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Growth and biochemical responses of wheat (*Triticum aestivum* L.) to different herbicides

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The present experiment was conducted to assess the effect of herbicides on growth and biochemical behaviour of wheat crop during the rabi season 2009-10 at Instructional farm of Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad, Uttar Pradesh, India. The experiment comprised one wheat variety HUW-468 and four herbicides treatments, isoproturon, phenoxaprop-p-ethyl, sulfosulfuron and one untreated check. All the plots were kept free of weeds manually throughout the growing season. Plant height, number of tillers plant^{-1} and leaf area index of wheat were reduced under herbicides treatments with respect to untreated check. Reduction in above growth characters was lowest with sulfosulfuron followed by phenoxaprop-p-ethyl and isoproturon. Although, the difference between untreated check and herbicides for grain yield, biological yield and harvest index was non significant but slightly lower values for above traits were observed with herbicides as compared to untreated check. Nitrate reductase, nitrite reductase activity and protein content in leaves were also reduced due to herbicides relative to untreated check, however sulfosulfuron caused least reduction. Catalase and peroxidase activities in leaves were significantly increased due to herbicides over untreated check; minimum increase was observed with sulfosulfuron, while isoproturon had maximum increase. Thus, it may be concluded that among herbicides used in the experiment, sulfosulfuron is the safest for weed control in wheat as it showed least adverse effect on growth, yield and biochemical parameters of crop.

Key words: *Triticum aestivum* L., herbicides, isoproturon, phenoxaprop-p-ethyl, sulfosulfuron, nitrate reductase, nitrite reductase, catalase, peroxidase.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important cereal crop and occupies a significant position in the economy of India. About one third of the world population is based on wheat crop for protein and caloric requirements (Khan,

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2003; Montazeri and Baghestani, 2005). It is foremost among cereals and as a main source of carbohydrates and protein for both human beings and animals; contains starch (60-90%), protein (11-16.5%), fat (1.5-2%), inorganic ions (1.2-2%) and vitamins (B-complex and vitamin E) (Guarda et al., 2004; Rueda-Ayala et al., 2011). Although, the soil and climatic conditions of India are favourable for wheat production but its per hectare yield is very low. Among various factors, weeds infestation is one of the main causes of low wheat yield not only in India but all over the world, as it reduces wheat yield by 37 to 50% (Waheed et al., 2009; Rao, 2000).

Weeds can be controlled manually which is laborious, time consuming, energy intensive and only possible on small scale. Mechanical means are economical but it controls only inter row weeds, not intra row weeds. In such situations, herbicides offer most ideal, practical, effective and economical means of reducing early weed competition and crop production losses. So, chemical method for controlling weeds is most effective, efficient, up-to-date and time saving (Ashiq et al., 2007). At present, a number of pre and post-emergence herbicides are used in wheat fields for controlling weeds and to enhance wheat grain yield. There are evidences suggesting that herbicides besides controlling weeds produced phytotoxic effect in crop. A decrease in chlorophyll (Wavare and Baste, 1989; Auge et al., 1990), protein (Mohammed et al., 2000; Aamil et al., 2004) and carbohydrate content (Nemat et al., 2001) have been found in wheat with herbicides. Reduction in nitrate reductase activity and nitrite reductase activity in leaves of wheat has been also reported by researchers (Klipper, 1979; Rao et al., 1988). In India particularly in eastern Uttar Pradesh, the most commonly used herbicides by farmers in wheat are isoproturon, phenoxaprop-p-ethyl and sulfosulfuron. The response of wheat for detoxification of above chemical compound may not be the same; for some herbicides it may possess rapid mechanism of detoxification than others. So, these herbicides may have differential phytotoxic effect on crop. Still, it is unclear which one is the safest, however due to their equal weed killing efficiency and cost farmers choose any of them. It was, therefore, felt necessary to evaluate the phytotoxic effect of above herbicides in terms of their effect on growth and biochemical components of wheat crop.

MATERIALS AND METHODS

The present investigation was carried out under field condition at Instructional Farm of Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad, Uttar Pradesh, India during rabi season 2009-10. The experiment consisted one wheat variety, HUW-468 and four herbicides treatments including one untreated check (Table 1). The experiment was laid out in a randomized complete block design (RCBD) with three replications. The crop was sown during last week of October using a seed rate of 100 kg ha^{-1} in rows 22.5 cm apart at an average depth of 5 cm with the help of a single row hand drill. Nitrogen (N), phosphorus (P) and potassium (K) were applied at the rate of 120, 60 and 60 kg ha^{-1} in the form of urea, diammonium phosphate and muriate of potash, respectively. Full P, K and half of the N were applied as basal dose at the time of sowing, while remaining N was top dressed in two equal splits; one at first irrigation (25 days after sowing) and the other at panicle initiation stage (90 DAS). The crop was irrigated five times; the first irrigation was done at 25 DAS (CRI stage) and subsequent irrigations were applied at four growth stages viz., tillering, booting, anthesis and dough stage. Suitable plant protection measures and other recommended agronomic practices were adopted for growing the crop. All the plots were kept free of weeds manually throughout the growing season. The post-emergence herbicides, isoproturon at 1.0 kg ha^{-1}, phenoxaprop-p-ethyl at 800 g ha^{-1}, and sulfosulfuron at 250 g ha^{-1} were sprayed at 30 DAS with the help of a hand operated knapsack sprayer fitted with flat fan nozzle using 600 L water per hectare. The growth observations were recorded on plant height, number of tillers per plant and leaf area index (LAI) at different growth intervals. Grain yield and biological yield was recorded at harvest of the crop. The height of main shoot of tagged plants was measured at 45, 60 and 90 days after sowing from ground level to the ligule of top most leaf prior to ear emergence and from ground level to the base of ear after ear emergence with the help of meter scale and average plant height was expressed in centimetre. The numbers of tillers were also counted on tagged plants at 45, 60 and 90 DAS and have been reported as number of tillers per plant. The leaf area index was calculated by dividing leaf area with ground area. A unit area of 1 m^2 was selected at random from two different sites of each plot for recording grain yield (kg ha^{-1}) and biological yield (kg ha^{-1}). The harvest index was computed by dividing the grain yield by biological yield and expressed as percent.

The total protein content in leaves was estimated at 45 and 60 DAS by the method of Lowry et al. (1951). Nitrate reductase and nitrite reductase activity in leaves was estimated at 45 and 60 DAS by the method of Jaworski (1971) and Ferrari and Varner (1971), respectively. Catalase and peroxidase activity in wheat leaves were assayed colorimetrically according to method given by Sinha (1872) and Galston and Kaur (1959), respectively. The statistical analysis of data was done by the method described by Panse and Sukhatme (1978).The comparison of the treatments means was made with the help of least significant differences (LSD) at 5% probability level.

RESULTS AND DISCUSSION

A slight decrease in plant height of wheat was found due to herbicides as compared to untreated check at all stages of observations which was statistically non significant (Table 2). Minimum decrease in plant height was recorded with sulfosulfuron followed by phenoxaprop-p-ethyl and Isoproturon at all stages of observation. Similar to our results decrease in plant height of wheat with the use of different herbicides has been reported by Shehzad et al. (2012). In the present experiment, it was also observed that sulfosulfuron caused least and non significant decrease in plant height which is supported by results of Davies et al.(2003) who found stimulatory effect of sulfosulfuron (0.1 µg L^{-1}) on shoot height of *Glyceria maxima* and *Myriophyllum spicatum*. Number of tillers per plant differed significantly due to herbicides at 60 and 90 DAS, whereas a non significant difference was found at 45 DAS (Table 2).

Table 1. Herbicide treatments used in experiment.

Herbicides	Application time	Trade name	Rate (kg a.i. ha^{-1})
Control	-	-	-
Isoproturon	At 30 DAS	Tolkan (50%WP)	1.0
Sulfosulfuron	At 30 DAS	Leader	0.25
Penoxaprop-p-ethyl	At 30 DAS	Puma super	0.80

Table 2. Effect of different herbicides on plant height, number of tillers/plant and leaf area index (LAI) of wheat (variety- HUW 468).

Herbicides	Plant height (cm)			No. of tillers/plant			Leaf area index		
	45 DAS	60 DAS	90 DAS	45 DAS	60 DAS	90 DAS	45 DAS	60 DAS	90 DAS
Control	21.84	62.44	90.48	3.53	5.86	3.39	1.65	3.51	4.06
Isoproturon	21.47	59.20	87.60	3.49	5.58	2.99	1.59	3.29	3.86
Sulfosulfuron	21.84	61.21	89.81	3.52	5.81	3.30	1.60	3.45	4.00
Penoxaprop-p-ethyl	21.72	60.28	88.78	3.49	5.73	3.18	1.60	3.41	3.95
SEm±	0.33	0.73	1.44	0.05	0.06	0.07	0.03	0.05	0.06
LSD at 5%	NS	NS	NS	NS	0.21	0.23	NS	NS	0.20

NS, Not significant.

Isoproturon recorded significant reduction in number of tillers per plant as compared to untreated check at 60 and 90 DAS, while sulfosulfuron being at par with phenoxyaprop-p-ethyl caused least and non significant reduction at both stages. A non significant decrease in leaf area index was found with herbicides relative to control at 45 and 60 DAS, whereas at 90 DAS, significant decrease was observed (Table 2). Isoproturon showed significant reduction in leaf area index, while sulfosulfuron and phenoxyaprop-p-ethyl had at par values with respect to control at 90 DAS. Decrease in number of tillers and leaf area index of wheat with herbicide treatments over untreated check indicates that herbicides besides controlling the weeds weaken the physical and biochemical defences of crop. This is evident from the results on biochemical parameters (Table 3) of this experiment. Sulfosulfuron does not directly interfere photosynthetic process, as isoproturon affect the light reaction and phenoxaprop-p-ethyl affect translocation of carbohydrates. This may be one of the possible reasons for less inhibitory effect of sulfosulfuron in comparison to other herbicides.

All the herbicide treatments decreased nitrate reductase activity in leaves over untreated check (Table 3). At 45 DAS, differences in nitrate reductase activity among sulfosulfuron and untreated check were non significant, however significant differences were found between Isoproturon, phenoxaprop-p-ethyl and untreated check. However at 60 DAS, only isoproturon showed significant reduction in nitrate reductase activity with respect to untreated check, while sulfosulfuron and phenoxaprop-p-ethyl showed at par values. This indicates that the reducing effect of phenoxaprop-p-ethyl on nitrate reductase activity was minimised at 60 DAS. Nitrite reductase activity was decreased significantly with all herbicide treatments relative to untreated check at 45 and 60 DAS (Table 3). At both the stages, minimum reduction was found with sulfosulfuron followed by phenoxaprop-p-ethyl and isoproturon. The reduction of NO_3 takes place in the leaves of plant. The nitrate reductase and nitrite reductase, enzymes associated with nitrate reduction are located in cytoplasm and chloroplast of leaves, respectively. The activity of both enzymes largely depends on energy (ATP) generated in light reaction and electron transfer from ferredoxin. Isoproturon inhibit light reaction by blocking the transfer of electron from PSII to pheophytin, which indirectly affect the activity of nitrate reductase and nitrite reductase. Secondly, isoproturon also causes photobleaching of pigments due to excess excitation of chlorophyll molecules. On the other hand, sulfosulfuron does not act directly on chloroplast, so has less adverse effect on the activity of both enzymes as compared to isoproturon and phenoxaprop-p-ethyl. Klepper (1979) reported that photosynthetic inhibitor herbicides interfere with the transfer of light energy and cause accumulation of nitrite due to decrease in activity of nitrite reductase. Similar to our findings Rao et al. (1988) found a decrease in activity of nitrate reductase and nitrite reductase in leaves of wheat with pyidazinone herbicide.

Herbicide treatments resulted in an increase in the activity of catalase and peroxidase enzymes over untreated check (Table 3). All the herbicides showed significant increase in catalase activity relative to untreated

Table 3. Effect of different herbicides on biochemical parameters of wheat (variety- HUW 468).

Herbicides	Nitrate reductase (µ mole NO_2 produced/g fresh wt./h)		Nitrite reductase activity (µ mole NO_2 reduced/g fresh wt./h)		Catalase activity (unit/g fresh wt.)		Peroxidase acivity (unit/g fresh wt.)		Protein content (%)	
	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS	45 DAS	60 DAS
Control	41.63	52.00	33.75	46.25	51.88	73.38	61.00	86.38	1.90	2.00
Isoproturon	35.38	46.25	28.38	39.75	59.25	81.00	67.75	95.75	1.78	1.96
Sulfosulfuron	39.88	50.50	31.88	43.13	54.75	76.63	63.88	90.13	1.84	1.99
Penoxaprop-p-ethyl	37.75	49.00	30.88	42.50	56.25	83.88	65.63	97.25	1.84	1.97
SEm±	0.52	1.09	0.33	0.58	0.68	1.24	0.89	0.96	0.03	0.03
LSD at 5%	1.78	3.76	1.13	2.00	2.36	4.29	3.07	3.32	0.09	NS

NS, Not significant.

check at 45 DAS. However at 60 DAS, differences between sulfosulfuron and untreated check were at par, while other herbicides had significant increase. The increase in peroxidase activity was highest with Isoproturon followed by phenoxaprop-p-ethyl and sulfosulfuron at both 45 and 60 DAS. Catalase and peroxidase enzymes are considered as reactive oxygen species (ROS) scavenging enzymes in the plant system which split the hydrogen peroxide (H_2O_2) in to water and oxygen. Super oxide radicals (O_2^-) and H_2O_2 anions are produced in plants under various abiotic and biotic stresses. These anions are toxic in nature, cause membrane damage and inactivation of various enzymes. Increase in catalase and peroxidase activity in wheat leaves due to herbicide treatments is an indicative of increased detoxification of H_2O_2 anions which are produced in response to stress caused by herbicides. Highest expression of both enzymes under isoproturon suggests about its more phytotoxic nature than other herbicides as these enzymes are substrate inducible, their activity increase due to enhanced production of H_2O_2 anions. Mohammad et al. (2000) also found an increase in catalase activity in wheat seedlings with herbicide, but found a decrease in peroxidase activity.

Leaf protein content was also adversely affected due to application of herbicides (Table 3). Isoproturon showed a significant decrease in protein content over untreated check, while other herbicides had non significant decrease at 45 DAS. However at 60 DAS, difference between untreated check and all herbicides was non significant. The key enzymes associated with synthesis of amino acids and proteins are targeted by the herbicides which may be one of the possible reasons for decreased protein content under herbicide treatments. Although, there was a non-significant variation in grain yield and biological yield between herbicides and untreated check but slightly lower values were observed with herbicide treatments with respect to untreated check (Table 4). Percent reduction in grain yield due to herbicides over untreated check was lowest with sulfosulfuron (0.47%), while Isoroturon had highest reduction (4.85%). Similarly, minimum percent reduction in biological yield with respect to untreated check was observed with sulfosulfuron (0.17%) followed by phenoxaprop-p-ethyl (2.26%) and isoproturon (4%). Difference in harvest index among untreated check and different herbicides was found non significant (Table 4). Yield is a summation of all metabolic processes and growth events during life cycle of a crop plant, so the adverse effects of herbicides on these processes as evident from the results of present experiment may affect crop yield. Our results are in close conformity with Nowicka (1991) who also observed a decrease in yield of different wheat varieties with chlorosulfuron and metoxuron. Similarly, Devlin et al. (1995) found 16% reduction in dry weight of wheat by 50 um CGA-26423 herbicide.

Conclusion

The study concludes that among the herbicides used in the experiment, sulfosulfuron is the safest for weed control in wheat as it had least adverse effect on growth, yield and biochemical parameters of crop.

Table 4. Effect of different herbicides on grain yield, biological yield and harvest index of wheat (variety- HUW 468).

Herbicides	Grain yield (q/ha)	Biological yield (q/ha)	Harvest index (%)
Control	46.76	101.77	45.95
Isoproturon	44.49 (4.85)	97.70 (4.00)	45.54
Sulfosulfuron	46.54 (0.47)	101.60 (0.17)	45.81
Penoxaprop-p-ethyl	45.54 (2.61)	99.47 (2.26)	46.00
SEm±	0.74	1.62	0.77
LSD at 5%	NS	NS	NS

Values in parentheses indicate percent decrease over control; NS, Not significant.

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