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Full Length Article

Herbicide Mixtures and Row Spacing Effects on Fenoxaprop Resistant *Phalaris minor* in Wheat

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Abstract

Widespread herbicide resistance in *Phalaris minor* Retz., is a major bottleneck towards sustainability of wheat-based cropping systems. Development and promotion of an integrated weed management program is crucial. Field trials were conducted in Faisalabad, Punjab, Pakistan during the winter of 2014–2015 and 2015–2016 to evaluate the effectiveness of herbicide mixtures on herbicide-resistant *P. minor* (resistant to fenoxaprop-P-ethyl) in wheat sown at 11.25- and 22.50-cm rows. Tank mixtures of clodinafop–propargyl + metribuzin, pinoxaden + sulfosulfuron, pinoxaden + metribuzin, and sulfosulfuron + clodinafop-propargyl at 75 and 100% of label dose/s provided effective control of *P. minor.* The herbicide mixtures performed better in 11.25-cm rows than in 22.50-cm spacing of wheat. Narrow row spacing (11.25-cm) reduced the number of seeds per spike and the dry shoot biomass (33–38%) of *P. minor* relative to the wider row spacing. Wheat growth and yield (up to 32%) were improved by herbicide mixtures in both growing seasons, and such an effect was more pronounced at the narrow row spacing. Narrowing spacing not only compensated for 25% less herbicide input but also increased wheat grain yield by 6% more than recommended spacing. Therefore, narrow row spacing and herbicide mixtures can help tackle herbicide-resistant *P. minor* in wheat fields.

Keywords: ACCase inhibitors; Herbicide resistance; Integrated weed management; Post-emergence herbicide mixtures

Introduction

Among the weed species which infest wheat crop, *Phalaris minor* Retz. (littleseed canarygrass), postures a serious threat to sustainable wheat production. This is widespread weed of winter crops in more than 60 countries worldwide (Travlos, 2012). It is most dominant in wheat fields of Bangladesh, India, Iran, Nepal and Pakistan. In Asia, it is the most serious problem in rice-wheat cropping systems (Hussain *et al.*, 2015). Wheat yield losses due to *P. minor* vary between 25 to 50% (Chhokar and Sharma, 2008). A dense infestation of about 2000–3000 plants m^{-2} may result in complete wheat crop failure (Chhokar *et al.*, 2006). An infestation of 40 plants m^{-2} can reduce yields of mid- and late-sown wheat by 28–34% (Hussain *et al.*, 2015). Its close resemblance to wheat plants during the early stages makes manual control difficult, causing increased infestation and rendering it difficult-to-control by non-chemical methods (Abbas *et al.*, 2016a, b). Thus, post emergence herbicides are most effective to control *P. minor* in wheat.

From the lens of herbicide resistance, endemic resistance in *P. minor* to acetyl-coA carboxylase (ACCase) inhibitors (A/1), photosystem II (PSII) inhibitors (urease and amides) and acetolactate synthase (ALS) inhibitors has made its control more complicated (Om *et al.*, 2004; Yadav *et al.*, 2016; Heap, 2018) including in Pakistan (Abbas *et al.*, 2017). Recently, the confirmation of cross resistance of up to three herbicide modes of action in *P. minor* has made the situation even worse for wheat growers (Yadav *et al.*, 2016). Integration of diverse chemical and non-chemical weed control methods could help reduce herbicide selection pressure for resistant biotypes. Such information has been disseminated and promoted for years (Owen, 2016). The use of herbicide mixtures is now regarded as a vital component of proactive and reactive resistance management (Beckie, 2006; Bailly *et al.*, 2012; Evans *et al.*, 2016). This is supported by resistance prediction models and field studies, which also showed that mixtures with dissimilar herbicide chemistries are more effective for resistance management than herbicide rotations (Diggle *et al.*, 2003;

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Beckie, 2006; Evans *et al.*, 2016; Lamichhane *et al.*, 2016). Herbicide mixtures are more effective in avoiding resistance in self-pollinated weed species, like *P. minor,* than in cross-pollinated weed species (Beckie, 2006). Metribuzin is a soil-residual broad spectrum herbicide that enters in plants through roots, and this can be used as a post emergence herbicide in wheat to control *P. minor* (Shaw and Wesley, 1991; Chhokar *et al.*, 2008). In addition to application of metribuzin alone, the mixture of metribuzin and fenoxaprop provided efficient control of *P. minor* in wheat; however, some phytotoxicity was observed on wheat crop (Singh *et al.,* 2005).

Mixing ALS inhibitors with MCPA provided effective control of various weed species including ball mustard [*Neslia paniculata* (L.) Desv.], kochia (*Kochia scoparia* L.), redroot pigweed (*Amaranthus retroflexus* L.), Russian thistle (*Salsola iberica* Sennen & Pau), field penny (*Thlaspi arvense* L.) and wild mustard (*Sinapis arvensis* L.) to manage and delay resistance (Beckie, 2006). Mixtures of various ACCase and ALS inhibitors are effective in controlling resistant black-grass population (*Alopecurus myosuroides* Huds.) in winter cereals (Bailly *et al.*, 2012). Combining herbicides with different sites of action is an excellent management tool to glyphosate or other herbicides (Evans *et al.*, 2016). In addition, mixing appropriate herbicide partners could be cost-effective in cases where the mixture is synergistic. For example, mixtures of propanil with piperophos and/or anilofos at reduced rates are used commonly in Costa Rica and Columbia to manage propanil-resistant *Echinochloa colona* (L.) Link. (Valverde *et al.*, 2000; Beckie, 2006). *Echinochloa crus-galli* (L.) Beauv. was synergistically controlled with propanil and anilofos mixtures at various doses in the rice fields of the southern United States (Beckie, 2006). Less use of herbicides reduces selection pressure for resistance evolution, but this will be acceptable only if it does not compromise efficacy (Diggle and Neve, 2001).

Integrated use of chemical weed control and cultural practices can enhance weed control efficacy and sustainability (Alsaadawi *et al.*, 2011). In contemporary agriculture, field crops, especially cereals, are sown in distinctly spaced crop rows with variable densities per unit land area (Chen *et al.*, 2008). Narrowing of crop row spacing allows early closure of crop canopy and reduces light interception by weeds (Matloob *et al.*, 2015). This results in less weed pressure and improved herbicide efficacy (Khaliq *et al.*, 2014). Drews *et al.* (2009) stated that weed growth was significantly suppressed in wheat sown at 12 cm as compared to that sown in 24-cm rows. Narrow row spacing averted weed growth and reproductive potential, thereby increasing the wheat yield (Fahad *et al.,* 2015). Reduced rates of ACCase inhibitors in combination with optimum seeding rate for the crop effectively controlled weeds in wheat, resistant or otherwise (Beckie and Kirkland, 2003). Thus, integrating reduced crop row spacing with reduced herbicide rates can help lessen the selection pressure for resistance without sacrificing weed control efficacy (Little and Tardif, 2005; Beckie, 2006; Abbas *et al.,* 2016c; Evans *et al.*, 2016).

Phalaris minor control with post-emergence herbicides in wheat is becoming increasingly complicated due to resistance evolution against ACCase- (clodinafop-propargyl, penoxaprop-P-ethyl, pinoxaden and fluazifop-P-butyl), PS II- (isoproturon), and ALS (iodosulfuron-methyl-sodium and mesosulfuron-methyl) inhibitors as well as multiple cross resistance against these groups in many countries (Heap, 2018). The recent confirmation of ACCase-resistant *P. minor* from Pakistan has increased the vulnerabilities of wheat production systems (Abbas *et al.*, 2016a). If this issue is not addressed, it is speculated that this weed may evolve rapid resistance to other herbicide molecules as well, rendering them less suitable as a weed management tool. To best of our knowledge the efficacy of herbicide mixtures alone or in conjunction with narrow row spacing in controlling resistant *P. minor* in wheat has not been determined. Recent studies under controlled conditions indicated that herbicide mixtures at various doses were effective against *P. minor* without causing phytotoxicity to wheat (Abbas *et al.*, 2016b). It is hypothesized that reduced doses of herbicide mixtures may be used in conjunction with narrow row spacing of wheat under field conditions to control resistant *P. minor*. A two-year field study was conducted to evaluate the effectiveness of different herbicide mixtures and row spacings of wheat in controlling herbicide-resistant *P. minor* under semi-arid conditions of Punjab, Pakistan.

Materials and Methods

Site Description

A field study was conducted at Agronomic Research Area, University of Agriculture (UAF), Faisalabad, Pakistan (31.25°N, 73.09°E, 184 m above sea level), for two consecutive growing seasons (2014–2015 and 2015–2016). The soil of experimental field was sandy clay-loam, pH 7.9, and with 0.71% organic matter. Total nitrogen, available phosphorus, and available potassium contents were 4.4 g kg^{-1}, 0.00512 g kg^{-1} and 0.127 g kg^{-1}, respectively. The bulk density and cation exchange capacity were 1.330 kg m^{-3} and 0.039 mol kg^{-1}, respectively. The climate is semi-arid with an average rainfall of 10–15 mm and relative humidity of 60% over the winter (November to March). Meteorological data were obtained from the AgroMet Observatory, Department of Crop Physiology, UAF (Fig. 1).

Experimental Detail

In both seasons, wheat was grown from November to April following transplanted rice to simulate rice-wheat rotation, which is the dominant cropping system in this region. Rice was manually harvested and crop residues

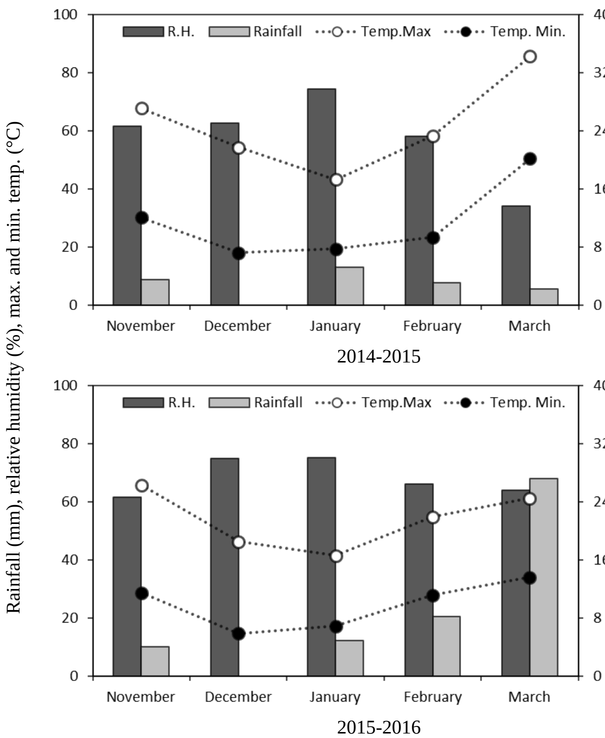

Fig. 1: Metrological data during the course of the present study (Source: AgroMet Observatory, Department of Crop Physiology, UAF

were removed from the field. For wheat seedbed preparation, the soil was tilled three times with mechanical cultivator followed by planking each time.

Wheat cultivar 'Glaxy-2013' was sown in the third week of November with a manually-pushed, single-row drill at two-row spacing (11.25 cm and 22.50 cm) using seed rate of 125 kg ha^{-1}. The length and width of each plot was 6 m and 2.7 m, respectively. Recommended fertilizer dose at 105-85-65 kg ha^{-1} (N: P: K) was applied in the form of urea (46% N), diammonium phosphate (46% P_2O_5 and 18% N) and sulfate of potash (50% K2O). Whole potassium and phosphatic fertilizers, and half of the nitrogen were applied as basal dose. The remaining half of the nitrogen (53 kg ha^{-1}) was top dressed in two equal splits at tillering and booting stages of wheat. The other agronomic practices were uniform for all the plots during experimental period.

The following herbicides were used during present study, clodinafop-propargyl, metribuzin, pinoxaden and sulfosulfuron. Detailed information about herbicides used in the mixtures has been given in Table 1. Progeny of already tested *P. minor* plants having uniform resistance (resistance index 6) to fenoxaprop-P-ethyl (Abbas *et al.*, 2016a) were used. *Phalaris minor* seeds in known numbers were sown in separate rows along with wheat crop. Weed plants were in separate rows between the rows of wheat to make them easily distinguishable from naturally grown native susceptible *P. minor* plants. Naturally occurring broad-leaved weeds were controlled by using bromoxynil plus MCPA at 490 g a.i. ha^{-1} a day after actual treatment application. However, all narrow-leaved weeds, except manually transplanted resistant *P. minor*, were removed by manual pulling. Resistant *P. minor* plants were exposed to variable doses of eight different herbicide mixtures (Table 2) a week after transplanting (once seedlings had established themselves). Labeled doses of herbicides (R) to control *P. minor* were considered as the 100% dose. Other doses were then calculated from these recommended doses. Treatments were applied at 4-5 leaf stage of *P. minor*. The herbicide mixtures were sprayed with a knapsack hand sprayer fitted with a flat fan nozzle (800067 nozzle) at a pressure of 207 kPa after volume calibration (320 L ha^{-1}).

Data Collected

The mortality percentage of *P. minor* was worked out after 21 days of mixtures spray while dry biomass (g m^{-2}), number of seeds per spike (calculated from 20 spikes per plot), and weed control index (%) were evaluated at maturity.

Weed control index (WCI) was calculated with the formula:

$$\mathrm{WCI} = \frac{(x - y)}{x} \times 100$$

Where, x = weed dry biomass in the weedy check and y = weed dry biomass in the mixture treated plot.

At physiological maturity wheat was harvested and threshed manually from each plot (2.7 × 6 m) to determine grain yield, which was presented as t ha^{-1}. Data regarding crop growth rate was taken at pre-anthesis, post-anthesis and grain filling stage during both the growing seasons to appraise the phytotoxic inhibition of wheat due to herbicide mixtures.

Net assimilation rate (g m^{-2} d^{-1}) (NAR) was calculated as proposed by Hunt (1978).

$$\mathrm{NAR} = \mathrm{TDM} / \mathrm{LAD}$$

Where TDM and LAD are the total dry matter and leaf area duration, respectively.

Experimental Design and Statistical Analyses

A randomized complete block design with split plot arrangement was used with three replications. Row spacing of wheat was assigned to the main-plots and herbicide mixtures to the sub-plots. Collected data were subjected to Fisher's analysis of variance technique and means were compared using Tukey's HSD test at the 5% probability level (Statistix 8.1, Analytical software, Statistix; Tallahassee, FL, USA, 1985–2003). Statistical analyses revealed significant cropping season effect; therefore, the

Table 1: Herbicides used in mixtures, mode of actions, and application rates used during this study

Common name	Trade name	Site of action	Chemical family	Recommended field rate (g a.i. ha^{-1})
Clodinafop-propargyl	Topik	ACCase inhibitor	Aryloxyphenoxy-propionate	55
Metribuzin	Sencor	Photosynthetic inhibitors at Photosystem ll	Triazinones	115
Pinoxaden	Axial	ACCase inhibitor	Phenylpyrazoline family	45
Sulfosulfuron	Outrider	Acetolactate synthase inhibitor	Sulfonylurea	30

Table 2: Interactive effect of different herbicide mixtures and row spacing on mortality (%) and number of seeds per spike of *P. minor*

Herbicide mixtures (g a.i. ha^{-1})	Mortality (%)				Number of seeds per spike	
	2014-2015		2015-2016		2014-2015	2015-2016
	11.25 cm	22.50 cm	11.25 cm	22.50 cm		
Clodinafop-propargyl+metribuzin (41+86)	9.80 ± 0.34 bc (95.67)	9.50 ± 0.34 de (90.00)	9.75 ± 0.39 b (94.67)	9.39 ± 0.25c (87.77)	10.67± 0.43B (114.00)	10.43± 0.34B (108.73)
Clodinafop-propargyl + metribuzin (55+115)	10.02 ± 0.00a (100.00)	10.02 ± 0.00 a (100.00)	10.02 ± 0.00 a (100.00)	10.02 ± 0.00a (100.00)	0.71 ± 0.00C (0.00)	0.71 ± 0.00C (0.00)
Pinoxaden + sulfosulfuron (34+23)	9.80 ± 0.45bc (95.00)	9.50 ± 0.65 e (89.00)	9.63± 0.20 bc (92.33)	9.31 ± 0.43c (86.33)	10.97± 0.43B (120.33)	10.79± 0.64B (116.47)
Pinoxaden + sulfosulfuron (45+30)	10.02 ± 0.00a (100.00)	10.02 ± 0.00 a (100.00)	10.02± 0.00 a (100.00)	10.02± 0.00a (100.00)	0.71 ± 0.00C (0.00)	0.71 ± 0.00C (0.00)
Pinoxaden + metribuzin (34+86)	10.02 ± 0.00a (100.00)	9.90 ± 0.19abc (96.67)	10.02± 0.00a (100.00)	9.70 ± 0.34a (93.67)	0.71 ± 0.00C (0.00)	0.71 ± 0.00C (0.00)
Pinoxaden + metribuzin (45+115)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02± 0.00a (100.00)	10.02± 0.00a (100.00)	0.71 ± 0.00C (0.00)	0.71 ± 0.00C (0.00)
Sulfosulfuron + clodinafop-propargyl (23+41)	9.90 ± 0.23ab (98.33)	9.70 ± 0.13cd (93.33)	9.87 ± 0.23b (97.00)	9.60 ± 0.43bc (91.67)	9.52 ± 0.46B (90.67)	9.21 ± 0.34B (84.86)
Sulfosulfuron + clodinafop-propargyl (30+55)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	0.71 ± 0.00C (0.00)	0.71 ± 0.00C (0.00)
Weedy check	0.71 ± 0.00f (0.00)	0.71 ± 0.00f (0.000)	0.71 ± 0.00d (0.00)	0.71 ± 0.00d (0.00)	14.54± 0.54A (211.33)	14.35± 0.34A (206.07)
Hand weeding (twice)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00 a (100.00)	10.02 ± 0.00a (100.00)	0.71 ± 0.00C (0.00)	0.71 ± 0.00C (0.00)

Means not sharing a letter in common differ significantly at 5% probability level by Tukey's HSD test. Data are the square root transformed values of means ± standard error. Figures in parenthesis are the original values

data is described separately for both the growing seasons. Data regarding all parameters except grain yield and net assimilation rate were transformed using square root transformation to achieve normality.

Results

Management of Resistant *P. minor*

Mortality (%): The two-way interaction of herbicide mixtures and row spacing was significant in both growing seasons (Table 2). The four herbicide mixtures namely clodinafop–propargyl + metribuzin, pinoxaden + sulfosulfuron, pinoxaden + metribuzin, and sulfosulfuron + clodinafop–propargyl at 100% of their labeled dose controlled *P. minor* 100% in both row spacings (11.25 and 22.50 cm) and in both cropping seasons. When these herbicide mixtures were applied at 75% of their respective labeled doses, the mortality of *P. minor* was still above 90% at the narrow-row spacing. The efficacy of these reduced rates was significantly reduced at the wide-row spacing.

Number of seeds per spike: The interactive effect of two wheat row spacing and different herbicide mixtures was not significant. However, herbicide mixture had a significant influence on number of seeds per spike of canary grass, and various herbicide mixtures significantly averted the seed production potential of *P. minor* as compared to the weedy check (Table 2). The surviving *P. minor* plants, in plots sprayed with reduced rates of herbicides, produced significantly less number of seeds than those in the non-treated check plots. The seed production potential of surviving *P. minor* was reduced by 43–59% across both growing seasons.

Dry biomass of *P. minor* (g m^{-2}): Dry biomass of *P. minor* was significantly influenced by the interactive effect of herbicide mixtures and row spacing in both growing seasons. *Phalaris minor* was killed 100% by all herbicide mixtures at the full dose. The biomass of *P. minor* that survived the reduced herbicide rates produced less biomass than the non-treated check under both narrow (11.25 cm) or recommended (22.50 cm) row spacing of wheat (Table 3). Maximum dry biomass (24.05–27.60 g m^{-2}) of *P. minor* was recorded in weedy check plots where wheat was sown at 22.50-cm rows without herbicides. The weedy check plots of wheat sown at 11.25-cm row spacing had 33–38% less dry biomass of *P. minor* compared to the 22.50-cm row spacing across two seasons.

Weed control index (%): Herbicide mixtures and row spacing of wheat manifested significant influence regarding weed control index (WCI) during both the growing seasons (Table 3). Maximum weed control efficiency (100%) was

Table 3: Influence of different herbicide mixtures, row spacing and their interaction on *P. min*or dry biomass (g m^{-2}) and grain yield of wheat

Herbicide mixtures (g a.i. ha^{-1})	*P. min*or dry biomass (g m^{-2})				Weed control index (%)			
	2014-2015		2014-2015		2014-2015		2015-2016	
	11.25 cm	22.50 cm	11.25 cm	22.50 cm	11.25 cm	22.50 cm	11.25 cm	22.50 cm
Clodinafop-propargyl + metribuzin (41+86)	1.69 ± 0.04c (2.35)	1.59 ± 0.21c (2.03)	2.00 ± 0.05c (3.53)	1.75 ± 0.04c (2.59)	9.32 ± 0.21c (86.46)	9.26 ± 0.24c (85.25)	9.54 ± 0.31bc (90.55)	9.14 ± 0.43c (82.99)
Clodinafop-propargyl + metribuzin (55+115)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)
Pinoxaden + sulfosulfuron (34+23)	1.67 ± 0.12c (2.33)	1.89 ± 0.11c (3.11)	1.62 ± 0.09c (2.20)	2.08 ± 0.15c (3.85)	9.63 ± 0.17b (92.42)	9.32 ± 0.43c (86.32)	9.62 ± 0.37b (91.99)	9.29 ± 0.25c (85.86)
Pinoxaden + sulfosulfuron (45+30)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)
Pinoxaden + metribuzin (34+86)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)
Pinoxaden + metribuzin (45+115)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)
Sulfosulfuron + clodinafop-propargyl (23+41)	1.02 ± 0.07d (0.75)	1.56 ± 0.08c (1.99)	1.16 ± 0.06d (1.24)	1.72 ± 0.21c (2.55)	9.89 ± 0.12ab (97.40)	9.31 ± 0.24c (86.23)	9.71 ± 0.31b (93.34)	9.24 ± 0.00c (85.06)
Sulfosulfuron + clodinafop-propargyl (30+55)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)
Weedy check	4.22 ± 0.19b (17.36)	4.94 ± 0.14a (24.05)	4.60 ± 0.23b (20.71)	5.27 ± 0.13a (27.60)	-	-	-	-
Hand weeding (twice)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	0.71 ± 0.00e (0.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)	10.02 ± 0.00a (100.00)

Means not sharing a letter in common differ significantly at 5% probability level by Tukey's HSD test. Data are the square root transformed values of means ± standard error. Figures in parenthesis are the original values

Table 4: Influence of different herbicide mixtures, row spacing and their interaction on grain yield of wheat

Herbicide mixtures (g a.i. ha^{-1})	Wheat grain yield (t ha^{-1})			
	2014-2015		2015-2016	
	11.25 cm	22.50 cm	11.25 cm	22.50 cm
Clodinafop-propargyl + metribuzin (41+86)	4.33 ± 0.21ef	4.04 ± 0.23g	4.66 ± 0.34gh	4.33 ± 0.23j
Clodinafop-propargyl + metribuzin (55+115)	4.78 ± 0.18c	4.53 ± 0.52de	5.15 ± 0.34c	4.86 ± 0.42de
Pinoxaden + sulfosulfuron (34+23)	4.75 ± 0.32c	4.38 ± 0.35ef	5.04 ± 0.24c	4.63 ± 0.24fg
Pinoxaden + sulfosulfuron (45+30)	4.55 ± 0.15de	4.27 ± 0.19f	4.78 ± 0.45def	4.44 ± 0.32i
Pinoxaden + metribuzin (34+86)	4.88 ± 0.40c	4.55 ± 0.36d	5.07 ± 0.42c	4.87 ± 0.54efg
Pinoxaden + metribuzin (45+115)	4.52 ± 0.26de	4.28 ± 0.41f	4.74 ± 0.24de	4.53 ± 0.15hi
Sulfosulfuron + clodinafop-propargyl (23+41)	5.24 ± 0.25a	5.03 ± 0.43b	5.69 ± 0.54a	5.36 ± 0.59b
Sulfosulfuron + clodinafop-propargyl (30+55)	4.56 ± 0.31de	4.38 ± 0.23ef	4.83 ± 0.15d	4.65 ± 0.43gh
Weedy check	4.02 ± 0.12g	3.84 ± 0.34h	4.23 ± 0.43j	4.04 ± 0.34k
Hand weeding (twice)	5.23 ± 0.45a	4.97 ± 0.21b	5.55 ± 0.32a	5.36 ± 0.12b

Means not sharing a letter in common differ significantly at 5% probability level by Tukey's HSD test. Data are the square root transformed values of means ± standard error. Figures in parenthesis are the original values

achieved in plots that were treated with clodinafop–propargyl + metribuzin, pinoxaden + sulfosulfuron, pinoxaden + metribuzin and sulfosulfuron + clodinafop–propargyl at 100% of the labeled dose, pinoxaden + metribuzin at 75% of the labeled dose, and subjected to manual weeding twice. These were closely followed by plots treated with clodinafop–propargyl + metribuzin, pinoxaden + sulfosulfuron and sulfosulfuron + clodinafop–propargyl at 75% of the labeled dose. These herbicide treatments scored WCI to the tune of 87–91, 92–93 and 94–97% when applied to wheat plots sown at 11.25 cm row spacing. The corresponding WCI amounted to 83–85, 86–87 and 85–86% in wheat plots sown at 22.50 cm row spacing, respectively.

Wheat Growth and Yield

Net assimilation rate (g m^{-2} d^{-1}) of wheat: Net assimilation rate (NAR) demonstrates the net photosynthetic productivity from functional leaves per unit area of land per day. Application of different herbicide mixtures significantly improved NAR of wheat as compared to weedy check under both row spacings during both growing seasons (Fig. 2). However, two herbicide mixtures including pinoxaden + sulfosulfuron and pinoxaden + metribuzin at 100% of labeled dose caused slight reduction in NAR as compared to 75% labeled dose of these herbicide mixtures. The difference in NAR across various treatments was more pronounced at pre-anthesis than post-anthesis and grain filling stages. Application of herbicide mixtures improved the NAR of wheat at both row spacing; yet at narrow row spacing (11.25 cm), NAR of wheat was higher compared with 22.50 cm row spacing during both the growing seasons.

Grain yield (t ha^{-1}) of wheat: The highest grain yield was produced in weed free and sulfosulfuron plus clodinafop-propargyl at 75% of recommended dose treated plots that was 5.39 and 5.16 t ha^{-1} at 11.25 cm and 22.50 cm row spacing of wheat, respectively. Plots with full weed

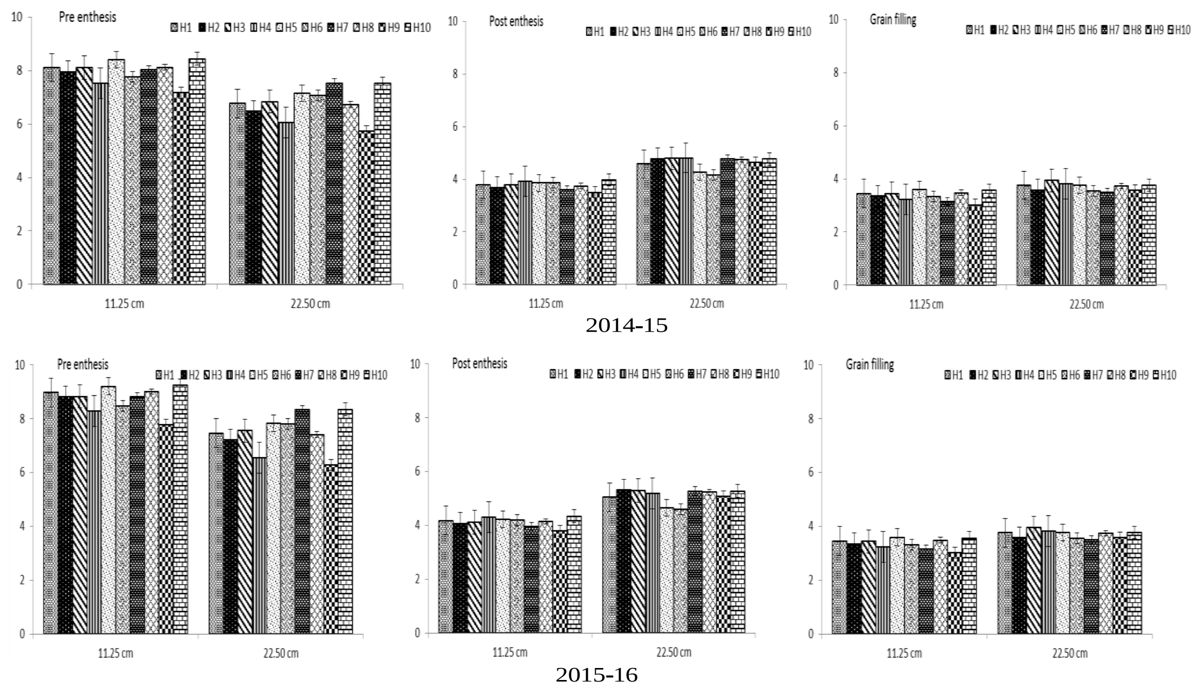

Fig. 2: Influence of different herbicide mixtures and row spacing (11.25 and 22.50 cm) on net assimilation rate (g m^{-2} day^{-1}) of wheat at pre enthesis, post enthesis and grain filling stage during 2014-15 and 2015-16. H_1: Clodinafop –propargyl + metribuzin (75% of R for each), H_2: Clodinafop-propargyl + metribuzin (R for each), H_3: Sulfosulfuron + clodinafop-propargyl (75% of R for each), H_4: Sulfosulfuron + clodinafop-propargyl (R for each), H_5: Pinoxaden + sulfosulfuron (75% of R for each), H_6: Pinoxaden + sulfosulfuron (R for each), H_7: Pinoxaden + metribuzin (75% of R for each), H_8: Pinoxaden + metribuzin (R for each), H_9: weedy check, H_{10}: weed free were used, R: recommended dose. Vertical bars represent standard errors of three replicates

competition produced minimum grain yield (4.12 and 3.94 t ha^{-1} at 11.25 and 22.50 cm row spacing, respectively). Interactive effect of different herbicide mixtures and two row spacing was found significant for both years (Table 4). All mixture treatments at both row spacing increased the grain yield of wheat compared to the weedy check during both years of study. Application of herbicide mixtures caused up to 31 and 32% increase in grain yield of wheat during 2014–2015 and 2015–2016, respectively. Overall effects revealed that at 11.25 cm row spacing wheat produced 6% more grain yield as compared to 22.50 cm row spacing.

Discussion

Herbicides used in these mixtures including clodinafop–propargyl, metribuzin, pinoxaden and sulfosulfuron are alternative narrow leave herbicides to manage fenoxaprop resistant *P. minor* in wheat. Tank mixtures comprising of compatible herbicides provided effective control of both susceptible and resistant weed populations (Beckie, 2006; Lagator *et al*., 2013; Evans *et al.,* 2016). We did not select fenoxaprop as a mixture component in any herbicide mixture to assure the efficacy of that mixture against fenoxaprop resistant *P. minor* (Beckie, 2006). In the present study, all mixtures effectively controlled *P. minor* even at 75% of the labeled dose for each mixture component and furnished about 90–100% control as is reported elsewhere (Abbas *et al.,* 2016b; Evans *et al.,* 2016). Differential growth and reproductive response of resistant *P. minor* to different herbicide mixtures can be justified by differences in the efficacy of herbicides used in the mixture and potential cross resistance of *P. minor* population (Shehzad *et al.,* 2012). It was also detected that surviving *P. minor* plants after mixtures spray produced significantly less dry biomass and seeds than non-treated ones. These findings support the notion that use of diverse herbicide molecules can help avert or delay resistance since multiple genes will be required to confer herbicide tolerance trait. Moreover, probability of occurrence of this phenomenon in a single plant is also extremely low (Yadav *et al.,* 2016). During the course of the present study, weed previously resistant to vulnerable herbicide/s was killed by the more efficient and robust herbicides or were at least rendered reproductively less fit than untreated plants. Reduction in seed production potential of *P. minor* can help reduce the weed seed bank in the long run. Recent studies revealed the importance of herbicide mixtures to slow down the evolution of resistance and to manage resistant weeds by reducing their fitness and negative cross-resistance (Beckie, 2006; Abbas *et al.*, 2016c; Evans *et al.*, 2016; Lamichhane *et al.*, 2016). Nevertheless, the component herbicides in the mixture should have different target sites, and modes of actions and similar weed control efficiency to cope with resistance development (Friesen *et al.*, 2000; Beckie, 2006) as used in the present study.

The higher cost of herbicide mixtures is a major concern for their use (Lagator *et al.*, 2013). However, the present study demonstrates that such costs can be reduced by reducing dose of herbicides mixtures and the reduction in weed control efficacy can be compensated by wheat row spacing. More weed control achieved under narrow row spacing (11.25 cm) as compared to recommended row spacing (22.50 cm) of wheat can be justified by the reduced competition for space, light and inputs (Rasmussen, 2004; Drews *et al.*, 2009). Crop competition has an important role in weed management (Sardana *et al.*, 2016). Integration of wheat row spacing with herbicides had been reported to reduce the herbicide dose to control *P. minor* in wheat (Bhullar and Walia, 2004; Sardana *et al.*, 2016). Thus, integration of cultural weed control such as the adjustment of row spacing with herbicide mixtures can potentially be used to reduce the doses of herbicide mixtures (Beckie, 2006; Little and Tardif, 2005; Lagator *et al.*, 2013; Sardana *et al.*, 2016). Use of lower rate of herbicide mixtures in conjunction with cultural weed control is the best strategy to delay resistance for sustainable weed control (Beckie and Kirkland, 2003).

The phytotoxic influence of mixtures on crop growth is considered a crucial factor in limiting basic restriction to their use in crop production. Our results revealed that there was no phytotoxicity of any herbicide mixture on the wheat, except slight reduction in NAR in the plots treated with pinoxaden + sulfosulfuron and pinoxaden + metribuzin at 100% product labeled doses. These findings supported by previous studies revealed that various herbicide mixtures can be used in field crops without any phytotoxicity (Bailly *et al.*, 2012; Collavo *et al.*, 2013; Evans *et al.*, 2016). This might be due to the complementary effect of these mixture combinations and varietal sensitivity of wheat to higher herbicide doses (Yadav *et al.*, 2016). Slight toxicity that was observed after spray was recovered by wheat plants later on, and non-significant influence was detected on the grain yield. Likewise, Mahajan *et al.* (2011), while working with rice, observed that herbicide induced growth inhibition and injury was transitory and crop recovered in a week still producing desired yields. Khaliq and Matloob (2012) opined that herbicides are phytotoxic molecules, the selectivity for crop plants and weed species achieved under field conditions is relative depending on several factors and their complex interactions. These authors concluded that even an herbicide cause phytotoxcity to crop plants, its use to manage weed species (especially resistant biotypes as is the case with present study) will still depend on the relative benefits as compared with other non-chemical weed control options for wheat crop. Efficient control of resistant *P. minor* yields less weed-crop competition for resources and little to no phytotoxicity to wheat plants with factors conducive to higher grain yield. Narrow row spacing without increasing total seed rate increased wheat grain yield due to less intra-plant competition between plants of the same row and provided more room for roots to uptake nutrient (Chen *et al.*, 2008). It also increased the water and nutrient use efficiency (Chen *et al.*, 2010) that leads to higher crop yield. Hussain *et al.* (2015) reported 28 to 43% reduction in wheat yield at the *P. minor* density of 40 plants m^{-2}. They also concluded 3 to 7 plants m^{-2} of *P. minor* as threshold level of this weed in wheat. Hussain *et al*. (2012), also revealed increase in wheat yield at narrow row spacing.

Conclusion

Post-emergence mixtures including clodinafop-propargyl + metribuzin, pinoxaden + sulfosulfuron, pinoxaden + metribuzin and sulfosulfuron + clodinafop-propargyl at 75or 100% of the labeled dose/s of each mixture can be effectively used in wheat to manage fenoxaprop resistant *P. minor*, especially at 11.25 cm row spacing of wheat. Narrowing the spacing of wheat rows by half from 22.50 to 11.25 cm not only compensated for 25% less herbicide input but also increased wheat yield by 6% more than recommended row spacing. Farmers should consider the integrated use of these herbicide mixtures and narrow row spacing (11.25 cm) of wheat to cope with the increasing challenge of *P. minor* resistance and to increase wheat yield. Moreover, these herbicide mixtures need to be marketed as pre-packaged commercial products to manage herbicide resistant *P. minor* or other related grassy weeds in wheat.

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