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CADMIUM INDUCED ALTERATION IN LEAF NUMBER, STEM GIRTH AND BRANCH NUMBER OF CHICKPEA

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Abstract

Every substance added to the soil that can adversely affect the soil function and the ability to produce a crop is known as contamination of the soil. They are considered an important source of environmental contamination due to their toxicity and their ability to accumulate. A waste from all industries, a chemical used in agriculture, came into contact with the soil the main recipient. The average number of leaves in respect of all treatments was not modified. The average stem girth was significantly reduced as compared to T0 with 32.06% and 21.78% when treated with a higher dose of putrescine (T6). The average branch number was significantly reduced as compared to T2 with 12.7% and 18.7% when treated with a higher dose of putrescine (T6).

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

Introduction

Chickpea also is known as Bengal gram, the most important pulse crop of India. 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 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). Legumes were known for their important source of balanced protein food for vegetarians and poor peoples, which makes a major part of the population (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; Gogia *et al*., 2014q). 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. 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. 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. Due to its toxicity and capacity to accumulate, they are considered as an important source of environmental contamination.The soil the primary recipient came on contact with a waste of from all the industries, a chemical used in agriculture (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; Kumar *et al*., 2018y; Kumar *et al*., 2018z; Kumar *et al*., 2018aa; Kumar *et al*., 2018bb; Kumar *et al*., 2018cc). 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 (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).Cadmium one of the most toxic heavy metals having an upper limit is 14.157 µg/g [6]. 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. 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. 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 ingemmation. Also, the reduction in the relative water content (RWC) and net photosynthetic rate. 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 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.

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 (26-30). 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 morphological parameters and the standard procedure adopted during study are given below:

Morphological Parameters

Leaf number

The number of leaves per plant was recorded by counting the leaves from top to bottom of the plant and the mean value of the plants selected in each treatment was expressed as number per plant.

Stem girth

The stem girth was recorded from the base of the plant to the tip of the stem of the plant at 60 and 90 days of time interval by using a digital Vernier caliper. Mean stem girth was calculated and expressed in the cm.

Branch Number

The number of branches per plant was recorded by counting the branch from top to bottom of the plant and the mean value of the plants selected in each treatment was expressed as the number per branch per plant.

Results and Discussion

Leaf Number (Plant^{-1})

The influence in the chickpea variety GPF-2, under the stress of cadmium of polyamine (putrescine), mycorrhiza, salicylic acid and their combination on leaf number was studied. Sixty days, and ninety days, after sowing (DAS) data have been collected (Table 2, Fig. 1).The average leaf numberwas found no change in terms of a number concerning all the treatments. Even though, scientist reported the changes in the same. In a case study of Kumar *et al.* in the year 2018-19, he reported that, conducted a pot experiment, in which mycorrhiza and putrescine were applied as the ameliorative agents for Cd toxicity. The combination of mycorrhiza and putrescine showed a better result for the mitigation of Cd casing toxicity in plant height and leaf number per plant.Kumar (2018a) reported that the combined application of putrescine and mycorrhiza in maize crop under cadmium toxicity.

The combination was suitable for mitigating Cadmium toxicity linked to internal nodal length and node number. Kumar (2018b) conducted a pot experiment, in which mycorrhiza and putrescine were applied as the ameliorative agents for Cd toxicity [24, 25]. The combination of mycorrhiza and putrescine showed a better result for the mitigation of Cd casing toxicity in plant height and leaf number per plant. Kumar P. (2018c) reported that there was a significant increase in chlorophyll in treatment T17 (0.15 % Cd $(NO_3)_2$ + 5mM Putrescine + mycorrhiza) for T12 (0.15 % Cd $(NO_3)_2$). Also, chlorophyll b in T17 has a significant effect with 10.90%, 7.09% and 8.05% increase for T12. Natarajan *et al.*, (2018) conducted a pot experiment tomato by using increasing concentration (10, 25, 50, 75 and 100mg Kg^{-1}) of cadmium in soil. The Cd treatment plant shows reduced growth in root and shoots length and biochemical components such as protein (except phenol and proline) content compared to control. Shoot length was higher compared to root in Cd-treated plant, but proline and phenol content in the root was higher compared to shoot. Osmolovskaya *et al.*, (2018) experimented to study the effect of cadmium at concentrations of 1 and 10 μM on biomass increment, mineral nutrient elements (potassium, calcium, and magnesium) accumulation, and oxalic acid pools in organs of *Amaranthuscruentus* L. plants growing underwater culture conditions was investigated.

Table 2 : Leaf Number (Plant^{-1}) of Chickpea during *Rabi*

Treatments	Leaf number (60 DAS)	Leaf number (90 DAS)
T0	13.333^{a}±0.333	14.333^{a}±0.333
T1	14.000^{a}±0.000	14.333^{a}±0.667
T2	13.333^{a}±0.333	14.333^{a}±0.333
T3	13.333^{a}±0.333	14.333^{a}±0.667
T4	13.000^{a}±0.000	14.333^{a}±0.333
T5	13.333^{a}±0.333	14.333^{a}±0.667
T6	13.667^{a}±0.333	14.000^{a}±0.577
T7	12.667^{a}±0.882	13.333^{a}±1.202
T8	13.333^{a}±0.333	14.333^{a}±0.667

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)

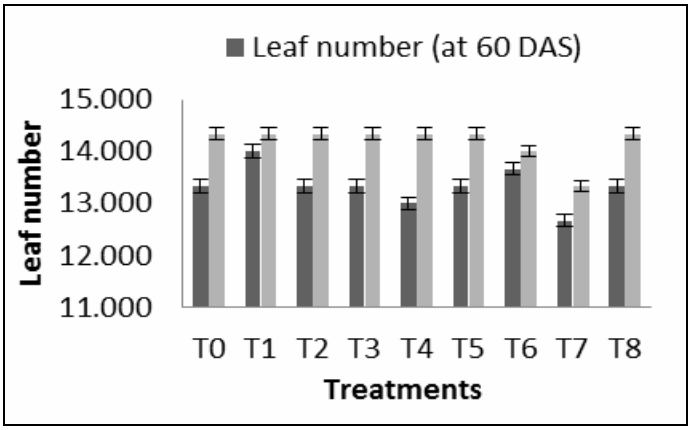

Fig. 1 : Leaf Number (Plant^{-1}) of Chickpea during *Rabi*

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)

Stem girth (cm)

In the chickpea variety GPF–2, under cadmium stress, the effect of polyamine (putrescine), mycorrhiza, and Salicylic acid and their combination was studied. After sowing (DAS) data were recorded in 60 and 90 days (Tables 3, Fig. 2).Clearly, in cadmium metal stress (T1) exposed compared to control (T0), the average stem girth was significantly reduced by 29.88 percent and 29.59 percent at dates of interval 60 and 90 DAS.Similarly, when plants were subjected to a higher dose of lead (T2), their stem circumference was significantly reduced, compared to control (T0) at the interval proposed with 32.18 and 2.09 percent, respectively.The mitigation effect was shown by the exogenous application of endomycorrhizal soil (T3) by increasing the stem girth to 6.45 percent and 4.48 percent, compared with T1 at interval dates proposed.Similarly, the stem girth was reduced significantly with 13.7% and 26.93% at the proposed interval date in the treatment T4 as opposed to T2. Compared to T1, the mitigation of stem girth with 8.96% and 26.55% on the proposed date of the interval was shown by the exogenous application of putrescin (T5). Compared to T0, with 32.06 percent and 21.78 percent in higher doses of putrescine (T6), average stem circumference was significantly reduced. Similarly, the stem length was less significantly increased with 25.64% and the proposed interval date when T7 was compared to T1. T7 was less significantly increased.When treated with a high dose of salicylic acid (T1), the average stem circle was reduced significantly as compared to T8, at 17.25% and 6.73%.

The most effective mitigation of cadmium was found with a polyamine (putrescine), which reduced the stem rim on the proposed interval date. An. M, *et al*., (2019) experimented on a test station at Shihezi University, China. The effect of four liquid modifiers (inorganic polymer compound modifier, organic-inorganic composite modifier, an organic polymer compound, andpoly-acrylate compound modifier) on plant growth, cadmium content, photosynthetic parameters and antioxidant enzymes in cotton under Cadmium stress (mg kg^{-1}). The result showed that the Cd-treated soil increases the Cd content in cotton plant and reduction in plant height, chlorophyll fluorescence parameter, antioxidant enzyme activity, net photosynthetic rate, and biomass. Scientistsstudied the screen bacterial strainsmost effective in increasing alfalfa growth and metalaccumulation in the presence of toxic levels of lead (Pb) and zinc (Zn)[25].

Table 3 : Stem Girth (cm) of Chickpea during *Rabi*

Treatments	Stem girth (60 DAS)	Stem girth (90 DAS)
T0	0.290^{a}±0.006	0.303a±0.003
T1	0.203^{bc}±0.003	0.213c±0.013
T2	0.197^{bc}±0.009	0.297a±0.003
T3	0.217^{bc}±0.012	0.223c±0.012
T4	0.170^{c}±0.006	0.217c±0.012
T5	0.223^{b}±0.015	0.290a±0.006
T6	0.197^{bc}±0.012	0.237^{bc}±0.012
T7	0.273^{a}±0.032	0.323^{a}±0.019
T8	0.163^{c}±0.009	0.277^{ab}±0.033

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)

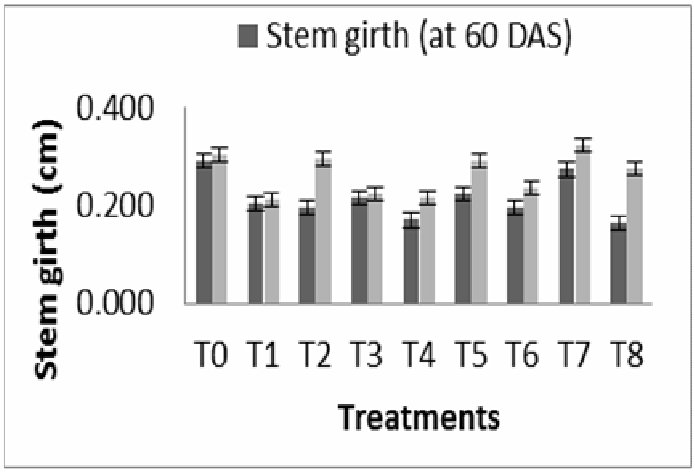

Fig. 2 : Stem Girth (cm) of Chickpea during *Rabi*

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)

Branch number

In chickpea variety GPF-2, under the cadmium stress, the effect of polyamines, mycorrhiza, salicylic acid and their combination on branch number was studied. Sixty and 90 days following sowing (DAS) data were recorded. (Table 4, Fig. 3).It is obvious that in comparison with control (T0) at 60 and 90 DAS intervals, the average number of branches was significantly reduced at 49.1% and 35.8 percent in cadmium metal stress (T1).Likewise, the branch number of plants exposed to a higher dose (T2) of lead was significantly decreased at the dates of the proposed interval, with 47.0 and 33.3 percent in comparison to control (T0).The mitigating effect was shown by the exogenous application of the endomycorrhiza (T3) in soil by a decrease of 23.29 percent and 29.7 percent compared to T1 at the suggested interval dates.Similarly, the number of branches in treatment T4 was significantly increased at 18.54 percent and the proposed interval date by 32.3 percent compared to T2. Compared to T1, at the proposed date of the interval, exogenous putrescin application (T5) indicated a 13.2% and 27.8% branch mitigation. When treated with a greater dose of putrescine (T6), the average number of branches was significantly decreased compared to T2 with 12.7 and 18.7 percent. Also, the branch number decreased considerably with 1.04% and 25.8% as at the proposed interval date when treatment T7 was compared to T1.When treated with a high salicylic acid dose, the average number of branches was significantly reduced compared to T8 with 2.25% and 16.3% (T2). The most effective mitigation effect against polyamine (putrescine) was demonstrated by reducing the branch number on the suggested interval date. Rady *et al*., (2019) experimented to analyze the effect of exogenous application of polyamine under lead (2.0mM) stress on growth and productivity of wheat. The seeds of wheat were soaked in 0.25mM Spm, 0.50 Spd or 1.0mM put, showed bettergrowth and yield attributes, RWC, MSI, leaf pigment and nutrient uptake compared to seeds soaked in water under 2.0mM lead stress. Among the polyamines, put showed the best result and thus it was recommended the soaking of wheat seed under lead stress.

Table 4 : Branch Number of chickpea during *Rabi*

Treatments	Branch number (60 DAS)	Branch number (90 DAS)
T0	24.667^{ab}±4.485	30.333^{ab}±2.603
T1	24.333^{ab}±3.844	29.000^{ab}±2.082
T2	18.000^{b}±2.309	21.667^{b}±5.044
T3	24.000^{ab}±4.163	31.667^{ab}±7.311
T4	24.667^{ab}±6.438	30.000^{ab}±6.658
T5	24.667^{ab}±1.202	29.667^{ab}±1.667
T6	32.333^{a}±1.202	36.667^{ab}±2.333
T7	24.667^{ab}±1.764	25.667^{ab}±3.180
T8	31.333^{a}±1.764	39.333^{a}±5.044

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)

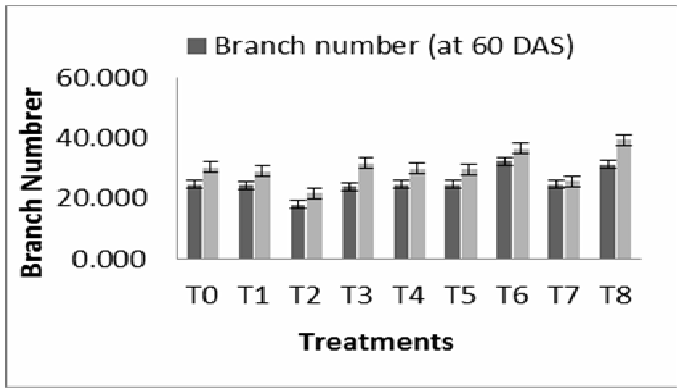

Fig. 3 : Branch number of Chickpea during *Rabi*

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-induced toxicity in chickpea mediated by increasing the branch number and stem girth in chickpea stem through their defensive role in plants. Growth regulators are compound which has been used to reduce 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 morphological 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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