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BIOTIC SYMBIOSIS AND PLANT GROWTH REGULATORS AS A STRATEGY AGAINST CADMIUM AND LEAD STRESS IN CHICKPEA

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Abstract

Climate change had not the only cause of stress in plants; both factors biotic and abiotic are responsible for the inducing stress in plants. These factors are severely affecting the productivity and distribution of plants. In the arid and semi-arid region, heavy metals are one of the major factors responsible for the abiotic stress cause reduction in yield. The degradation of natural resources has perhaps one of the worst things that humanity has ever done in its progress and civilization journey. For a long time, both, biotic and abiotic stress affecting the land and water resources continuously, due to anthropogenic activity. When plants exposed to a higher dose of lead (T2) then its catalase activity was significantly increased with 47.13 and 42.85% as compared to control (T0) on the dates of the proposed interval. The average SOD activity was significantly reduced as compared to T0 with 5.88 and 38.85% when treated with a higher dose of putrescine (T6).
Keywords: Agriculture, Biotic, Cadmium, Density, Economy, Forage, Lead

Introduction

Chickpea has a rich amount of protein, minerals, and folate. Chickpea ranked second after soybean seed in protein content; it also contains a good amount of dietary fiber and a good source of carbohydrates for the patient or people suffering from diabetes insulin sensitivity (Kumar and Dwivedi, 2018a; Kumar *et al.,* 2018b; Kumar *et al.*, 2018c; Kumar and Dwivedi, 2018d; Kumar *et al.*, 2018e; Kumar and Pathak, 2019f; Kumar *et al.*, 2019g). In chickpea, poly-saturated fats present in a low amount. Consumption of chickpea makes the bone strong because it has iron, potassium, calcium, magnesium, manganese, zinc, and vitamin-K in chickpea, low sodium salt present which helps in the control of blood pressure (Siddique and Kumar, 2018h; Siddique *et al.,* 2018i; Pathak *et al.,* 2017j; Prakash *et al.,* 2014L; Kumar *et al.,* 2014n). According to International Crops Research Institute, In India, chickpea seeds produced at Hyderabad consist of the highest protein amount 21.1per cent, 61.5 percent total carbohydrate, 4.56 percent fat and 6.1 percent fiber. The seeds also contain a rich amount of minerals like calcium niacin and iron (Kumar 2013o; Kumar and Dwivedi 2015p; Gogia *et al.,* 2014q; Kumar 2014r; Kumar *et al.,* 2012s; Mishra *et al.,* 2012t; Kumar *et al.,* 2011u; Kumar *et al.,* 2011v). The density criteria for the heavy metals range from above 3.5 g/cm^3 to above 7 g/cm^3. Heavy metals are natural elements of the world's crust, yet their geochemical and biochemical balance has changed drastically through indiscriminate human activities. Any substance added into the soil which can harm the soil functioning and ability to yield a crop knows as soil contamination (Kumar and Pathak, 2016w; Pathak *et al*., 2016x; Kumar, *et al.*, 2018y; Kumar *et al.*, 2018z; Kumar *et al.*, 2018aa). Due to its toxicity and capacity to accumulate, they are considered as an important source of environmental contamination (Kumar *et al.*, 2018bb; Kumar *et al.*, 2018cc; Singh *et al.,* 2020a; Singh *et al.*, 2020b; Sood *et al.*, 2020; Bhadrecha *et al.,* 2020; Singh *et al.*, 2020c; Sharma *et al.*, 2020; Singh *et al.*, 2020d; Bhati *et al.*, 2020; Singh *et al.*, 2019; Sharma *et al.*, 2019). The soil the primary recipient came on contact with a waste of from all the industries, a chemical used in agriculture. Polyamines (Pas) are those compounds which consist of two or more primary amine group, have low molecular mass and present in free form; i.e. putrescine, spermidine, and spermine. Polyamines are present in almost all living organisms and also in the plant). Polyamines are helpful in growth and development, also respond during abiotic or biotic stress, the Pas are present in trace amounts like putrescine but in mammal's spermidine and spermine are present. Cadmium one of the most toxic heavy metals having an upper limit is 14.157 µg/g (Kumar *et al*., 2018bb; Kumar *et al*., 2018cc; Singh *et al.,* 2020a; Singh *et al.*, 2020b; Sood, *et al.*, 2020; Bhadrecha *et al.,* 2020; Singh *et al.*, 2020c; Sharma *et al.*, 2020; Singh *et al.*, 2020d; Bhati *et al.*, 2020; Singh *et al.*, 2019; Sharma *et al.*, 2019). Effects of Cd, according to Sharmila *et al.* 2017, when mustard exposed to Cd^{2+} affects the growth of the plant and reduces the activity of photosystem II with a rise in the level of proline. Affect the oxidative phosphorylation in mitochondria and water uptake (Pathak *et al.*, 2017j; Prakash *et al.*, 2017k; Kumar and Mandal, 2014L; Kumar *et al.*, 2014m; Kumar *et al.*, 2014n); Linear increase in amount and production of MDA and H_2O_2 during stress in roots of chickpea inhibits the plant growth by stimulating ROS; affects the leaves, shoot, Significant reduction in the amount of nitrogen, phosphorus and chlorophyll were observed with an increase in the concentration of Cadmium; affects the translocation and storage of sugar in sweet sorghum; reduces the internodal space and internode number in maize. Lead (Pb) is one of the non – essential trace elements that mainly accumulate due to anthropogenic activities in agricultural soils (Kumar *et al.*, 2014L). The upper limits of leads are 61.87 µg/g (Kumar *et al.*, 2018a; Kumar *et al.,* 2018b; Kumar *et al.*, 2018c; Kumar *et al.*, 2018d; Kumar and Purnima *et al.*, 2018e; Kumar and Pathak 2019f; Kumar *et al.*, 2019g). The increased levels of Pb in the soil increase the concentration of Pb in plants growing in these soils and ultimately increases the risk of Pb toxicity in food crops. Lead toxicity induces the effects chlorophyll, affects concentration and catabolism of IAA, and stimulates ROS production and also POD activity, reduced total nitrogen and total phosphorus in the

plant reduction in gemmation (Kumar, 2013o; Pathak *et al.*, 2016x; Kumar *et al.*, 2018y; Kumar 2018z; Kumar *et al.*, 2018cc). Also, the reduction in the relative water content (RWC) and net photosynthetic rate (Siddique and Kumar, 2018h; Siddique *et al*., 2018i; Pathak *et al*., 2017j; Prakash and Kumar, 2017k; Kumar and Mandal, 2014L; Kumar *et al*., 2014m; Kumar *et al*., 2014n; Kumar, 2013o; Kumar and Dwivedi, 2015p). The symbiosis of plant roots with fungi occurs in various forms known as mycorrhiza. Arbuscular mycorrhizal fungi (AMFs) are major soil microorganisms that are key to enabling plant nutrient uptake, particularly in low-input farming, vegetation, and rhizoremediation processes, in various agroecosystems (Gogia, N., *et al.,* 2014q, Kumar 2014r; Kumar *et al.,* 2012s; Mishra *et al.,* 2012t; Kumar *et al.,* 2011u; Kumar *et al.,* 2011v; Kumar and Pathak, 2016w; Pathak *et al.,* 2016x). Salicylic acid (SA) a compound which has been used to reduces the heavy metals toxicity in plants, which helps in the regulation of plant growth. Reduces the heavy metals uptake, protects the membrane integrity and provides stability and by scavenging the reactive oxygen species which activates the antioxidant defenses mechanism and improves the photosynthesis (Pathak *et al.*, 2017j; Prakash *et al.*, 2017k; Kumar *et al.*, 2014L; Kumar *et al.*, 2014n).

Materials and Methods

This was the pot for the experiment with a 30 cm diameter and a 25 cm height and 10 kg of soil each with a small hole underneath it. Under the work plan, targeted pots with Endomycorrhiza have been inoculated. The exogenous use of cadmium (100 ppm) by Cadmium sulfate and Lead (100 ppm) by Lead chloride on the plant creates heavy metal stresses. Fifteen days interval application with Putrescine (1ppm) and Salicylic Acid (1ppm). Two phases such as 60 DAS and 90 DAS were measured in the respective pots. (Table 1).

Table 1 : Name of the Treatments and symbol used respectively

Name of Treatments	Symbol Used for Respective Treatments
Control	T-0
Cadmium(100 ppm)	T-1
Lead(100 ppm)	T-2
Cadmium + Mycorrhiza	T-3
Lead + Mycorrhiza	T-4
Cadmium + Putrescine	T-5
Lead + Putrescine	T-6
Cadmium + Salicylic Acid	T-7
Lead + Salicylic Acid	T-8

Design and Layout of Experiment

In a completely randomized (CRD) design, the experiment was developed. Eight treatments were available, including control. Three times every treatment has been replicated.

Observation Recorded

The observations were recorded two stages such as 60 DAS, and 90 DAS. The recorded observations of enzymatic parameters and the standard procedure adopted during the study are given below:

Catalase activity (EC. 1.11.1.6)

The activity of enzyme catalase was measured according to the protocol given by Aebi *et al.* (1983). The enzyme activity is assayed by estimating the residual H_2O_2 in the reaction mixture.

Reagents

- M Phosphate buffer, pH 6.4
- 1% (v/v) H_2O_2

Procedure

100 mg leaf samples were taken and uniformed in a chilled pestle and mortar in 5 ml of a 0.1 M phosphates buffer. At 10,000 g for 20 minutes at 4 °C, the crude extract was centrifuged. Until the enzyme test was completed, the enzyme extract was kept at a low temperature. The enzyme's activity was tested with 2.6 ml, a buffer of 0.1 M phosphate, an extract of 0.1 ml and a 1% H_2O_2 of 0.2 ml. At room temperature, the reaction mixture was quickly mixed. In a reaction mixture instead of enzyme extraction, a blank was made similarly, adding 0.1 M phosphate buffer. At an interval of 15 seconds for 2 minutes, changes in absorption at 240 nm (Ţ A240) were observed. Extinction coefficient 43.6 of H2O2 decomposition was used to estimate the activity of an enzyme per gram fresh weight. It was also estimated based on mg protein and expressed as follows:

EU mg^{-1} protein = δ $A_{240/min} \times 1000 / 43.6 \times$ mg protein ml^{-1} reaction mixture

The EU was expressed on a per g fresh weight basis as well as based on per mg protein (specific activity).

Superoxide dismutase (SOD) (EC. 1.15.1.1)

In the protocol Dhindsa *et al.* (1981) the function of the superoxide dismutase enzyme was measured. The SOD test is based on EDTA, L-methionine and Nitro-blue tetrazoliumformazone formation inhibitions. At the end of the reaction, the color formed at 560 nm can be extracted in butanol.

Reagents

- Potassium phosphate buffer (0.1M, pH 7.5)
- The solution of potassium dihydrogen phosphate 0.1M (Solution A) and dipotassium hydrogen phosphate 0.1M (Solution B) were prepared by dissolving 13.6 go^{-1} and 17.4 l^{-1} salts, respectively. 16 ml of solution A and 84 ml of solution B were mixed and the pH of the mixed solution was adjusted to pH 7.5
- L-methionine (200 mM) L –methionine (0.298 g) was dissolved in distilled water and the volume was made to 10 ml.
- Nitro blue tetrazolium (NBT) (2.25 mM) NBT (0.0184) was dissolved in distilled water and the volume was made to 20 ml and kept in the airtight vial.
- EDTA (3 mM): EDTA (0.0560 g) was dissolved in distilled water and the volume was made to 50 ml.
- Riboflavin (60 µM): Riboflavin (0.0023g) was dissolved in distilled water and the volume was made to 100 ml with distilled water and stored in an amber colour bottle at 4°Cin a refrigerator.
- Sodium carbonate (1.5M): Sodium carbonate (15.9 g) was dissolved in distilled water and the volume was made to 100 ml.

Procedure

The sample leaves (100 mg), with an extraction buffer of 5 mL (0.1 M phosphate buffer, pH 7.5 containing 0.5 mM EDTA), were homogenized. In 10,000 g for 10 minutes, the homogeneous product was centrifuged into a centrifuge cooling machine (REMI, C-24). The supernatant was collected after centrifugation and used as an enzyme source. In test tube 3 replicates of each enzyme sample were used, 3 µL of the reaction mixture containing 0.1 x 1.5 x 1.5 m sodium carbonate, 0.2 mL of 200 x 200 mM methionine, 0.1 x 2.25 mM NBT, 01 x 3 mM EDTA, 1.5 mL of 100 mM potassium phosphate buffer, 1 ml of distilled water and 0.1 ml enzyme extract. Two enzyme-free tubes have been taken as control. The reaction began by adding 0.1 ml (60 µM) riboflavin to all test pipe sets and placing 2 sets for 15 minutes below a light source of both fluorescent lamps (one in which enzyme was added and the other in which no enzyme is additionally added). The reaction was stopped by switching off the light and covering the sets of tubes by a black cloth. The set of tubes without enzyme extract developed the maximum colour. Under the light source, but with enzyme extract, a non-irradiated set was kept dark and was not colored and served as blank. A spectrophotometer (ELICO, SL 196) recorded the absorption of all the sets of the tube at 560 nm. Enzyme unit (EU) was calculated as per the formula is given below:

EU = Absorbance without enzyme in light - (Absorbance with an enzyme in light- Absorbance in Dark) / Absorbance without enzyme in light /2

The EU was expressed on a per g fresh weight basis as well as based on per mg protein (specific activity).

Results and Discussion

Catalase activity (EU mg^{-1} protein)

Polyamine (putrescine), mycorrhiza, salicylic acid, and their combination were examined under cadmium and lead stress in chickpea vary GPF-2. The 60 and 90 days of sowing (DAS) data were recorded (Table 2 & Fig. a). It is obvious that, with cadmium metals (T1) as opposed to control (T0) dates of 60 and 90 DAS interval, the average catalase activity increased by 49.29 and 50.21 percent substantially. Similarly, the catalase activity was significantly increased with plant exposure to a higher lead (T2) dose compared with the control (T0) at a 47.13 and 42.85 percent time intervals. The mitigation effect by reducing the catalases activity with 39.77 and 11.99 percent compared to T1 on the proposed interval dates has been shown by exogenous application of endomycorrhizal (T3). Similarly, the catalase activity decreased considerably with 35.64 and 28.12% at the proposed interval date when the treatment T4 was compared with the treatment T2. Compared to T1, the exogenous use of putrescine (T5) shows decreasing trends in catalase activity and catalase mitigation at the suggested interval date at 33.70 and 15.63 percent. When treated with a higher dose of putrescin (T6), the median catalase was significantly less than T2 at 30.98% and 24.37%. Likewise, the catalase activity of T7 was significantly decreased on its proposed interval date when compared with T1 at 35.70 and 17.91% (particularly compared to T1). When treated with a higher dose of salicylic acid (T2), the average catalase was significantly reduced in comparison to T8 at 35.54 and 10.50 percent. The salicylic acid showed the best mitigation effect against the cadmium and lead by increasing catalase on the proposed date of interval. Kuramshina *et al.* (2016) reported that the presence of cadmium in plant seeds inoculated with *Bacillus subtitles* increases the activity of POD, catalase and non-protein thiols content, but a decrease in lipid peroxidation. It was found that the seed inoculated bacteria reduces the metal content in plant shoot. Tohidi *et al.* (2018) experimented by using four different concentrations of Ni (0, 60, 120, and 180mg kg^{-1} soil), and two levels of mycorrhiza (inoculated and non-inoculated) on wheat. The reduction in seed number per spike, test weight, chlorophyll a and b and seed yield per plant due to Ni stress, also increase in catalase (CAT) enzyme activity (Kumar and Harsavardhn *et al.*, 2018y; Kumar and Yumnam *et al.*, 2018z; Kumar *et al.*, 2018aa; Kumar *et al.*, 2018bb; Kumar *et al.*, 2018cc; Singh *et al.,* 2020a; Singh *et al.*, 2020b; Sood *et al.*, 2020; Bhadrecha *et al.,* 2020; Singh *et al.*, 2020c; Sharma *et al.*, 2020; Singh *et al.*, 2020d; Bhati *et al.*, 2020; Singh *et al.*, 2019; Sharma *et al.*, 2019).

Table 2 : Catalase activity (EU mg^{-1} protein) of chickpea during *Rabi*

Treatments	Catalase activity (60 DAS)	Catalase activity (90 DAS)
T0	$7.982^{c} \pm 0.175$	$8.157^{d} \pm 0.561$
T1	$15.742^{a} \pm 0.220$	$16.384^{a} \pm 0.957$
T2	$15.099^{a} \pm 0.404$	$14.274^{b} \pm 0.184$
T3	$9.480^{b} \pm 0.485$	$14.419^{b} \pm 0.447$
T4	$9.717^{b} \pm 0.470$	$10.260^{c} \pm 0.243$
T5	$10.436^{b} \pm 0.208$	$13.822^{b} \pm 0.744$
T6	$10.420^{b} \pm 0.165$	$10.795^{c} \pm 0.176$
T7	$10.122^{b} \pm 0.404$	$13.448^{b} \pm 0.206$
T8	$9.732^{b} \pm 0.485$	$12.775^{b} \pm 0.576$

where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T0-Control; T1-Cadmium (100ppm); T2- Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid(1 ppm); T6-Lead + Salicylic acid (1 ppm); T7-Cadmium +Putrescine(1 ppm); T8-Lead +Putrescine (1 ppm)

Superoxide dismutase activity (SOD) (EU per gram fresh weight)

In Chickpea variety GPF-2, under cadmium and lead stress the effects of polyamine (putrescine), mycorrhiza, salicylic acid, and their combination on MDA levels were studied. The 60 and 90 days after sowing (DAS) data were recorded (Table 3 & Fig. b). The average SOD decreased by 18.09 and 39.15% on dates of interval 60 and 90 DAS when exposed by cadmium metal stress (T1). Similarly, the SOD activity was significantly reduced when the plant was exposed to a higher dose of lead (T2), with 17.48% and 38.45% compared to control (T0) at dates of the interval proposed. The effect of mitigation by decreasing SOD activity by 14.07 and 37.61 percent was demonstrated by the exogenous application of endomycorrhiza (T3) on the soil compared with T0 on the proposed interval dates.

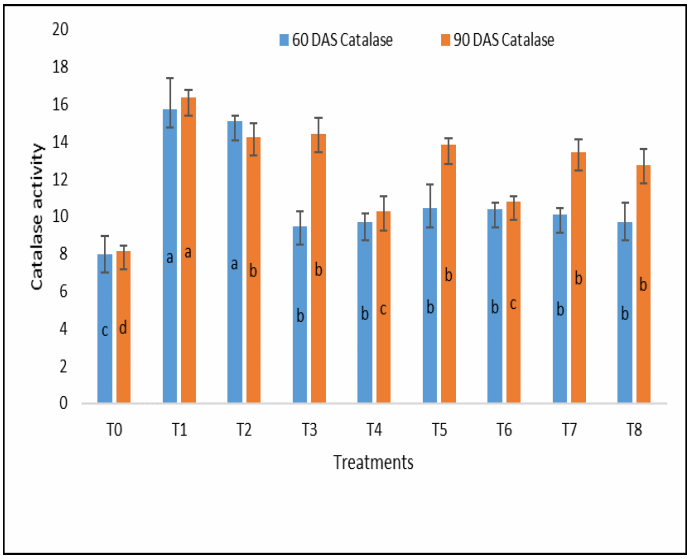

Fig. a : Catalase activity (EU mg^{-1} protein) of chickpea during *Rabi*

where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T0-Control; T1-Cadmium (100ppm); T2- Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid(1 ppm); T6-Lead + Salicylic acid (1 ppm); T7-Cadmium +Putrescine(1 ppm); T8-Lead +Putrescine (1 ppm)

Similarly, when compared to T0, the T4 treatment, the MDA content decreased substantially at the proposed interval date by 10.87 and 38.39 percent. Compared to T0, the exogenous application of putrescine (T5), with 11.46 and 36.83 percent at the proposed interval date, showed decreasing trends in SOD activity. The SOD average in treatments with a higher dose of putrescine (T6) decreased considerably compared to T0 with 5.88 and 38.85%. Similarly, when treatment T7 was compared with T0 the MDA was decreased significantly with 4.96 and 34.96% on the proposed date of interval. The average SOD activity was significantly reduced as compared to T8 with 2.74 and 22.37% when treated with a higher dose of salicylic acid (T0). The salicylic acid showed the best mitigation effect against the cadmium and lead by increasing SOD activity on the proposed date of interval. Ali (2017) conducted a pot experiment to investigate the effect of SA to enhance the tolerance of mung bean plant to aluminum (0.0, 1.0 or 10.0mM) stress. The aluminum causes a reduction in growth (length, the dry and fresh mass of shoot and root), water content, water use efficiency, photosynthesis rate, and chlorophyll content.

It also causes an increase in antioxidant enzymes (CAT, SOD, proline, and POD) in the shoot and root by aluminum toxicity. The application of SA results in good growth and stimulation of antioxidants caused due to aluminum toxicity. Sadeghipour (2016) reported that the lead toxicity significantly reduces the chlorophyll content, relative water content (RWC) and net photosynthetic rate but increase in lipid peroxidation and catalase, superoxide dismutase (SOD), glutathione reductase, ascorbate peroxidase, and proline content.

Table 3 : SOD activity (EU per gram fresh weight) of chickpea during *Rabi*

Treatments	SOD activity (60 DAS)	SOD activity (90 DAS)
T0	$0.018^{c} \pm 0.009$	$0.050^{bc} \pm 0.019$
T1	$0.121^{a} \pm 0.014$	$0.040^{bc} \pm 0.021$
T2	$0.101^{ab} \pm 0.018$	$0.103^{a} \pm 0.009$
T3	$0.091^{ab} \pm 0.025$	$0.023^{bc} \pm 0.007$
T4	$0.049^{bc} \pm 0.015$	$0.040^{bc} \pm 0.015$
T5	$0.048^{bc} \pm 0.023$	$0.025^{bc} \pm 0.012$
T6	$0.081^{ab} \pm 0.026$	$0.062^{b} \pm 0.009$
T7	$0.016^{c} \pm 0.008$	$0.052^{bc} \pm 0.008$
T8	$0.048^{bc} \pm 0.014$	$0.015^{c} \pm 0.007$

where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T0-Control; T1-Cadmium (100ppm); T2- Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid(1 ppm); T6-Lead + Salicylic acid (1 ppm); T7-Cadmium +Putrescine(1 ppm); T8-Lead +Putrescine (1 ppm)

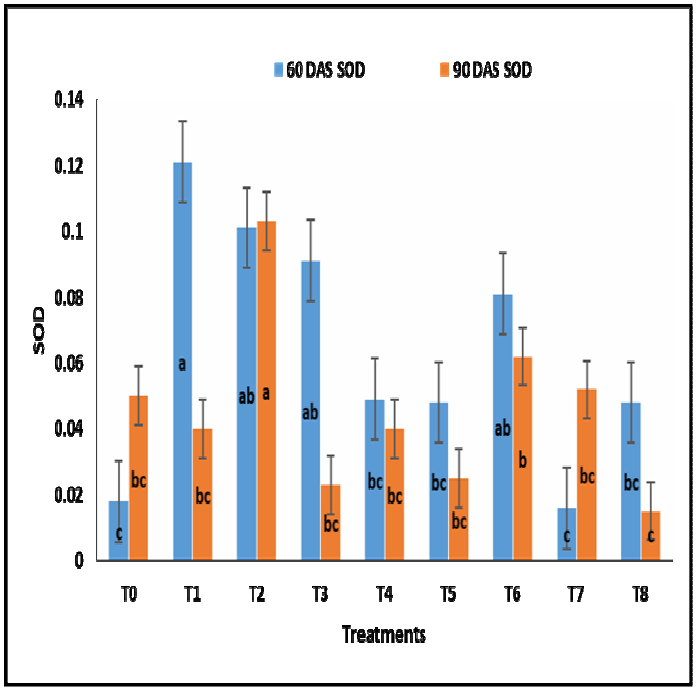

Fig. b : SOD activity (EU per gram fresh weight) of chickpea during *Rabi*

where, DAS: Days after sowing, Data are in the form of Mean±SEM at p>0.05, T0-Control; T1-Cadmium (100ppm); T2- Lead (100ppm); T3-Cadmium + mycorrhiza; T4- Lead + Mycorrhiza; T5- Cadmium + Salicylic acid(1 ppm); T6-Lead + Salicylic acid (1 ppm); T7-Cadmium +Putrescine(1 ppm); T8-Lead +Putrescine (1 ppm)

Conclusion

Growth regulators and fungi significantly alleviate the toxicity of cadmium to chickpeas by increasing the defensive role of carotenoids and anthocyanin pigments in chickpea. Polyamines are present in almost all living organisms and also in the plant). Polyamines are helpful in growth and development, also respond during abiotic or biotic stress, the Pas are present in trace amounts like putrescine but in

mammal's spermidine and spermine are present. The symbiosis of plant roots with fungi occurs in various forms known as mycorrhiza. *Arbuscular mycorrhizal* fungi (AMFs) are major soil microorganisms that are key to enabling plant nutrient uptake, particularly in low-input farming, vegetation, and rhizoremediation processes, in various agroecosystems. Salicylic acid (SA) a compound which has been used to reduces the heavy metals toxicity in plants, which helps in the regulation of plant growth.

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Author Contributions

The study was designed by P.K. and M.N, the biochemical protocolizations were established, experiments were carried out and the data analyzed and interpreted were collected. The paper has been written by P.K and M.N.

Conflict of Interest

The authors declare no conflict of interest.

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