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Research Article

OXIDATIVE STRESS AND ANTIOXIDANT METABOLIC ENZYMES RESPONSE OF MAIZE (*Zea mays* L.) SEEDLINGS TO A BIOTIC STRESS (ALACHLOR) CONDITION

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Abstract- In the present investigations, an endeavor was been made to appraise the alachlor efficiency on biochemical behavior of maize seedlings. Biochemical parameters significantly influenced by different doses alachlor application. The total starch, catalase, polyphenol oxidase and peroxidase content were significantly enhanced by ~ 53, 28, 85 34 and 31, 17, 26, 81% with 4, 8, 12 and 15 days old maize seedlings in both root shoot axis and endosperm respectively compared control ~ 44, 25, 37, 33 and 38, 33, 10, 77% in root shoot axis and endosperm respectively. Whereas, the reducing sugar and α-amylase content was decreased ~ 80, 91 and 61, 18% in both root shoot and endosperm from 4 to 15 days old maize seedlings, respectively compared to control. The results showed that the higher concentration of herbicide adversely affects the biochemical parameters, which are associated with seed germination and seedling growth of maize.

Keywords- Herbicide, Biochemical, Peroxidase, α-amylase, Alachlor, Maize, Greenhouse

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Introduction

Extreme uses of herbicides considerably manipulate all aspects of primary and secondary metabolism of the maize crop. Nowadays intensive agricultural practice uses of several herbicides are directly applied to the soil-plant system influenced the herbs, weeds and other aggressive plants, which were growing along with the main crop. The worldwide food deficiency is a severe comprehensive problem and hence to meet the intensifying demands of hastily growing world population for food to be augmented [1]. The injudicious application of agrochemicals forms an essential part of the crop production technology that makes it possible for the farmers to feed the ever-growing population. The use of pesticide at high rate may cause toxicity problem, which can deleteriously affect the plant growth and development, therefore reduction in the photosynthetic activity can delay the time of fruit harvest and affect the crop quality [2]. One of the possible ways to enhance the agricultural productivity with the effective pest management because~ 45% of annual food production is lost due to pest infestation, to sustain the agricultural productivity, herbicides are being used, which is an important member of pesticide family. The imbalanced and extensive use of pesticide negatively influenced the soil-plant-environment system. Such chemicals create diverse environmental problem via biomagnifications [3]. Maize is the third most important cereal crop in the worldwide after wheat and rice with a production of 590 million tonnes and productivity of 4229 kg/ha which occupies an area of 139 million ha.[4]. The herbicide alachlor (2-chloro-N-(2, 6 diethylphenyl) -N-(methoxymethyl) acetamide), is widely used as a selective herbicide to control the annual grasses. Moreover, the relevance of such chemicals in agricultural plants has a detrimental effect on their biochemical changes during growth and development. The several herbicides generate active oxygen species (AOS) either by direct involvement in the radicle production or by inhibition of biosynthetic pathways [5]. A number of enzymes, such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) [6] work as an antioxidant enzyme to scavenge the stress effects, CAT, APX and a variety of other peroxidases catalyze the breakdown of H_2O_2[7]. The plants have a well developed anti-oxidative machinery to prevent cellular membranes from toxic effects caused by reactive oxygen species [8]. Reactive oxygen species (ROS) are responsible for multi-stress induced damages to cellular structures [9,10]. Under the stress conditions, plants may alter the ROS scavenging enzymes, such as CAT and SOD [11]. Thus we made an attempt that the application of different doses of herbicide alachlor on biochemical parameters of maize seedling.

Materials and Methods

Maize (*Zea mays* L.) cultivar NAC-6002 was obtained from Zonal station, University of Agricultural Sciences, Naganahalliy, Mysore. The herbicide alachlor was obtained from Monsanto Company. The present experiment was conducted with five concentrations of herbicide (1.0, 2.5, 5.0, 7.5 and 10.0 ppm) of 10 ml each concentration along with control. The seeds were sown in pots with a diameter of 20 cm previously filled with fertile soil, followed by sowing spray the different concentrations of herbicide on the soil, for control sets treat with only distilled water. The 4, 8, 12 and 15- day-old seedlings were selected and root-shoot and endosperm were separated used for biochemical study includes starch, reducing sugar, α-amylase, catalase, peroxidase and polyphenol oxidase.

Starch content

In brief, 0.1 g sample was homogenized in hot 80% ethyl alcohol and centrifuged at 3,000 rpm for 10 minutes. Residue was washed repeatedly with hot 80% ethanol, the residue was dried over a water bath. To the residue 5.0 ml of water and 6.5 ml of perchloric acid was added. The extracts were kept at 4°C for 20 minutes, centrifuge and save the supernatant. The extraction was repeated using fresh perchloric acid and centrifuge. All the supernatants were pooled and make up to 100 ml with distilled water. 0.2 ml of the sample was pipette and the volume was made up to 1.0 ml with distilled water. 4.0 ml of anthrone reagent was added to each tube and heated for 8 minutes in a boiling water bath. The test tubes were cooled rapidly and the intensity of green to dark green colour was read at 630 nm. The amount of starch content in the sample will be calculated using the standard graph prepared by glucose [12].

Reducing sugar content

The 100 mg of the sample was used for the extraction of sugars with hot 80% ethyl alcohol. The contents were centrifuged at 3,000 rpm for 10 minutes. The supernatant was retained and evaporated by keeping it on a water bath at 80° C. 10 ml water was added to dissolve the sugars. 1.0 ml of the extract was pipette out in to the tubes and the volume was made up to 3 ml with distilled water. 3.0 ml of DNS reagent was added to each test tube. The content was mixed thoroughly and kept in a boiling water bath for 5 minutes. When the contents of the tubes are still warm 1.0 ml of 40 % Rochelle salt solution was added. Test tubes were cooled under running tap water and intensity of dark red colour was read at 510 nm. The amount of reducing sugars present in the sample was calculated by using standard graph prepared with glucose. The reducing sugar content was determined by using a spectrophotometer [13].

Estimation of α-amylase

The α-amylase content was estimated according to [14]. 1.0 g of sample was extracted with 10ml of ice-cold phosphate buffer (pH 6.8) centrifuged at 3,000 g at 4°C for 20 minutes and supernatant was used for the estimation of enzyme. 1 ml of enzyme extract was taken in a test tube and 1.0 ml of starch solution was added, incubated at 37°C for 30 minutes. The reaction was stopped by adding 2 ml of DNS reagent. The test tubes were kept in a boiling water bath for 5 minutes. While the tubes are still warm, 1.0 ml of 40 % potassium sodium tartarate solution was added and cooled under running tap water. The volume was made up to 10 ml with distilled water. The colour developed was read at 540nm against the reagent blank. The amount of α-amylase present in the sample will be calculated by using standard graph prepared with maltose.

Catalase content

Take 1.0 g of plant tissue was ground in a chilled mortar and pestle in the presence of 10 ml of cold 0.1 M phosphate buffer (pH 7.0).The homogenate was cooled centrifuged at 10,000 rpm for 30 minutes. The sediments were stirred with cold phosphate buffer and extraction was repeated twice. The supernatants were combined after each centrifugation and the clear supernatant was used as the source of enzyme. 2.5 ml of 0.1 M phosphate buffer was taken in an experimental cuvette along with 0.14 ml of 1 % hydrogen peroxide and 0.2 ml of enzyme extract was added. Cuvette containing enzyme solution with H2O2 but devoid of PO4 buffer was serving as blank. The change in absorbance was recorded at 240 nm for 3 minutes at 30 second intervals. The absorbance was adjusted to zero using the extract and buffer. The time required for a decrease in absorbance from 0.45-0.4 was recorded at 240 nm and the amount of catalase present in the sample was determined [15].

Estimation of Peroxidase:

The Peroxidase content was estimated following the method of [16]. 0.2 g of sample was ground well with a pre-cooled mortar and pestle with 10 ml of 0.1M phosphate buffer (pH 7) and centrifuged for 30 minutes at 10,000 g in cool centrifuge. The supernatant was used as an enzyme source. A mixture of 2 ml of phosphate buffer, 1 ml of 1% hydrogen peroxide, 1 ml of pyrogallol and 1 ml of tissue extract was incubated at 25° for 5 minutes. Then the reaction was stop by adding 0.5 ml of 5 % of sulphuric acid. The amount of purpurogallin formed will be determined by taking the absorbance at 420 nm for every 30 second for three minutes. A standard graph was prepared using purpurogallin and amount of peroxidase present in the sample was determined.

Estimation of Polyphenol oxidase:

The Polyphenol oxidase content was estimated following the method of [16]. 0.2 g of sample was homogenized with 10 ml of 0.1M phosphate buffer (pH 6.8). The contents were centrifuged at 4°C for 30 minutes at 10,000 g in cool centrifuge. Supernatant was used as a source for enzyme. 2.0 ml of phosphate buffer, 1 ml of pyrogallol and 1 ml of extract was taken in a test tube and incubate at 25°C for 5 minutes. The reaction was terminated by adding 0.5 ml of 5% sulphuric acid. The amount of purpurogallin formed was determined by taking the absorbance at 420 nm for every 30 seconds for three minutes. A standard graph was prepared using purpurogallin and amount of Polyphenol oxidase present in the sample was determined.

Statistical analysis

The obtained data were subjected to statistical analysis using SPSS package ver. 14.00 using Tukey's mean range test at 5% level of significance.

Results

Effect of alachlor on starch content

Results showed that the starch content was found to be increased with increasing concentrations of herbicide treatments in both root shoot axis and endosperm when compared to control. The starch content in root shoot axis of 4, 8, 12 and 15 day seedling increased from 3 % to 81 %, 18 % to 82, 9 % to 89 % and 31 % to 98% from 1.0 to 10 ppm concentrations, respectively compared to control. The starch content of endosperm at 4, 8, 12 and 15 day seedling increased from 10 % to 78 %, 16 % to 81 %, 16 % to 86 % and 27 % to 89% from 1.0 to 10 ppm concentrations, respectively compared to control. The amount of starch was found to be decreased in both root shoot axis [~ 53%] and endosperm [~ 34%] as the day's proceeds from 4 to 15 day seedlings at higher concentrations [Fig-1a and 1b].

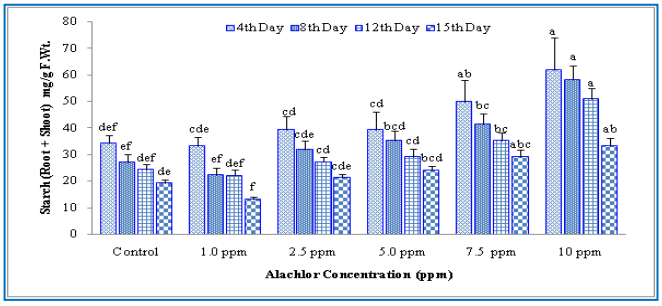

Fig-1a Effects of different concentrations of alachlor and growth interval of maize seedling on starch content in root and shoot of maize. Means followed by different alphabets differ significantly as established by SPSS package ver. 14.00 according to Tukey's mean range test at 5% level.

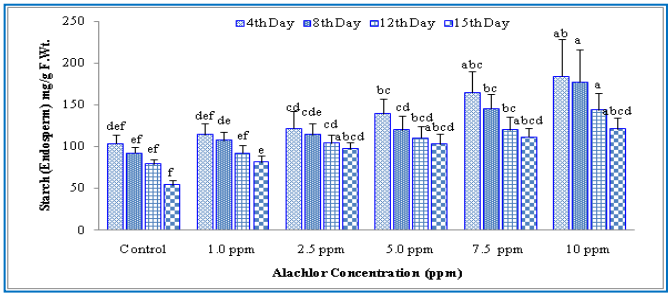

Fig-1b Effects of different concentrations of alachlor and growth interval of maize seedling on starch content in endosperm of maize. Means followed by different alphabets differ significantly as established by SPSS package ver. 14.00 according to Tukey's mean range test at 5% level.

Effect of alachlor on reducing sugar content

Data showed that the reducing sugar content significantly varied among the treatment with days after sowing. The amount of reducing sugar in root shoot axis increased and in endosperm decreased from 4 to 15 day's seedlings. The reducing sugar content in root shoot axis of 4, 8, 12 and 15 day seedling decreased from 23 to 74%, 12 to 65%, 20 to 59% and 10 to 59% from 1.0 to 10 ppm concentrations, respectively, when compared to control. The reducing sugar content of endosperm in 4, 8, 12 and 15 day seedling decreased from 16 to 70%, 7 to 61%, 20 to 61% and 7 % to 68 % from 1.0 to 10 ppm concentrations, respectively compared to control. The amount of reducing sugar significantly increased in root shoot axis and decreased in endosperm as the day's proceeds from 4 to 15 days from 13.14 to 28.87, 13.03 to 22.16, 8.14 to 19.09, 6.45 to 17.14, 4.31 to 13.05 and 7.34 to 3.01, 7.12 to 2.67, 6.01 to 2.14, 4.13 to 1.96, 2.65 to 1.02 mg/g fresh weight from 1.0, 2.5, 5.0, 7.5 and 10.0 ppm, respectively [Fig-2a and 2b].

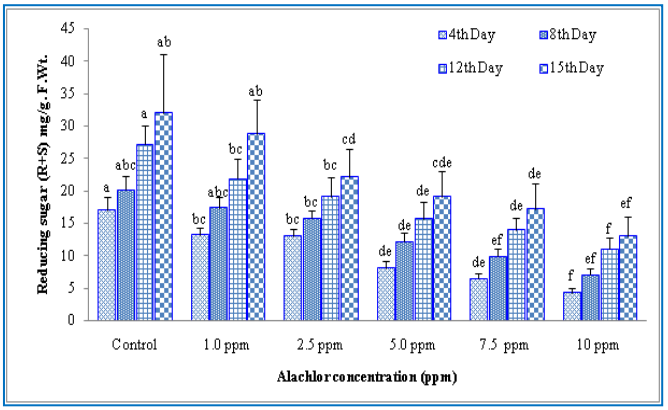

Fig-2a Effects of different concentrations of alachlor and growth interval of maize seedling on reducing sugar content in root shoot of maize. Means followed by different alphabets differ significantly as established by SPSS package ver. 14.00 according to Tukey's mean range test at 5% level.

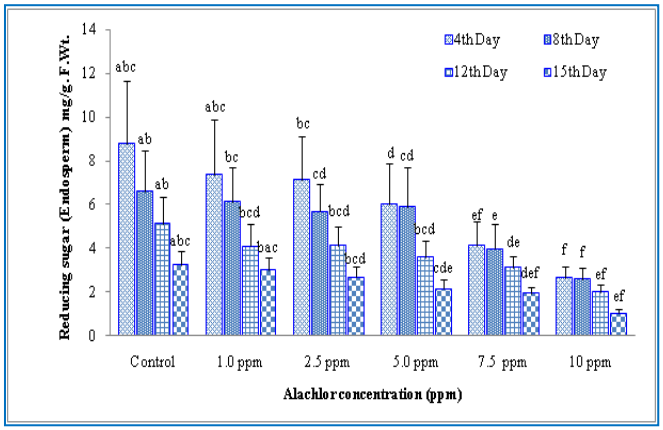

Fig-2b Effects of different concentrations of alachlor and growth interval of maize seedling on reducing sugar content in endosperm of maize. Means followed by different alphabets differ significantly as established by SPSS package ver. 14.00 according to Tukey's mean range test at 5% level.

Effect of alachlor on α-amylase activity

Results showed that the α-amylase activity in root shoot axis and endosperm was significantly affected with herbicide treatment respectively. The α-amylase content in both root shoot axis and endosperm was found to be decreased as the concentration increased from 1.0 to 10.0 ppm when compare to control [Fig-3a and 3b]. As the days of treatment increased the quantity of α-amylase in root shoot axis and in endosperm is slightly increased from 4 to 15 days seedlings 0.41 to 0.62, 0.4 to 0.59, 0.34 to 0.51, 0.29 to 0.49, 0.21 to 0.41 and 2.93 to 3.19, 2.84 to 3.09, 2.12 to 2.71, 1.75 to 2.02, 1.02 to 1.21 mg starch hydrolysed/gm fresh weight/min. from 1.0, 2.5, 5.0, 7.5 and 10.0 ppm respectively. On 4, 8, 12 and 15 day in root shoot axis and endosperm the α-amylase content decreased from 30 to 64%, 29 to 56%, 24 to 55%, 23 to 49 % and 3 to 66%, 6 to 64%, 7 to 65%, 9 to 65% from 1.0 to 10.0 ppm concentrations, respectively compared to control.

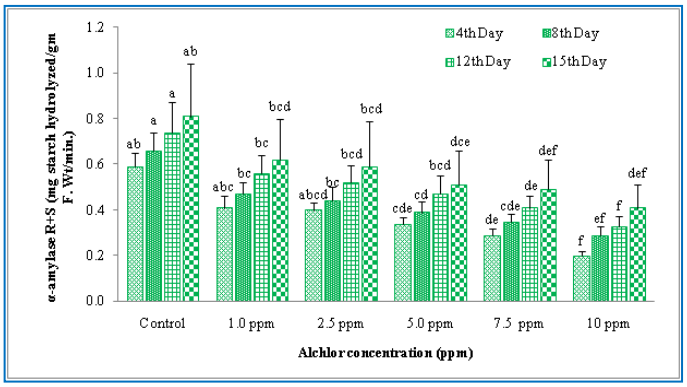

Fig-3a Effects of different concentrations of alachlor and growth interval of maize seedling on α-amylase content in root and shoot of maize. Means followed by different alphabets differ significantly as established by SPSS package ver. 14.00 according to Tukey's mean range test at 5% level.

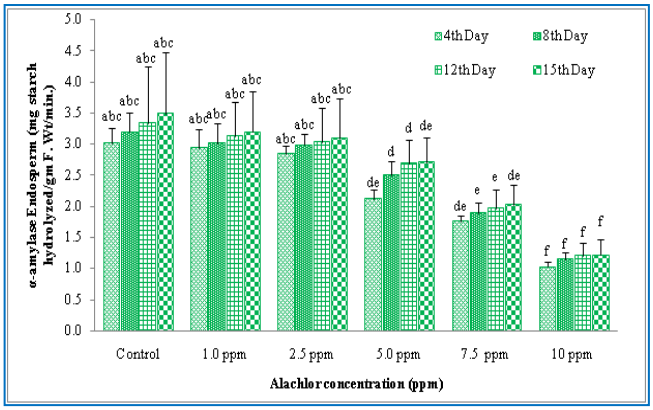

Fig-3b Effects of different concentrations of alachlor and growth interval of maize seedling on α-amylase content in endosperm of maize. Means followed by different alphabets differ significantly as established by SPSS package ver. 14.00 according to Tukey's mean range test at 5% level.

Effect of alachlor on catalase activity

The results showed that the different concentrations of herbicide efficacy on catalase activity of root-shoot axis and endosperm of maize were significantly influenced [Table-1]. Catalase activity in root shoot axis and endosperm was found to be increased as the concentration increased from 1.0 to 10.0 ppm, compared to control. Whereas, the catalase activity in root shoots axis was found to be increased and in endosperm it is decreased from 4 to 15 days seedlings. As the days of treatment increased the catalase content in root shoot axis and in endosperm was increased and decreased from 10 to 48%, 7 to 50%, 10 to 42%, 8 to 41% and 10 to 35%, 8 to 37%, 24 to 51%, 32 to 84% from 4 to 15 days seedlings from 1.0 to 10.0 ppm, respectively.

Effect of alachlor on peroxidase activity

The results showed that the peroxidase activity in root shoot axis and in endosperm was significantly influenced by the concentration of the herbicide increased from 1.0 to 10 ppm respectively, when compared to control. However, peroxidase activity in root shoots axis and in endosperm of maize seedlings was found to be increased from 4 to 15 days old seedlings. As the days of treatment increased the peroxidase content in root shoot axis and in endosperm is found to be increased from 14 to 19%, 23 to 26%, 29 to 41%, 55 to 60%, 64 to 68% and 15 to 18%, 30 to 31%, 40 to 42%, 42 to 44%, 45 to 47% from 1.0, 2.5, 5.0, 7.5 and 10.0 ppm respectively. On 4, 8, 12 and 15 day in root shoot axis and endosperm the peroxidase increased and decreased from 14 to 64%, 19 to 74%, 19 to 83%,

18 to 68% and 15 to 72%, 24 to 58%, 14 to 47%, 15 to 40% from 1.0 to 10 ppm concentrations, respectively compared to control [Table-1].

Effect of alachlor on Polyphenol oxidase

Results showed that the polyphenol oxidase activity in root shoot axis and endosperm significantly increased as the concentration of herbicide increased from 1.0 to 10 ppm concentration. As the days of treatment increased the polyphenol oxidase in root shoot axis and in endosperm was found to be increased from 4 to 15 days On 4, 8, 12 and 15 day in root shoot axis and endosperm the polyphenol oxidase increased from 63 to 213%, 14 to 107%, 27 to 160%, 16 to 116 %and 40 to 160%, 29 to 143%, 44 to 178%, 23 to 108 % from 1.0 to 10.0 ppm concentrations, respectively compared to control [Table-1].

Table-1 *Effect of different concentrations of herbicide alachlor on catalase, peroxidase and polyphenol oxidase content of maize Seedlings*

Concentration ppm	4th Day	8th Day	12th Day	15th Day	4th Day	8th Day	12th Day	15th Day
	Root shoot axis				Endosperm			
Catalase mg H_2O_2 oxidized/g F.Wt./min.								
Control	0.63 ± 0.01^{d}	0.69 ± 0.01^{e}	0.75 ± 0.01^{c}	0.77 ± 0.01^{d}	0.51 ± 0.01$_{c}$	0.49 ± 0.01^{c}	0.41 ± 0.01^{d}	0.31 ± 0.01^{e}
1.0	0.69 ± 0.01^{d}	0.74 ± 0.02^{d}	0.79 ± 0.01^{c}	0.83 ± 0.02^{c}	0.56 ± 0.01^{b}	0.53 ± 0.01^{c}	0.51 ± 0.01^{c}	0.41 ± 0.01^{d}
2.5	0.74 ± 0.03^{c}	0.8 ± 0.02^{c}	0.91 ± 0.02^{b}	0.94 ± 0.02^{b}	0.59 ± 0.02^{b}	0.56 ± 002^{b}	0.52 ± 0.01^{c}	0.47 ± 0.01^{c}
5.0	0.81 ± 0.02^{b}	0.86 ± 0.02^{b}	0.93 ± 0.02^{cb}	0.97 ± 0.02^{b}	0.62 ± 0.04^{a}	0.59 ± 0.02^{ab}	0.56 ± 0.03^{b}	0.51 ± 0.03^{b}
7.5	0.86 ±	0.91 ± 0.04^{b}	0.97 ± 0.04^{b}	1.07 ± 0.04^{a}	0.65 ± 0.04^{a}	0.62 ± 0.05^{a}	0.59 ± 0.03^{a}	0.53 ± 0.03^{b}
10.0	0.93 ± 0.05^{a}	0.97 ± 0.05^{a}	1.03 ± 0.05^{a}	1.09 ± 0.05^{a}	0.69 ± 0.06^{a}	0.67 ± 0.05^{a}	0.62 ± 0.05^{a}	0.57 ± 0.05^{a}
Peroxidase moles H_2O_2 oxidized/g F.Wt./min.								
Control	1.74 ± 0.86^{d}	2.09 ± 0.95^{e}	2.12 ± 0.84^{f}	2.34 ± 0.76^{e}	0.74 ± 0.09^{a}	0.98 ± 0.08^{a}	1.18 ± 0.25^{a}	1.34 ± 0.42^{a}
1.0	1.98 ± 0.88^{c}	2.48 ± 1.01^{d}	2.54 ± 0.92^{e}	2.78 ± 0.91^{e}	0.63 ± 0.07^{b}	0.74 ± 0.06^{b}	1.02 ± 0.09^{b}	1.14 ± 0.21^{b}
2.5	2.14 ± 0.91^{b}	2.69 ± 1.05^{c}	2.79 ± 1.02^{d}	2.94 ± 0.92^{d}	0.51 ± 0.05^{c}	0.68 ± 0.06^{c}	0.88 ± 0.09^{c}	1.03 ± 0.08^{c}
5.0	2.24 ± 1.02^{b}	2.89 ± 1.07^{c}	3.14 ± 1.06^{c}	3.29 ± 0.14^{c}	0.44 ± 0.05^{d}	0.59 ± 0.05^{d}	0.74 ± 0.06^{d}	0.91 ± 0.07^{d}
7.5	2.69 ± 1.06^{a}	3.08 ± 1.06^{b}	3.42 ± 1.06^{b}	3.72 ± 1.42^{b}	0.38 ± 0.02^{e}	0.47 ± 0.05^{e}	0.71 ± 0.04^{d}	0.84 ± 0.04^{e}
10.0	2.85 ± 1.09^{a}	3.64 ± 1.11^{a}	3.89 ± 1.15^{a}	3.94 ± 1.51^{a}	0.21 ± 0.01^{f}	0.41 ± 0.02^{f}	0.63 ± 0.03^{e}	0.81 ± 0.03^{e}
Polyphenol oxidase mg purpurogallin /gm F.Wt./min.								
Control	0.08 ± 0.01^{d}	0.14 ± 0.01^{c}	0.15 ± 0.01^{d}	0.19 ± 0.01^{c}	0.05 ± 0.01^{c}	0.07 ± 0.01^{c}	0.09 ± 0.02^{c}	0.13 ± 0.03^{d}
1.0	0.13 ± 0.01^{c}	0.16 ± 0.01^{c}	0.19 ± 0.01^{d}	0.22 ± 0.02^{c}	0.07 ± 0.01^{c}	0.09 ± 0.02^{c}	0.13 ± 0.02^{b}	0.16 ± 0.03^{c}
2.5	0.17 ± 0.02$_{c}$	0.21 ± 0.02^{b}	0.26 ± 0.02$_{b}$	0.28 ± 0.02$_{b}$	0.09 ± 0.02^{b}	0.11 ± 0.02^{b}	0.15 ± 0.12^{b}	0.18 ± 0.02$_{b}$
5.0	0.19 ± 0.02^{b}	0.23 ± 0.02^{b}	0.29 ± 0.03^{b}	0.33 ± 0.03^{b}	0.09 ± 0.02^{b}	0.12 ± 0.03^{b}	0.18 ± 0.03^{b}	0.22 ± 0.05^{b}
7.5	0.21 ± 0.03^{b}	0.27 ± 0.03^{a}	0.33± 0.03^{b}	0.38 ± 0.03^{a}	0.13 ± 0.03^{a}	0.15 ± 0.03^{a}	0.23 ± 0.05^{a}	0.27 ± 0.04^{a}
10.0	0.25 ± 0.03^{a}	0.29 ± 0.04^{a}	0.39 ± 0.04^{a}	0.41 ± 0.05^{a}	0.13 ± 0.03^{a}	0.17 ± 0.04^{a}	0.25 ± 0.05^{a}	0.27 ± 0.04^{a}

[11]Mean ±SD followed by same superscript are not statistically significant between the concentrations when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level

Discussion

Modern agriculture practices enhance the use of pesticides. However, excessive use may lead to pollute environment, so causing toxic effects on crops [17]. In general herbicides kills the plants by disturbing essential physiological or biochemical events associated with the germination and growth process, through a specific interaction with a single molecular target in the plant [18]. These results provide new insights into the effects of alachlor herbicide on maize physiology through the analysis of many parameters. Starch is the major constituent of carbohydrate in higher plants. It is a foremost storage product of many seeds and storage organs. Hydrolytic enzymes degrade starch into sugars during the germination process and the seed reserve gets hydrolyzed [19]. The alachlor herbicide induced starch accumulation in both root shoot axis and endosperm. The accumulation of starch under abiotic stress has been reported by [20] water deficit, cadmium treatment [21] suboptimal temperature [22, 23] salt stress [24]. According to [25] reported that lower concentrations of herbicide atrazine significantly promoted starch content in P. Stratiotes due to higher rate of photosynthesis and low rate of respiration.

The hydrolytic enzymes degrade starch into sugars during germination process, which leads to an increase in reducing sugar content and serves as a source of energy. In the present study the reducing sugar content of root-shoot axis and endosperm of maize significantly decreased with increased in herbicide concentrations. This result is in agreement with those observed under a biotic stress condition [26]. The production of amylase in the germinating seeds is under the control of either embryo by exogenous application of gibberellic acid. α-amylase is omnipresent in plants and their role in starch degradation is well known in cereal grains [27]. The decrease may be due to the inhibition in the biosynthesis of gibberellicacid or due to low synthesis of α-amylase or to inhibition of the enzyme activity [28]. The decrease in α-amylase activity is due to herbicide which impairs the degradation and mobilization of seed reserves. Gibberlic acid (GA3) is known to induce the synthesis of α-amylase. Thus the decrease GA3 activity could be a loss of gibberellic acid or protein synthesis. Since gibberellic acid also controls the metabolism of ribonucleic acid during the production of hydrolytic enzymes and protein synthesis, the blockage.

One of the markers used in the oxidative stress tolerance in plants is ROS [29]. Plants protect cells and sub cellular structures from the effect of ROS by enzymes such as catalase, peroxidase, polyphenol oxidase and non-enzymatic antioxidant namely ascorbate [30]. Stress induces production of ROS, which leads to oxidative stress. These toxic ROS may react with macromolecules, proteins and lipid components of membranes, causing damage through lipid peroxidation resulting in increased permeability of the cell membrane. In the present investigation a significant increase was found in the catalase activity of root-shoot axis and endosperm of maize seedlings. Activities of CAT and peroxidases are widely known to be responsible for the enzymatic suppression of H2O2 [31]. Moreover the activities of SOD, POD and CAT increased significantly in wheat and rice plants by application of 1, 2, 4-trichlorobenzene and in bitter ground by application of dimethoate [32, 33 and 34]. The increase in catalase activity may be due to the toxic substances produced during the development of plant or due to osmotic stress. The accumulation of antioxidant defense activity is still a complex phenomenon, which needs to be elucidated. The result of several studies reveled that increased activity of antioxidant defense is well correlated with reduced lipid

peroxidation content which has been reported in methamidophos stressed Chinese cabbage [35] and freezing stressed wheat plants [36]. Use of herbicide ahead of the critical limit draw the plant under chemical stress, under this circumstance there is release of free radicals of oxygen due to peroxidation of fatty acids, damaging the integrity of the membrane, creating exudation of soluble sugar in rhizosphere [37]. Our results are in line with [38] who observed higher peroxidase activity in younger plant sunflower. The increase in peroxidase activity may be due to increased synthesis of enzymes useful for adaptation of membrane lipids. Increase in peroxidase activity could be due to formation of H_2O_2, which could release the enzyme from the membrane structure [39]. The significant decrease in peroxidase activity was observed in Vicia faba with increase in the concentration of glyphosate herbicide and duration [40].

PPO catalyze the oxygen dependent oxidation of phenols to quinones, reactive species that can covalently modify and cross-link a variety of cellular nucleophiles via 1, 4 addition mechanism and or undergo reverse dis-proportionation to semiquinone radicals [41]. The activity of antioxidant enzymes is improved in order to decrease membrane injure beside active oxygen radicals. The physiological studies bare the undesirable effects of alachlor on the plant metabolism due to the inhibition caused by the herbicide which in turn affect the behavior of a range of enzymes.

Conclusions

The findings of the present study showed that biochemical and anti-oxidative changes occur early in plant development during germination processes. The herbicide application at higher concentrations caused suppression of growth by in turn inhibiting the essential physiological processes, which are required for plant development. The effects of herbicides were also shown by an increase in the antioxidative enzymes *viz*. peroxidase, polyphenol oxidase and catalase. Starch, reducing sugar and α-amylase content showed significant variation among the treatments in both root shoot axis and endosperm. Therefore, the present results disclose that the toxicity of herbicide at higher dosage to maize seedlings where biochemical processes which are associated with seed germination and seedling growth were found to be severely affected.

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