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WEED MANAGEMENT AND HERBICIDE RESISTANT WEEDS: A CASE STUDY FROM WHEAT GROWING AREAS OF PAKISTAN

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Abstract

Evolution of herbicide resistance in weeds is among the serious challenges that agriculture face today. In Pakistan herbicide use over last 6 decades has enabled earlier planting of short-season crops and improved weed control in wheat as well as other cropping systems. This has greatly favored the use of more and more herbicides. Conversely, this exercise increased the evolution of resistant biotypes. Wheat being Pakistan's number one staple crop faces daunting weeds problem. In order to understand the status of herbicide resistant weeds in wheat fields, survey was conducted for two consecutive years (2015-2017) in different regions of Pakistan. A questionnaire was designed to collect data from three hundred farmers randomly selected in all four provinces of Pakistan on herbicides used in wheat, history of crop cultivation regimes and weed species that have seized to respond to field rates of herbicides. Seed of suspected biotypes were collected from the survey sites and preserved in our gene pool for the confirmation of the resistance in the laboratory by bioassay and molecular level in future. Farmers (96%) perceived that *Phalaris minor* Retz. was the most important annual weed of wheat crop followed by *Avena fatua* L. (94%). Customarily, three aryloxyphenoxy propionate herbicides, clodinafop-propargyl, fenoxaprop-P-ethyl and diclofop-methyl were used to control different grass weeds such as *P. minor, A. sativa* L. and *A. fatua*. In farmers' opinion, high yield of wheat was associated with the continuous use of these herbicides. Resistance-suspicious weeds were reported from all studied areas (Punjab, KPK, Sindh, and Baluchistan provinces) in fields with the herbicide use history of five to seven years or more. This study suggests that for long-term avoidance of herbicide resistance, in our wheat growing systems we will require embracing practices that decrease selection pressures favoring resistant weeds. Achieving these goals requires an understanding of the evolution and dynamics of resistant populations. It is recommended that for the effective management of resistant populations of *P. minor* and *A. fatua*, farmers' education on ecologically sustainable weed management is critically important. This study will provide a basis for examining the questions that are relevant to understanding herbicide resistance evolution in Pakistan and that may help determine appropriate weed management strategies.

Key words: Herbicide Resistance, Wheat herbicides, Wheat yield in Pakistan.

Introduction

Pakistan being top ten wheat producing country globally is producing far lower than its potential yield due to severe weed infestation, as a major biological constraint. Weeds infestation in major crops in Pakistan causes yield losses up to 36 million tons, accounting for about 3 billion USD annually (Mushtaq & Cheema, 2007).

Phalaris minor (littleseed canarygrass) causes 30-50% yield loss in wheat depending upon crop conditions, density of the weed, soil fertility, cultural practices, and duration of competition (Chhokar & Sharma, 2008; Jabran *et al*., 2010). Bhan & Sushil (1998) reported wheat yield losses up to 80% because of littleseed canarygrass infestation. With an increased density of this weed (0 to 200 plants m^{-2}), wheat yield loss was increased by 33% (Duary & Yaduraju, 2005). High levels of infestation, i.e., 2000-3000 plants m^{-2} may cause complete crop failure (Chhokar *et al.,* 2006). Herbicide resistance is reported to be dependant upon rise in temperature due to global change in climate, as high temperature leads to quick detoxification of herbicides (Matzrafi *et al*., 2016).

In recent past, herbicide resistance has emerged as the greatest concern of contemporary agriculture which relies primarily on synthetic inputs for its sustainability. Fenoxaprop-p-ethyl has been used for more than two decade due to its availability and affordability in Pakistan (Jabbar & Mallick, 1994). Resistance evolution in littleseed canarygrass against ACCase inhibitors has been reported by Owen *et al.,* 2007, Delye *et al*., 2011and Gherekhloo *et al.,* 2012. Resistance of little seed canarygrass to isoproturon was first reported in India during 1995 (Malik & Singh, 1995).

There is ever growing consensus that littleseed canarygrass has evolved resistance to different herbicides including ACCase inhibitors, photosystem II (PS-II) inhibitors, and acetolactate synthase (ALS) inhibitors in many countries including Australia, India, Iran, Israel, Mexico, South Africa, and the United States (Heap, 2018). Multiple resistance (against three and two herbicide sites of action) in littleseed canarygrass against ACCase, ALS, and PS II inhibitors has been confirmed in India and South Africa (Pieterse & Kellerman, 2002; Chhokar & Sharma, 2008).

Phalaris minor Retz. (littleseed canarygrass) is of a Mediterranean origin , and has spread to numerous parts of the world (Anderson, 1961; Baldini, 1995; Singh *et al*., 1999). It is a notorious grassy weed of barley and wheat in more than 60 countries worldwide including, Australia, USA, South Africa, Canada, India and Pakistan (Chhokar *et al*., 2008; Travlos, 2012; Hussain *et al.,* 2015). It is categorized as the most bothersome grassy weed in wheat (Holm *et al*., 1979; Singh *et al*., 1999; Jabran *et al.,* 2010) inflicting significant reduction in the grain yield (Malik and Singh, 1995; Afentouli & Eleftherohorinos, 1996). Different acetyl-CoA carboxylase (ACCase) inhibitors, including fenoxaprop-p-ethyl, clodinafop–propargyl, and pinoxaden have been used to control *Phalaris minor* in wheat (Yadav *et al*., 2016; Abbas *et al.*, 2016).

Avena fatua is another harmful grassy weed of cereal crops. Prompt germination, crop mimicry and seed capability to persist in soil for longer durations signify a severe economic risk to crop yields (Medd & Pandey, 1990: Jones and Medd; 1997). Diclofop-methyl (since 1978) and succeeding associated acetyl-CoA carboxylase (ACCase)- inhibiting herbicides are of great importance in its control (Abbas *et al*., 2016). In various crops for selective control of *A. fatua*, with these herbicides has been broadly used and herbicide resistance evolution has been caused due to their constant and prevalent use in main crop growing areas worldwide (Owen *et al.*, 2007; Heap, 2008). Like *A. fatua,* the control of *P. minor* is also totally dependent on early crop-selective, post-emergence herbicides, with ACCase and ALS-inhibiting herbicides (Beckie *et al*., 2002). The resistance threat is surely aggravating due to the increasing dependency on the same target-site herbicides. In Mediterranean regions, the condition is less fast than Europe, due to the presence of broader variability of herbicides and the biotypes cultivated (Sattin, 2005; Travlos *et al*., 2012). From Greece, *A. sterilis* L. and some other biotypes resistance has been reported (Travlos & Chachalis, 2010, 2011). It has been reported that many *P.minor* populations have developed resistance to ACCase inhibitor herbicides (Afentouli & Georgoulas, 2002; Heap, 2018). The response of fenoxaprop-resistant littleseed canarygrass to diverse herbicide molecules like clodinafop-propargyl, metribuzin, pinoxaden, and sulfosulfuron was also evaluated in further dose response bioassays. All accessions manifested variable resistance to fenoxaprop, clodinafop. However, metribuzin, pinoxaden, and sulfosulfuron were still effective in controlling fenoxaprop-resistant canarygrass (Abbas *et al*., 2017). While, in some cases wheat crop is harvested while unripe and used as fodder due to lack of substitute herbicides (Malik & Singh, 1995). However, less evidence has been reported regarding *P. minor* in Pakistan, hence the current study was initiated as several reports illustrated that *P. minor* control is becoming progressively challenging in the main cereal-producing areas of Pakistan with the objectives 1) to find out the efficacy of different herbicides usedto control *P.minor* in Pakistan (clodinafop-propargyl, fenoxaprop-p-ethyl, and sulfosulfuron + mesosulfuron methyl) 2) to establish the speculated resistance and cross resistance to the aforesaid herbicides. It is need of the day that ecologically sustainable weed management is focused and farmers, land managers, industry personnel and policy makers to focus on weeds within whole farming systems rather than one unit (weeds alone).

Materials and Methods

Survey data collection: The survey work was conducted during wheat growing periods from the start of the wheat crop in October till its maturity in May during the years 2015-2017. The distinctive cereal producing areas in Punjab, Khyber Pakhtunkhwa, Sindh and Baluchistan, Pakistan were selected for herbicide resistance survey and collection of seeds (Fig. 1). A questionnaire was used to interview three hundred farmers from all over Pakistan. Information regarding use of herbicides, their effect and control of target weeds were collected. Farmers were asked regarding the difficultiesin controlling grass weeds. Commencing in the southern sides (where seed development arose first) and systematic northwards just before the 2016-17 grain harvest. Three separate sub regions were made from every region for lay out of the sampling sites. In each sub region, 3 to 5 fields were sampled. Seeds were primarily collected from herbicide-treated wheat fields. For the use of susceptible control populations seeds from fields that had never been treated with herbicides were collected for comparison. In total, at random more than 60 wheat fields were visited in the four selected areas. Based on survey a tabulated data was arranged to summarize the resistant weeds reported in the area.

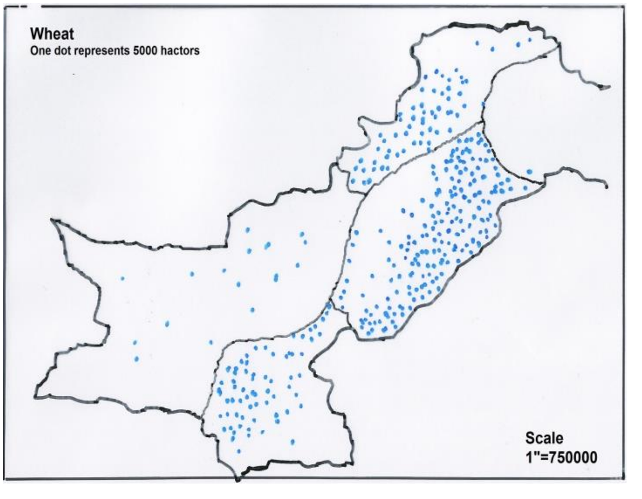

Fig. 1. Map of Pakistan the dots represents the area where wheat isgrown and data was collected randomly.

Results and Discussion

Despite the very common usage of herbicides and other control strategies practiced yearly in crop fields (counting the 2015-17 growing seasons), *P. minor* and *A. fatua* were found to infest many, but by no means all, crop fields all over Pakistan. The pattern of their infestation was diverse according to locality and rainfall. The southern areas, mainly coastal regions (Sindh), noticed very low *A. fatua* influx. Paterson (1974) working in the Western Australian grain belt also reported the irregular distribution of wild oat and associated with rainfall and soil type. However *P. minor* (little seed canary grass) and other weeds were commonly distributed. It is highlighted that this survey was conducted very late in the growing season, and practically early-season herbicide applications were performed for weed control (maximum herbicide vulnerable vegetation have been controlled). Thus the surveys highlight only the occurrence of weed flora getting maturity. As predictable, the survey shown, that wheat (*Triticum aestivum* L.) was the prevailing growing crop in the fields.

Social status and some land use attributes of the respondents of Pakistan:

Regardless of area, on the average, the respondents were in their mid-forties (Table 1). The oldest respondent was 57 years and the youngest was 27 years old. On average the respondents attained ten years of educational experience, it shows that at least they got high school certificate. The highest education of the respondents was Ph.D. and lowest was grade 1.

Data related land tenure status confirms 73% respondents have their own land and remaining (27%) were the non-owner tenants (Table 1).

Regardless of area, respondents were dependent on canal system for irrigation (57%) followed by tube wells (21%).

With regards to respondent's characteristics, all (100%) confirmed crop rotation. Further data includes for intercropping, which highlights 44% respondents follow intercropping. Regarding major constraints in wheat production (Table 2a, 2b), the majority of the respondents (55%) in Sindh identify weeds as their No. 1 enemy followed by Baluchistan (41%) and Punjab (39%). In Khyber Pakhtunkhwa 36% of the interviewed report the non-availability of quality seeds. Agrochemicals were claimed to be constraint in low percentages across different provinces. Moreover other constraints like diseases, disasters etc., were also indicated by the respondents in Punjab (32%) and Baluchistan (31%).

Herbicides stand on top regarding weed management. A significant number of respondents in Punjab (66%) found herbicides to be the best for controlling weeds in wheat, followed by Khyber Pakhtunkhwa (63%). Crop rotation was also identified as very helpful in management of weeds. Hand weeding is practiced in low land areas. Regarding weed management, in Punjab 42% of the respondents assure that herbicides give best weed control as compared to other management practices. Moreover, in all four provinces, fast action of herbicides was confirmed by the respondents. A total of 29% of the correspondents also elaborated that herbicides are non-laborious, thereby meaning that herbicides save time and labor for farmers.

Herbicides and resistant weeds in Pakistan: Phenoxaden, Mesosulfuran-methyll, and Phenoxaprop-p-ethyl are used in high percentage in fields, 39% and 27% in Punjab, respectively followed by Khyber Pakhtunkhwa province (Table 3). *Phalaris minor* was reported to be the most severe among all the weeds of wheat (42%) in Punjab followed by Baluchistan (33%). While, *Avena fatua* and *Rumex dentatus* are more severe in Khyber-Pakhtunkhwa with 32% and 30%, respectively. In Sindh province, *Chenopodium murale* was noticed with aggressive growth (29%).

Regarding herbicide resistance, few weeds were noticed which were not controlled by herbicides. Regardless of geographical area, highest complaints were reported against *P. minor* L. followed by *A. fatua* and *Rumex dentatus* by more than 20%. In Sindh, 21% respondents replied about *Chenopodium murale* alone.

Table 1. Social status and some land use attributes of the respondents of Pakistan.

Character	Punjab	Khyber Pakhtunkhwa	Sindh	Baluchistan	Mean
Average age (yr.)	48	47	52	50	49
Youngest age	27	26	30	29	28
Oldest age	57	65	68	64	63
Average education attainment	10	10	10	10	10
Highest	PhD	MSc	PhD	PhD	PhD
Lowest	Grade 5	Grade 1	Grade 1	Grade 1	Grade 1
Land tenure status (%)					
Owner	70	80	60	80	73
Non owner	30	20	40	20	27
Source of irrigation (%)					
Canals	82	71	35	39	57
Tube wells	10	12	40	23	21
Rain	8	5	5	14	8
Others	0	12	20	24	14
Crop rotation	100	100	100	????	100
Intercropping	28	48	52	47	44

Table 2a. Wheat area by province (FAOSTAT 2016).

Province	Area (Million Hectares)	Percentage of Total Area
Punjab	6.75	74.6
Sindh	1.17	12.9
KPK	0.74	8.2
Baluchistan	0.39	4.3
Total	9.05	100

Table 2b. Major constraints in wheat production and weed management in Pakistan.

	Major constraints to wheat production (%)			
	Weeds	**Quality seeds**	**Agrochemicals**	**Others**
Punjab	39	27	6	32
Khyber Pakhtunkhwa	31	36	5.5	28
Sindh	55	22	5	18
Baluchistan	41	23	5	31
Mean	41.5	27	5.3	27.2
Best weed management practices (%)				
	Herbicides	**Crop rotation**	**Hand weeding**	
Punjab	66	20	14	
Khyber Pakhtunkhwa	63	17	20	
Sindh	53	14	33	
Baluchistan	52	21	27	
Mean	58.5	18	23.5	
Reason for using herbicides (%)				
	Best control	**Fast action**	**Non laborious**	
Punjab	42	33	27	
Khyber Pakhtunkhwa	40	31	29	
Sindh	39	32	29	
Baluchistan	37	35	28	
Mean	39.5	32.7	28.2	

Survey of the selected areas of Pakistan revealed that *P. minor* (96%) was one of the most resistant weed among all followed by *A. fatua* L. (Table 3). Again *A. fatua* L., *Cynodon dactylon* ,*Chenopodium album* L., *Convolvulus arvensis* L. and *Rumex dentatus* L. were found uncontrolled by herbicides used with percentage 94, 92, 88, 83 and 78, respectively (Table 3). In all fields, wheat plants were noticed in healthy con4ditions regardless of the use of herbicides. *P. minor* Retz was found more resistant as compared to wild oat and other weeds. In Punjab, the resistance was higher as compared to other areas. Screening of the *P. minor* populations collected from the five regions revealed prevalent resistance to the wheat-selective ACCase-inhibiting herbicide diclofop. In studied *P. minor* over 85% biotypes were overall susceptible to the other studied herbicides. The survey revealed high resistance of *P. minor* and *A. fatua* L., to the one or more herbicides, in the surveyed regions (Ilias & Travlos, 2012). The data in Table 4 exhibits fenoxaprop-ethyl is categorized a selective chemical herbicide used in wheat crop for *grassy weed*s. Maximum yield of wheat is attributed to continuous use of herbicides which boosts the progress of resistant *weed* biotypes. Varied management practices like integration of good varietal selection, herbicide rotation, and crop rotation can be durable approaches of avoiding resistance in *P. minor* (Yasin *et al.,* 2011). Field screening of *P. minor* accords with the herbicides fenoxaprop and clodinafop, presented ominously fewer *P. minor* accords to be resistant to fenoxaprop and clodinafop related to diclofop. Similar results are reported fromother countries accordingly (Om *et al.*, 2004; Chhokar *et al*., 2008), possibly as a result of the prolonged selection pressure phase forced from the higher sequential use of diclofop. Prior registration of clodinofop (from other herbicides before more than two decades) and its wide range of usage is the cause of its high use (Owen *et al.,* 2007). The absence of herbicide and crop rotations has been calculated the leading cause of progressed resistance in several regions all over the world (Tal *et al.,* 1996; Travlos & Chachalis, 2010). Numerous diclofop resistant populations were also found resistant to fenoxaprop or clodinafop. But, the level of resistance in these accords was different to these two herbicides in reaction, with a greater resistance index for fenoxaprop associated with clodinafop conferring to the ordering by Bourgeois *et al*., (1997).

Table 3. Farmer's response on major herbicides and their suspected resistant weeds in wheat.

	Major herbicides						
	Clodinafop-propargyl (Topik)	Phenoxaprop-p-ethyl (Puma Super)	Phenoxaden and Mesosulfuran-methyll (Axial, Atlantis)	Phenoxy herbicide (MCPA)	Hydroxybenzonitrile (Bromoxynil + MCPA)	Thifensulfuron-methyl and Tribenuron-methyl (Affinity)	Phenoxaprop-p-ethyl + Hydroxybenzonitrile (Puma super+Buctril)
Punjab	4%	27%	39%	13%	12%	2%	3%
Khyber Pakhtunkhwa	3%	31%	4%	3%	7%	25%	27%
Sindh	13%	27%	38%	7%	12%	2%	1%
Baluchistan	19%	22%	23%	9%	8%	17%	2%
Mean	9.75%	26.75%	26%	8%	9.75%	11.5%	8.25%

	Weeds in severity									
	Phalaris minor L.	*Avena fatua* L.	*Rumex dentatus*	*Convolvulus arvensis* L.	*Lathyrus aphaca* L.	*Eleusinef lagellifera* Nees.	*Chinupodium morale* L.	*Silibum marianum*	*Lolium rigidum*	*Melilotus alba*
Punjab	40%	23%	17%	4%	9%	5%	2%	2%	0%	0%
Khyber Pakhtunkhwa	22%	32%	30%	4%	1%	1%	3%	3%	4%	0%
Sindh	32%	11%	1%	1%	2%	3%	29%	6%	0%	17%
Baluchistan	34%	22%	20%	3%	2%	1%	5%	8%	3%	2%
Mean	32%	22%	17%	3%	3.5%	2.5%	4.75%	4.75%	1.75%	4.75%

	Resistant weeds							
	Phalaris minor L.	*Avena fatua* L.	*Rumex dentatus*	*Convolvulus arvensis* L.	*Lathyrus aphaca* L.	*Lolium rigidum*	*Chenopodium murale*	*Melilotus alba*
Punjab	40%	20%	19%	5%	7%	0%	7%	2%
Khyber Pakhtunkhwa	29%	25%	21%	7%	2%	6%	8%	2%
Sindh	31%	16%	2%	2%	10%	0%	21%	18%
Baluchistan	33%	22%	23%	3%	3%	6%	9%	1%
Mean	33.25%	20.75%	16.25%	4.25%	5.5%	3%	11.25%	5.75%

Table 4. Weeds, herbicides used and their effect.

S.#	Scientific name	Family	English name	Herbicide used	Resistance incident
1.	*Phalaris minor* Retz.	Poaceae	Littleseed canary grass	Topik, Acetachlore, Axial, Sulfosulfuron	96%
2.	*Avena fetua* L.	Poaceae	Wild oat	Topik, Puma super	94%
3.	*Cynodon dactylon* (L.)	Poaceae	Bermuda Grass	Puma super, Topik, Affinity	92%
4.	*Chenopodium album* L.	Chenopodiaceae	Lamb'squarters	Bromoxynil	88%
5.	*Convolvulus arvensis* L.	Convolvulaceae	Field bindweed	Bromoxynil	83%
6.	*Rumex dentatus* L	Polygonaceae	Curly Dock	Bromoxynil, MCPA	78%

Conclusion and Recommendation

Our study has found that in Pakistan wheat systems non-judicious herbicide application has increased productivity losses from weeds populations becoming resistant to several wheat herbicides. In the first decade of the millennium, annual rice and wheat yields in Pakistan fluctuated by up to 1.31 and 0.57 t ha^{-1} at the national level (FAOSTAT 2016), which is far below the average yield produced by China, USA and Germany. The probability of weed population shifts and evolution of herbicide resistance in a number of weed species is increasing every year with selection pressure of herbicide application. These weeds gone unmanaged challenges, if not addressed, could become a key factor affecting wheat production. Due to increasing concerns over herbicide resistance, weed population shifts, ecological hazards, and human health-related issues, integration of chemical, manual, or mechanical weed control means are thought to be the most successful approach for controlling weed species in major agronomic crops (Riaz *et al*., 2006; Ali *et al*., 2013a). For example, PRE herbicides integrated with inter-culture or hand weeding was observed to be the most efficient option, as compared to herbicide alone (Ali *et al*., 2013a). The integration of reduced herbicide application with agronomic practices, such as competitive cultivar selection, high planting densities, reduced row spacing, altered row orientation, non-conventional crop rotations, and intercropping-based cropping systems, has helped in suppressing competitive weed species in wheat. Allelopathic plants can be used to manage the resistant weeds, for instance *Carica papaya* has been reported to cause 48% germination inhibition in *P. minor* (Anwar *et al.*, 2019). Although, more research is needed to understand the ramifications of herbicide resistance and plan appropriate and actionable adaptation responses in Pakistan.

In particular as per wheat grower response in the present study it is simply concluded that management of *P minor, A.fatua, Rumex dentatus* and *Chenopodium murale* is becoming a great problem in wheat. Herbicide rotation is present in low percentage. Our findings suggest the importance of reviewing existing pesticide policies that aim to increase cereal farmers' resilience in Pakistan, and more broadly in South Asia. A number of studies have estimated the herbicide resistance in major food crops in the Indo-Gangetic plains, with considerable emphasis on Punjab (Abbas *et al*., 2017). However, potential agronomic and extension strategies are needed to be tailored to and proposed for further investigation. Further studies including lab. experiments are recommended to find out the existence and severity of resistance, cross resistance and multiple resistance. Alternate sources of management should be applied in order to combat resistance.

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