a	100 ± 0.00a	100 ± 0.00a	100 ± 0.00a

Here the required quantity of herbicide (clodinafop-propargyl-55 gram a.i. per hac) is denoted by "X" .Means' comparison is represented in the similar column of table and the value of means having same letters which are statistically equal to each other at five percent probability level. The tabulated data is in the arrangement of means ± standard error

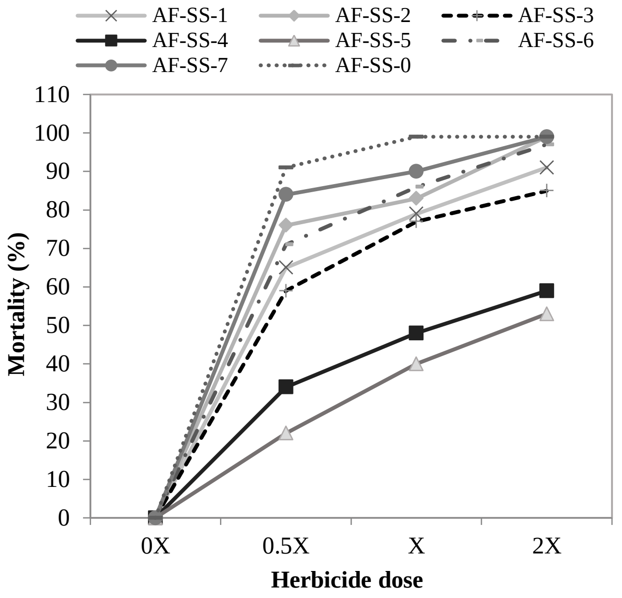

Fig. 1. Dose-response curve for the efficacy of different doses of clodinafop-propargyl on the mortality percentage of selected *A. fatua* biotypes three weeks after its application.

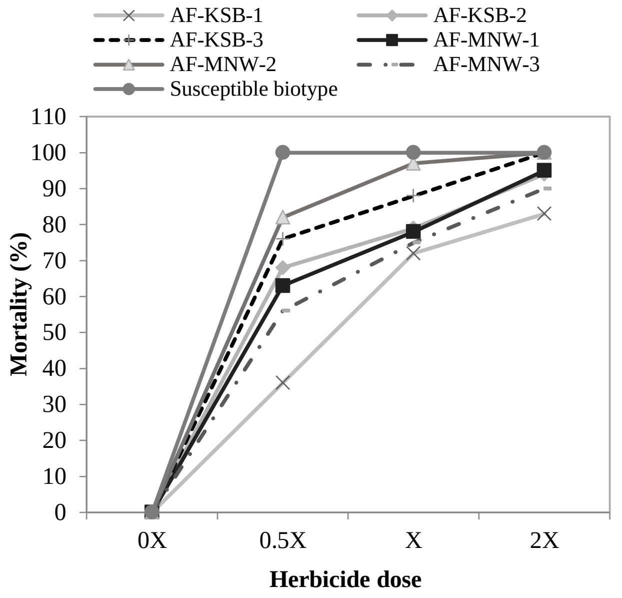

Fig. 2. Dose-response curve for the efficacy of different doses of clodinafop-propargyl on the mortality percentage of selected *A. fatua* biotypes three weeks after its application.

Table 5. Biomass (% of the control) of selected *A. fatua* biotypes three weeks after the application of clodinafop-propargyl.

A. fatua biotypes	Biomass (% of the control)			
	0X	0.5X	1X	2X
AF-KSB-1	100 ± 0.00a	81 ± 2.37a	66 ± 1.29ab	49 ± 2.02a
AF-KSB-2	100 ± 0.00a	56 ± 2.78cd	42 ± 2.45c	33 ± 1. 47b
AF-KSB-3	100 ± 0.00a	52 ± 3.72cd	44 ± 2.58c	26 ± 3.32c
AF-MNW-1	100 ± 0.00a	59 ± 2.38c	46 ± 4.01c	31 ± 3.43bc
AF-MNW-2	100 ± 0.00a	37 ± 0.34 de	26 ± 2.16d	0.0 ± 0.0d
AF-MNW-3	100 ± 0.00a	68 ± 3.24bc	51 ± 1.52bc	39 ± 0.45ab
Susceptible biotype	100 ± 0.00a	0.0 ± 0.0f	0.0 ± 0.0e	0.0 ± 0.0d

Here the required quantity of herbicide (clodinafop-propargyl-55 gram a.i. per hac) is denoted by "X". Means' comparison is represented in the similar column of table and the value of means having same letters which are statistically equal to each other at five percent confidence level. The tabulated data is in the arrangement of means ± standard error

Table 6. Origin, location, and field history, clodinafop-propargylrequired doses to kill 50% plants (LD_{50}) and resistance index (RI) of different biotypes of *A. fatua.*

Biotypes	Origin	Locations	Field history		Clodinafop-propargyl	
			Cropping system	Herbicide use (years)		
				Clodinafop-propargyl	LD_{50} (g a.i per hac) [a]	Resistance index (RI)[b]
AF-KHB-1	Khushab	32°17'- 32°27' N, 72°21' - 72°29' E	Rice-Wheat	5-6	36.66	5.25
AF-KHB-2	Khushab	31°52'-31°63' N, 71°53'-71°58' E	Rice-Wheat	>3	27.50	3.94
AF-KHB-3	Khushab	32°19'- 32°25' N, 71°54'- 71°59' E	Maize-Wheat	4	24.99	3.58
AF-MNW-1	Mianwali	32°34'-32°39' N, 71°31'- 71°42' E	Rice-Wheat	4-5	27.65	3.96
AF-MNW-2	Mianwali	32°16'- 32°22' N, 71°21'- 71°24' E	Maize-Wheat	3-4	17.43	2.49
AF-MNW-3	Mianwali	32°52'-32°59' N, 70°54'-70°60' E	Cotton-Wheat	4	29.50	4.23
Susceptible	Sargodha	31°49'-31°53' N, 72°32'- 72°39' E	Susceptible	0	6.97	-

[a] LD_{50} was calculated through conducting probit analysis in JMP 11 statistical softewear

[b]RI was determined by dividing the value of LD_{50} dose (g a.i. ha^{-1}) for resistant biotype by the value of LD_{50} dose for susceptible biotype

Discussion

Results of this study revealed widespread resistance in *A. fatua* against commonly used herbicide such as clodinafop-propargylmostly in wheat-growing regions. Our results are strongly supported by Owen *et al*., (2007) who indicated that the use of the same herbicide for the long period-imposed selection pressure caused resistance to develop and to select resistant biotypes. Resistance status in *A. fatua* against clodinafop-propargyl, and other marketed herbicides has been recorded in several countries over the world (Uludag *et al*., 2007, 2008; Adamczewski *et al*., 2013; Heap, 2020). These findings are also in line with Friesen *et al*., (2000) who assessed the herbicides resistance in *A. fatua* biotypes against fenoxaprop-P-ethyl, flamprop, and imazamethabenz. Similarly, Stokłosa & Kieć (2006) conducted lab and field experiments and the results of their study are related to our findings, they exposed that in the laboratory test, ≥50% survived plants and in field condition, ≥50% flowering plants of *A. fatua*species were predicted as the herbicides resistant plants. Our results are in line with Owen & Powles (2009) who explained that about 71% biotypes of *A. fatua* were found highly resistant to ACCase-inhibiting herbicides and comparatively less resistant to other herbicides of this inhibiting group and the reductions in the dry biomass of resistant biotypes were less than susceptible biotypes. Another same experiment was conducted by Friesen *et al*., (2000) who reported that resistant biotypes showed 7 to 8 times more resistance than susceptible biotypes to the applied herbicides which were measured by the ratio of recommended doses to reduce the dry biomass accumulation by 50% growth reduction. They described that the dry biomass of resistant biotypes were more than susceptible plants. Our results are supported by findings of Nandula&Messersmith (2001) who worked on resistance confirmation of *A. fatua* and found that there was a clear difference between the value of $LD_{50,}$ and 50% growth reduction (GR50) ratio of resistant and susceptible biotypes against ACCase-inhibitors and acetolactate synthase (ALS)-inhibitors. Similarly, Friesen *et al*., (2000) explained that resistant *A. fatua* biotypes showed different responses to fenoxaprop-P-ethyl herbicide and the resistance level for two biotypes was 2.0 fold and 2.9 fold for the remaining biotype.

Conclusion

The findings from these experiments concluded that among seven *A. fatua* biotypes that were collected from three different districts, three biotypes showed resistance to clodinafop-propargyl and the conducted survey showed that herbicide resistance in *A. fatua* biotypes has developed against clodinafop-propargyl in selected wheat-producing areas. The resistance against herbicides is expected due to the minimum practices of herbicide and crop rotation. Availability of less amount of herbicides with new sites of actions is necessary to evaluate the resistance level of various weeds against commonly marketed herbicides and to use alternate practices of chemical weed control for sustainable agriculture. However, resistance status against other marketed herbicides could also create if the farmers are growers will not follow the suitable using practices to avoid the development of resistance against different herbicides.

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