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Effects of Foliar Application of Selenium in Maize (*Zea mays* L.) under Cadmium Toxicity

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ABSTRACT: Cadmium (Cd) is the most toxic element present in the soil, air and water that causes deleterious effects on the physico-chemical and antioxidant processes of maize plant. Selenium (Se) is an essential micronutrient for humans, animals and plants as it reduces the harmful effects of cadmium toxicity. The interactive effects of Se and Cd interactions on plant growth and metabolism are not fully clear. In the present study, we assessed whether Se could alleviate the toxic effects of Cd on growth and metabolism of maize. To measure the role of foliar application of selenium (Se) in mitigation of cadmium toxicity during vegetative growth in maize a pot experiment was conducted at research area of Regional Agriculture Research Institute, Bahawalpur by using complete randomized design (CRD) with three replications of maize. Cd was applied at the rate of 750 mg/kg sand and 1500 mg/kg sand after the completion of germination. However, foliar application of Se was applied at the rate of 0.2 mM, 0.4 mM and 0.6 mM. The data was recorded for morphological (shoot-root lengths and their fresh weights), biochemical (chlorophyll contents, TAA, TSS, TSP and NPK % age) and antioxidant parameters (POD, SOD, APX and CAT).Data obtained showed variability in results but suitably increases the morphological, biochemical and anti-oxidant parameters obtained by the application of treatment number T9 in which Se was applied at the rate of 0.6 mM without Cd, while lowest results were obtained by Cd toxicity which reduced these parameters of growth and metabolism when treatment number T2 applied at the rate of 1500 mg/kg sand. Findings represent that Cd toxicity reduced the physico-chemical and antioxidant parameters while, the foliar application of Se counter the toxic effects of cadmium in maize plant.

Keywords: cadmium toxicity, foliar application, maize, selenium,

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INTRODUCTION

Among cereal crops maize (*Zea mays* L.) is a main crop that can grow in many types of soils and different environmental conditions. It remained main staple food of people worldwide for a long time (Hossain *et al*., 2016). Maize has a wide range of uses as its grain is a main source of starch (72%), proteins (10%), vitamins A and B (3-5%), oil (4.8%), sugar (3.0%), fiber (5.8%), and ash (1.7%) 100g of fresh grain of maize contain 361 calories of energy, 9.4g protein, 4.3g fat, 74.4g carbohydrate, 1.8g fiber, 1.3g ash, 10.6% water, 140mg vitamins, 9mg calcium, 290mg phosphorus and 2.5mg iron (Arain, 2013). Maize can be used as fodder, feed for animals and in feed preparation.

Maize also caused maximum conversion of dry substances to meat, milk and eggs compared to other food grains. Heavy metal contamination of soil is one of the major problems that negatively affect plant growth and produce (Meers *et al*. 2010). The main sources of the heavy metal pollution are municipal waste disposal practices, at liberty mining and extensive use of agrochemicals which result in the addition of large amounts of heavy metals throughout world (Gupta *et. al.,* 2018). Cadmium is taken up readily and exerts adverse physiological effects in plants (Gupta *et. al.,* 2018; Choppala *et al.* 2014). It binds to the sulfhydryl groups (SH) of proteins and thus alters the activity of many enzymes (Hall, 2002).

The exposure to higher Cd concentrations usually causes growth inhibition, disturbance of the redox control of the cell by over production of reactive oxygen species (ROS) and interferences with mineral uptake, stimulation of secondary metabolism, alteration of membrane functions and eventually causes cell death (Hasan *et al.* 2009; Choppala *et al*. 2014). Cadmium also affects the photosynthetic parameter (chlorophyll content, gas exchange and chlorophyll inflorescence) of cotton seedlings, the seedlings show important reduction in Chl*a* and *b*, photosynthetic rate, stomatal conductance and transpiration rate (Liu *et al*., 2014). An important improvement in proline and ascorbic acid content is noticed in leaves of plants that face Cd stress (Pandey *et al*., 2011).

The physico-chemical and anti-oxidative properties of selenium (Se) have raised the curiosity of biologists in recent past. Research shows that selenium promotes the plant growth and may act as heavy metal opponent as it is a necessary micronutrient with some physiological and anti-oxidative properties (Nawaz *et al*., 2014). Se increase the plant growth at low concentrations and enhance the capacity of anti-oxidant system by increasing the anti-oxidant enzyme activity (Hasanuzzaman *et al*., 2010). Se makes better plant tolerance to DS by regulating water status (Yao *et al.,* 2009), increasing chlorophyll in leaves of plants (Dong *et al.,* 2013) protecting against cadmium toxicity (Elkahoui *et al.,* 2004), reducing damage caused by, oxidative stress of UV-radiation (Valkama *et al.,* 2003) and enhancing chlorophyll content under light stress (Seppänen *et al.,* 2003). The present investigation operated to manage or reduce the deleterious effects of Cd on maize plant which has huge economic value, worldwide. However, foliar application of Selenium Se was applied at the vegetative stage (6^{th} leaf) of maize that minimizes the Cd toxic effects on morphological, bio-chemical and antioxidant processes in *Zea mays.*

MATERIAL AND METHODS

A. Experimental Site and Conditions

The cadmium (Cd) toxicity was reduced by foliar application of selenium (Se), for which a pot experiment was conducted to examine the physio-chemical and antioxidant activity in *Zea mays*. The experiment was conducted in spring 2018 in the Regional Agricultural Research Institute of Bahawalpur, Pakistan. The experiments were performed in a complete block design (CRD) with factorial arrangement in three replications. Local seeds of the plant hybrids were collected from the Bahawalpur Seed Market, for sowing. The Cd was applied after completion of germination along with control and the different treatments of foliar Se were also applied at 6^{th} leaf stage of hybrid maize varieties. Sand used as a growth medium which was sun dried, ground, sieved and mixed well to avoid any plant residues and 3kg sand was filled carefully in each pot. Five seeds were sown in each pot and then watered with distilled water. In the beginning all pots were kept at field capacity level for obtaining good germination and emergence. Later, the water was applied according to the requirement for the experiment. Before applying cadmium, the plants were thinned out and three healthy plants were kept in each pot. Recommended doses of NPK were applied in solution form at the time of planting, but N was applied every 2 weeks. At 6^{th} leaf stage the foliar spray of different treatment of Se was applied. The subsequent condition was maintained for one week after applying the supplemental foliar Se. Plants were grown up to 40 days and data regarding various morphological, biochemical and antioxidant parameters were recorded using standard recommended methods.

B. Parameters Recorded

Morphological parameters such as shoot length (cm) and root length (cm) were recorded with the help of ruler while the shoot fresh weight (g) and root fresh weight (g) were recorded with the help of digital electrical balance.

Physiological parameters: Chlorophyll contents were recorded with the help of SPAD meter. A relevant measurement of the amount of chlorophyll present in a leaf based on measurement with a SPAD meter produced by Minolta. Unit of chlorophyll content is umol/meter^2.

Biochemical parameters: Total free amino acids were determined according to Hamilton and Van Slyke (1973). Fresh plant leaves (0.5g) were chopped and extracted with phosphate buffer (0.2M) having pH 7.0. Took 1 mL of the extract in 25 mL test tube, added 1 mL of pyridine (10%) and 1mL of ninhydrin (2%) solution in each test tube. Ninhydrin solution was prepared by dissolving 2g ninhydrine in 100 mL distilled water. The test tubes with sample mixture, heated in boiling water bath for about 30 min. Volume of each test tube was made up to 50 mL with distilled water. Read the optical density of the colored solution at 570nm using spectrophotometer. Developed a standard curve with Leucine and calculated free amino acids using the formulae given below:

$$\text{Total amino acids } (\mu g\, g^{-1} \text{ fresh wt.}) = \frac{\text{Graph reading of sample} \times \text{Volume of sample} \times \text{Dilution factor}}{\text{Weight of fresh tissue} \times 1000}$$

Total soluble Protein was determined using the method of Lowry *et al.* (1951), one mL of the leaf extract from each treatment was taken in a test tube. The blank contained 1 mL of phosphate buffer (pH 7.0). One mL of solution C was added to each test tube. The reagents in the test tube were thoroughly mixed and allowed to stand for 10 min at room temperature.

Then 0.5 mL of Folin-Phenol reagent (1:1 diluted) was added, mixed well and incubated for 30 min. at room temperature. The optical density (OD) was read at 620 nm on a spectrophotometer (Hitachi, 220, Japan).

Total soluble Sugar was determined according to the method of Yemm and Willis (1954), plant extract was taken in 25 mL test tubes and 6 mL another one reagent was added to each tube, heated in boiling water bath for 10 min. The test tubes were ice-cooled for 10 min. and incubated for 20 min. at room temperature (25^{o}C). Optical density was read at 625 nm on a spectrophotometer (Hitatchi, 220, Japan). The concentration of soluble sugars was calculated from the standard curve developed by using the above method.

N,P,K were measured on the ground dry matter, the nitrogen (N) concentration was determined according to AOAC by the Nessler method (1960), phosphorus (P) was measured according to Jackson (1973), using a chloride reduction molybdenum phosphorescence method, and potassium (K) was measured by a flame photometer (CORNING M410).

Antioxidants: The activities of SOD, POD, CAT, and ascorbate peroxidase (APX) were determined spectrophotometrically. For determining SOD activity we used a method which was based on measurement of one of the products of the SOD reaction, hydrogen peroxide. Hydrogen peroxide is quantitated using a couple reactions where horse radish peroxidase catalyzes the formation of a fluorescent product. Substrate for SOD was provided by reduction of oxygen during the auto-oxidation of riboflavin in the presence of UV light. The activity of POD was determined by measuring peroxidation of hydrogen peroxide with guaiacol as an electron donor (Chance and Maehly, 1955). Catalase activity was assayed by measuring the conversion rate of hydrogen peroxide to water and oxygen molecules, following the process described by Chance and Maehly (1955). Ascorbate peroxidase (APX) activity was measured by monitoring the decrease in absorbance of ascorbic acid at 290 nm (extinction coefficient 2.8 mM cm^{-1}) in a 1 ml reaction mixture containing 50 mM phosphate buffer (pH 7.6), 0.1 mM Na-EDTA, 12 mM H_2O_2, 0.25 mM ascorbic acid and the sample extract as described by Cakmak, (1994).

RESULTS

A. Effect on physio-chemical parameters

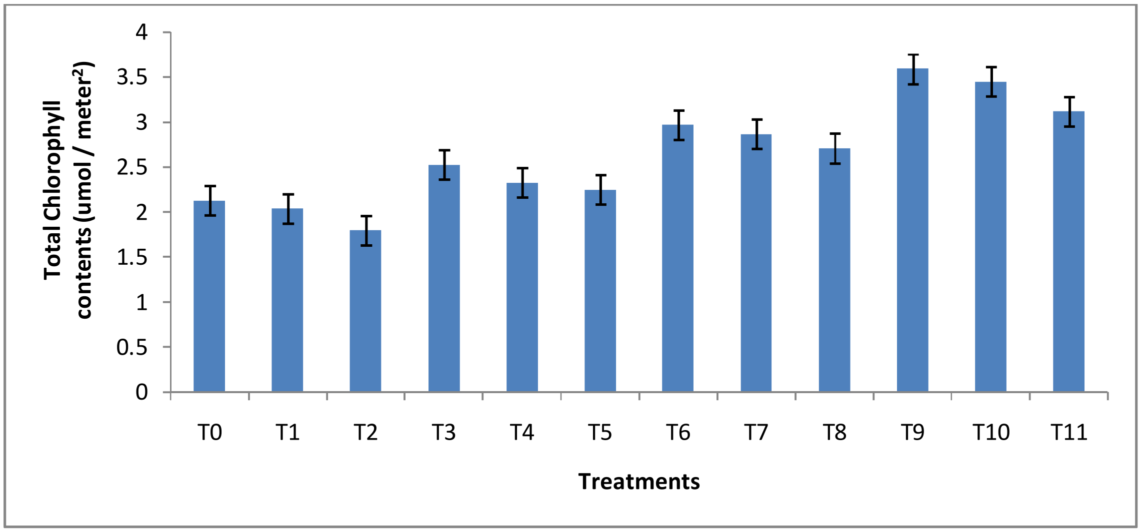
Morphological parameters. Data showed many variations among all maize plants which were used for the experiment (Table 1). Cadmium (Cd) stress affects the morphology and plant growth. The shoot length, root length, shoot fresh weight, root fresh weight of maize was reduced by the application of different doses of Cd which are respresenetd by treatment number T_1 and T_2 while the application of different rates of foliar selenium (Se) enhanced them under control as well as Cd stress treatments (Table 1). The maximum shoot length, root length, shoot fresh weight and root fresh weights were observed in treatment number T_9 where folair application of Se was applied at the rate of 0.6 mM. Data showed that different quantity of cadmium (Cd) reduced the parameters of morphlogy as treatment number T_2 performed more reduced growth of root under Cd applied at the rate of 1500 mg/kg sand than other treatments. While application of different rates of foliar Selenium (Se) enhanced these lengths. More enhanced length was observed in treatment number T_9 that was treated with 0.6 mM Se without Cd.

Table 1. Effect of different treatments of foliar application of selenium on morphological parameters of Maize under Cd stress.

Treatments	Shoot Length	Root Length	Shoot Fresh Weight	Root Fresh Weight
T_0 (T_0Cd_0)	9.6500 CD	7.7000 I	2.8500 BC	2.0300 EFG
T_1 (T_0Cd_1)	9.2000 D	6.7000 J	2.7200 C	1.8750 FG
T_2 (T_0Cd_2)	7.0000 E	6.5000 J	2.2300 D	1.7833 G
T_3 (T_1Cd_0)	10.700 BC	10.700 F	2.8500 BC	2.1900 DEF
T_4 (T_1Cd_1)	10.400 CD	9.9000 G	2.8500 BC	2.0850 EFG
T_5 (T_1Cd_2)	10.350 CD	9.1000 H	2.8500 BC	2.0350 EFG
T_6 (T_2Cd_0)	11.000 BC	11.300 D	2.9000 ABC	2.5300 BC
T_7 (T_2Cd_1)	10.950 BC	11.050 DE	2.9000 ABC	2.5050 BCD
T_8 (T_2Cd_2)	10.800 BC	10.900 EF	2.9000 ABC	2.2200 CDE
T_9 (T_3Cd_0)	12.550 A	22.700 A	3.2000 A	3.0800 A
T_{10} (T_3Cd_1)	12.100 AB	12.300 B	3.0900 AB	2.7750 AB
T_{11} (T_3Cd_2)	11.000 BC	11.650 C	3.0350 ABC	2.7200 B

B. Effect on physio-chemical parameters

Total Chlorophyll Contents (umol /meter^2). Cadmium stress adversely affects the plant growth as well as plant physiology that were mitigated by foliar application of selenium. A noticeable decrease was observed in chlorophyll contents of maize plants. Treatment number T_1 (Cd applied @ 750 mg/kg sand) and treatment number T_2 (Cd applied @ 1500 mg/kg sand) showed reduction in the chlorophyll contents as compared to control plant (Fig. 1). Application of foliar selenium (Se) increased the total chlorophyll contents. High chlorophyll contents observed in treatment number T_9 under Se applied at the rate of 0.6 mM without Cd followed by treatment number T_{10} and treatment number T_{11} under Se applied at the rate of 0.6 mM with Cd applied at the rate of 750 mg/kg sand and 1500 mg/kg sand respectively (Fig. 1).

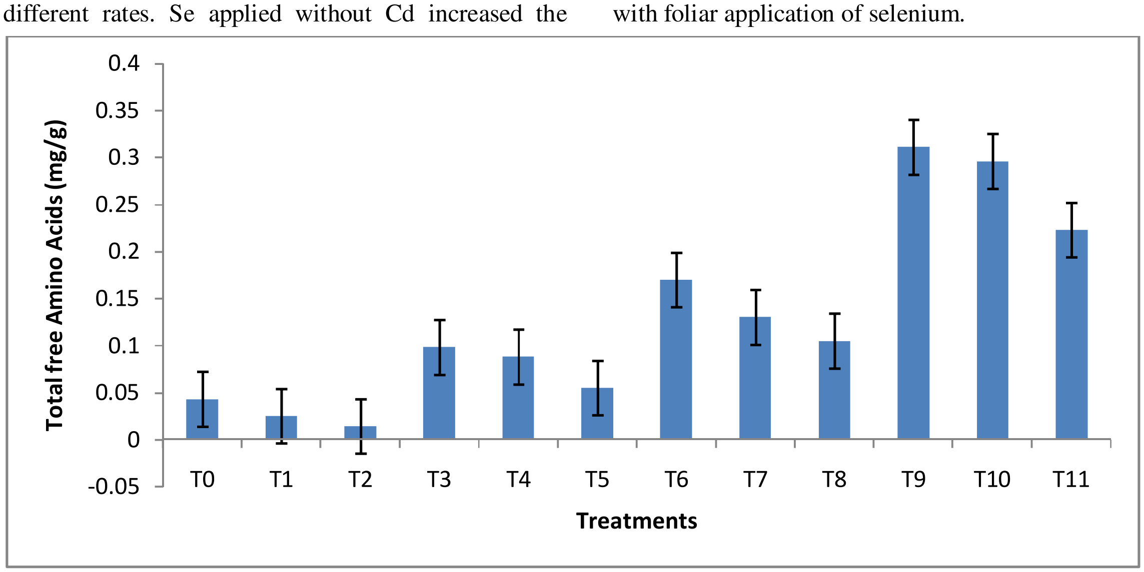


Treatments: T_0 = Control, T_1= Cd applied @ 750 mg/kg sand, T_2= Cd applied @ 1500 mg/kg sand, T_3= Se applied @ 0.2 mM without Cd, T_4= Se applied @ 0.2 mM and Cd applied @ 750 mg/kg sand, T_5= Se applied @ 0.2 mM and Cd applied @ 1500 mg/kg sand, T_6= Se applied @ 0.4 mM without Cd applied, T_7= Se applied @ 0.4 mM and Cd applied @ 750 mg/kg sand, T_8= Se applied @ 0.4 mM and Cd applied @ 1500 mg/kg sand, T_9= Se applied @ 0.6 mM without Cd applied, T_{10}= Se applied @ 0.6 mM and Cd applied @ 750 mg/kg sand, T_{11}= Se applied @ 0.6 mM and Cd applied @ 1500 mg/kg sand.

Fig. 1. Total chlorophyll contents of maize plants under Cd stress with foliarapplication of Se.

Total free Amino Acids (mg/g). Maize plants that were treated with cadmium without selenium showed reduction in total free amino acid contents (Fig. 2) and in case of treatment number $T_{2,}$ showed reduction in total free amino acids under Cd at the rate 1500 mg/kg sand followed by treatment number T_1(Cd @ 750 mg/kg sand). Foliar application of selenium (Se) enhanced the contents of total free amino acid. Variations were observed in Se effect applied at different rates. Se applied without Cd increased the total free amino acids. As shown in Fig.2,treatment number T_9 showed highest total free amino acid contents under Se applied at the rate of 0.6 mM without Cd followed by treatment number T_{10} under Se applied at the rate of 0.6 mM with Cd applied at the rate of 750 mg/kg sand.

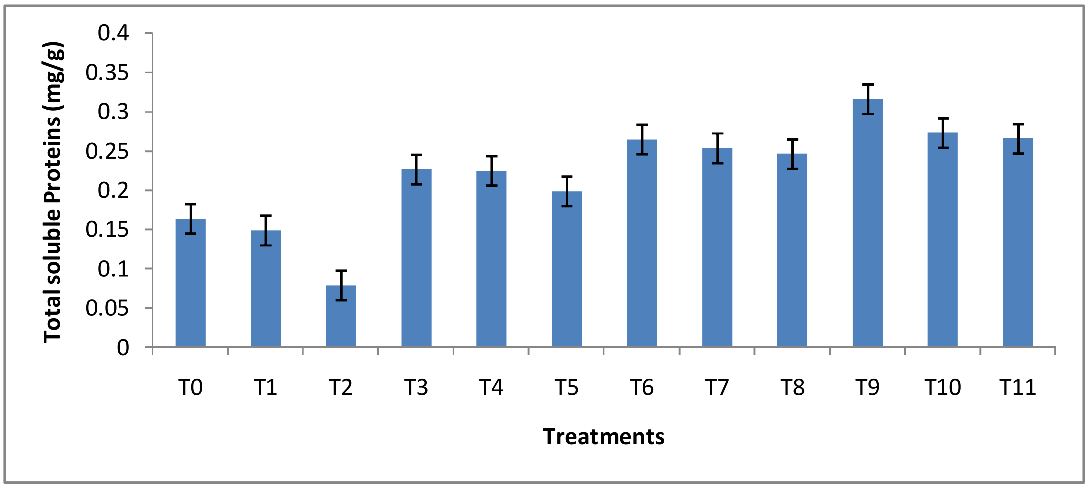
Total soluble Proteins (mg/g). Total soluble protein contents of maize plants reduced by application of different doses of cadmium (Cd) and its toxicity combat with foliar application of selenium.



Treatments: T_0= Control, T_1= Cd applied @ 750 mg/kg sand, T_2= Cd applied @ 1500 mg/kg sand, T_3= Se applied @ 0.2 mM without Cd, T_4= Se applied @ 0.2 mM and Cd applied @ 750 mg/kg sand, T_5= Se applied @ 0.2 mM and Cd applied @ 1500 mg/kg sand, T_6= Se applied @ 0.4 mM without Cd applied, T_7= Se applied @ 0.4 mM and Cd applied @ 750 mg/kg sand, T_8= Se applied @ 0.4 mM and Cd applied @ 1500 mg/kg sand, T_9= Se applied @ 0.6 mM without Cd applied, T_{10}= Se apllied @ 0.6 mM and Cd applied @ 750 mg/kg sand, T_{11}= Se applied @ 0.6 mM and Cd applied @ 1500 mg/kg sand.

Fig. 2. Effect of different treatments of foliar applied selenium on total free amino acids of maize under Cd stress.

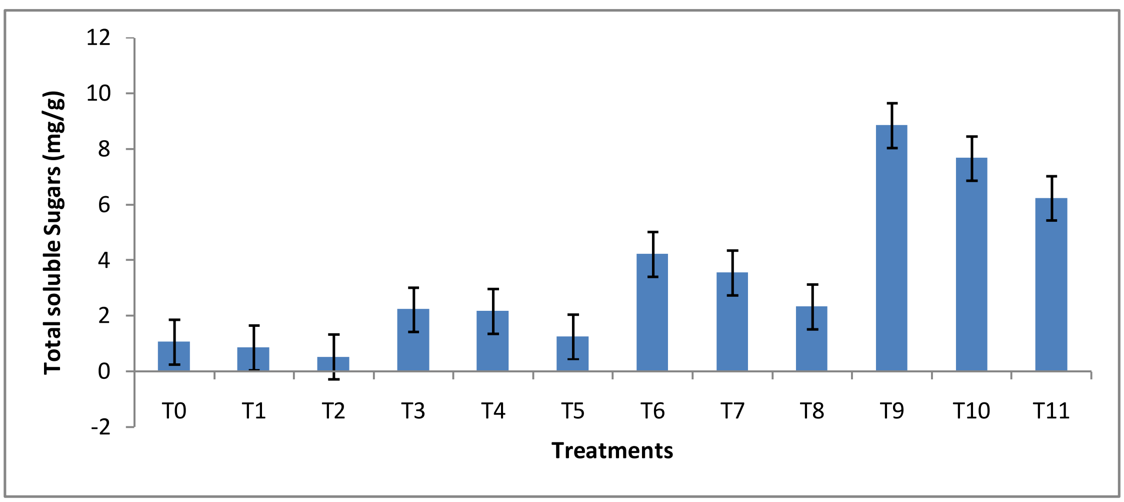
As compared to control plants maximum reduction in total soluble protein content was observed in treatment number T_2 followed by treatment number T_1 under Cd applied at the rate of 1500mg/kg sand and 750 mg/kg sand respectively without selenium. Foliar application of selenium (Se) improved the total soluble protein content of maize plants both in control and Cd stress conditions while the maximum total soluble protein content was recorded in treatment number T_9 (Se applied @ 0.6 mM without Cd) followed by treatment number T_{10} and treatment number T_{11} respectively when folair application of Se was applied at the rate of 0.6 mM (Fig. 3).



Treatments: T_0= Control, T_1= Cd applied @ 750 mg/kg sand, T_2= Cd applied @ 1500 mg/kg sand, T_3= Se applied @ 0.2 mM without Cd, T_4= Se applied @ 0.2 mM and Cd applied @ 750 mg/kg sand, T_5= Se applied @ 0.2 mM and Cd applied @ 1500 mg/kg sand, T_6= Se applied @ 0.4 mM without Cd applied, T_7= Se applied @ 0.4 mM and Cd applied @ 750 mg/kg sand, T_8= Se applied @ 0.4 mM and Cd applied @ 1500 mg/kg sand, T_9= Se applied @ 0.6 mM without Cd applied, T_{10}= Se applied @ 0.6 mM and Cd applied @ 750 mg/kg sand, T_{11}= Se applied @ 0.6 mM and Cd applied @ 1500 mg/kg sand.

Fig. 3. Effect of different treatments of foliar applied selenium on total soluble proteins of maize under Cd stress.

Total soluble Sugars (mg/g). The results showed in maximum reduction in total soluble sugar contents in treatment number T_2 that was treated with Cd (applied at the rate of 1500 mg/kg sand) without Se followed by treatment number T_1 that was treated with Cd (applied at the rate of 750 mg/kg sand) without Seas compared to control plant (Fig. 4) however, foliar application of selenium (Se) improved the total soluble sugars content of maize plants both in control and Cd stress conditions.

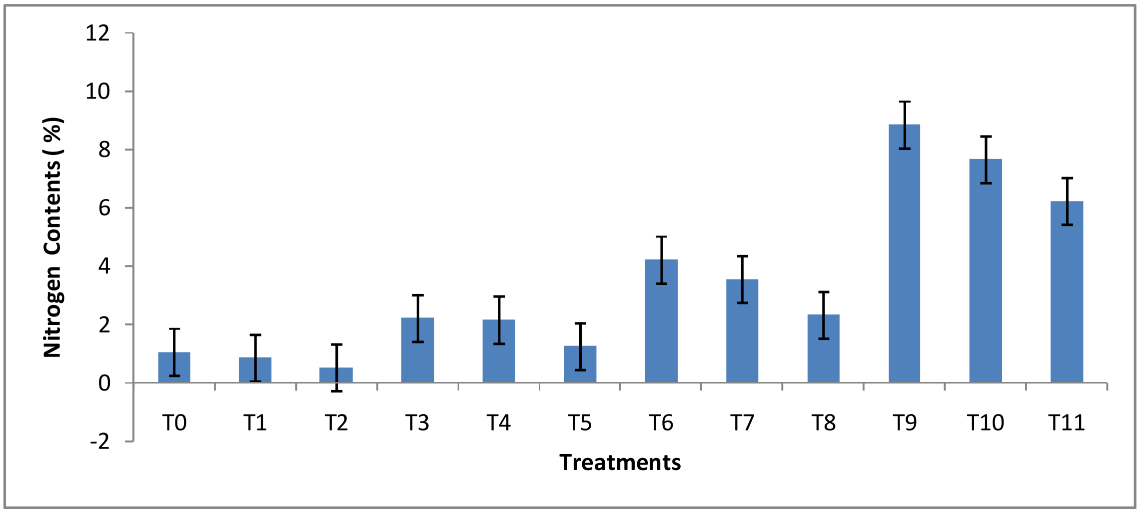


Treatments: T_0= Control, T_1= Cd applied @ 750 mg/kg sand, T_2= Cd applied @ 1500 mg/kg sand, T_3= Se applied @ 0.2 mM without Cd, T_4= Se applied @ 0.2 mM and Cd applied @ 750 mg/kg sand, T_5= Se applied @ 0.2 mM and Cd applied @ 1500 mg/kg sand, T_6= Se applied @ 0.4 mM without Cd applied, T_7= Se applied @ 0.4 mM and Cd applied @ 750 mg/kg sand, T_8= Se applied @ 0.4 mM and Cd applied @ 1500 mg/kg sand, T_9= Se applied @ 0.6 mM without Cd applied, T_{10}= Se applied @ 0.6 mM and Cd applied @ 750 mg/kg sand, T_{11}= Se applied @ 0.6 mM and Cd applied @ 1500 mg/kg sand.

Fig. 4. Total soluble sugar of maize plants under Cd stress with foliar application of Se.

The maximum total soluble sugars content was recorded in treatment number T_9 followed by treatment number T_{10} andtreatment number T_{11} respectively when folair application of Se was applied at the rate of 0.6 mM (Fig. 4).

Nitrogen content (%). Variations were observed in nitrogen contents in maize plants that were treated with Cadmium (Cd) with and without application of foliar selenium (Se). Cd stress reduced nitrogen contents when treated with Cd without Se. Maximum reduction was recorded in treatment number T2 that was treated with Cd (applied at the rate of 1500 mg/kg sand) without Se followed by treatment number T1 that was treated with Cd (applied at the rate of 750 mg/kg sand) without Se as compared to control plant T0 (Fig.5). Application of foliar Se enhanced the nitrogen contents in maize plants. Different concentrations were used i.e., 0.2 mM, 0.4 mM and 0.6 mM. Maximum nitrogen contents were recorded in treatment number T9 that was treated with Se applied at the rate of 0.6 mM without Cd (Fig. 5).



Treatments: T_0= Control, T_1= Cd applied @ 750 mg/kg sand, T_2= Cd applied @ 1500 mg/kg sand, T_3= Se applied @ 0.2 mM without Cd, T_4= Se applied @ 0.2 mM and Cd applied @ 750 mg/kg sand, T_5= Se applied @ 0.2 mM and Cd applied @ 1500 mg/kg sand, T_6= Se applied @ 0.4 mM without Cd applied, T_7= Se applied @ 0.4 mM and Cd applied @ 750 mg/kg sand, T_8= Se applied @ 0.4 mM and Cd applied @ 1500 mg/kg sand, T_9= Se applied @ 0.6 mM without Cd applied, T_{10}= Se applied @ 0.6 mM and Cd applied @ 750 mg/kg sand, T_{11}= Se applied @ 0.6 mM and Cd applied @ 1500 mg/kg sand.

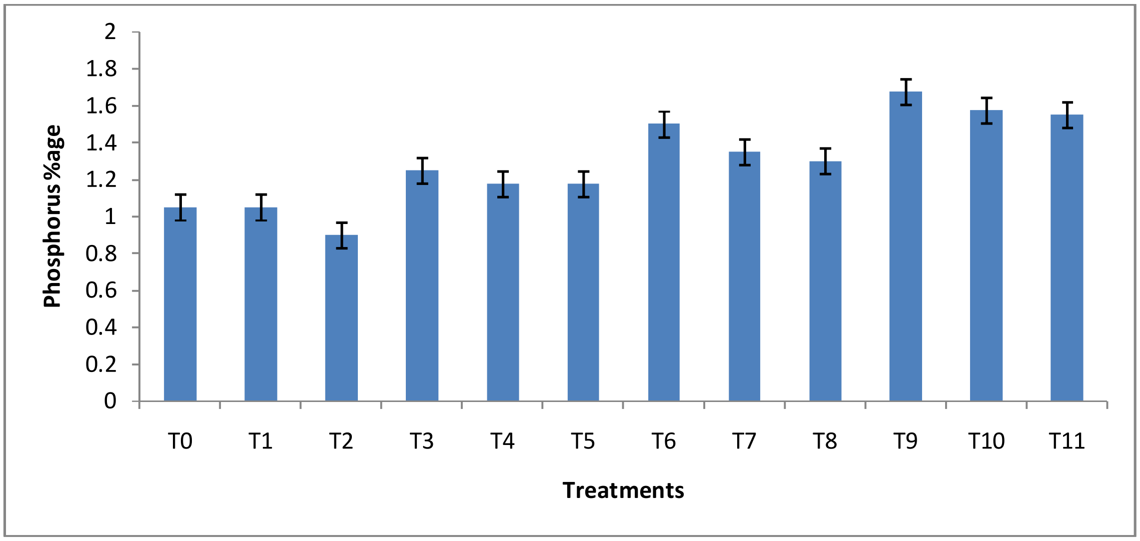
Fig. 5. Nitrogen contents percentage, under Cd stress with foliar application of Se.

Phosphorus content (%). In this data we study about phosphorus contents in maize plants that were showed variations according to cadmium (Cd) applied with and without selenium. Maximum reduction was recorded in treatment number T_2 which was treated with Cd applied at the rate of 1500 mg/kg sand without Se as compared to control plant treatment number T_0. Application of foliar Se improved the phosphorus contents when maize plants treated with different concentrations like 0.2 mM, 0.4 mM and 0.6 mM. Maximum phosphorus content recorded in treatment number T_9 that was treated with Se (applied at the rate of 0.6 mM without Cd) and then treatment numbers T_{10} and T_{11} where Cd applied at the rate of 750 mg/kg sand and1500 mg/kg sand with 0.6 mM foliar Se respectively (Fig. 6).

Potassium content (%). Data for potassium content of maize plants with and without application of selenium (Se) under cadmium (Cd) stress also showed variations in results. The data for potassium content figure out the effect of different doses of Cd affected the phosphorus content of maize plants. The potassium content of maize plants was significantly reduced by the application of different doses of Cd. Maximum reduction in potassium content was observed in treatment number T_2 followed by treatment number T_1 as compared to control plant T_0. Foliar application of selenium (Se) improved the potassium content of maize plants both in control and Cd stress conditions.The maximum potassium content was recorded in treatment number T_9 and treatment number T_{10} followed by treatment number T_{11} when folair application of Se was applied at the rate of 0.6 mM (Fig. 7).

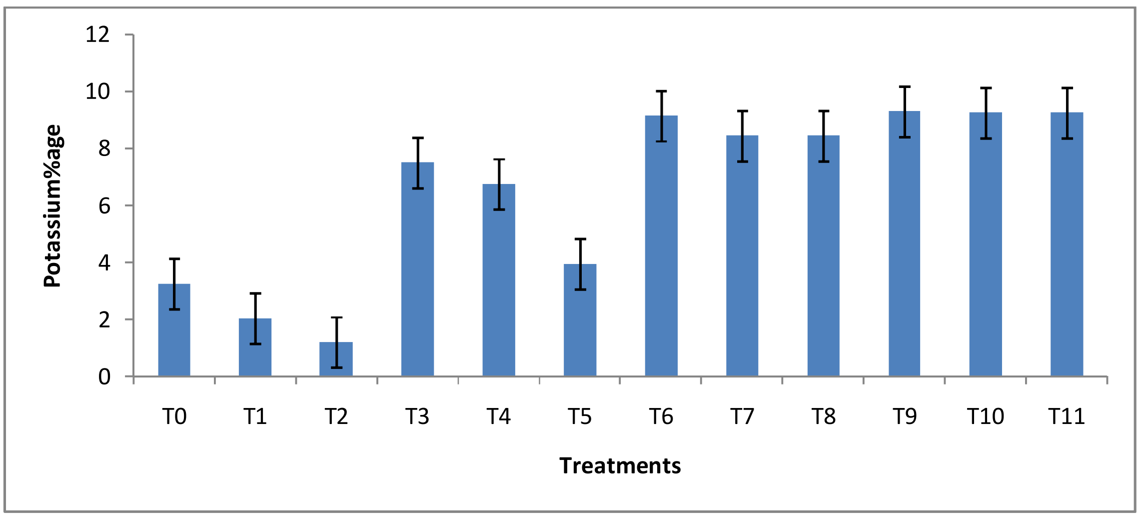
C. Effect on Antioxidants

Superoxide Dismutase (unit/g). Cadmium (Cd) stress affected the antioxidant system of plants. We found that the quantity of Superoxide Dismutase (SOD) in maize plants which were treated with different doses of Cd and the given data showed that Cd reduced the SOD in maize plants that were treated with Cd without selenium. Maximum reduction was observed in treatment number T_2 which was treated with Cd applied at the rate of 1500 mg/kg sand without Se and then in treatment number T_1 which was treated with Cd applied at the rate of 750 mg/kg sand without Se as compared to control plant T_0.



Treatments: T_0= Control, T_1= Cd applied @ 750 mg/kg sand, T_2= Cd applied @ 1500 mg/kg sand, T_3= Se applied @ 0.2 mM without Cd, T_4= Se applied @ 0.2 mM and Cd applied @ 750 mg/kg sand, T_5= Se applied @ 0.2 mM and Cd applied @ 1500 mg/kg sand, T_6= Se applied @ 0.4 mM without Cd applied, T_7= Se applied @ 0.4 mM and Cd applied @ 750 mg/kg sand, T_8= Se applied @ 0.4 mM and Cd applied @ 1500 mg/kg sand, T_9= Se applied @ 0.6 mM without Cd applied, T_{10}= Se applied @ 0.6 mM and Cd applied @ 750 mg/kg sand, T_{11}= Se applied @ 0.6 mM and Cd applied @ 1500 mg/kg sand.

Fig. 6. Phosphorus content of maize plants under Cd stress with foliar application of Se.

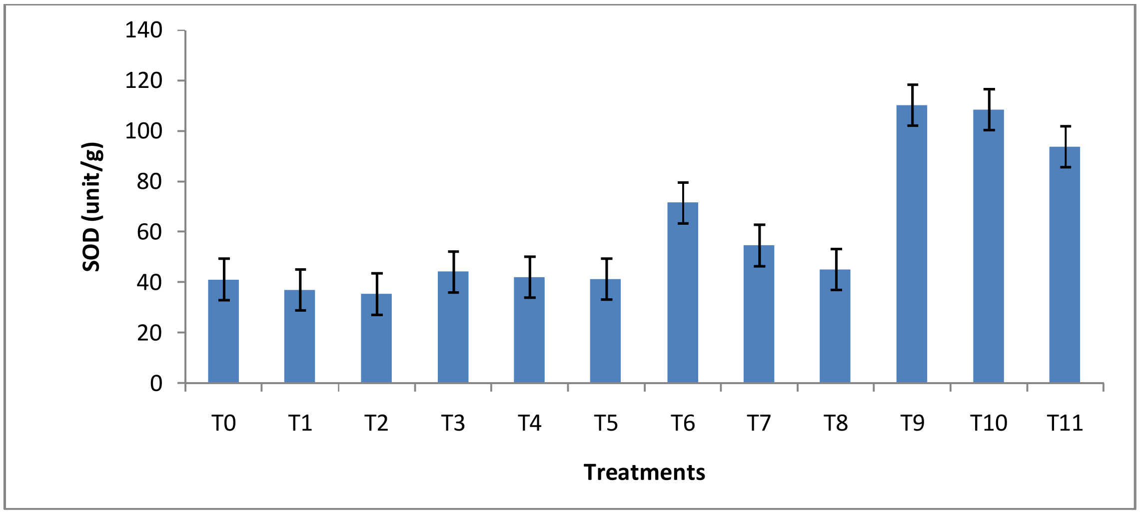


Treatments: T_0= Control, T_1= Cd applied @ 750 mg/kg sand, T_2= Cd applied @ 1500 mg/kg sand, T_3= Se applied @ 0.2 mM without Cd, T_4= Se applied @ 0.2 mM and Cd applied @ 750 mg/kg sand, T_5= Se applied @ 0.2 mM and Cd applied @ 1500 mg/kg sand, T_6= Se applied @ 0.4 mM without Cd applied, T_7= Se applied @ 0.4 mM and Cd applied @ 750 mg/kg sand, T_8= Se applied @ 0.4 mM and Cd applied @ 1500 mg/kg sand, T_9= Se applied @ 0.6 mM without Cd applied, T_{10}= Se applied @ 0.6 mM and Cd applied @ 750 mg/kg sand, T_{11}= Se applied @ 0.6 mM and Cd applied @ 1500 mg/kg sand.

Fig. 7. Potassium content of maize plants under Cd stress with foliar application of Se.

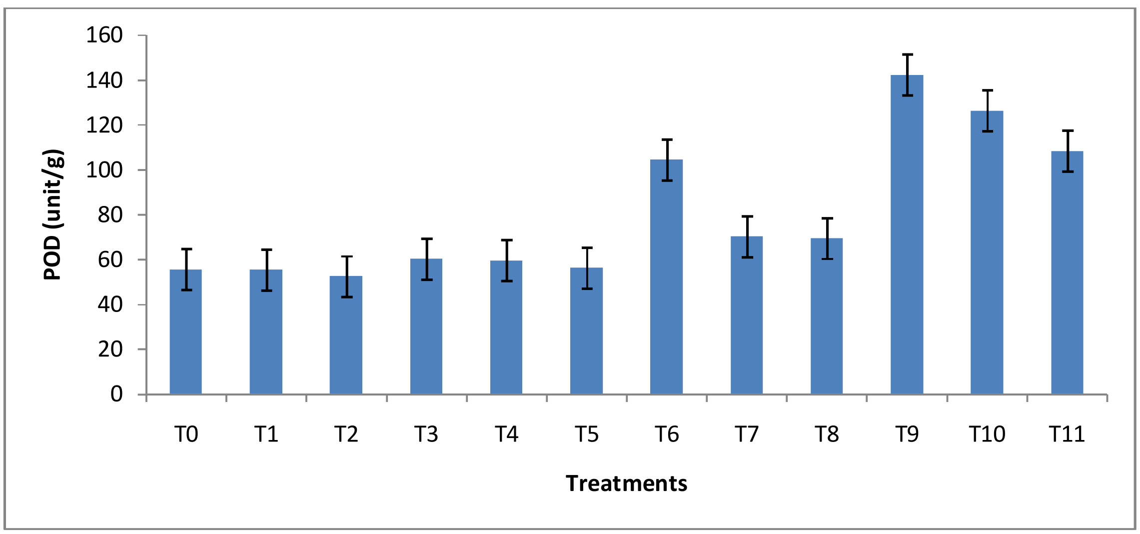
Application of foliar Se enhanced the SOD in maize plants that were treated with Se. As data showed improvement in treatment number T_6 that was treated with Se applied at the rate of 0.4mM without Cd applied. Maximum SOD improved by foliar application of Se was recorded in treatment number T_9 that was treated with Se applied at the rate of 0.6 mM without Cd applied (Fig. 8).

Peroxidase (unit/g). We observed quantity of Peroxidase (POD) in maize plants which were treated with different doses of Cd. The given data showed that Cd decreased the POD in maize plants that were treated with Cd without selenium. Maximum reduction was observed in treatment number T_2 which was treated with Cd applied at the rate of 1500 mg/kg sand without Se followed by treatment number T_1 which was treated with Cd applied at the rate of 750 mg/kg sand without Se. Application of foliar Se increased the SOD in maize plants that were treated with foliar Se. As data showed a little bit improvement treatment number T_3 that was treated with Se applied at the rate of 0.2 mM without Cd applied and in treatment number T_6 that was treated with Se applied at the rate of 0.4 mM without Cd applied.



Treatments: T_0= Control, T_1= Cd applied @ 750 mg/kg sand, T_2= Cd applied @ 1500 mg/kg sand, T_3= Se applied @ 0.2 mM without Cd, T_4= Se applied @ 0.2 mM and Cd applied @ 750 mg/kg sand, T_5= Se applied @ 0.2 mM and Cd applied @ 1500 mg/kg sand, T_6= Se applied @ 0.4 mM without Cd applied, T_7= Se applied @ 0.4 mM and Cd applied @ 750 mg/kg sand, T_8= Se applied @ 0.4 mM and Cd applied @ 1500 mg/kg sand, T_9= Se applied @ 0.6 mM without Cd applied, T_{10}= Se applied @ 0.6 mM and Cd applied @ 750 mg/kg sand, T_{11}= Se applied @ 0.6 mM and Cd applied @ 1500 mg/kg sand.

Fig. 8. SOD of maize plants under Cd stress with foliar application of Se.



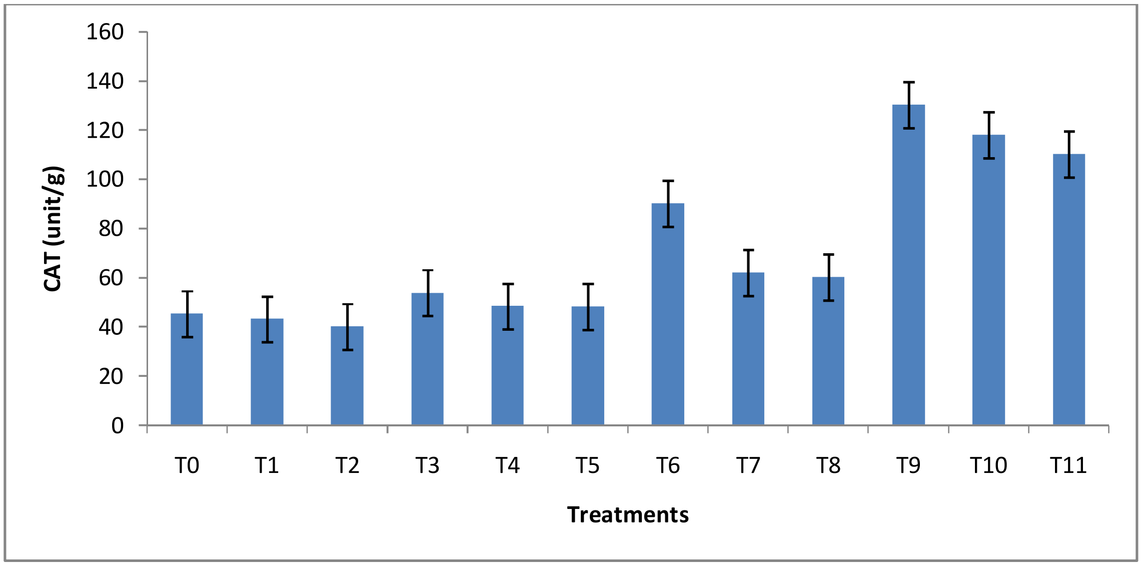
Treatments: T_0= Control, T_1= Cd applied @ 750 mg/kg sand, T_2= Cd applied @ 1500 mg/kg sand, T_3= Se applied @ 0.2 mM without Cd, T_4= Se applied @ 0.2 mM and Cd applied @ 750 mg/kg sand, T_5= Se applied @ 0.2 mM and Cd applied @ 1500 mg/kg sand, T_6= Se applied @ 0.4 mM without Cd applied, T_7= Se apllied @ 0.4 mM and Cd applied @ 750 mg/kg sand, T_8= Se applied @ 0.4 mM and Cd applied @ 1500 mg/kg sand, T_9= Se applied @ 0.6 mM without Cd applied, T_{10}= Se applied @ 0.6 mM and Cd applied @ 750 mg/kg sand, T_{11}= Se applied @ 0.6 mM and Cd applied @ 1500 mg/kg sand.

Fig. 9. POD of maize plants under Cd stress with foliar application of Se.

Maximum SOD was recorded in treatment number T_9 that was treated with Se applied at the rate of 0.6 mM without Cd applied (Fig. 9).

Catalase (unit/g). Analysis of variance of the data for the quantity of Catalase (CAT) of maize plants with and without application of selenium (Se) under cadmium (Cd) stress showed variations in results. This data for quantity of CAT showed that the different doses of Cd affected the CAT of maize plants. The CAT of maize plants was significantly reduced by the application of differeent doses of Cd.

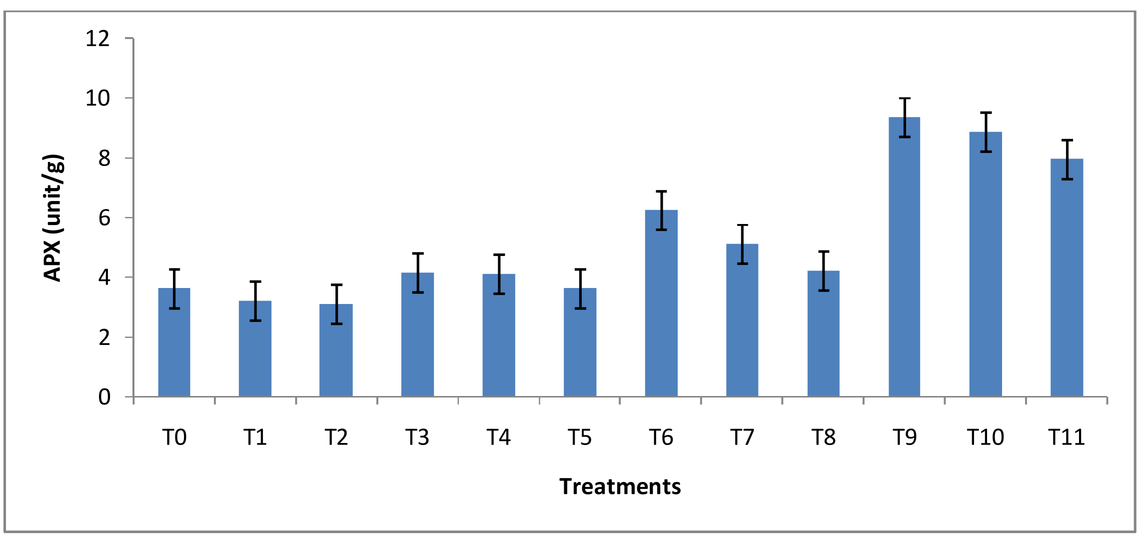
Maximum reduction in CAT was observed in treatment number T_2 followed by treatment number T_1 as compared to control plant T_0. Foliar application of selenium (Se) improved the CAT of maize plants both in control and Cd stress conditions.The maximum CAT was recorded in treatment number T_9 and treatment number T_{10} followed by treatment number T_{11} when folair application of Se was applied at the rate of 0.6 mM (Fig. 10).



Treatments: T_0= Control, T_1= Cd applied @ 750 mg/kg sand, T_2= Cd applied @ 1500 mg/kg sand, T_3= Se applied @ 0.2 mM without Cd, T_4= Se applied @ 0.2 mM and Cd applied @ 750 mg/kg sand, T_5= Se applied @ 0.2 mM and Cd applied @ 1500 mg/kg sand, T_6= Se applied @ 0.4 mM without Cd applied, T_7= Se applied @ 0.4 mM and Cd applied @ 750 mg/kg sand, T_8= Se applied @ 0.4 mM and Cd applied @ 1500 mg/kg sand, T_9= Se applied @ 0.6 mM without Cd applied, T_{10}= Se applied @ 0.6 mM and Cd applied @ 750 mg/kg sand, T_{11}= Se applied @ 0.6 mM and Cd applied @ 1500 mg/kg sand.

Fig. 10. CAT of maize plants under Cd stress with foliar application of Se.

Ascorbate Peroxidase (unit/g). Analysis of variance of the data for the quantity of Ascorbate Peroxidase (APX) of maize plants with and without application of selenium (Se) under cadmium (Cd) stress showed variations in results. This data for APX showed that the different doses of Cd affected the APX of maize plants. The APX of maize plants was significantly reduced by the application of diffeerent doses of Cd.



Treatments: T_0= Control, T_1= Cd applied @ 750 mg/kg sand, T_2= Cd applied @ 1500 mg/kg sand, T_3= Se applied @ 0.2 mM without Cd, T_4= Se applied @ 0.2 mM and Cd applied @ 750 mg/kg sand, T_5= Se applied @ 0.2 mM and Cd applied @ 1500 mg/kg sand, T_6= Se applied @ 0.4 mM without Cd applied, T_7= Se applied @ 0.4 mM and Cd applied @ 750 mg/kg sand, T_8= Se applied @ 0.4 mM and Cd applied @ 1500 mg/kg sand, T_9= Se applied @ 0.6 mM without Cd applied, T_{10}= Se applied @ 0.6 mM and Cd applied @ 750 mg/kg sand, T_{11}= Se applied @ 0.6 mM and Cd applied @ 1500 mg/kg sand.

Fig. 11. APX of maize plants under Cd stress with foliar application of Se.

Maximum reduction in APX was observed in treatment number T_2 that was treated with Cd applied at the rate of 1500 mg/kg sand without Se followed by treatment number T_1 that was treated with Cd applies at the rate of 750 mg/kg sand without Se appliesd as compared to control plant T_0. Foliar application of selenium (Se) improved the APX of maize plants both in control and Cd stress conditions.The maximum APX was recorded in treatment number T_9 and treatment number T_{10} followed by treatment number T_{11} when folair application of Se was applied at the rate of 0.6 mM (Fig. 11).

DISCUSSION

Maize (*Zea mays* L.) is a very crucial food crop that is used as a food source and now has become the most significant unprocessed material for animal feed (Mazen *et. al.,* 2018). The results gathered in this study show that Cd stress influnce the growth of maize plantsas in case of morphological parameters like shoot length, root length, shoot fresh weight, and root fresh weight andshowed reduction in growth because of their treatment with different concentrations of cadmium (Cd 750 mg/kg sand and Cd 1500 mg/kg sand). Higher concentration of Cd showed more reduction than low concentration of Cd whilefoliar application of selenium enhanced the morphological parameters. Se effects more strongly in the absence of Cd treatment and highprogress in morphological characters is observed at 0.6 mM concentration of selenium. Studies represents different concentrations of Cd did not adversely affect the growth of *T. erecta* in terms of root length, shoot length, fresh mass of root, dry mass of root, fresh mass of shoot, dry mass of shoot and leaf area, although, Cd at higher concentrations was found to cause reduction in levels of all morphological parameters (Shah *et al*., 2017). Similar results were also noticed by Turgut *et al*., (2004) where they found that increasing Cd concentration set a severe phytotoxicity that caused stunted growth in *Helianthus annuus*.

Cadmium (Cd) stress also decreased the chlorophyll contents in maize when treated with different concentrations (Cd 750 mg/kg sand and Cd 1500 mg/kg sand). Kilic and Kilic (2017) demonstrated in his experiment that increased level of Cd in the medium increases the degradation of chlorophyll. Photosynthesis rate decreased due to highest concentration of Cd which lowers the chlorophyll content. At 0.6mM concentration of Se, chlorophyll contents of maize showed an enhancement as compared with control or Cd effected. Similar results by (Wang *et al.,* 2011), showed that by increasing the concentration of selenium, the carotenoid content in the leaves of plants increased, which may be a factor in the improvement of chlorophyll growth by selenium (Tarighaleslami *et. al*., 2017).

Biochemical parameters under high concentration (750 mg/kg sand 1500 mg/kg sand) are also affected by cadmium stress. Total soluble sugar, total amino acids, total soluble proteins, N, P, K % age, SOD, APX, POD and CAT of maize plant show more reduction when treated with Cd @ 1500 mg/kg sand. Narwal and Singh 1993, observed that amino acid content increased in maize treated with Cd. High cadmium concentrations in cells, either increased the hydrolysis of protein or stop the utilization of amino acids, so affecting the average account of proteins in the cells (Tandon and Srivastava, 2004). Foliar application of selenium revealed a notable increase in biochemical activities of maize. Total soluble sugars, total soluble proteins, total amino acids, nutrients (N, P and K) % age and antioxidant activities showed increase at 0.6 mM concentration of selenium without Cd applied. SOD, POD and CAT are three recognizable and vital antioxidant enzymes that play a significant role in antioxidant defense system. At low concentration of Se, SOD and POD activities increased. In termination, cadmium (Cd) toxicity proves harmful to the morphological, biochemical and antioxidant parameters while foliar application of selenium enhanced all these para meters capably and minimizes the toxic effects of Cd and we concluded that Se and Cd had both synergistic and antagonistic effects on different attributes of maize.

CONCLUSIONS

We observed that Cd cause toxicity in *Zea mayes* L. and reduces the morphological, biochemical and antioxidant parameters when present in high concentrations, which ultimately turn down growth of the plant. While positive effects of Se foliar spray on the plant was found to be associated with Se-mediated regulation of physico-chemical and antioxidant processes. At treatment number T_9 with high amount of Se and less Cd availability we found Se acting in antagonism toward Cd toxicity which normalizes the plant growth and metabolism. Moreover, foliar application of Selenium also increased the Se content in shoot, which may be exploited as a viable and effective approach to increase Se concentration in fodders.

Conflict of Interest: All authors declare no conflict of interest.

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