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IDENTIFYING OPTIMUM HERBICIDE MIXTURES TO MANAGE AND AVOID FENOXAPROP-P-ETHYL RESISTANT *Phalaris minor* IN WHEAT[1]

*Identificação de Misturas Eficazes de Herbicidas para o Controle de **Phalaris minor** Resistente ao Fenoxaprop-p-Ethyl no Trigo*

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ABSTRACT - Use of herbicide mixtures has been advocated as most effective strategy for avoidance and management of herbicide resistant weeds. Effect of twelve selected treatments of four herbicides (clodinafop-propargil, metribuzin, pinoxaden and sulfosulfuron) two-way mixtures at different doses was investigated against fenoxaprop-p-ethyl resistant and susceptible populations of *P. minor* grown along the wheat plants. In repeated experiment, herbicides mixtures were applied at 3 to 4 leaf stage of *P. minor* under greenhouse conditions. All the herbicide mixtures were effective to control resistant as well as susceptible *P. minor*. Mixtures having 75% lethal dose of each mixture component provided best control against *P. minor*. Mixtures with 50% lethal dose of each herbicide also provided more than 80% control of *P. minor*. Surviving *P. minor* plants after exposure to herbicide mixtures showed reduced growth and seed production potential. No mixture combination produced phytotoxic effects on wheat plant up to 75% of lethal dose of each mixture component. Mixtures including clodinafop-propargil + metribuzin, pinoxaden + sulfosulfuron and pinoxaden + metribuzin at 100% dose of each mixture component produced minor phytotoxic effects on wheat plants and caused no reduction in terms of ultimate growth and grain yield. However, mixture of sulfosulfuron + clodinafop-propargil at 100% dose of each component was phytotoxic to wheat and caused significant reduction in term of growth and grain yield. So, farmers can use these mixtures even at 75% of recommended dose of mixture component to control susceptible and resistant *P. minor* in wheat.

Keyswords: Herbicide mixtures, resistance management, resistance avoidance, *Triticum aestivum*, erva-cabecinha.

*RESUMO - O uso de misturas de herbicidas tem sido visto como a estratégia mais eficaz para prevenção e controle de plantas daninhas resistentes a herbicidas. Os efeitos de 12 tratamentos, selecionados entre quatro herbicidas (clodinafop-propargil, metribuzin, pinoxadene e sulfosulfuron) com misturas de duas vias em doses diferentes, foram estudados para as populações de **P. minor** resistentes e suscetíveis ao fenoxaprop-p-ethyl e que cresciam ao longo de plantas de trigo. Em experimentos repetidos, as misturas de herbicidas foram aplicadas em três a quatro estádios de crescimento da folha de **P. minor** em casa de vegetação. Todas as misturas de herbicidas foram eficazes para controlar plantas de **P. minor** resistentes e suscetíveis. As misturas com 75% de dose letal de cada componente ofereceram melhor controle de **P. minor**. As misturas com 50% da dose letal de cada herbicida também proporcionaram controle de mais de 80% de **P. minor**. As plantas que sobreviveram após serem expostas às misturas de herbicidas apresentaram deficiência de crescimento e de produção de sementes. Nenhuma combinação das misturas gerou efeitos fitotóxicos para o trigo em até 75% da dose letal de cada componente. As misturas com clodinafop-propargil + metribuzin, pinoxadene + sulfosulfuron e pinoxadene + metribuzin a 100% da dose de cada componente produziram leves efeitos fitotóxicos em plantas de trigo e não causaram redução em termos de crescimento final e rendimento de grãos. No entanto, a mistura de sulfosulfuron + clodinafop-propargil a 100% da dose de cada componente foi fitotóxica ao trigo e causou redução significativa do crescimento e produtividade de grãos. Portanto, os agricultores podem utilizar essas misturas, mesmo a 75% da dose recomendada de componente, para controlar plantas de **P. minor** suscetíveis e resistentes ao trigo.*

Palavras-chave: misturas de herbicidas, controle da resistência, impedimento da resistência, *Triticum aestivum*, *littleseed* alpista.

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INTRODUCTION

Wheat (*Triticum aestivum*) is a staple food of Pakistani people and it is considered a key component of global food security. Among the challenges of wheat production sustainability, the issue of herbicide resistant weeds is the most relevant. *Phalaris minor* (littleseed canary grass) is a major issue and the most challenging weed of wheat crop and found in more than 60 countries around the world (Travlos, 2012). Depending upon the intensity of littleseed canarygrass, emergence time, competition period with wheat, agronomic practices and climatic conditions yield losses in wheat due to this grass may vary from 25 to 50% (Chhokar and Sharma, 2008) and may result in complete wheat crop failure, due to heavy infestation (2,000-3,000 plants m^{-2}) (Chhokar et al., 2006). Duary and Yaduraju (2005) have claimed that as the density of littleseed canarygrass increased from 0 to 200 plants m^{-2}, wheat grain yield reduced by 32.6%. Due to its morphological resemblances with wheat crop, *P. minor* control is totally herbicide dependent (Chhokar et al., 2008). Unfortunately, *P. minor* resistance against most of the commonly used herbicides has been increasing in many countries around the world (Heap, 2015). Recently cross resistance problems in *P. minor* have also been reported (Heap, 2015). Therefore, alternative strategies to help avoid the development of resistance and to manage resistant *P. minor* is crucial for the sustainability of wheat.

The use of herbicide mixtures with two or more than two sites of chemistries is an important strategy to manage resistant weeds and to delay the development of herbicide resistance (Lagator, 2013). Based on compounded resistance, frequency models and field experiments, herbicide mixtures have been proved more effective to delay and avoid resistance than herbicide rotation or sequence (Diggle et al., 2003; Beckie, 2006). Quick evolution of resistance occurred due to the selection pressure imposed by exposure to single herbicide with same mode of action. The advantage of using herbicides with more than one mode of action is that resistant biotypes selected by one herbicide were killed by partner herbicide in the mixture (Diggle et al., 2003). The mixtures are also an effective strategy to control herbicide resistant weeds by exploring the reduced fitness of resistant weeds and due to negative cross resistance (Beckie, 2006). Partner herbicides must have different modes of action to make herbicide mixtures more efficient in delaying and preventing resistance (Friesen et al., 2000; Beckie and Reboud, 2009). Tank mixture of acetolactate synthase (ALS) herbicides with MCPA have proved very effective to manage and delay resistance development in different weed species. Delay in resistance was more prominent in weed species that were self-pollinated in nature (Guia..., 2005; Beckie, 2006).

However, form a growers' standpoint, use of mixtures is normally not preferred as they increase the cost of weed control and may damage crop plants (Hart and Pimentel, 2002). From a researcher perspective, it is a very important and effective strategy to manage the problem of resistance development in weeds to ensure the sustainability of the chemical weed control method, which is cheaper and extensively used as weed control method. Additionally, it has been reported that herbicide synergistic mixtures may be successfully applied at lower individual rate than recommended, to avoid and manage resistance (Little and Tardif, 2005; Caseley et al., 2013). Use of cost effective synergistic mixtures of different herbicides is common under field conditions to manage and avoid resistant weeds in crops (Little and Tardif 2005; Beckie 2006; Caseley et al., 2013). However, no information has been found in the literature on the use of herbicide mixtures to manage *P. minor* in wheat. Therefore, this research aimed to evaluate the efficacy of different herbicide mixtures at range of doses to control fenoxaprop-p-ethyl resistant and susceptible biotypes of *P. minor*. Additionally, the effects of herbicide mixtures on the growth and yield of wheat plants were also investigated.

MATERIALS AND METHODS

Studies were conducted twice in a greenhouse at the Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan, during 2014-2015. The greenhouse was located about 31.25° N latitude, 73.09° E longitude and altitude of 184 m. The mean minimum and maximum temperatures at the experimental site were 25 ± 2 °C and 33 ± 2 °C, respectively. Herbicides free soil was collected from research fields near the experimental site. Each tray (45 x 30 x 30 cm) was filled with 15 kg sandy loam soil having 1.10% organic matter content and pH of 8.0-8.5. The experiment was laid out in completely randomized design with factorial arrangement having four replications and reshuffled each week in order to achieve uniform growth conditions for all trays. Three rows of wheat variety Glaxy-2013 were sown in each metal tray. *P. halaris minor* seeds were sown between wheat rows. To start, 1.2 liter of water was applied to each tray and then trays were kept moist throughoutout the experimental period.

The herbicides used in this study were: Clodinafop –propargyl [Acetyl-CoA carboxylase (ACCase) inhibitor], metribuzin (Photosystem II inhibitor), pinoxaden (ACCase inhibitor), sulfosulfuron (ALS inhibitor). The herbicides were used in different combination (Table 1). The herbicide mixtures were sprayed using a CO_2 pressurized backpack sprayer fitted with TeeJet 8003VS nozzle at 30 psi pressure that sprayed about 20 gallons of water per acre. Sprayer calibration was done and amount of spray solution were calculated for one square matter. All replications trays of same treatment were placed in one square matter and spray was carried out. Data regarding weed mortality, plants height and dry biomass for both *P. minor* and wheat were recorded at three weeks after herbicide application and at maturity. The dry biomass, spike length, number of grains per spike, 100 grain weight and grain yield per plant for wheat and number of seeds per spike of *P. minor* were also recorded at maturity.

Table 1 - Herbicide mixtures at range of doses for *Phalaris Minor*

Treatment	Herbicides mixture
CM1	Clodinafop-propargil 50% of R + metribuzin 50% of R
CM1.5	Clodinafop-propargil 75% of R + metribuzin 75% of R
CM2	Clodinafop-propargil 100% of R + metribuzin 100% of R
PS1	Pinoxaden 50% of R + sulfosulfuron 50% of R
PS1.5	Pinoxaden 75% of R + sulfosulfuron 75% of R
PS2	Pinoxaden 100% of R + sulfosulfuron 100% of R
PM1	Pinoxaden 50% of R + metribuzin 50% of R
PM1.5	Pinoxaden 75% of R + metribuzin 75% of R
PM2	Pinoxaden100% + metribuzin 100% of R
SC1	Sulfosulfuron 50% of R + clodinafop-propargil 50% of R
SC1.5	Sulfosulfuron 75% of R + clodinafop-propargil 75% of R
SC2	Sulfosulfuron 100% of R + clodinafop-propargil 100% of R
WC	Weedy check
C	Manual weed control

R represents the recommended doses of herbicides e.i. Clodinafop-propargil (C), metribuzin (M), Pinoxaden (P) and Sulfosulfuron (S). CM Treatments, for example, refer to the mixture of clodinafop-propargil and metribuzin at 50% (CM1), 75% (CM1.5) and 100% (CM2) of R of each mixture herbicide, respectively.

Our objective was to compare the efficacy of different mixtures at range of doses to control *P. minor* in wheat. Therefore to analyze data and comparison of all treatments, Fisher's analysis of variance techniques was carried out using Tukey's honest test at 5% probability level (Steel et al., 1997). Pooled data were used for analysis because repeated experiment gave statistically similar results.

RESULTS AND DISCUSSION

Our results showed that herbicide mixtures can be successfully used to control fenoxaprop-p-ethyl resistant and susceptible *P. minor*. There was no difference in the response of fenoxaprop-p-ethyl resistant and susceptible population of *P. minor* to the tested herbicides mixtures. Same efficacy of mixtures against resistant and susceptible populations of *P. minor* was due to no cross resistance in *P. minor* populations against selected mixtures. During this study fenoxaprop-p-ethyl was not used as a mixture component in any of the mixtures. Effective control of resistant weed populations with herbicide mixtures has been reported in previous findings (Beckie and Reboud, 2009).

Results on the response of fenoxaprop-p-ethyl resistant population and wheat plants to the herbicide mixtures application have been described.

Effect of herbicides mixtures on *P. minor*, data were collected three weeks after mixtures application

Results showed that herbicides mixtures combinations (CM1.5, CM2, PS1.5, PS2, PM1.5, PM2, SC1.5, SC2 and C) containing 75 and 100% doses of each mixture herbicide caused 100% mortality of *P. minor*. Mixtures at low doses including CM1, PS1, PM1 and SC1 caused 85.00, 84.33, 81.00 and 81.67 percent mortality respectively (Table 2). However, plant height and dry biomass of surviving plants were significantly reduced due to mixtures application as compared to control plants (Table 2).

Effect of herbicides mixtures on *P. minor*, data were collected at maturity

Results about plant height at maturity revealed that toxicity caused by mixtures application sustained up to plant maturity and significantly reduced the plant height (46.53, 48.14, 42.80 and 37.41 cm) as compared to control (70.30 cm) (Table 3). Dry biomass of mixture exposed plants was also significantly lower than plants not treated with any mixture (C). Minimum dry biomass as compared to other mixtures treatments was produced in SC1 (1.95 g) and maximum biomass was produced by control plant (C) (Table 3). Seed production potential of mixtures exposed plants was also considerably reduced as compared to control. Plants exposed to CM1, PS1, PM1 and SC1 produced 53.32, 67.41, 58.37 and 43.17 seeds per plant respectively. However, *Phalaris minor* plants of control pots (C) produced 178.03 seeds per plant (Table 3). Mixtures were effective when components were used at or lower than 75% to their recommended dose. When mixtures were used at 50% of each herbicide recommended dose, decrease in control efficacy was observed. Our results support previous findings, which suggested the use of mixtures components at close to or at their recommended doses for efficient weed control (Russell, 2005; Beckie, 2006; Lagator, 2013). It was also observed that plants that survived after being exposed to low doses of herbicides mixtures had significantly reduced growth and lesser seeds than control. Reduced growth and low seed production of *P. minor* plant treated with herbicide above the doses caused hormesis is supported by Abbas et al. (2016).

Herbicides mixtures of two or more than two different herbicides are recommended to slow down the development of resistance and to control resistant weeds, due to their reduced fitness and negative cross resistance (Beckie, 2006). However, herbicides in the mixtures

Table 2 - Efficacy of herbicide mixtures to control *P. minor*, data were collected two weeks after spray

Herbicide mixture	Mortality (%)	Plants height (cm)	Biomass (g per plant)
CM1	85.00 ± 4.47b	9.67 ± 0.44bc	1.80 ± 0.08b
CM1.5	100.00 ± 0.00a	--	--
CM2	100.00 ± 0.00a	--	--
PS1	84.33 ± 5.39b	10.57 ± 0.60b	2.00 ± 0.11b
PS1.5	100.00 ± 0.00a	--	--
PS2	100.00 ± 0.00a	--	--
PM1	81.00 ± 3.22b	7.76 ± 0.31c	1.87 ± 0.06b
PM1.5	100.00 ± 0.00a	--	--
PM2	100.00 ± 0.00a	--	--
SC1	81.67 ± 2.58b	8.13 ± 0.50c	1.95 ± 0.11b
SC1.5	100.00 ± 0.00a	--	--
SC2	100.00 ± 0.00a	--	--
WC	0.00 ± 0.00c	21.17 ± 1.22a	2.37 ± 0.12a
C	100.00 ± 0.00a	--	--

The means marked with same letter do not differ significantly at 5% confidence level. Data are the means ± standard error.

Table 3 **-** Efficacy of herbicide mixtures to control *P. minor*, data were collected at maturity

Herbicide mixture	Plants height (cm)	Biomass (g per plant)	No. of seeds per plant
CM1	46.53 ± 2.20b	2.40 ± 0.10b	53.32 ± 2.53bc
CM1.5	--	--	--
CM2	--	--	--
PS1	48.14 ± 2.81b	2.49 ± 0.13b	67.41 ± 3.95b
PS1.5	--	--	--
PS2	--	--	--
PM1	42.80 ± 1.79bc	2.62 ± 0.09b	58.37 ± 2.46bc
PM1.5	--	--	--
PM2	--	--	--
SC1	37.41 ± 2.39c	1.95 ± 0.11c	43.17 ± 2.77c
SC1.5	--	--	--
SC2	--	--	--
WC	70.30 ± 4.12a	3.49 ± 0.18a	178.03 ± 9.64a
C	--	--	--

The means marked with same letter do not differ significantly at 5% confidence level. Data are the means ± standard error.

should have different mode of action and have same weed control efficacy to avoid resistance evolution (Friesen et al., 2000; Guia..., 2005; Beckie, 2006) as used in this present study. Different herbicides mixtures have been successfully used to control different types of weeds without development of resistance even after twenty years (Wrubel and Gressel, 1994). However, mixtures are not preferred because it may increase the cost of weed control and also result in crop damage. In present studies mixtures synergistically control *P. minor* even at lower doses of mixtures components (50% or 75% of R for each herbicide). At 75% doses of each mixture herbicides 100% weed control efficacy was achieved (Table 2). Use of mixtures at lower doses will reduce the cost of weed control. Literature exposed that herbicides mixtures may be applied at lower individual rate of each mixture component in mixture than recommended to avoid resistance (Little and Tardif, 2005; Beckie, 2006; Caseley et al., 2013).

Effect of mixtures application on wheat growth, data were collected three weeks after mixtures application and at maturity

Results of data collected two weeks after mixtures application showed that herbicides mixtures inhibited growth of wheat plants at higher doses (100% or R of each herbicide in mixture) e.i. CM2, PS2, PM2 and SC2. However, at 50% and 75% of R in mixture did not cause significant reduction in plant height and dry biomass of wheat (Table 4). Results of data collected at maturity showed that inhibitory response of herbicide mixtures did not remain with time. Herbicides mixtures did not caused reduction in plant height at maturity except PM1 and SC1. Dry biomass of wheat also showed no significant effect of any mixture application at any doses (Table 4).

Effect of mixtures application on grain yield and yield contributing parameters of wheat

The effect of mixtures application on wheat yield was investigated by collecting data on grain weight and yield contributing parameters of wheat including spike length, number of grains per spike and 100 grain weight. Results on spike length showed no significant inhibitory effect of any mixture application on spike length (Table 5). Herbicide mixtures caused no significant reduction on number grain produced per spike except SC2 and WC which caused reduction in number of grain produced per spike (Table 5). Results of 100 grain weight revealed that mixtures applications did not affect grain weight expect SC1.5 and SC2 (Table 5). Minimum 100 grain

Table 4 **-** Effect of herbicide mixtures on wheat growth, data were collected three weeks after spray and at maturity

Herbicide mixture	Three weeks after spray		At maturity	
	Plants height (cm)	Biomass (g per plant)	Plant height (cm)	Biomass (g per plant)
CM1	29.07 ± 1.37a	2.52 ± 0.11abc	60.11 ± 2.53a	3.64 ± 0.15^{NS}
CM1.5	29.27 ± 1.06a	2.52 ± 0.08abc	61.51 ± 2.04a	3.68 ± 0.12
CM2	23.89 ± 1.66bc	2.23 ± 0.14bcd	58.31 ± 3.64a	3.57 ± 0.23
PS1	29.16 ± 1.70a	2.45 ± 0.13abcd	62.58 ± 3.26a	3.51 ± 0.19
PS1.5	30.74 ± 1.12a	2.55 ± 0.08abc	62.48 ± 2.06a	3.64 ± 0.12
PS2	23.41 ± 1.62bc	2.19 ± 0.14cd	57.36 ± 3.58a	3.37 ± 0.21
PM1	30.15 ± 1.26a	2.63 ± 0.10a	58.37 ± 2.20a	3.59 ± 0.13
PM1.5	27.32 ± 0.99ab	2.61 ± 0.08ab	58.58 ± 1.96a	3.62 ± 0.11
PM2	22.28 ± 1.05c	2.39 ± 0.10abcd	39.74 ± 1.78b	3.53 ± 0.15
SC1	27.81 ± 1.78ab	2.49 ± 0.15abcd	61.18 ± 3.83a	3.49 ± 0.22
SC1.5	28.87 ± 1.68a	2.45 ± 0.13abcd	59.69 ± 3.10a	3.43 ± 0.18
SC2	20.20 ± 1.17c	2.12 ± 0.11d	45.24 ± 2.36b	3.30 ± 0.17
WC	28.87 ± 1.68a	2.40 ± 0.13abcd	56.80 ± 2.95a	3.39 ± 0.18
C	29.94 ± 1.58a	2.56 ± 0.12abc	64.73 ± 3.04a	3.65 ± 0.17

The means marked with same letter do not differ significantly at 5% confidence level. Data are the means ± standard error. NS = non-significant ($p \leq 0.05$).

Table 5 **-** Effect of herbicide mixtures on wheat grain yield and yield contributing parameters, data were collected at maturity

Herbicide mixture	Spike length (cm)	No. of grain per spike	100 grain weight (g)	Grain yield (g per plant)
CM1	8.70 ± 0.39^{NS}	44.59 ± 2.11a	3.47 ± 0.15a	1.39 ± 0.06ab
CM1.5	8.63 ± 0.30	43.92 ± 1.60ab	3.39 ± 0.11a	1.36 ± 0.05ab
CM2	8.48 ± 0.57	43.02 ± 2.99ab	3.31 ± 0.21ab	1.31 ± 0.08ab
PS1	8.55 ± 0.48	43.32 ± 2.53ab	3.30 ± 0.17ab	1.51 ± 0.07ab
PS1.5	8.76 ± 0.30	41.97 ± 1.53ab	3.42 ± 0.11a	1.46 ± 0.53ab
PS2	8.53 ± 0.58	39.20 ± 2.73abc	3.37 ± 0.21a	1.46 ± 0.09ab
PM1	8.33 ± 0.33	43.77 ± 1.83ab	3.26 ± 0.12ab	1.48 ± 0.05ab
PM1.5	8.74 ± 0.30	45.88 ± 1.67a	3.34 ± 0.10ab	1.53 ± 0.05a
PM2	8.59 ± 0.39	42.65 ± 2.02ab	3.27 ± 0.14ab	1.37 ± 0.06ab
SC1	8.50 ± 0.52	39.33 ± 2.51abc	3.28 ± 0.19ab	1.44 ± 0.08ab
SC1.5	8.57 ± 0.48	38.51 ± 2.25abc	3.28 ± 0.17ab	1.30 ± 0.06b
SC2	8.58 ± 0.49	33.69 ± 1.96c	2.70 ± 0.14c	0.86 ± 0.05c
WC	8.56 ± 0.48	36.58 ± 2.13bc	2.80 ± 0.14bc	0.93 ± 0.05c
C	8.65 ± 0.44	45.41 ± 2.40a	3.41 ± 0.16a	1.45 ± 0.06ab

The means marked with same letter do not differ significantly at the 5% confidence level. Data are the means ± standard error. NS = non-significant ($p \leq 0.05$).

weight (2.70 g) was obtained in plants that were treated with SC2 which was followed by WC (2.80 g). Mixtures application effect on grain yield per plant was also not significant for CM2, PS2 and PM2 mixtures at their varied doses (Table 5). However, SC2 caused significant reduction in grain yield per plant (0.86 g), which was followed by WC (0.93).

The phytotoxic effect of herbicide mixtures on crop plants is one of the major constraints to the use of herbicides mixtures in crop production. Our results showed that three types of herbicides mixtures including Clodinafop-propargil + metribuzin, Pinoxaden + sulfosulfuron and Pinoxaden + metribuzin can be effectively used to control *P. minor* without phytotoxic effect on wheat plants (Tables 4 and 5). However, mixture of Sulfosulfuron and clodinafop-propargil caused phytotoxic effect at higher doses (100% of R for each mixture component). Our findings reinforce previous research on the control of weeds in rice field using herbicide mixtures at varied doses with no phytotoxic effect on crop plants (Beckie, 2006). Inhibited growth and biomass reduction due to exposure of herbicide mixtures was recovered by wheat plant and no significant effect was observed at maturity (Table 5). Recovery to phytotoxic effects of herbicides has been reported for wheat plant by Hosseini et al. (2011) and Bhullar et al. (2012).

Finally, herbicide mixtures e.i. clodinafop-propargil + metribuzin, pinoxaden + sulfosulfuron and pinoxaden + metribuzin can be effectively used at range of doses to avoid and control resistant *P. minor* in wheat crop with no phytotoxic effects on wheat. Sulfosulfuron + clodinafop-propargyil was phytotoxic to wheat and caused reduction in grain yield. However, the use of multiple herbicides in agriculture must be considered in the light of economic and environmental concerns of herbicide mixtures.

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