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FIRST REPORT ON *AVENA FATUA* RESISTANCE TO FENOXAPROP-P-ETHYL AND EFFICACY OF HERBICIDE MIXTURES FOR ITS EFFECTIVE CONTROL IN WHEAT

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SUMMARY

Effective herbicide resistance management in *Avena fatua* is a crucial challenge to sustain wheat production. Using classic bioassay confirmed fenoxaprop-P-ethyl resistance in *A. fatua* biotypes in four districts of Punjab, Pakistan (Khushab, Mianwali, Bhakkar, and Sargodha). The efficacy testing of herbicide mixtures comprising clodinafop-propargyl plus metribuzin, pinoxaden plus sulfosulfuron, pinoxaden plus metribuzin, and sulfosulfuron plus clodinafop-propargyl had concentrations at 100%, 75%, and 50% of the recommended dose for each herbicide to control *A. fatua* resistant to fenoxaprop-P-ethyl. Results indicated that *A. fatua* displayed widespread resistance to fenoxaprop-P-ethyl, ranging from 4.12 to 5.49, in all the surveyed districts. Every mixture of herbicides demonstrated 100% mortality at 100% and 75% of the authorized dose, demonstrating high efficacy in suppressing the weeds. At 100% and 75% of the recommended dose, every tested herbicide mixture showed 100% mortality, indicating high efficacy in controlling weeds. At 50% doses, clodinafop-propargyl plus metribuzin exhibited higher efficacy in controlling weeds than other herbicide mixtures. It is the first report of *A. fatua* from Punjab, Pakistan, stating resistance to fenoxaprop-P-ethyl. The improved herbicide combinations will be beneficial in minimizing the possible emergence of cross-resistance, decreasing wheat production losses, and efficiently managing *A. fatua*.

Keywords: ACCase resistance, classical bioassay, herbicide resistance, wheat sustainability, wild oat

Key findings: Empirical studies validate the extensive dispersion of fenoxaprop-P-ethyl-resistant *A. fatua* in major wheat-producing regions, such as Khushab, Mianwali, Bhakkar, and Sargodha, Pakistan. All tested herbicide mixtures effectively suppressed both susceptible and fenoxaprop-P-ethyl-resistant *A. fatua* biotypes. The combination of metribuzin and clodinafop-propargyl displayed the best efficacy in controlling *A. fatua*.

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INTRODUCTION

The emergence of herbicide resistance severely hampered globally sustainable crop production (Clay, 2021). Chemical weed management began earlier and was more intensive in industrialized countries, where several weed species have already acquired resistance to herbicides (Heap, 2024). A particularly problematic weed of wheat and other cereal crops, wild oat (*Avena fatua* L.) severely reduces wheat yields (Bajwa *et al*., 2017). This plant has increased over numerous nations worldwide. As it resembles a wheat crop, using herbicides is the most inexpensive, efficient, and successful strategy to manage this weed (Bajwa *et al*., 2017). Controlling *A. fatua* in wheat fields has employed a variety of herbicides. The commonly used herbicides in wheat to control *A. fatua* include clodinafop-propargyl, diclofop-methyl, fenoxaprop-ethyl, flamprop-methyl, mesosulfuron-methyl, cycloxydim, pinoxaden, propaquizafop, and imazamethabenz-methyl. Different countries have recorded the development of herbicide resistance in *A. fatua* against these herbicides (Bajwa *et al*., 2017; Heap, 2024).

Herbicide resistance has become an issue of great magnitude to farmers in Pakistan (Abbas *et al*., 2016; Abbas *et al*., 2024). In recent times, numerous weed species have exhibited resistance to different herbicides. A 2016 report, for example, stated the fenoxaprop-P-ethyl-resistant *Phalaris minor* was the first instance of a resistant weed from Pakistan (Abbas *et al*., 2016a). Raza *et al*. (2021) indicated that *A. fatua* resistance to clodinafop-propargyl developed, a widely used herbicide to control leaved weeds in wheat fields. Furthermore, Abbas *et al.* (2024) evaluated the bispyribac-sodium-resistant *Echinochloa colona* in Punjab rice fields, highlighting its significant threat to sustainable rice production.

The continuous rise in herbicide resistance is one of the threats facing sustainable agriculture, especially since chemical weed control remains very common among Pakistani farmers (Matloob *et al*., 2020). This dependence on chemical weed control could result in severe crop yield losses brought about by uncontrolled weeds due to improperly handling the herbicide resistance. Recently, after using fenoxaprop-ethyl, wheat growers had trouble with uncontrolled *A. fatua* in their fields.

Fenoxaprop-ethyl is an effective and frequently used herbicide in wheat fields, with earlier enrolment in Pakistan (Abbas *et al*., 2016a). Identification and management of herbicide-resistant weeds are crucial. Among various approaches to resistance management, using herbicide mixtures is a highly successful strategy for controlling resistant weeds and reducing further evolution of endurance (Abbas *et al*., 2016b; Abbas *et al*., 2018a, b). Early detection of resistance is essential to minimize uncontrolled spread through seeds of resistant biotypes and yield losses from weed infestation (Abbas *et al*., 2024).

A published material on *A. fatua*'s status concerning its resistance within Pakistan is nonexistent. Therefore, this study aimed to assess fenoxaprop-ethyl resistance among *A. fatua* biotypes collected from four districts, Khushab, Mianwali, Bhakkar, and Sargodha, renowned for wheat production in the Punjab province. In addition, an evaluation of different herbicidal mixtures will distinguish the management of fenoxaprop-ethyl-resistant *A. fatua* populations in wheat fields. Early detection of herbicide-resistant *A. fatua* and optimization of management strategies is crucial for effectively controlling and reducing the further spread of resistant biotypes.

MATERIALS AND METHODS

Classical bioassay for resistance confirmation

A repeated dose-response bioassay continued to confirm resistance in *A. fatua* against fenoxaprop-P-ethyl under greenhouse conditions at the University of Sargodha, Sargodha, Pakistan. The experiment employed a complete randomized design with four replicates. Five different herbicide concentrations comprised T_1: 0X (control), T_2:

0.5X, T_3: 1X, T_4: 2X, and T_5: 4X, where X is a recommended dose (93.75 g a.i. ha^{-1}) of herbicide.

The collection of resistant-suspected biotype seeds of *A. fatua* commenced in four districts, including Khushab, Mianwali, Bhakkar, and Sargodha, Pakistan. The selection of locations consisted of six from Khushab, eight from Mianwali, five from Bhakkar, and nine from Sargodha, Pakistan. Resistance susceptible biotypes with no exposure to herbicide were also part of the collection from each district. The gathered seeds in the survey remained stored in a dry place in paper bags at room temperature until sowing. Fifteen seeds sown in each plastic pot remained until after emergence, with 10 plants maintained per pot. Twenty-one days after emergence (at the 3–4 leaf stage), *A. fatua* plants received sprays with different herbicide doses. The herbicide application used a CO2 pressurized sprayer equipped with a TeeJet 8003VS nozzle, operating at 30 psi, with an approximate spray volume of 186 liters per hectare. Three weeks after spraying, the mortality percentage recording utilized a 0%–100% rating scale, where 0 indicates no effect of treatment and 100 implies complete control. Determining the resistant index depended on mortality percentage, LD_{50}, and resistance level (Abbas *et al.,* 2016a). The data regarding mortality underwent probit analysis using inverse prediction of logistic 3P in JMP 13 to calculate the herbicide rate necessary to kill 50% of (LD50) of each biotype.

$$LD50 = c/(1+\exp[-a\{dose-b\}])$$

Where: a is the growth rate, b is an inflection point, and c is an asymptote.

The resistance level of various resistant *A. fatua* biotypes takes the label resistance index (RI), determined as a ratio of LD_{50} of resistance by the LD_{50} of the susceptible biotype (Abbas *et al.,* 2016a).

Optimization of herbicide mixtures

A repeated greenhouse study ensued to evaluate the efficacy of herbicide mixtures in controlling the fenoxaprop-P-ethyl-resistant *A. fatua*. Four different herbicide mixtures for testing included clodinafop-propargyl plus metribuzin, pinoxaden plus sulfosulfuron, pinoxaden plus metribuzin, and sulfosulfuron plus clodinafop-propargyl. Each mixture application had three doses: 100%, 75%, and 50% of the recommended dose for each herbicide. The recommended field rate of herbicides to control *A. fatua* was at 100% dose. The recommended doses for clodinafop-propargyl, metribuzin, pinoxaden, and sulfosulfuron were 55, 425, 45, and 50 g a.i. ha^{-1}, respectively. The second factor was *A. fatua*-resistant and susceptible biotypes. The *A. fatua* biotype, AF-SG-42, with a maximum RI (5.34), became the resistant biotype. The considered susceptible biotype from Sargodha, AF-SG-SS, served as a susceptible biotype in this study. The sown 15 seeds in each plastic pot were near the surface. Pots contained soil with well-decomposed farmyard manure at a 1:1 ratio. Ten plants remained in each pot after emergence. Mixture application continued at the 3–4 leaf stage of *A. fatua* plants. Herbicide spraying utilized a CO2 pressurized sprayer equipped with a TeeJet 8003VS nozzle, operating at 30 psi, with an approximate spray volume of 186 liters per hectare.

Mortality percentage determination applied the same procedure in experiment 1. For dry biomass attainment, carefully uprooted plants proceeded to oven drying at 70 °C to achieve a constant weight. Plant harvesting at maturity had the number of seeds per spike counted manually.

Statistical analysis

Both experiments had two repeats. The results of repeated experiments showed no significant difference. Therefore, mean values of repeated studies were items for statistical analysis. The data analysis used Fisher's analysis of variance technique (Steel *et al.,* 1997), with treatment's means compared by Tukey's test at a 5% significance level.

RESULTS

Fenoxaprop-P-ethyl-resistant *A. fatua* in Khushab

Mortality (%)

All biotypes showed differences in mortality under different herbicide doses (Table 1), with the highest mortality recorded with the susceptible standard (AF-KB-SS). Among biotypes under investigation, AF-KB-04 resulted in the highest mortality (100%) of *A. fatua* at the recommended dose, whereas AF-KB-26 showed significantly the lowest mortality (40%). At double the recommended dose (2X), the biotype AF-KB-04 gave the maximum mortality (100%), followed by AF-KB-02 (96.67%) against the minimum mortality by AF-KB-26 (60%), followed by AF-KB-19 (76.67%). At four times higher doses (4X), all the biotypes bared complete mortality except AF-KB-26 (70%), AF-KB-19 (90%), and AF-KB-06 (93.33%). All the biotypes provided an increase in mortality with an increase in herbicide dose. The complete visual injury appeared except for AF-KB-26 (73.33%), AF-KB-19 (92%), and AF-KB-06 (92.33%). All the biotypes displayed increasing visual injury with an escalation in herbicide dose.

LD-50 and Resistance Index (%)

The data about LD_{50} and resistance index (RI) is available in Table 1. The susceptible standard (AF-KB-SS) showed significantly lower LD_{50} values. The biotype AF-KB-26 gave the highest values of LD_{50} (139.15 g a.i. ha^{-1}) and resistance index (4.49), followed by AF-KB-26 with LD_{50} of 93.23 g a.i. ha^{-1} and resistance index of 3.01; however, was statistically at par with AF-KB-06 (91.17 g a.i. ha^{-1} LD_{50} and 2.94 resistance index).

Fenoxaprop-P-ethyl-resistant *A. fatua* in Mianwali

Mortality (%)

All biotypes differed in percent mortality compared with the control. The biotype AF-MW-36 showed the lowest mortality values at all herbicide levels (Table 2). At the recommended dose (1X), none of the biotypes gave 100% mortality, while a minimum (36.67%) was notable with AF-MW-36. When increasing the dose of herbicide to double the recommended dose (2X), the mortality of all the biotypes increased, but only AF-MW-07 and AF-MW-18 showed 100% mortality. The treatment receiving the dose four times the recommended dose (4X) resulted in the utmost mortality compared with all other herbicide doses, and 100% mortality was evident in all biotypes except AF-MW-13 and AF-MW-36. The lowest mortality appeared with biotype AF-MW-36 (73.33%).

LD-50 and Resistance Index (%)

The data about LD_{50} and resistance index (RI) for district Mianwali appears in Table 2. The susceptible standard (AF-MW-SS) showed significantly lower LD_{50} values. The biotype AF-MW-36 showed the highest values of LD_{50} (128.57 g a.i. ha^{-1}) and resistance index (4.14), followed by AF-MW-13 with the LD_{50} of 94.25 g a.i. ha^{-1} and resistance index of 3.04. The lowest resistance index value and LD_{50} manifested in biotype AF-MW-03 (2.35 and 73.49 g a.i. ha^{-1}).

Fenoxaprop-P-ethyl-resistant-*A. fatua* in Bhakkar

Mortality (%)

All biotypes significantly increased the percent mortality compared with the check. At the recommended dose and double the recommended dose (2X), none of the biotypes except the susceptible standard resulted in 100% mortality, with a minimum mortality (60.67%) observed in AF-BK-26 (Table 3). The mortality increased when raising the herbicide dose in all biotypes, and at four times the herbicide dose (4X), all biotypes showed complete mortality except AF-BK-06 and AF-BK-26. The biotype AF-BK-26 resulted in the lowest mortality (23.33% to 80%) at all herbicide doses.

Table 1. The mortality (%), LD_{50}, and RI in selected *A. fatua* biotypes of district Khushab.

Biotypes	Doses of herbicides					LD-50	Resistance Index (%)
	0.0X	0.5X	1X	2X	4X		
AF-KB-02	0.00 ± 0.00a	53.33± 0.03 a	73.33±0.98bc	96.67 ± 0.99 a	100.00 ± 0.00a	71.85 d	2.32 d
AF-KB-04	0.00 ± 0.00a	40.00± 0.71a	80.00± 0.67 b	100.00 ± 0.96a	100.00 ± 0.67a	69.67 d	2.25 d
AF-KB-06	0.00 ± 0.00a	43.33±1.23a	56.667± 1.02d	80.00 ± 0.67 b	93.33±0.56ab	91.17 b	2.94 b
AF-KB-12	0.00 ± 0.00a	46.67± 1.23 a	66.67 ±1.03cd	90.00 ± 0.65 ab	100.00 ±0.56a	78.04 c	2.52 c
AF-KB-19	0.00 ± 0.00a	43.33± 0.98 a	60.00 ± 0.67 d	76.67 ± 0.98 b	90.00 ±0.87b	93.23 b	3.01 b
AF-KB-26	0.00 ± 0.00a	30.00±1.01 b	40.00 ±0.89 e	60.00 ± 0.09 c	70.00 ± 0.90c	139.15 a	4.49 a
AF-KB-SS	0.00 ± 0.00a	55.00± 0.03 a	100.00 ±0.00a	100.00 ± 0.00a	100.00 ± 0.00a	30.97 e	----

X represents the field rate of fenoxaprop-P-ethyl. Means in the same column with different letters are statistically different at the 5% confidence level. The data present means ± standard error.

Table 2. The mortality (%), LD_{50}, and RI of selected *A. fatua* biotypes of district Mianwali.

Biotypes	Doses of herbicides					LD-50	Resistance Index (%)
	0.0X	0.5X	1X	2X	4X		
AF-MW-03	0.00 ± 0.00a	33.33± 0.19 c	61.67 ±0.95 bc	80.00± bc	100.00 ± a	79.14 d	2.55 d
AF-MW-07	0.00 ± 0.00a	40.00 ± 0.20 bc	61.67 ±0.12 bc	100.00 ± a	100.00 ± a	73.49 f	2.37 f
AF-MW-09	0.00 ± 0.00a	35.00± 0.23 c	50.00± bcd 0.98	88.33± ab	100.00 ± a	83.13 c	2.68 c
AF-MW-13	0.00 ± 0.00a	31.67 ±0.99 c	48.33± 1.23 cd	68.33± cd	90.00± b	94.25 b	3.04 b
AF-MW-18	0.00 ± 0.00a	50.00± 0.95 b	66.67 ±1.23 b	100.00 ± a	100.00 ± a	75.82 d	2.44 e
AF-MW-26	0.00 ± 0.00a	31.67 ±0.96 c	45.00±2.23 cd	68.33± cd	100.00± a	94.77 b	3.06 b
AF-MW-33	0.00 ± 0.00a	41.67 ±1.02 bc	58.33± bc	78.33± bc	100.00 ± a	81.79 c	2.64 c
AF-MW-36	0.00 ± 0.00a	20.00±0.56 d	36.67 ± d	56.67 ± d	73.33± c	128.57 a	4.14 a
AF-MW-SS	0.00 ± 0.00a	65.00 ± a	100.00 ± a	100.00 ± a	100.00 ± a	31.05 g	----

X represents the field rate of fenoxaprop-P-ethyl. Means in the same column with different letters are statistically different at the 5% confidence level. The data present means ± standard error.

Table 3. The mortality (%), LD_{50}, and RI of selected *A. fatua* biotypes of district Bhakkar**.**

Biotypes	Doses of herbicides					LD-50	Resistance Index (%)
	0.0X	0.5X	1X	2X	4X		
AF-BK-03	0.00 ± 0.00a	43.33 ± 0.61 b	50.00 ± 0.87 c	90 ± 1.34ab	100 ± 0.00a	65.70 d	2.19 d
AF-BK-06	0.00 ± 0.00a	46.67 ± 0.98 ab	56.67 ±0.87 bc	66.67 ± 1.34 c	93.33 ± 0.67a	97.77 b	3.26 b
AF-BK-13	0.00 ± 0.00a	43.33 ±1.45 b	60.00 ± 1.23 bc	90 ± 0.78 ab	100 ± 0.00 a	82.85 c	2.76 c
AF-BK-21	0.00 ± 0.00a	50.00 ±1.56 ab	66.67 ± 1.451 b	83.33 ± 0.67 b	100 ± 0.00 a	82.40 c	2.75 c
AF-BK-26	0.00 ± 0.00a	23.33 ± 0.78 c	46.67 ± .56 c	60.67 ±1.45 c	80 ± b	123.73 a	4.12 a
AF-BK-SS	0.00 ± 0.00a	60.00 ± 0.67a	100 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a	30.01 e	----

X represents the field rate of fenoxaprop-P-ethyl. Means in the same column with different letters are statistically different at the 5% confidence level. The data present means ± standard error.

LD-50 and Resistance Index (%)

The results regarding LD_{50} and RI exhibited that AF-BK-26 from selected biotypes of *A. fatua* revealed more resistance to fenoxaprop-P-ethyl (Table 3). However, the RI differed depending on the biotype. The AF-MW-06 showed slight resistance to fenoxaprop-P-ethyl. LD_{50} values of resistant biotype (AF-BK-26) was 123.73g a.i. ha^{-1}, and its resistance index was 4.12.

Fenoxaprop-P-ethyl resistant-*A. fatua* in Sargodha

Mortality (%)

All biotypes varied in mortality %. In AF-SG-10 and AF-SG-42 biotypes, percent mortality was very low, even at 4X. However, the *A. fatua* biotypes AF-SG-02, AF-SG-32, AF-SG-38, AF-SG-47, AF-SG-52, and AF-SG-SS displayed higher mortality (100%) at 4X (Table 4). With the increase in herbicide dose, the mortality % was significant for all the biotypes.

LD-50 and Resistance Index (%)

The results regarding LD_{50} and RI are in Table 4. The data showed that AF-SG-42 biotypes of *A. fatua* showed the highest LD_{50} and RI to fenoxaprop-P-ethyl, followed by AF-SG-10. All the biotypes under investigation showed a resistance index greater than two, indicating a lesser resistance level. Two biotypes, AF-GS-10 and AF-SG-42, gave a resistance index of more than five.

Efficacy of herbicide mixtures to control *A. fatua*

The herbicide mixtures significantly affected the resistance and susceptible biotype of *A. fatua* (Table 5). The clodinafop-propargyl plus metribuzin application at 100% of R for each herbicide in the mixture (T_1) resulted in 100% mortality for both biotypes under investigation. However, the minimum mortality (0.00%) was evident with the weedy check (T_{13}). Among biotypes, the maximum mortality (94.69%) was prominent for the susceptible biotype and the minimum (93.48%) for the resistant biotype. The interactive effect of herbicide mixture and *A. fatua* biotypes also emerged as significant. The maximum mortality was noteworthy with the susceptible biotype at clodinafop –propargyl plus metribuzin (100% of R for each), while the minimum mortality (0.00%) by resistance biotype under the weedy check.

Various herbicide mixture combinations statistically affected the biomass of *A. fatua* biotypes (Table 5). The application of herbicide mixtures in the mixture at 100% of the recommended rate and 75% of the recommended rate caused 100% mortality to *A. fat*ua biotypes; hence, no data regarding the biomass of *A. fatua* biotypes was notable. The data was evident only from treatments with some survivors. Among surviving plants, the maximum biomass (5.53–5.58 g) was markable in the weedy check (T_{13}). However, the minimum (3.37–3.42 g) was with clodinafop–propargyl + metribuzin (50% of R for each) (T_3). No significant difference in the biomass of surviving plants surfaced after applying herbicide mixtures.

Similarly, various herbicide mixture combinations statistically affected the number of seeds per plant of *A. fatua* biotypes (Table 5). The seed production data recording for the surviving plants ensued after they matured following herbicide application. A significantly higher number of seeds emerged in the weedy check, ranging from 166 seeds per plant for herbicide-resistant biotypes to 165 seeds for herbicide-susceptible biotypes. Among applications of herbicide mixtures, a minimum seed of 42.75 to 39.50 per plant resulted in plants treated with clodinafop-propargyl plus metribuzin at 50% of the recommended rate. Plants treated with sulfsulfuran plus clodinafop –propargyl at 50% of the recommended dose produced substantially more seeds (61.75 to 64.75 seeds per plant) than other herbicide mixtures.

DISCUSSION

The study uncovered extensive resistance to fenoxaprop-P-ethyl in *A. fatua*. Biotypes

Table 4. The mortality (%), LD_{50}, and RI of selected *A. fatua* biotypes of district Sargodha.

Biotypes	Doses of herbicides					LD-50	Resistance Index (%)
	0.0X	0.5X	1X	2X	4X		
AF-SG-02	0.00 ± 0.00a	30.00± 1.23 bc	45.00± 0.45 cd	63.33± 0.45 def	93.33± 0.23 a	94.68 c	3.05 c
AF-SG-10	0.00 ± 0.00a	18.33± 0.45 d	36.67 ± 0.56 d	53.33± 0.56 ef	73.33 ± 0.49 b	157.96 b	5.09 b
AF-SG-26	0.00 ± 0.00a	40.00 ± 0.39 b	60.00± 0.67 b	73.33± 0.58 cde	98.33 ± 0.12 a	87.95 d	2.84 d
AF-SG-32	0.00 ± 0.00a	37.00± 0.15 b	53.33± 0.12 bc	80.00 ± 0.12 abcd	95.00 ± 0.45 a	88.66 d	2.86 d
AF-SG-38	0.00 ± 0.00a	35.00± 0.26 b	53.33± 0.67 bc	80.00± 0.69 abcd	100.0±0.00a	82.85 e	2.67 e
AF-SG-41	0.00 ± 0.00a	30.00± 0.76 bc	50.00± bcd0.49	76.67 ± 0.59 bcd	96.68 ± 0.87 a	89.66 c	2.89 d
AF-SG-42	0.00 ± 0.00a	20.00± 0.69 cd	36.66± 0.76 d	50.00± 0.67 f	72.00± 0.18 b	165.53 a	5.34 a
AF-SG-47	0.00 ± 0.00a	33.33± 0.87 b	56.67 ± 0.69 bc	92.50 ± 0.12 ab	100.00 ± 0.00 a	77.18 f	2.49 f
AF-SG-52	0.00 ± 0.00a	31.66±0.78 b	48.33± 1.45 bcd	86.66± 1.56 abc	100.00 ± 0.00 a	82.17 e	2.65 e
AF-SG-SS	0.00 ± 0.00a	57.50± 1.48 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00± 0.00 a	31.05 g	----

X represents the field rate of fenoxaprop-P-ethyl. Means in the same column with different letters are statistically different at the 5% confidence level. The data present means ± standard error.

Table 5. Effect of herbicide mixtures on mortality (%), dry biomass (g $plant^{-1}$), and number of seeds ($plant^{-1}$) of fenoxaprop-P-ethyl resistant and susceptible biotypes of *A. fatua*.

Herbicide mixture	Resistance Biotype	Susceptible biotype	Resistance Biotype	Susceptible biotype	Resistance Biotype	Susceptible biotype
T_1	100.00 a	100.00 a	--	--	--	--
T_2	100.00 a	100.00 a	--	--	--	--
T_3	82.29 bc	85.00 b	3.42 b	3.37 b	42.75 ef	39.50 f
T_4	100.00 a	100.00 a	--	--	--	--
T_5	100.00 a	100.00 a	--	--	--	--
T_6	79.00 de	83.00 b	3.47 b	3.42 b	51.00 de	48.75 de
T_7	100.00 a	100.00 a	--	--	--	--
T_8	100.00 a	100.00 a	--	--	--	--
T_9	77.75 ef	82.50 bc	3.54 b	3.48 b	55.25 cd	50.75 de
T_{10}	100.00 a	100.00 a	--	--	--	--
T_{11}	100.00 a	100.00 a	--	--	--	--
T_{12}	76.25 f	80.50 cd	3.93 ab	3.86 ab	64.75 b	61.75 bc
T_{13}	0.00 g	0.00 e	5.58 a	5.53 a	166.25 a	165.00 a
T_{14}	100.00 a	100.00 a	--	--	--	--
Mean	93.48 B	94.69 A	3.99A	3.92 A	76.00 A	73.15 B

Means in the same column with different letters are statistically different at the 5% confidence level. T_1= CP + ME (100%), T_2= CP + ME (75%), T_3= CP + ME (50%), T_4= PI + SU (100%), T_5= PI + SU (75%), T_6= PI + SU (50%), T_7= PI + ME (100%), T_8= PI + ME (75%), T_9= PI + ME (50%), T_{10}= SU + CP (100%), T_{11}= SU + CP (75%), T_{12}= SU + CP (50%), T_{13}= Weedy check, T_{14}= Manual weed control (two hand weeding). (CP= Clodinafop –propargyl, ME= metribuzin, PI= Pinoxaden, SU= sulfsulfuran).

collected from four districts in Punjab, Pakistan (Khushab, Mianwali, Bhakkar, and Sargodha) exhibited resistance, though the degree of resistance varied by biotype and location. These locations had prolonged use of fenoxaprop-P-ethyl to control *A. fatua* in wheat fields, and the continuous use without rotation caused the development of resistance (Abbas *et al.,* 2016a,b). Widespread resistance in *A. fatua* against different herbicides with various modes of action has excellent documentation globally (Heap, 2024). The first herbicide resistance reported in Pakistan was the fenoxaprop-P-ethyl resistance in *P. minor* (Abbas *et al.,* 2016a). As *A. fatua* is also a weed associated with wheat crops, the herbicide selection pressure imposed on *P. minor* to develop resistance similarly affected *A. fatua*.

Further, the selected locations for collecting resistant-suspected seeds had a long history of wheat production. Different histories of herbicide use, wheat production, and other production practices caused variations in resistance levels (Travlos and Chachalis, 2010). The high seed production capacity of *A. fatua* and its seed maturity before wheat are also significant causes in increasing the proportion of resistant seeds in the soil weed seed bank, thus enhancing the number of resistant *A. fatua* in the field, developing herbicide resistance (Abbas *et al.,* 2016a).

Resistance in *A. fatua* refers to alterations in the ACCase enzyme, with mutations in the gene responsible for encoding this, leading to the plant's insensitivity to fenoxaprop-P-ethyl (Gherekhloo *et al.,* 2012). Reports from other countries have also documented varying levels of resistance in *A. fatua* against fenoxaprop-P-ethyl (Heap, 2024). The differences in resistance levels among the collected biotypes may be because of their distinct evolutionary history of herbicide selection pressures, as the biotypes came from locations with varying histories of wheat cultivation and herbicide use. Additionally, fields in diverse regions employed distinct approaches for weed control, both herbicidal and non-herbicidal. The variability in resistance levels might be due to different resistance mechanisms, as various resistance mechanisms are responsible for the herbicide resistance development in *A. fatua* (Beckie *et al.,* 2012; Abbas *et al.,* 2016a).

Utilizing herbicide mixtures not only effectively controlled the resistant *A. fatua* biotypes but also provided satisfactory control over other resistant biotypes. Our findings corroborate previous research, which advocates using herbicide mixtures at or near their recommended doses to achieve optimal weed control (Abbas *et al.,* 2016b; Abbas *et al.,* 2018a, b). Furthermore, it was noticeable that plants surviving after applying low doses of herbicide mixtures exhibited remarkably inhibited growth and seed production compared with the control. This reduction in growth and seed production in herbicide-treated *P. minor* plants at suboptimal doses is consistent with the findings of Abbas *et al*. (2016).

Herbicide combinations are proposals to mitigate resistance evolution and effectively manage resistant weeds (Barbieri *et al.,* 2022). Moreover, mixtures effectively decrease the cross-resistance likelihood (Abbas *et al.,* 2016b; Barbieri *et al.,* 2022). The herbicides in these mixtures must have distinct modes of action and similar weed control efficiency to prevent the evolution of resistance (Abbas *et al.,* 2018a; Barbieri *et al.,* 2022), as employed in the current study. Various herbicide mixtures have proven applicable in controlling different weed species without leading to resistance for a long time (Wrubel and Gressel, 1994). Despite their benefits, herbicide mixtures are often less preferred due to potential increases in weed control costs and possible crop damage. However, herbicide mixture integration can proceed with other non-chemicals like wheat row spacing to reduce herbicide doses in the mixtures (Abbas *et al.,* 2018b). Integrating herbicide mixtures with allelopathic crop mulches proved beneficial in reducing the herbicide doses in the amalgam and lessening wheat toxicity (Abbas *et al.,* 2018a).

In this study, herbicide mixtures provided synergistic control of *A. fatua* even at reduced doses (50% or 75% of the recommended rate for each herbicide). At 75% of the field rate for each component, the mixtures achieved 100% weed control efficacy. Utilizing these blends at lower doses can lessen weed control costs. Research has shown that spraying herbicide combinations can be at lower rates of each herbicide than the recommended rates to prevent resistance (Abbas *et al.,* 2018a, b).

CONCLUSIONS

Research revealed that fenoxaprop-P-ethyl-resistant *A. fatua* is widespread across chief wheat-producing districts, including Khushab, Mianwali, Bhakkar, and Sargodha, Pakistan. All the tested herbicide mixtures effectively controlled fenoxaprop-P-ethyl-resistant and susceptible *A. fatua* biotypes. Among herbicide mixtures, clodinafop–propargyl plus metribuzin provided more weed control ability than the others. Hence, *Avena fatua* control can be successful in wheat using herbicide mixtures. This approach also helps mitigate the development of further resistance and potential cross-resistance.

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