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EFFECTS OF 2, 4-D HERBICIDE ON THE BIOCHEMICAL CHARACTERISTICS OF *TRITICUM AESTIVUM* L.

Saima Ashraf* and Ghulam Murtaza

Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad-13100, Pakistan

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Abstract

Dichlorophenoxy acetic acid herbicide was used to check its effect on biochemical characteristic of wheat (*Triticum aestivium*). It was sprayed on wheat on tillering stage and samples were collected on milking stage. The samples were used to estimate protein, sugar, phenolic compound and activity of catalase and ascorbate peroxidase. 0.03 M concentration showed high protein and sugar contents. 0.04 M showed highest activity of catalase and ascorbate peroxidase activity.

Introduction

Among the pesticides most widely used are herbicides. For the control of weeds herbicides are effective and economical because weeds are the most important pest in crop production. Certain herbicides affect metabolic pathways and systems unique to plants but absent in animals. (Periyanayagi and Senthilkumar 2015). Herbicide interference with the morphology, physiology and biochemical pathways of treated plants varies according to the characteristic actions of the herbicide and depends upon the degree of tolerance or susceptibility of the crop plant species. Mode of action of herbicide is not well documented but it was examined that once an herbicide reaches the targeted site of treated plants, the biochemical processes are affected. Herbicides differ in their site of action and may have more than one site of action in plants.

Dichlorophenoxy acetic acid (2,4-D), a chlorinated phenoxy compound, functions as a systemic herbicide and is used to control many types of broad leaf weeds (Periyanayagi and Senthilkumar 2015). The herbicide 2,4-D had been used in crop fields quite for a long time. Depending on the type of cereal crop, the weed spectrum, cultural practices and climatic factors, 2,4-D might be applied as salts, esters, amines or free acid formulations. The herbicide application is usually made when weeds and cereal crop plants are small (Kumar and Singh 2010).

Wheat is the member of Poaceae, tribe hordeae, genus *Triticum* and species *aestivum.* Wheat crop has ability to grow everywhere so it was cultivated in all areas such as tropical, subtropical and temperate areas (FAO 2002). Most of the people used wheat (*Triticum aestivum* L.) as a major source of food. It has rightly been called "The King of Cereals, in addition food, wheat is used in a number of ways for a large population (Khan *et al*. 2011). Wheat (*T. aestivum*) is a staple food in Pakistan and plays a vital role in its economy. Weeds are one of the factors that are effect the wheat crop production and reduce yield. Many agronomic practices are used to eliminate weeds. 2,4-D is a common herbicide used in the fields to remove the weeds. The present study is conceived to analyze the impact of 2,4-D on a biochemical aspect of wheat.

Materials and Methods

Five genotypes *viz.,* AAS-2011, Punjab-2011, NARC-2011, NARC-2009 and Millat-2011 were selected to test their response toward the effect of 2, 4-D herbicide. RCBD with three replications was used. Three different concentrations of 2, 4-D were used. Fully developed flag

*Author for correspondence: <s.ashraf084@gmail.com>.

leaves was sampled for biochemical analysis at milking (Zadoks scale 70) stage (Zadoks *et al*. 1974).

Protein contents from the samples were determined by following the method of Bradford (1976). The quantity of protein was estimated from samples by reading the absorbance at 595 nm. Sugar content was estimated according to Dubois *et al*. (1956). The absorbance was taken at 420 nm. Standard curve of glucose was used to calculate the concentration of unknown sample. Total phenolics was determined following the method of Julkenen-Titto (1985). The mixture was vortexed and absorbance was read at 750 nm using a spectrophotometer. Total phenolic contents were calculated from the standard curve made of gallic acid.

The activity of ascorbate peroxidase was deteremined according to Bartoli *et al*. (1999). The activity of enzyme was analyzed by following the decrease in the absorbance (265 nm). Catalase activity was assayed according to Aebi (1984). The disintegration of H_2O_2 was directly observed at 240 nm by following the decrease in extinction. The catalase activity was measured by measuring per minute difference in extinction.

ANOVA was used to test the significance of the obtained data and then DMRT was performed to compare differences among means.

Results and Discussion

Significant results in wheat varieties from each other were observed with respect to protein during 2013-14 and 2014-15 (Table 1). In case of herbicide treatment, 0.03 M application of 2,4-D herbicide increased protein content and 0.04 M decrease protein content. The high concentration (5 ppm) of 2,4-D treated Bhendi seedlings showed a reduction in protein (Periyanayagi and Senthilkumar 2015) while El-tayeb and Zaki (2009) reported that soluble protein was decrease significantly with increase the concentrations of 10 - 4 % 10 - 5% and 10 - 6% round up herbicide in leaves, root and stem of *Vicia faba*.

Varieties, concentration of 2, 4-D showed significant difference regarding sugar during both years of study (Table 2). 0.03 M application of 2,4-D increased sugar content and 0.04 M decreased sugar content.

High concentration of 2,4-D (5 ppm) showed reduction in sugar in *Abelmoschus esculentus* seedlings (Periyanayagi and Senthilkumar 2015). Prensser *et al*. (1984) investigated that application of herbicide Malathion decreased the soluble sugar content in *Vicia faba* plants. Our result are in accordance with that of Periyanayagi and Senthilkumar (2015) and Prensser *et al*. (1984). This is probably due to the inhibition of photosynthetic machinery especially the Hill reaction and reduction in chlorophyll content of the leaf also decline in protein (Periyanayagi and Senthilkumar 2015).

Results showed that significant difference was observed among five genotypes of wheat by applications of different concentrations of 2,4-D with respect to phenolic compound during both growing seasons (Table 3).

An increase in phenolic compound was observed in soybean plants treated with the commonly used herbicide glyphosate ((Marchiosi *et al*. 2009). Our results are in agreement from previous results. Which might have resulted from the formation of excessive amounts of free radicals in the leaves (Michałowicz and Duda 2009).

Genotypes of wheat found to be significantly different from each other in case of catalase activity during both growing seasons. Herbicide treatments resulted in an increase in the activity of catalase enzyme over untreated check. Mohammad *et al*. (2000) also reported an increase in catalase activity in wheat seedlings with herbicide. El-Tayeb and Zaki (2009) reported that catalase activity was increased significantly with the increased concentrations of roundup herbicide in leaves and root of *Vicia faba*.

Table 1. Effects of 2,4-D herbicide on protein (mg/g L FW) in flag leaves of wheat varieties at milking stage.

Conc.→	2013 -2014					2014 -2015				
Varieties↓	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	0.01 M	0.03 M	0.04 M	Means
AAS-2011	2.030 c	2.156 b	2.276 a	1.977 d	2.110A	2.042 c	2.154 b	2.264 a	1.973d	2.108A
Punjab-2011	1.748 g	1.618 i	1.838 e	1.405k	1.652C	1.831 f	1.758 g	1.997 d	1.645 h	1.803B
NARC-2011	1.834 e	1.779 f	1.974 d	1.649 h	1.809B	1.736 g	1.623 h	1.842 f	1.439i	1.660C
NARC-2009	1.477 j	1.775 h	1.656 h	1.257 l	1.541 E	1.439 i	1.742 g	1.635h	1.244j	1.515D
Millat-2011	1.830 e	1.642 h	1.969 b	1.117m	1.640D	1.855 f	1.638 h	1.930 e	1.122k	1.636C
Means	1.784 B	1.794 D	1.943 A	1.48 1 C		1.781 B	1.783 B	1.930 A	1.485 C	

Values having similar letters are non-significant to each other using DMRT at 0.05% probability level.

Table 2. Effects of 2,4-D herbicide on sugar (mg/g LFW) in flag leaves of wheat varieties at milking stage.

Conc. →	2013 -2014					2014 -2015				
Varieties↓	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	0.01 M	0.03 M	0.04 M	Means
AAS-2011	0.668 h	0.840 d	0.976 c	0.779 e	0.816 B	0.670 f	0.842 c	0.981 b	0.777 d	0.817 B
Punjab-2011	0.578 j	0.440 k	0.615 i	0.328 n	0.492 E	0.965 b	1.039 a	1.064 a	0.820 c	0.972 A
NARC-2011	0.685 h	0.357 m	0.757 ef	0.415 l	0.553 D	0.625 g	0.761de	0.732 e	0.558 h	0.669 C
NARC-2009	0.964 c	1.031 b	1.077 a	0.825 d	0.974 A	0.565 h	0.453 i	0.621 g	0.339 k	0.494 E
Millat-2011	0.620 i	0.752 f	0.724 g	0.556 j	0.663 C	0.684 f	0.356 k	0.759 de	0.424 j	0.556 D
Means	0.703 B	0.685 C	0.830 A	0.581 D		0.702 B	0.690 B	0.831 A	0.584 C	

Values having similar letters are non-significant to each other using DMRT at 0.05% probability level.

Table 3. Effects of 2,4-D herbicide on phenole (mg/gl FW) in flag leaves of wheat varieties at milking stage.

Conc. →	2013 -2014					2014 -2015				
Varieties↓	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	0.01 M	0.03 M	0.04 M	Means
AAS-2011	0.710 c	0.662 g	0.669g	0.957 b	0.749b	0.520 d	0.535 d	0.652 b	0.741a	0.612 a
Punjab-2011	0.653 gh	0.562 i	0.683 fg	0.625 h	0.631d	0.452 f	0.568 c	0.373 hi	0.466 ef	0.465 b
NARC-2011	0.660 g	0.867c	0.427 j	0.767 e	0.680 c	0.365 hi	0.316 k	0.357 i	0.398 g	0.359 d
NARC-2009	0.143 k	0.815d	0.760 e	0.558 i	0.569 e	0.306 k	0.314 k	0.381 h	0.338 j	0.335 e
Millat-2011	0.741e	1.007a	0.942 b	0.671 g	0.840a	0.560 C	0.285l	0.472 e	0.376h	0.423 c
Means	0.581 d	0.783a	0.696 c	0.716 b		0.441 b	0.404 c	0.447 b	0.464 a	

Values having similar letters are non-significant to each other using DMRT at 0.05% probability level.

Table 4. Effects of 2,4-D herbicide on catalase (µmole H_2O_2 reduced min/mg/protein) in flag leaves of wheat varieties at milking stage.

Conc. →	2013-2014					2014-2015				
Varieties↓	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	0.01 M	0.03 M	0.04 M	Means
AAS-2011	0.435d	0.420d	0.552b	0.643a	0.513a	0.458 c	0.342 f	0.263 gh	0.338 ef	0.350b
Punjab-2011	0.468c	0.352f	0.273hi	0.366ef	0.365b	0.425 d	0.404d	0.532 b	0.621 a	0.495a
NARC-2011	0.217k	0.265hi	0.257i	0.298g	0.259d	0.259 ghi	0.341ef	0.267 gh	0.323 f	0.298c
NARC-2009	0.214k	0.206k	0.281h	0.234 j	0.234e	0.229hi	0.230hi	0.232 hi	0.252 ghi	0.236d
Millat-2011	0.185l	0.460 c	0.372e	0.276h	0.323c	0.201 i	0.480bc	0.201 de	0.310fg	0.346b
means	0.304 c	0.341b	0.347b	0.363a		0.315 c	0.359 ab	0.337bc	0.369 a	

Values having similar letters are non-significant to each other using DMRT at 0.05% probability level.

Table 5. Effects of 2,4-D herbicide on ascarbate peroxidese (µmole H_2O_2 oxidized min/mg/protein) in flag leaves of wheat varieties at milking stage.

Conc. →	2013-2014					2014-2015				
Varieties↓	0 M	0.01 M	0.03 M	0.04 M	Means	0 M	0.01 M	0.03 M	0.04 M	Means
AAS-2011	0.570 d	0.368 i	0.618c	0.252 j	0.452 cd	0.574cd	0.356 g	0.240h	0.610 bc	0.445b
Punjab-2011	0.479f	0.767b	0.470fg	0.516e	0.558 b	0.409 fg	0.597 bcd	0.413 fg	0.365 g	0.446b
NARC-2011	0.745b	0.471fg	0.921a	0.538e	0.669a	0.640	0.365 g	0.433ef	0.812 a	0.562a
NARC-2009	0.372i	0.435h	0.489f	0.448gh	0.436d	0.483e	0.543d	0.556cd	0.599 bcd	0.545a
Millat-2011	0.237j	0.577d	0.577d	0.470 fg	0.465 c	0.293h	0.578cd	0.469e	0.574cd	0.465b
Means	0.481 c	0.524b	0.615a	0.445d		0.469b	0.488 b	0.422c	0.592 a	

Values having similar letters are non-significant to each other using DMRT at 0.05% probability level.

Data concerning ascorbate peroxidase activity (Table 5) showed varieties and concentrations to be significant during both growing seasons.

Foliar application of both 50 µM oxyfluorfen and 100 µM paraquat in rice plants increased ascorbate peroxidase (Kim and Jung 2013). El-tayeb and Zaki (2009) reported that ascorbate peroxidase activity was increased significantly with the increased concentrations of roundup herbicide in leaves and root of *vicia faba.*

Results showed an improvement in biochemical attributes of wheat varieties (NARC-2011, Millat-2011 and Aas-2011) when 0.01, 0.03 and 0.04 M concentrations of 2,4-D herbicide were applied.

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