

Growth, Physiological and Biochemical Responses of Mung Bean (*Vigna radiata* L.) to Cadmium Polluted Soil

Rida Hayat¹, Imran Khan², Muhammad Umer Chattha², Muhammad Umair Hassan³, Wei Jian^{4*}, Jameel Mohammed Al-Khayri^{5*}, Mohammed Ibrahim Aldaej⁵, Muhammad Naeem Sattar⁶, Adel Abdel-Sabour Rezk^{5,7}, Mustafa Ibrahim Almaghasla^{8,9}

¹ Department of Botany, University of Agriculture Faisalabad, 38000 Faisalabad, Pakistan

² Department of Agronomy, University of Agriculture Faisalabad, 38000 Faisalabad, Pakistan

³ Research Center on Ecological Sciences, Jiangxi Agricultural University, Nanchang, China

⁴ School of Agriculture, Jilin Agricultural University, China

⁵ Department of Agricultural Biotechnology, College of Agriculture and Food Sciences, King Faisal University, 31982 Al-Ahsa, Saudi Arabia

⁶ Central Laboratories, King Faisal University, PO Box 420, 31982 Al-Ahsa, Saudi Arabia

⁷ Department of Virus and Phytoplasma, Plant Pathology Institute, Agricultural Research Center, 12619 Giza, Egypt

⁸ Department of Arid Land Agriculture, College of Agriculture and Food Sciences, King Faisal University, 31982 Al-Ahsa, Saudi Arabia

⁹ Plant Pests, and Diseases Unit, College of Agriculture and Food Sciences, King Faisal University, 31982 Al-Ahsa, Saudi Arabia

* Corresponding author's e-mail: weijian@jlau.edu.cn, jkhayri@kfu.edu.sa

ABSTRACT

Cadmium (Cd) toxicity is an alarming issue for our agricultural soils and serious threat to crop productivity. The concentration of Cd in our soils is continuously increasing which is posing serious threat to plants, animals and humans. Mung bean is a conventional pulse crop cultivated all over the world. Thus, this study's goal was to evaluate response of mung bean seedlings in terms of growth, physiology, and biochemistry to varying degrees of Cd stress. The investigation examined various Cd levels, including control, 5, 10 and 15 mg Cd/kg of soil. The results indicate that mungbean growth, physiological and biochemical components was negatively impacted by Cd stress. Results depicted that Cd (15 mg/kg) reduced the growth attributes photosynthetic pigments (Chl. a, b and carotenoids), total soluble proteins (TSP) and free amino acids (FAA) and increased the malondialdehyde (MDA), hydrogen peroxide (H₂O₂), and electrolyte leakage (EL). Interestingly, activities of all four antioxidants (ascorbic acid, catalase, ascorbate peroxidase and peroxidase) increased with increase in Cd toxicity.

Keywords: antioxidants, cadmium, chlorophyll, growth, oxidative stress markers.

INTRODUCTION

Soil heavy metals (HMs) pollution is a major concern across the globe and concentration of HMs in environment is continuously increasing owing to industrial effluents, sewage sludge, chemical fertilizers and municipal trash [Rady et al., 2023]. The rise in HMs

concentrations in soil and water can cause severe threat to humans, animals, and plants owing to their non-degradable nature [Sahay and Gupta, 2017]. Heavy metals damage plant metabolic processes and cause growth limitation and even plant death [Sahay and Gupta, 2017]. Plant plasma membranes allow heavy metals to pass through, where they combine with oxygen

and sulfur to interact with proteins and DNA [Rady et al., 2023] Cadmium (Cd), is a metal and it is absorbed by plant roots and cause leaf chlorosis, reduction in photosynthesis and transpiration, poor root growth and plant death in severe cases [Wei et al., 2021]. Additionally, Cd in plants is eventually enters into humans by food chain which is serious threat for humans [Tyagi et al., 2020; Ma et al., 2023].

Cadmium toxicity can impede the plants growth and development and roots are considered as primary site for propagation of Cd pollution in plant species [Yang et al., 2021]. Cadmium toxicity also reduce the nutrient and water uptake Cd, which negatively affect plant growth [Shaari et al., 2022]. Cadmium toxicity, also disrupts the plant metabolic and morphological processes and resulting in nutrient deficiency [Almuwayhi, 2021] Furthermore, plant cells, mitochondria, nuclei, and the synthesis of chlorophyll are also harmed by Cd toxicity [Zhu et al., 2023]. Additionally, excessive reactive oxygen species (ROS) produced by Cd stress damage proteins, DNA and membranes by causing lipid peroxidation [Shaari et al., 2022]. However, Bplants also turn on the antioxidant defense mechanism, which lowers ROS production convert the (H_2O_2) and superoxide (O^{-2}) to mitigate Cd toxicity [Zulficar et al., 2022].

After absorbing Cd through their roots, plants move the metal to other plant parts through specific and non-specific transporters of other nutrients [Pacheco et al., 2023; Yousefi et al., 2023]. Additionally, under Cd stress, plants experience various modifications that impact signaling pathways, osmolyte synthesis, and secondary metabolite synthesis [Pacheco et al., 2023]. The accumulation of higher concentration of Cd is plants also damage the membranes that resulting in increased electrolyte leakage [Kavian et al., 2023]. Besides this over ROS also damage phospholipids and resulting in cell lysis [Kohli et al., 2017; Sachdev et al., 2023]. Additionally, Cd toxicity also limits the final production and deteriorate the product quality and cause health associated risks in humans [Islam and Sandhi, 2022]. The mung bean is a widely grown crop used for grain, vegetable, and animal feed. This crop is sensitive to a variety of biotic and abiotic stresses that can cause significant yield reductions. Thus, the purpose of this study was to ascertain the growth, physiological and biochemical responses of mung bean against Cd toxicity.

MATERIALS AND METHODS

Growth conditions

The present pot study was conducted University of Agriculture Faisalabad, Pakistan. Mung bean NIAB-MUNG 2016 was utilized in this study and ten seed was placed in each pot. The soil was taken from Agronomy Field and it was clay loam with pH 7.82, organic matter 8.42 g kg^{-1} , EC 0.94 dS m^{-1} , available phosphorus 9.64 mg kg^{-1} , potassium 168 mg kg^{-1} , and total nitrogen 0.33 g kg^{-1} . The pots having capacity of 8 kg with 24 cm were filled with soil. Additionally, pots were routinely checked and irrigation was applied in accordance with crop needs, and all other management procedures remained constant.

Experimental treatments

The experiment was compromised of different levels of cadmium as control, 5, 10 and 15 mg Cd/kg of soil which was design in completely randomized design with four replications.

Determination of growth parameters

The plants were randomly taken from the pots to determine the growth traits. Once the roots and shoots were separated, they were weighed to obtain their fresh weight and subsequently dried in the oven (65°C) to determine their dry weights.

Determination of physiological traits

The chlorophyll and carotenoids content was determined by the method Arnon [1949]. Fresh leaves (0.3 g from each pot) were homogenized in 3 ml of methanol to obtain the extract and absorbance was determined at 480, 645, 663 nm. Fresh leaf sample were extracted from plant and weighed (FW) and then leaves were dipped distal H_2O for 24 h. Then took out the sample from water and removed the excess water with paper towel and final weight of sample was calculated immediately and leaf RWC was determined with following formula:

To determine EC: 0.5 g leaves were taken soaked in water for 24 hours and EC1 was taken. After that the leaves were auto-calved for 120 minutes, and they were allowed to come to equilibrium and EC2 was taken and EL%

measurements were made with following equation: $EL\% = (EC1/EC2) \times 100$.

Determination of osmolyte

The Bradford [1976] standard procedure was applied to ascertain the soluble protein concentration. Fresh leaf samples weighing 0.5 g were ground in 5 ml of phosphate buffer and centrifuged at 8000 rpm for 10 minutes in order to determine the TSP. After adding 1 mL of supernatant and 3 mL of Bradford reagent, the mixture was incubated for 10 minutes, and an absorbance was done at 595 nm. For FAA, we took 0.5 ml of fresh leaf samples and extract was obtained after adding the phosphate buffer. Next, 0.5 ml of extract was added to each test tube along with 1 ml of ninhydrin and pyridine. The test tubes were then placed in a water bath at 90°C for 30 minutes. After the tubes cooled, the volume was increased to 15 ml, and a spectrophotometer was used to measure the absorbance at 570 nm. To determine anthocyanin: 0.5 g plant sample was grinded with 5ml potassium phosphate buffer and absorbance was noted at 535 nm.

Determination of oxidative stress markers

One milliliter of the supernatant was combined with 20% thiobarbituric acid (TBA) reagent in 20% trichloroacetic acid (TCA) and heated for fifty minutes in order to measure the MDA content. Then, absorbance was determined at 532 nm and 600 nm by using spectrophotometer. For determination of hydrogen peroxide (H_2O_2) concentration, 0.5 ml plant sample was grinded in 5 ml of trichloroacetic acid (TCA) and centrifuged it to get supernatant. After adding 1 ml of IM of KI and 100 μ l of potassium buffer to a test tube mixture containing 1 ml of plant sample, the mixture was allowed to sit at room temperature for 30 minutes and absorbance was made at 390 nm.

Determination of antioxidant activities

The concentration of ascorbis acid (AsA) was determined by the method of Mukherjee and Choudhuri [1983]. 0.5 g of leaf sample was homogenized with ml of 10% tri-chloroacetic acid solution and centrifuged for 10 min at 8000 rpm. After centrifugation (0.5 ml) DTC reagent added in 1 ml supernatant and then incubated for 3 h at 37°C. After that, samples were quickly cooled,

and the absorbance at 520 nm was measured. To determine POD, 100 μ l plant sample was taken and mixed with 100 μ l of H_2O_2 and Guaiacol. Then total POD contents were measured at 520 nm by spectrophotometer and expressed in units/ μ g pro pod activity. APX contents was determined by Naknano and Asada [1981]. We took 50 mM potassium phosphate buffer, 0.1 mM hydrogen peroxide and 0.1 Mm EDTA with 0.7 ml of enzyme extract. By adding more hydrogen peroxide, the APX content was ascertained, and the absorbance at 290 nm was recorded. To determine catalase, 500 μ l buffer was added with 100 μ l H_2O_2 in the mixture and shake it gently. Following agitation, 0.1 ml of enzyme extract was added, and after 30 minutes, the absorbance was noted at 240 nm.

Statistical analysis

The studied traits on growth, physiological and biochemical traits was analyzed by analysis of variance (ANOVA) technique and least significant difference test was used to separate the treatment means. All the studied traits were expressed as the means of four replications with \pm standard error.

RESULTS

Growth traits

The findings showed that cadmium stress inhibited root and shoot growth. The growth parameters showed a decreasing tendency with increase in Cd toxicity and the greatest reduction in growth traits was noted at the highest level of Cd stress (15 mg/kg). Cd toxicity (15 mg/kg) decreased the shoot and root length by 59.5% and 57.6% followed by 10 and 5 mg/kg (Table 1). Similarly, Cd toxicity (15 mg/kg) also reduced the SFW and SFW by 47.9% and 52.7% than control. Moreover, same treatments decreased the leaves per plant by 64.0% and shoot and root dry weight by 27.1% and 32.2% (Table 1). The overall ranking of different Cd stress levels in decreasing growth traits was observed as: 15>10>5>0 mg/kg.

Photosynthetic pigments and leaf water status

Cd toxicity caused a marked reduction in photosynthetic pigments. Under 15 mg/kg Cd stress, the Chl-a, Chl-b, and Car were decreased by 1.63%,

Table 1. Effect of different levels of Cd stress on growth traits of mung bean crop

Cd levels	SL (cm)	RL (cm)	LP	SFW (g)	RFW (g)	SDW (g)	RDW (g)
Control	13.44±1.23	6.16±0.74	7.11±0.99	1392.56±70.57	72.6±20	205±4.02	12.8±1.66
5 mg/kg	9.33±1.11	4.77±0.82	5.44±0.83	1137.11±72.6	65.11±2.46	187.5±3.53	8±1.15
10 mg/kg	7.66±0.81	3.38±0.56	3.55±0.68	751.44±132.72	54.44±3.23	167.4±4.78	5.66±0.94
15 mg/kg	5.44±0.76	2.61±0.65	2.55±0.49	514.22±49.59	44.77±3.15	140.4±7.61	2.22±0.91
LSD≤0.05P	1.00	0.686	0.788	0.559	0.106	0.096	0.104

Note: the values are means (n=4) with ± SE; S – shoot length, RL – root length, LP – leaves per plant, SFW – shoot fresh weight, RFW – root fresh weight, SDW – shoot dry weight, RDW – root dry weight.

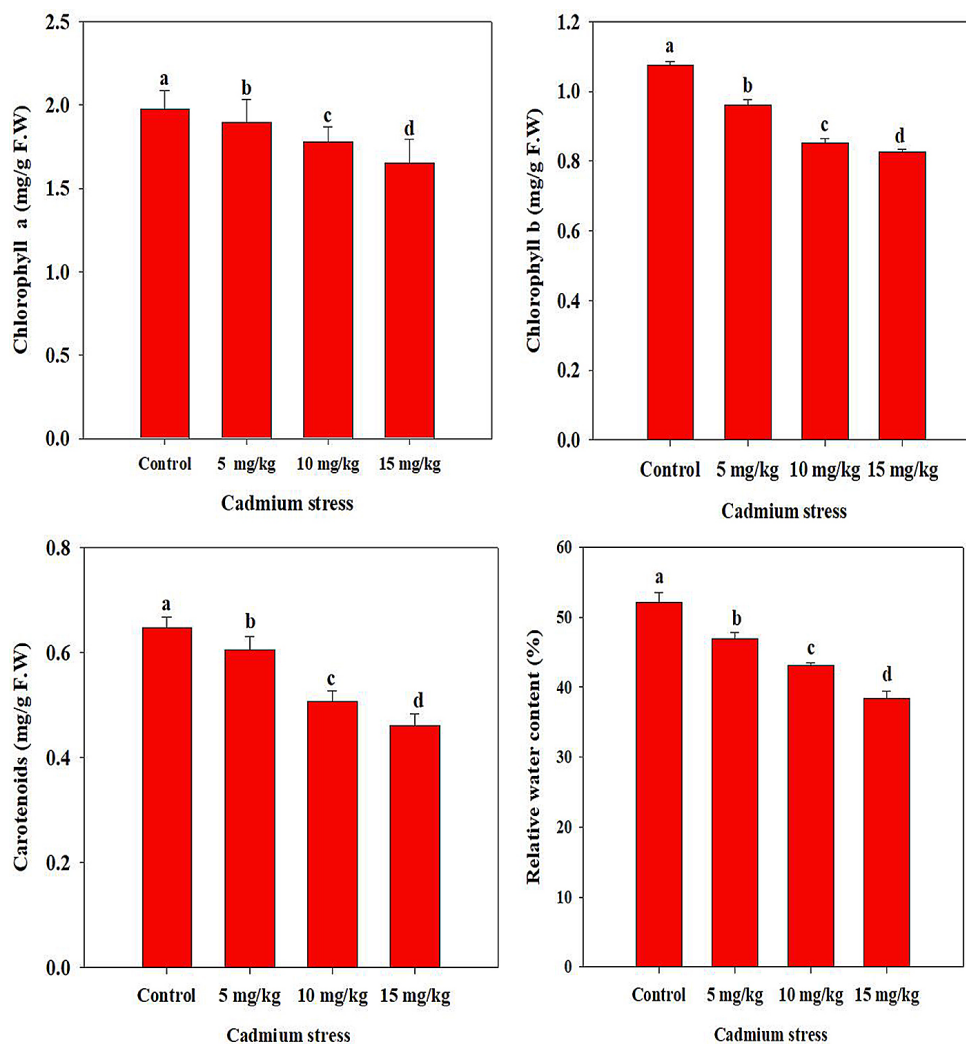


Figure 1. Effect of different concentration of Cd stress on chlorophyll-a, chlorophyll-b, carotenoid and relative water contents of mung bean plants. The values indicating means (n=4) with SE and different letter indicating significant differences at $p < 0.5$

4.96%, and 6.06%, respectively, compared to no Cd stress. This decrease in Chl-a, Chl-b, and Car was decreased with decrease in Cd stress and minimum reduction in chlorophyll a, chlorophyll b and carotenoids contents was seen under control (Figure 2). Leaf RWC also showed substantial reduction under varying Cd stress levels. The leaf RWC was

decreased by 99.0%, 13.7% and 22.4% under 5, 10, and 15 mg/kg of Cd toxicity (Figure 2).

Oxidative stress markers

The findings demonstrated a significant increase in electrolyte leakage, MDA, and H₂O₂

contents due to cadmium stress. The maximum concentration of these oxidative stress markers was observed at higher Cd stress (15 mg/kg) followed by 10 and 5 mg/kg and minimum concentration of electrolyte leakage, MDA and H_2O_2 was recorded in control conditions (Figure 3).

Osmolyte accumulation and antioxidant activities

The results given in Figure 1 indicate that Cd stress seriously decreased the TSP and FAA (Figure 1). A reduction of 14.8%, 27.5% and 40.9%

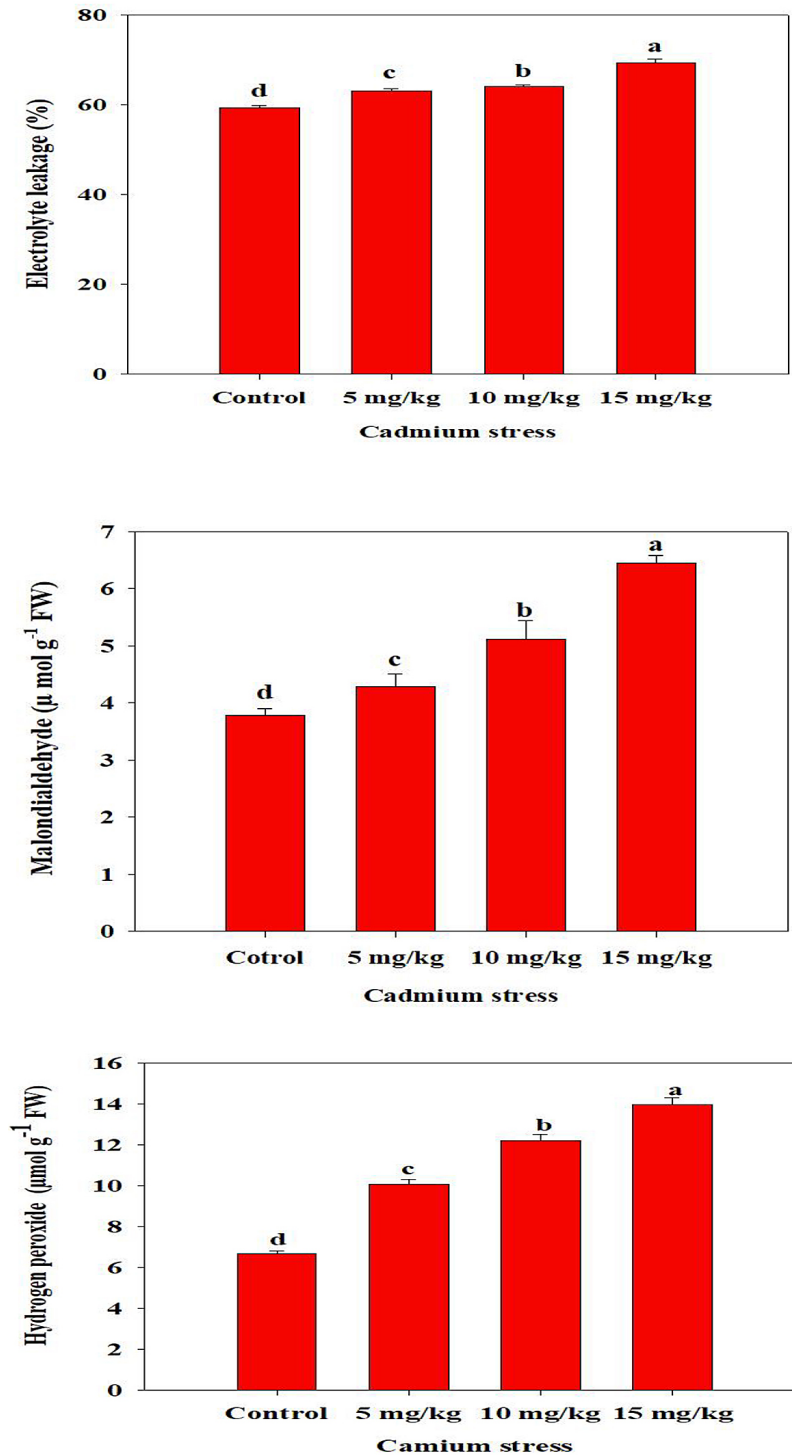


Figure 2. Effect of different concentration of Cd stress on electrolyte leakage, MDA and H_2O_2 concentration of mung bean plants. The values indicating means ($n=4$) with SE and different letter indicating significant differences at $p < 0.5$

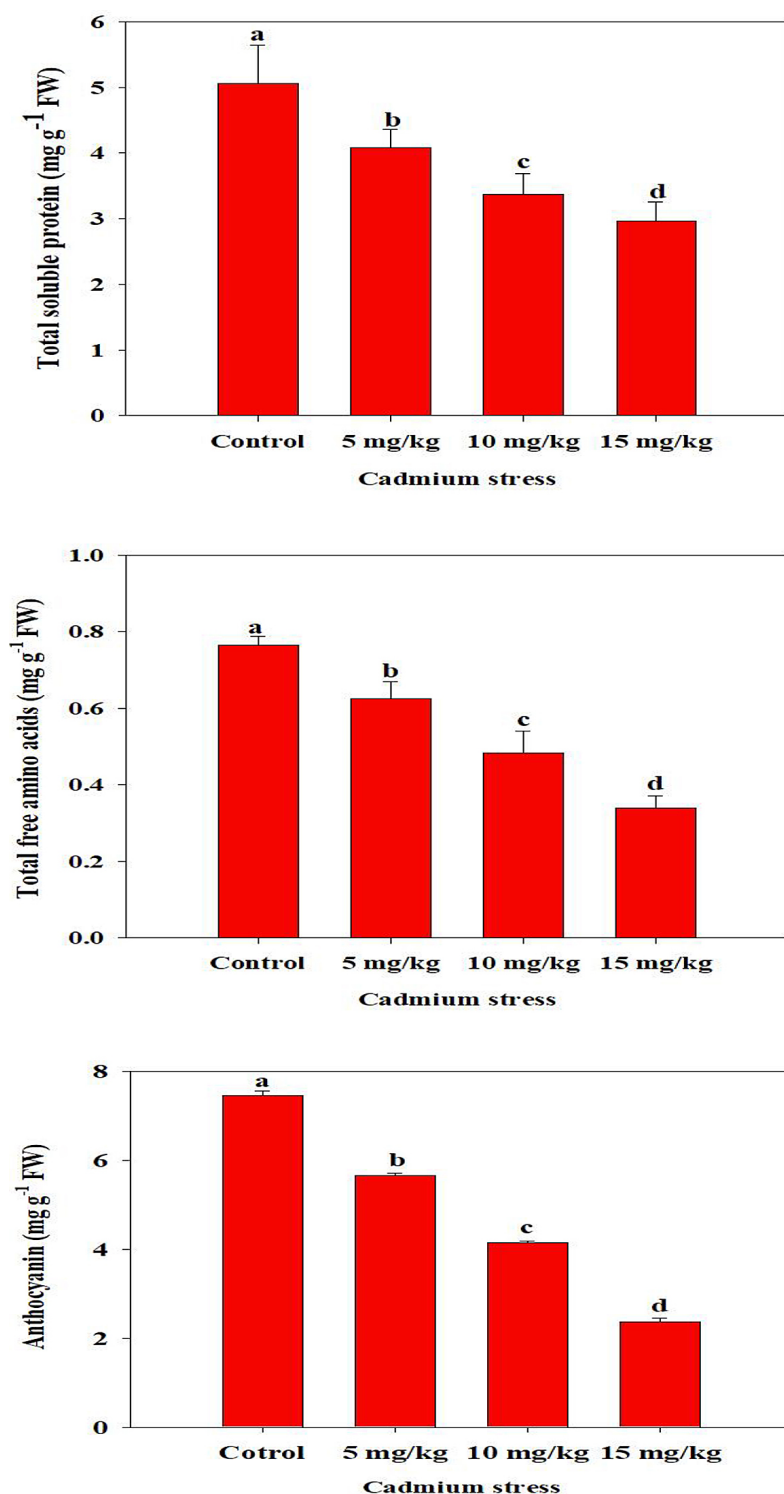


Figure 3. Effect of different concentration of Cd stress total soluble proteins, free amino acids and anthocyanin concentration of mung bean plants. The values indicating means (n=4) with SE and different letter indicating significant differences at $p < 0.5$

in TSP was observed at Cd stress of 5, 10 and 15 mg/kg Cd stress (Figure 2). Likewise, concentration of FAA was also decreased under Cd stress and maximum reduction was seen at higher level of Cd stress (15 mg/kg). The activities of all the studied antioxidant showed a substantial increase

under Cd stress which suggests that plants withstand the harmful effects, by up-regulating their antioxidant activity. There was 84.0% increase in activity of APX at lower Cd toxicity (5 mg/kg) which further enhanced to 146.6% and 282.4% at 10 mg/kg and 15 mg/kg cadmium. Similarly, in

case of CAT activity was also increased by 3.30%, 70.0% and 121.6% at 5, 10 and 15 Cd toxicity. Under Cd stress, ascorbic acid and POD activities also markedly increased and maximum increased was observed at higher Cd stress (15 mg/kg) and lowest AsA and POD activities was observed under normal conditions. The overall effect of different Cd treatments on antioxidant activities was observed as: 15>10>5>0 mg/kg (Figure 3).

DISCUSSION

Cd showed detrimental effects on both plant growth and physiological processes of mung-bean. The results of this study showed that Cd stress decreased biomass and growth, which could be ascribed to Cd damages to photosynthetic apparatus and plant structure. These findings are in consistent with results of Saidi et al. [2013] they also found significant reduction in plant growth with Cd toxicity. Cd stress also changed the biomass allocation of mung bean plants: the decline in above ground biomass was higher as compared to underground biomass probably due to Cd induced limitation in acquisition of nutrients needed for plants. These findings are same with outcomes of Kaya et al. [2020] they also found that Cd toxicity significantly reduced the above ground biomass as compared to below ground biomass. The toxic effects of Cd intensify the competition among different plant parts for nutrients. Therefore, to obtain more nutrients and continue growing normally, as a result, plants maintain their belowground biomass than their aboveground biomass. Besides this, Cd toxicity also limits as well as inhibit the photosynthesis which reduce the biomass production and consequently reduce the plant photosynthesis [Nie et al., 2023].

Photosynthesis is an important for assimilates production, however, Cd negatively inhibit the photosynthesis [Song et al., 2019]. Chlorophyll is an important photosynthetic pigments that shows plant's ability to for absorption and transfer of light energy. In present study Cd stress significantly decreased the chlorophyll and carotenoid synthesis, however, a linear reduction was seen with increasing the Cd concentration. Cd toxicity disrupt the uptake of nutrients (Mg, Fe, K and P) and it also damage the photosynthetic apparatus thereby resulting in reduction in chlorophyll contents [Dobrikova and Apostolova, 2019]. Besides this, Cd stress also destroy the chlorophyll structure which leads to reduction in photosynthesis

[Dobrikova et al., 2021]. Thus, Cd induced reduction chlorophyll synthesis reduce the plant photosynthetic efficiency and leads to substantial decrease in mung-bean growth which is consistent with study findings of Dobrikova et al. [2021].

Cd stress induced deleterious impacts on plants by disturbing plants physiological and biochemical functions [Wang et al., 2022]. MDA is an important indication of lipid per-oxidation and its synthesis is up-regulated in plants in response to different abiotic stress [Kohli et al., 2019]. Cd stress increased the production of MDA, and H_2O_2 which damage the membranes as evidenced by higher electrolyte leakage. This indicates that Cd stress has a certain impact on peroxidation of membranes and resulting in damage to cellular membranes. However, in response to Cd stress plants activate protective enzymes which reduces the membranes peroxidation therefore, maintains membranes integrity [Cuypers et al., 2023]. APX, CAT, POD and SOD are primary antioxidant enzymes and activity of these enzymes is increased in response to HMs concentration. However, an excessive concentration of HMs destroyed the systems of protective enzymes, which decreased the activity of antioxidant enzymes [Chaâbene et al., 2018]. Furthermore, the complicated process of antioxidant enzyme resistance to heavy metals (HMs) is influenced by HM concentration and plant species [Ozfidan-Konakci et al., 2018]. In present study, activity four antioxidants (APX, CAT, POD and SOD) significantly increased with increasing Cd toxicity (Figure 4). This indicates that these antioxidant enzymes were the main protective enzymes involved in scavenging of ROS. The results also indicated mung bean plants adapt to increase ROS and enhances its Cd tolerances by increasing antioxidant activities (APX, CAT, POD and SOD) under Cd stress, therefore, maintain plant functioning [Liu et al., 2019].

Plants use osmotic adjustment as one of their key defense mechanisms against the harmful effects of Cd. Different osmolytes, such as FAA and TSP are crucial in reducing the toxic impacts HMs [Abd_Allah et al., 2017]. Stress and normal protein production are adversely impacted by cadmium stress. Improved plant resistance to stress conditions can result from soluble protein's ability to raise the quantity of functional proteins necessary to sustain their functioning [Zhang et al., 2018]. TSP and FAA was declined under diverse concentration of Cd stress and linear decrease in TSP and FAA concentration was seen with increasing Cd

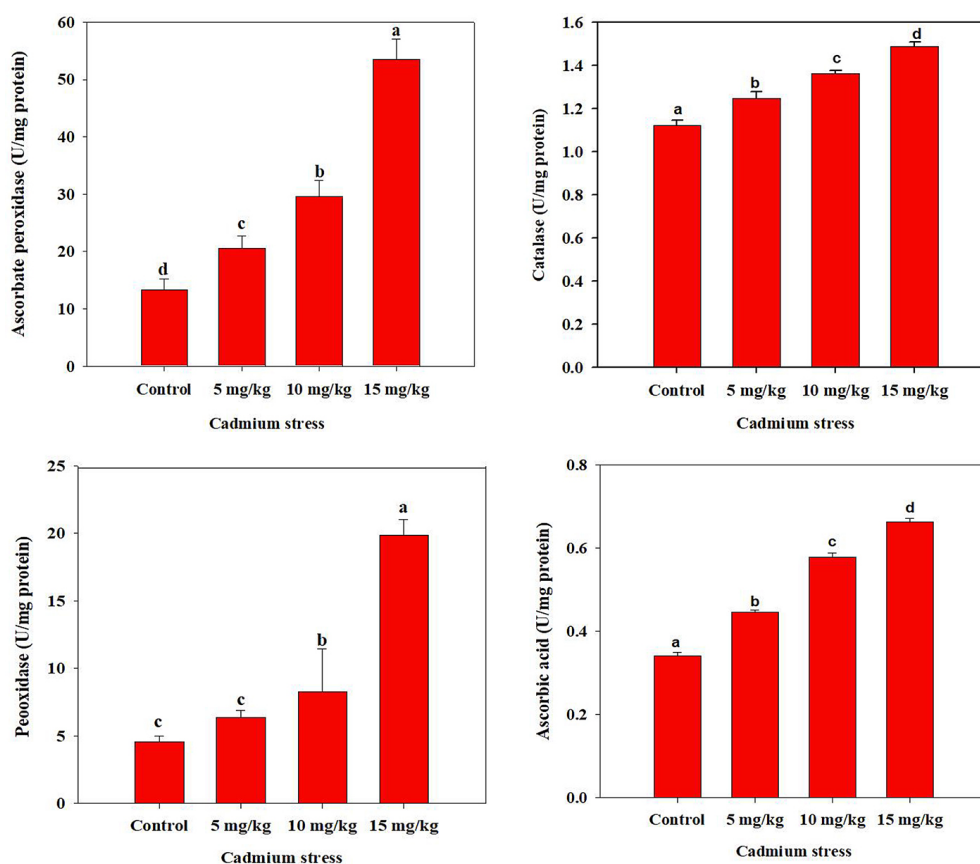


Figure 4. Effect of different concentration of Cd stress APX, CAT, POD and AsA activity of mung bean plants. The values indicating means ($n=4$) with SE and different letter indicating significant differences at $p < 0.05$

concentration in growth medium. This decrease could be attributed to destruction of chloroplasts and subsequently decrease in photosynthesis. This is in consistent with study findings of Ge and Jiao [2012] as they also found a substantial decrease in synthesis of protein under Cd stress. When Cd concentration is low and mild, plants production more antioxidant as well as stress proteins. However, the synthesis of the protein system is harmed by an increase in Cd stress. Furthermore, Cd stress suppresses photosynthesis and lowers the concentration of a protein involved in ATP-dependent activities, which lowers the synthesis of TSP and FAA [Kavian et al., 2023].

CONCLUSION

Cadmium stress negatively affected the growth traits of mung bean it reduced the root and shoot length, their biomass, and leaves per plant. The contents of MDA, H_2O_2 , and EL showed an increasing trend with increase Cd concentration in growth medium resulting in damage to cellular membranes. The contents of chlorophyll and

carotenoid, TSP and FAA declined under Cd toxicity. The activities of all four antioxidants (AsA, APX, CAT, and POD) demonstrated an increasing trend with increasing Cd concentration. These outcomes will contribute to increase the knowledge about growing the mung bean in Cd polluted soils. This will also provide guidelines to growth better and healthier crop production in Cd polluted soils.

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