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EFFECTS OF HEAVY METALS AND MICROBES ON LEAF SUGAR AND PROTEIN OF CHICKPEA: A TIME COURSE ANALYSIS

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Abstract

The soil the primary recipient came on contact with a waste of from all the industries, a chemical used in agriculture. Heavy metals are natural elements of the world's crust, yet their geochemical and biochemical balance has changed drastically through indiscriminate human activities. The soil the primary recipient came on contact with a waste of from all the industries, a chemical used in agriculture.In comparison with control (T0) at the date of the proposed interval, the plant was subject to a greater dose of lead (T2) and its overall soluble sugar was significantly reduced by 16.78 and 10.57%. It is clear that, compared to control (T0) at dates of 60 and 90 DAS of intervening, the average total solvent protein decreased significantly at 18.09 and 39.15% when exposed to cadmium metal stress (T1).

Keywords: Abiotic, Biotic, Cadmium, Density, Economy, Forage.

Introduction

Legumes were known for their important source of balanced protein food for vegetarians and poor peoples, which makes a major part of the population (Kumar, P., Dwivedi, P. (2018a), Kumar, P., Kumar S. *et al.* (2018b), Kumar, P., Misao, L., *et al.*, 2018c, Kumar P, Dwivedi, P. 2018d, Kumar, P. and Purnima *et al.*, 2018e, Kumar, P. Pathak, S. 2019f, Kumar, P. Siddique, A. *et al.*, 2019g, Siddique, A. Kumar, P. 2018h, Siddique, A., Kandpal, G., Kumar P. 2018i). Production of the pulses varies with the crop density and adaption, prevailing agro-climatic condition. Globally, the total area under pulses has 851.91 lakh ha having a production of 774.73 lakh tonnes. Pulses are grown in about 198 countries globally, but dry beans cultivated only in 152 countries, which consist of 35.95 percent area of the total world area. Chickpea consists of 139.81 lakh ha of the area with the production of 137.31 lakh tons. Lentil consists of 45.24 lakh ha of the area with the production of 48.27 lakh tons. Pigeon pea consists of 70.33 lakh ha of the area with the production of 48.27 lakh tons. Pea consists of 69.32 lakh ha of the area with the production of 48.90 lakh tons. Beans consist of 306.13 lakh ha of the area with the production of 245.16 lakh tons. Chickpea also is known as Bengal gram, the most important pulse crop of India (Pathak, S., Kumar, P., P.K Mishra, M. Kumar, M. 2017j, Prakash, A., P. Kumar, 2017k., Kumar, P., Mandal, B., 2014L , Kumar, P., Mandal, B., Dwivedi P., 2014m., Kumar, P., Kumar, P.K., Singh, S. 2014n, Kumar, P. 2013o., Kumar, P., Dwivedi, P. 2015p, Gogia, N., Kumar, P., Singh, J., Rani, A. Sirohi, Kumar, P. 2014q, Kumar, P., 2014r., Kumar, P., Dwivedi, P., Singh, P., 2012s, Mishra, P.K., Maurya, B.R., Kumar, Pp. 2012t, Kumar, P., Mandal, B., Dwivedi, P. 2011u. Kumar, P., Mandal, B., Dwivedi, P. 2011v). India having ranks first for the chickpea production of 98.80 lakh tons with an area of 99.27 lakh ha. Pakistan stood second having 6.29 lakh tons of production in 9.50 lakh ha area. Iran stood third having 2.62 lakh tons of production in 5.94 lakh ha area. Australia stood fourth having 6.29 lakh tons of production in 5.08 lakh ha area. Turkey stood fifth having 4.50 lakh tons of production in 3.88 lakh ha area (Kumar, P., Pathak, S. 2016w., Pathak, S., Kumar, P., Mishra, P.K., Kumar, M. 2016x., Kumar, P., Harsavardhn, M. *et al.*, 2018y. Kumar, P., Yumnam, J. *et al.*, 2018z, Kumar, P., Pandey, A.K., *et al.*, 2018aa, Kumar, P., Kumar, S. *et al.*, 2018bb, Kumar, P., Krishna, V., *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). In India, the highest chickpea production recorded in Madhya Pradesh, 40.62 lakh tons production and 34.46 lakh ha of the area of the total. In terms of area 15.41 lakh ha Maharashtra stood second but for production has third 11.98 lakh tons. As Rajasthan second in production 14.47 lakh tons but area wise third 15.37 lakh ha. Highest yield recorded in Telangana 1459 kg/ha, followed by Gujrat 1201 kg/ha, West Bengal 1163 kg/ha and lowest in Karnataka 578 kg/ha. Density criteria for the heavy metals range from above 3.5 g/cm3 to above 7 g/cm3. Any substance added into the soil which can harm the soil functioning and ability to yield a crop knows as soil contamination. Due to its toxicity and capacity to accumulate, they are considered as an important source of environmental contamination.Cadmium one of the most toxic heavy metals having an upper limit is 14.157 µg/g. 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; 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 amount of nitrogen, phosphorus and chlorophyll were observed with an increase in concentration of Cadmium; affects the translocation and storage of sugar in sweet sorghum; reduces the internodal space and internodes number in maize [Kumar, P., Pathak, S. 2016w., Pathak, S., Kumar, P., Mishra, P.K., Kumar, M. 2016x, Kumar, P., Harsavardhn, M. *et al.*, 2018y. Kumar, P., Yumnam, J. *et al.*, 2018z, Kumar, P., Pandey, A.K., *et al.*, 2018aa., Kumar, P., Kumar, S. *et al.*, 2018bb, Kumar, P., Krishna, V., *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 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 (Pb) is one of the non – essential trace elements that mainly accumulate due to anthropogenic activities in agricultural soils. The upper limits of leads are 61.87 μg/g. 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 germination. Also, the reduction in the relative water content (RWC) and net photosynthetic rate.

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, andspermine. 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, andrhizoremediation processes, in various agroecosystems. Salicylic acid (SA) a compound thathas 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.

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 biochemical parameters and the standard procedure adopted during the study are given below:

Total soluble sugar (Anthrone method)

The total soluble sugar content in the plant sample was estimated following the method proposed by Sadasuvam and Manickam (1992). The Anthrone reaction is the basis of a rapid and convenient method for the determination of total soluble sugar in the plant sample. Carbohydrates are dehydrated by conc. H_2SO_4 to form furfural. Furfural condenses with Anthorne to from blue-green coloured complex which is measured calorimetrically at 630 nm.

Reagents

- Ethanol (80%)
- Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice-cold 95% sulphuric acid. Prepare fresh before use.
- Standard glucose: Stock-dissolve 100mg of glucose in 100ml water, working standard-10 ml of the stock diluted to 100ml with distilled water.

Procedure

Until all leaf tissues were fully digested, 100 mg of the leaf sample was homogenized with 10 ml of ethanol. Afterward, the sample extract was centrifuged for 15 minutes at 5000 rpm. Extract volume was obtained by adding 100 ml of distilled water. Every test tube received 1 ml of the extract and 6 ml of the anthrone reactant was added. The tube was then put 10 minutes on a boiling water bath and allowed to cool down in running water. Similarly, a blank was made, but without a sample of the leaf. A spectrophotometer after some time developed a blue color in the test tubes and measured the intensity of the blue colour at 620 nm. A standard curve was calculated to calculate the amount of sugar in the sample. The 10 mg of the glucose was dissolved in 100 ml of distilled water or working standard was prepared by dilution of 10 ml of standard glucose stock with 100ml of distilled water. From this stock solution, different concentration of the sugar solution was prepared by taking 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the stock solution in a separate test tube. The final volume of these test tubes was made to 3 ml by adding distilled water and thereafter 6 ml of the anthrone reagent were added to each test tube. They were boiled in a water bath as described above. The solution was cooled and the intensity of the blue colour was read at 620 nm. The standard curve was prepared by plotting the absorbance value on the y-axis, against the concentration of the sugar in solution on the x-axis.

Total soluble protein content

The method developed by Bradford, (1976) was followed. The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Both hydrophobic and ionic interaction stabilizes the anionic form of the dye causing a visible colour change. The assay is useful since the extinction coefficient of a dye-albumin complex solution is constant over a 10-fold concentration range.

Reagents

- Sodium phosphate buffer (pH 7.4)

Solution A: To prepare the Sodium phosphate buffer, 13.9 g of 0.1 M sodium dihydrogen phosphate (NaH_2PO_4) was dissolved in distilled water and the volume was made up to 1000 ml.

Solution B: To prepare the Sodium phosphate buffer, 26.82 g of 0.1 M disodium hydrogen phosphate (Na_2HPO_4) was dissolved in distilled water and the volume was made up to 1000 ml.

The solution A and the solution B were mixed in the ratio of 19:81 and the final pH (7.4) was adjusted with the help of pH meter.

- Dye concentration: Dissolve 100mg of Coomassie brilliant blue G 250 in 50 ml of 95% ethanol. Add 100 ml of concentrated orthophosphoric acid. Add distilled water to a final volume of 200 ml. Store in an amber bottle in the refrigerator, the solution is stable at least six months. Mixed concentrated dye solution with distilled water at the ratio of 1:4. Filter with Whatman No. 1 paper if any precipitate occurs.

Procedure

It was taken 100 mg of plant sample into a morter. Added 10 ml cold extraction. The mortar was stowed in the ice bucket and cursed by the pestle until fine slurry was produced.The homogenates were centrifuged for 15 minutes at 15,000 rpm. The supernatant has been collected and used to extract crude protein. Diluted 5ml dye, crude leaf protein 0.2 ml extract, distilled water 0.8 ml; mix well and allow the colour to develop for at least five minutes, not more than 30 minutes. When it binds to proteins, the red dye turns blue, read the absorption at 595 nm in the spectrophotometer.The standard curve has been prepared with 0.1-1.0 ml of BSA. By comparing the absorbing value on the y-axis to the sugar concentration in solution on the X-axis, the standard curvature had been prepared. The total soluble protein in mg / g of the specimen is expressed.

Results and Discussion

Total Soluble Sugar (mg g^{-1} fresh weight)

The effects in the cadmium stress of the chickpea type GPF-2 were examined of polyamine (putrescine), mycorrhiza, Salicylic acid and its combination of total soluble sugar (mg g-1 fresh weight). 60 and 90 days after sowing (DAS) data were collected (Tab. 2 & Fig. a).In cadmium metal stress (T1) exposed to control (T0) dates of 60 and 90 DAS interval, the average total soluble sugar was significantly lower with 15.59 and 13.42% compared with control (T0). Similarly, the plant was substantially reduced in total soluble sugar with 16.78 and 10.5% compared with control (T0) on the dates of the proposed interval when exposed to a higher dose of lead (T2). The mitigating effect of the exogenous application in the soil (T3) was demonstrated by a decrease of 14.97 and 2.87 percent of total soluble sugar as compared to T0 at the proposed interval dates. Similarly, the total soluble sugar decreased by 13.37% and 3.02% on the proposed interval date with treatment T4 compared to T2.

In comparison to T0, there was a decreasing trend for exogenous use of putrescine (T5) with overall soluble sugar and mitigation with 8.42% and 3.34% of overall soluble sugar at the proposed interval date. Compared to T0 with 7.42 and 1.72 percent, the average total soluble sugar was significantly reduced when treated with a higher dose of putrescin (T6). Also, the total soluble sugar decreased significantly when T7 was compared with T0, at the proposed interval date with 2.69 and 15.5% (particularly compared with T1). The mean total soluble sugar for treatment with higher doses of salicylic acid (T0) was significantly reduced in comparison to T8 with 2.42 and 0.65 percent. The best effect on cadmium was found in salicylic acid, which increased the total soluble sugar at the proposed interval date. Kumar and Dwivedi (2018) conducted a pot experiment on maize variety BIO-9544, to study the effect of putrescine and glomus mycorrhiza on cadmium toxicity reference to sugar and protein. Found that the T17 (0.15 % Cd $(NO_3)_2$ + 5mM Putrescine + mycorrhiza) showedasignificant increase in total sugar content by 4.22%, 5.03% and 4.18% concerning T12 (0.15 % Cd $(NO_3)_2$) and concluded that Pu^+ and mycorrhiza can be used against Cd-induced toxicity.

Table 2 : Total soluble sugar (mg g^{-1} fresh weight) of chickpea during *Rabi*

Treatments	Total Soluble Sugar (60 DAS)	Total Soluble Sugar (90 DAS)
T0	$819.333^{a} \pm 0.601$	$633.600^{b} \pm 0.586$
T1	$691.533^{h} \pm 0.441$	$552.667^{g} \pm 0.726$
T2	$681.800^{i} \pm 0.153$	$570.833^{f} \pm 0.882$
T3	$696.600^{g} \pm 0.586$	$620.000^{d} \pm 0.866$
T4	$709.733^{f} \pm 0.561$	$619.000^{d} \pm 0.764$
T5	$750.300^{e} \pm 0.569$	$616.333^{e} \pm 0.601$
T6	$758.500^{d} \pm 0.577$	$627.333^{c} \pm 0.441$
T7	$797.267^{c} \pm 0.674$	$638.333^{a} \pm 0.726$
T8	$799.500^{b} \pm 0.462$	$634.167^{b} \pm 0.882$

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)

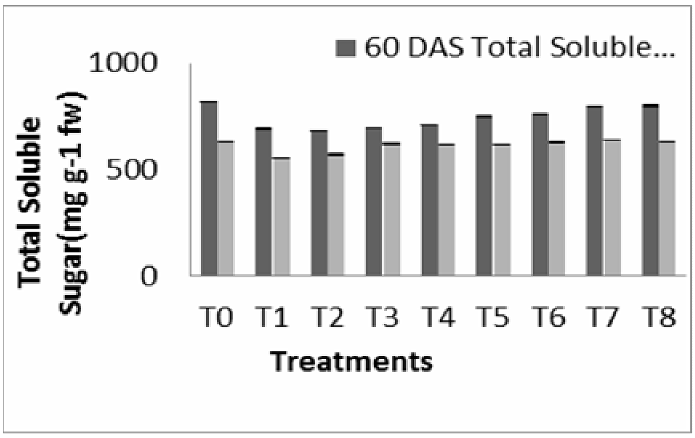

Fig. a. : Total soluble sugar (mg g^{-1} fresh weight) of chickpea during *Rabi*

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)

Total Soluble Protein (mg g^{-1} fresh weight)

The effect on the total soluble protein content of polyamine (putrescin), mycorrhiza, salicylic acid, and their combination was studied in the cadmium-stressed chickpea variant GPF-2. Sixty and ninety days after sowing (DAS) (Table 3 & Fig. b) were data recorded. It is clear that in cadmium metal stress (T1) as compared to control (T0) at 60 and 90 DAS interval dates, the total soluble protein average has significantly decreased at 18.09 and 39.15 percent. Similarly, the total soluble protein content of plants with a higher dose of lead (T2) was considerably decreased, compared to control (T0), at 17.48 and 38.45 percent on the proposed interval. The mitigation effect was shown by the exogenous application of endomycorrhiza to soil (T3) by a decrease in the total soluble protein content of 14.07% and 37.61% as compared to T0 at the proposed interval dates. The total soluble protein content decreases with 10.87 percent and 38.39 percent on the suggested date of intervals were also significant when T4 treatment was compared with T0.

The exogenous application of putrescine (T5) showed a decreased trend with 11.46 and 36.83 percent over the proposed interval date of the total soluble protein content and catalase mitigation compared to T0.When treated with a high dose of putrescin (T6), the average total soluble protein content was significantly reduced compared to T0, at 5.88 and 38.85%. Similarly, the total soluble protein was considerably decreased with T7 when compared to T0 at the proposed interval with 4.96 and 34.96%. Compared with T8 in treatment with a 2.74 and 22.37 percent higher salicylic acid dose (T0), the average total soluble protein content was significantly lower. At the interval suggested, the salicylic acid was the best mitigating effect against cadmium and lead to an increase in total soluble protein. Kumar and Dwivedi (2018)conducted a pot experiment on maize variety BIO-9544, to study the effect of putrescine and glomus mycorrhiza on cadmium toxicity reference to sugar and protein. Found that the T17 (0.15 % Cd $(NO_3)_2$ + 5mM Putrescine + mycorrhiza) showedasignificant increase in total sugar content by 4.22%, 5.03% and 4.18% concerning T12 (0.15 % Cd $(NO_3)_2$) and concluded that Pu^+ and mycorrhiza can be used against Cd-induced toxicity [23, 24 25 and 29]. Kumar (2018a)reported that the combined application of putrescine and mycorrhiza in maize crop under cadmium toxicity [31 and 32]. The combination was suitable for mitigating Cadmium toxicity linked to internal nodal length and node number[28, 29 and 30].

Table 3 : Total soluble protein (mg g^{-1} fresh weight) of chickpea during *Rabi*

Treatments	Total Soluble Protein (60 DAS)	Total Soluble Protein (90 DAS)
T0	$91.718^{a} \pm 0.903$	$33.664^{ab} \pm 0.190$
T1	$75.122^{e} \pm 0.079$	$32.381^{b} \pm 0.207$
T2	$75.681^{e} \pm 0.155$	$32.754^{ab} \pm 0.548$
T3	$78.808^{d} \pm 0.108$	$32.919^{ab} \pm 0.587$
T4	$81.747^{c} \pm 0.313$	$32.505^{b} \pm 0.509$
T5	$81.201^{c} \pm 0.103$	$33.333^{ab} \pm 1.030$
T6	$86.323^{b} \pm 0.221$	$32.257^{b} \pm 0.207$
T7	$87.164^{b} \pm 0.149$	$34.327^{a} \pm 0.180$
T8	$91.201^{a} \pm 0.895$	$32.257^{b} \pm 0.323$

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)

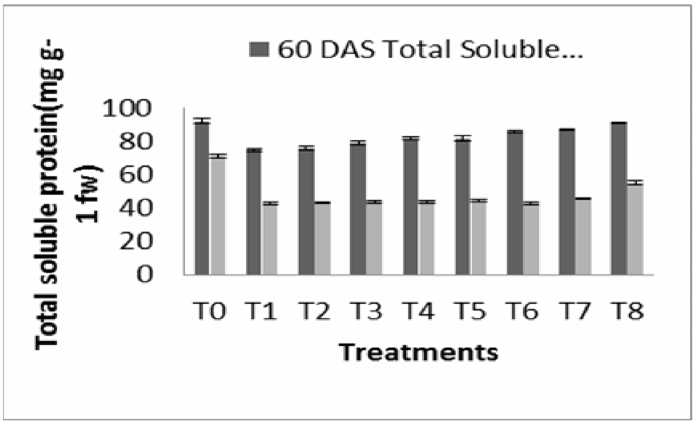

Fig. b : Total soluble protein (mg g^{-1} fresh weight) of chickpea during *Rabi*

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

Polyamines, SA, Mycorrhiza, and Rhizobium provide significant mitigation of cadmium and lead-induced toxicity in chickpea mediated by increasing through their defensive role in plants. It will happen because 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. 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.

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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., M.N. and T.K.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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