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EFFECT OF CYTOCHROME P_{450} INHIBITORS ON ACCASE RESISTANT *Phalaris minor* BIOTYPES

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Phalaris minor Retz. is a major issue in wheat growing areas of Pakistan because it has evolved resistance to ACCase inhibitors herbicides like fenoxaprop due to enhanced metabolic activity to detoxify herbicides. Cytochrome P_{450} inhibitors such as 1-aminobenzotriazole (ABT) and malathion are expected to increase the toxicity of herbicides against ACCase resistant weeds. In this regard, a wire house experiment was carried to explore the effect of cytochrome P_{450} inhibitors on ACCase resistant *P. minor* biotypes. Seeds of nine ACCase resistant *P. minor* biotypes and susceptibility were sown in pots (5 seeds per pot). After seedling establishment (at 3-4 leaf stage), different doses of cytochrome P_{450} inhibitors (ABT at 50, 100 and 150 µM and malathion at 500, 1000 and 1500 g a.i. ha^{-1}) were applied 30 minutes before spray of fenoxaprop at 67.5 g a.i. ha^{-1}. Outcomes exhibited that cytochrome P_{450} inhibitors improved the herbicidal activity of fenoxaprop, significant decrease in fresh and dry weight of resistant biotypes were achieved. Maximum decrease in weight (fresh and dry) was noted for ABT at 50 µM, 100 µM and 150 µM+ fenoxaprop at 67.5 g a.i ha^{-1}. Similarly, percent decrease in dry weight and mortality was also maximum when ABT at 50 µM, 100 µM and150 µM+ fenoxaprop at 67.5 g a.i ha^{-1} was used. Results conferred the resistance potential of *P. Minor* biotypes against fenoxaprop and P_{450}inhibitors could be used to synergize the activity of fenoxaprop.
Keywords: Fenoxaprop, Malathion, Mortality, *Phalaris minor*, Resistance.

INTRODUCTION

Phalaris minor is a winter annual self-pollinated grassy weed and prevails in all regions of the world, particularly in tropics and sub-tropics (Yasin *et al*., 2011). Its germination in Pakistan starts in November to January and gets maturity in March-April. In Pakistan, *P. minor* is the most problematic and noxious weed in wheat crop (Yasin *et al.*,2011) and causes significant yield losses (25-50%) in wheat depending on emergence time, density, competition period of *P. minor*, control measures and weather conditions (Chhokar *et al.*, 2008). With the passage of time, *P. minor* developed tolerance against particular herbicides because of continuous application of herbicides (Gherekhloo *et al.*, 2012) possessing same mode of action (Owen *et al.*, 2007). According to Malik and Singh (1995), first case was documented in India during 1991 regarding *P. minor* tolerance to herbicide but now, it has resistance to several herbicides generally Acetyl CoA Carboxylase (ACCase) and acetolactate synthase (ALS) inhibitors in various countries of the world including South Africa, Australia, Israel, India, Iran and USA (Heap, 2020).

Over the past 25 years, the genetic and biochemical bases of resistance in *P. minor* against herbicides have been studied by several researchers and they documented that resistant biotypes can show or possess one to several co-existing mechanisms that develop resistance in *P. minor*. Resistant *P. minor* population has both types of resistance (target and non-target-site resistance). Target-site resistance develops due to mutation in genes coding (Powles and Yu, 2010; Heap, 2020) for herbicides target-site enzyme (prevents the binding with herbicides) or by overproduction of target enzyme (Yu and Powles, 2014). While, non-target-site resistance prevents the translocation of particular herbicides reaching to target site thus increases the metabolism of herbicides (Tranel and Wright, 2002; De´lye, 2013). Herbicides do not give more mortality rate due to under dose, poor application methods, adverse environmental factors, etc. Unfortunately, most of the farmers in Pakistan use herbicides at low doses. Low dose of herbicide encourages the resistance because at low dose of herbicide, some plants survive due to genetic traits, which confer their survival at existing lower doses of herbicides (Yu and Powles, 2014)). Metabolic herbicide resistance is wide spread in the grass weeds like *P. minor*, *Lolium rigidum* Guad., *Alopecurus myosuroides* Huds., and *Echinochloa phyllopogon* (Stapf) Koso-Pol. etc. (Yu and Powles, 2014, Heap, 2020;).

Metabolic resistance in plants increases due to increased activity of endogenous enzymes like cytochrome P_{450}, glutathione S-transferases (GSTs), glucosyl transferases (GTs) etc. (Carey *et al.*, 1997). These enzymes have ability to metabolize herbicides (Kreuz *et al.*, 1996; Edwards and Dixon, 2000; Siminszky, 2006). The P_{450s}, GSTs and GTs enzymes belong to super families of enzymes, which have significant role in primary and secondary metabolism

(Werck-Reichhart *et al.,* 2000; Morant *et al.,* 2003), and, by chance, few of them attained toxification of herbicides. For example, several P_{450s} can catalyze herbicides alkyl-hydroxylation or aryl-hydroxylation (Cole and Edwards, 2000; Yuan *et al.,* 2007). Some herbicides inactivated by GST-catalyzed glutathione conjugation. Cytochrome P_{450} enzymes are responsible for metabolic-based resistance against multiple herbicides in rigid ryegrass (*Lolium rigidum*), rice barn yard grass (*Echinochloa phyllopogon*) and black-grass (*Alopecurus myosuroides*) (Fischer *et al*., 2000; Hall *et al*., 1997; Preston *et al*., 1996; Yu *et al*., 2009).

Cytochrome P_{450} inhibitors such as piperonyl butoxide (PBO), 1-aminobenzotriazole (ABT) and malathion are generally used to increase toxicity of herbicides against ACCase resistant weeds (Elmore *et al.,* 2015). The ABT can decrease metabolism and enhance the toxicity of fenoxaprop to herbicide-resistant biotypes of *P. minor*. Malathion is another cytochrome P_{450} inhibitor that has been used to antagonize cytochrome P_{450} monooxygenase-mediated chlorsulfuron and pendimethalin resistance in rigid ryegrass *(L. rigidum)*(Christopher *et al.,* 1994; Tardif and Powles, 1999). Alike, piperonyl butoxide (a cytochrome P_{450} inhibitor) has been used to detect resistance due to metabolism by PBO-sensitive cytochrome P_{450} enzyme (Kwon and Penner, 1995). The addition of these, inhibitors was reported to strongly enhance herbicide phytotoxicity toward bispyribac-resistant late water-grass (*E. phyllopogon*) plants (Fischer *et al*., 2000).Fenoxaprop is ACCase inhibiting herbicides and applied to control *P. minor* in wheat crop but due to continuous application of fenoxaprop, *P. minor* is now exhibiting resistance to this herbicide in Pakistan (Abbas *et al.,* 2017). Cytochrome P_{450} inhibitors like ABT and malathion are expected to enhance the herbicides toxicity against ACCase resistant weeds Therefore, a wire house experiments was conducted to explore the effect of cytochrome P_{450} inhibitors on ACCase resistant *P. minor* biotypes.

MATERIALS AND METHODS

Phalaris minor populations showing resistance to ACCase herbicides were evaluated for metabolic resistance during 2016-2017 growing season in wire house at Department of Agronomy, University of Agriculture, Faisalabad, Punjab, Pakistan. Completely randomized design was used with three replications. Seeds of *P. minor* PM MBD1, PM MBD 2, PM MBD 3, PM DPS, PM FSD 2, PM NS, PM SH and PM SGD1 *P. minor* biotypes were sown in pots (13×10×6 cm). Pots were filed with sieved soil and farmyard manure (2:1 w/w) was mixed with soil before filling of pots and 5 kg soil was filed in each pot. The pots were placed in a wire house with a temperature of 20±2°C and 14 h photoperiod. In each pot, 5 seeds were sown however after emergence; 3 healthy plants were kept for response. When plants reached 3-4 leaf stage, malathion with different doses such as 500, 1000 and 1500 g a.i. $ha^{-1}$was sprayed. Similarly, 1-aminobenzotriazole (ABT) with various doses of 50, 100 and 150 µM was also sprayed 30 minutes before application of fenoxaprop at 67.5 g a.i. ha^{-1}. Herbicide treatments were applied using a backpack sprayer fitted with TeeJet 8003VS nozzle at 30 psi pressure that sprayed about 187 L ha^{-1}. Control treatments were also kept for comparison. Separate experiment was conducted for each biotype.

***Observation*:** At 21 days of emergence, herbicides spray was used on these plants. After 3 weeks of herbicides spray, fresh plants (above ground parts) were taken and sundried and then their dry weight was taken. Percent biomass reduction over control was calculated by following formula. For this purpose, the surviving plants were harvested and oven-dried for 48 h at 70°C.

$$\text{Percent biomass reduction} = \frac{Wc - Wt}{Wc} \times 100$$

Where Wc is dry weight of control plant (untreated plants) and Wt is dry weight of treated plants.

After 3 weeks of treatments, the data regarding mortality percentage were calculated for each treatment using the formula of Kandhro *et al.* (2015) and Abbas *et al.* (2016).

$$\text{Mortality \%} = \frac{Wt - Ws}{Wt} \times 100$$

Where Wt mean total number of *P. minor* plants before spray while Ws represents number of surviving *P. minor* plants after spray

***Statistical Analysis*:** All the collected data were analyzed using M State C and treatments means were compared by least significant difference test at 5% probability level (Steel *et al.,* 1997).

RESULTS

Results of study showed that from different *P. minor* biotypes, PM MBD1, PM MBD2 and PM NS showed significance resistance against fenoxaprop at 67.5 g a.i. ha^{-1} because there was no difference in their dry weight when compared with the control treatments (Table 1). While fenoxaprop at 67.5 g a.i.ha^{-1} clearly decreased dry weight in susceptible biotypes while PM FSD2, PM MBD3, PM DPS, PM SH and PM SGD1 showed less reduction in dry weight. Results showed that application of ABT at 50, 100 and 150 µM + fenoxaprop at 67.5 g a.i.ha^{-1} was more effective than sole application of fenoxaprop or fenoxaprop + malathion at 500, 1000 and 1500 g a.i ha^{-1}. Spray of fenoxaprop at 67.5 g a.i. ha^{-1} + ABT at 150 µM decreased dry weight in the resistant (PM MBD1 (89%), PM MBD2 (67%), PM FSD2 (63%), PM MBD3 (68%), PM DPS (66%),PM NS (76%), PM SH (79%), PM SGD1 (17%)and susceptible (99%) biotypes of *P. minor*. Likewise, application of fenoxaprop at 67.5 g a.i.ha^{-1} + ABT at 100 µM also reduced dry weight of PM MBD1 (87%), PM MBD2 (59%), PM MBD3 (68%), PM DPS (64%) and PM SGD 1 (16%) biotypes and their dry weight was at par with treatment

Table 1. Effect of cytochrome P_{450} inhibitors and fenoxaprop on dry weight (mg/plant) of *P. minor* biotypes

Treat-ments	PM MBD1		PM MBD 2		PM FSD 2		PM MBD 3		PM DPS		PM NS		PM SH		PM SGD1		Susceptible	
	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE
T_1	91.6a	86.8a	36.9a	33.6a	27.6a	16.0c	83.3a	60.0c	35.0a	16.6def	128.3a	125.6a	48.9a	35.1b	339.1a	312.0f	93.3a	28.4e
T_2	41.0b	34.3bcd	29.7b	27.1bc	20.6b	14.3cd	75.0b	42.3d	31.6ab	15.8efg	112.1c	110.3c	45.6a	32.3bc	325.0b	307.0g	83.3b	6.0f
T_3	40.6b	28.5cd	26.0c	24.3cde	20.0b	13.0de	40.0d	33.0ef	30.0b	14.3e-h	119.1b	108.2c	32.6b	24.5de	323.6bc	301.9h	80.1bc	3.2fg
T_4	35.1bc	27.3de	25.0cd	22.0de	11.0f	10.6f	29.3gh	31.0fg	25.0c	13.0fgh	85.3d	59.3E	28.2cd	18.6f	322.3bc	295.0i	79.3bcd	1.0g
T_5	20.3ef	14.0fgh	22.0de	17.0fg	19.6b	14.0d	35.0e	30.0g	21.3cd	12.6fgh	53.6ef	41.6hi	23.0e	14.0g	319.6cd	293.0i	78.5cd	1.0g
T_6	19.3fg	11.6h	20.6ef	15.3gh	19.3b	11.6ef	31.6fg	27.0h	20.6cd	11.7gh	51.6fg	38.3i	21.0ef	11.6g	317.33de	285.00j	76.6cd	0.83g
T_7	12.0gh	10.3h	16.5g	12.3h	12.0ef	10.3f	30.0g	26.7h	19.0de	10.0h	46.6gh	30.5j	20.3ef	10.3g	315.0ef	282.0j	75.6d	0.80g
LSD	7.45		3.70		1.68		2.90		4.97		6.46		4.23		4.62		4.12	

T_1 = control, T_2 = Malathion at 500 g a.i. ha^{-1}, T_3 = Malathion at 1000 g a.i. ha^{-1}, T_4 = Malathion at 1500 g a.i. ha^{-1}, T_5 = 1 - aminobenzotriazole at 50 µM ha^{-1}, T_6 = 1-aminobenzotriazole at 100 µM ha^{-1}, T_7 = 1-aminobenzotriazole at 150 µM ha^{-1}. C = control, FPE = Fenoxaprop-p-ethyl at 67.5 g a.i.ha^{-1}. LSD = Least significant difference

receiving spray of fenoxaprop at 67.5 g a.i.ha^{-1} + ABT at 150 µM. Furthermore, dry weight of PM SH biotype in response to fenoxaprop (67.g a.i. ha^{-1}) + ABT (50 µM) was also at par with that of fenoxaprop at 67.5 g a.i. ha^{-1} + ABT at 150 µM (Table 1).

In case of percent reduction in dry weight over control, maximum reduction (99%) was noted in susceptible biotype sprayed with ABT at 50, 100 and 150 µM + fenoxaprop at 67.5 g a.i. $ha^{-1}$and fenoxaprop at 67.5 g a.i. ha^{-1} + malathion at 1500 g a.i. ha^{-1} (Table 2). Minimum percent reduction in dry weight over control was recorded in PM SGD1 (10-17%). Whereas PM MBD1, PM MBD2, PM FSD2, PM MBD3, PM DPS, PM NS and PM SH showed 63-89, 26-67, 48-63, 49-68, 54-66, 14-76 and 33-79% dry weight reduction, respectively. Likewise, susceptible biotype showed maximum mortality (92-99%) when ABT at 50, 100 and 150 µM ha^{-1}+ fenoxaprop at 67.5 g a.i. ha^{-1} and malathion at 1500 g a.i. ha^{-1} + fenoxaprop at 67.5 g a.i. ha^{-1}were used (Table 3). In PM MBD1 and PM FSD2, ABT at 150 µM ha^{-1}+ fenoxaprop 67.5 g a.i. ha^{-1}caused 90 and 68% mortality, respectively while in PM MBD2 and PM DPS showed 75 and 53% mortality, respectively. Alike, PM MBD3, PM SH and PM SGD1 showed 84, 81and 37% mortality, respectively at ABT 150 µM ha^{-1} + fenoxaprop (67.5 g a.i. ha^{-1}).Whereas Fenoxaprop alone caused minimum mortality, which ranged from17-45%

Table 2. Percent decrease (%) in dry weight of *P. minor* biotypes over control due to cytochrome P_{450} inhibitors and fenoxaprop

	PM MBD1		PM MBD 2		PM FSD 2		PM MBD 3		PM DPS		PM NS		PM SH		PM SGD1		Susceptible	
	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE
T_1	-	5	-	9	-	42	-	28	-	71	-	13	-	34	-	8	-	70
T_2	55	63	19	26	25	48	10	49	10	54	5	14	3	33	4	10	11	94
T_3	56	69	30	34	28	53	52	60	14	55	7	16	33	50	5	11	14	97
T_4	62	70	32	40	60	62	65	63	29	60	34	54	42	61	5	13	16	99
T_5	78	85	40	54	29	49	58	64	39	63	58	68	53	71	6	14	15	99
T_6	79	87	44	59	30	58	62	68	41	64	60	70	57	66	6	16	18	99
T_7	87	89	55	67	57	63	64	68	46	66	64	76	58	79	7	17	19	99

T_1 = control, T_2 = Malathion at 500 g a.i. ha^{-1}, T_3 = Malathion at 1000 g a.i. ha^{-1}, T_4 = Malathion at 1500 g a.i. ha^{-1}, T_5 = 1-aminobenzotriazole at 50 µM ha^{-1}, T_6 = 1-aminobenzotriazole at 100 µM ha^{-1}, T_7 = 1-aminobenzotriazole at 150 µM ha^{-1}. C = control, FPE = Fenoxaprop-p-ethyl at 67.5 g a.i.ha^{-1}LSD = Least significant difference.

Table 3. Effect of cytochrome P_{450} inhibitors and fenoxaprop on mortality (%) of *P. minor* biotypes.

	PM MBD1		PM MBD 2		PM FSD 2		PM MBD 3		PM DPS		PM NS		PM SH		PM SGD1		Susceptible	
	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE	C	FPE
T_1	0.0g	20f	0.0i	24gh	0.0g	45e	0.0f	28e	0.0g	36d	0.0i	18h	0.0k	19i	0.0l	17e	0.0f	67c
T_2	54e	54e	23h	30fg	27f	49de	30e	32e	27f	37d	17h	21gh	15j	37h	9.0k	19e	11e	92b
T_3	55e	67d	32f	35ef	29f	55cd	32e	34e	27ef	41c	18h	24g	34h	52ef	10jk	22d	12e	96a
T_4	63d	68d	34f	42d	31f	56bcd	34e	38de	30ef	43c	37f	56e	45g	59d	12ij	26c	12e	99a
T_5	79bc	86ab	41de	47cd	34f	61abc	37de	53c	30e	48b	58de	70b	50f	69c	13hi	31b	13e	99a
T_6	78c	87a	52c	61b	46de	65ab	49cd	72ab	34d	49b	63cd	75a	54e	76b	14gh	36a	36d	99a
T_7	89a	90a	65b	75a	60ab	68a	66b	84a	36d	53a	66bc	80a	58d	81a	15fg	37a	38d	99a
LSD	7.97		6.83		9.39		12.19		3.31		4.98		3.35		2.22		3.08	

T_1 = control, T_2 = Malathion at 500 g a.i. ha^{-1}, T_3 = Malathion at 1000 g a.i. ha^{-1}, T_4 = Malathion at 1500 g a.i. ha^{-1}, T_5 = 1-aminobenzotriazole at 50 µM ha^{-1}, T_6 = 1-aminobenzotriazole at 100 µM ha^{-1}, T_7 = 1-aminobenzotriazole at 150 µM ha^{-1}. C = control, FPE = Fenoxaprop-p-ethyl at 67.5 g a.i.ha^{-1}LSD = Least significant difference

Table 4. Field record of *P. minor* biotypes

***P. minor* biotypes**	**Wheat fields and herbicide use (years) from where seeds of *P. minor* biotypes were collected**		
	Wheat	**Fenoxaprop-p-ethyl**	**Location**
PM MBD 1	> 20.0	> 8.00	Mandi-bhaao-u-din
PM MBD 2	> 20.0	3.00	Mandi-bhaao-u-din
PM FSD 2	> 20.0	5.00	Faisalabad
PM SGD 1	10.0	6.00	Sargodha
PM MBD 3	> 20.0	>10.00	Mandi-bhaao-u-din
PM SH	> 20.0	10.00	Sangla hill
PM NS	> 20.0	10.00	Nankana sahib
PM DPS		10.00	Dinpur Shakargarh
S = Susceptible	00.0	0.00	From all locations for comparison

in all biotypes except susceptible biotypes where it caused 67% mortality (Table 3).

DISCUSSION

Our results depicted that different *P. minor* biotypes collected from different areas of Punjab, Pakistan showed resistance against fenoxaprop (ACCase inhibitor herbicide) but pre-herbicide application of cytochrome P_{450} inhibitors [malathion and 1-aminobenzotriazole (ABT)]+fenoxaprop significantly decreased dry weight and increased mortality percentage in *P. minor* biotypes (Tables 1-3). These results are in line with those of Abbas *et al.* (2016) and show that *P. minor* biotypes had developed resistance against fenoxaprop in Pakistan. On the basis of dry weight and mortality %, there was difference in resistance level of biotypes and it might be due to different resistance mechanisms and herbicides selection (Maneechote *et al.*, 1994; Gherekhloo *et al.*, 2011; Travlos *et al.*, 2011, Abbas *et al.*, 2016 and Abbas *et el.*, 2017.). Due to continuous spray of herbicides with similar mode of action *P. minor* has developed resistance. Similar outcomes were reported by Abbas *et al.* (2017) who reported that *P. minor* biotypes had tolerance to ACCase inhibitors. Similarly, Owen *et al*. (2007) also documented that there was resistance in *P. minor* population against ACCase inhibitors. Malik and Singh (1995) first confirmed *P. minor* resistance to isoproturon in India. Resistance biotypes have various mechanisms such as ACCase enzyme modification, mutation in genes and expression of genes which causes resistance in *P. minor* against fenoxaprop (Gherekhloo *et al*., 2012). Furthermore, resistance plants have high level of endogenous enzymes such as glucosyl transferases, cytochrome P_{450} and other enzymes (Carey *et al.*, 1997), which quickly metabolize the herbicides (Edwards and Dixon, 2000; Siminszky, 2006) and some of them work as detoxifier to herbicides. For instance, several P_{450}s enzymes can catalyze herbicides alkyl-hydroxylation or aryl-hydroxylation (Maneechote *et al*., 1994; Yuan *et al.*, 2007).

Cytochrome P_{450} inhibitors (malathion and ABT) decreased dry weight and increased mortality in *P. minor* biotypes (Tables 1-3) presumably due to their specific metabolic processes catalyzed and oxidized by different enzymes present in cytochrome P_{450}(Kemp *et al*., 1990). The main function of ABT is the inhibition of degradation process and works synergistically with herbicides to kill plants (Cabanne *et al*., 1987; Kemp *et al*., 1990). The enzymes present in cytochrome P_{450} inhibitors (malathion and ABT) have heme group, which damage the active site in substrates that binds to heme group of cytochrome P_{450} enzymes and make them unavailable for oxidation. Cabanne *et al*. (1987) reported that 1-aminobenzotriazole (ABT) inhibited the metabolism of chlorotoluron and isoproturon in wheat. It can be assumed that the mono-oxygenase enzymes present in *P. minor* biotypes are involved in the breakdown of fenoxaprop while cytochrome P_{450} inhibitors worked synergistically with fenoxaprop to kill *P. minor* plants.

Conclusion: *P. minor* is troublesome weed in rice-wheat growing areas of Pakistan and main threat to wheat productivity and sustainability. This investigation showed that *P. minor* has resistance to fenoxaprop. However, addition of cytochrome P_{450} inhibitors effectively controls the *P. minor*. Other methods of weed control should be used to control this weed and application of ACCase herbicides should be minimized.

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