Research Article

Cadmium (Cd) Induced Morpho-physiological Changes in *Brassica juncea* L. Seedlings

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ABSTRACT

The level of Cadmium (Cd) in agricultural soil and water is increasing due to various sources such as phosphate fertilizers, sewage sludge, mining, etc. Cd is one of the most harmful heavy metals to plants, and it can indirectly lead to cell death by producing reactive oxygen species (ROS). To investigate the effects of various concentrations of Cd (0, 0.1, 0.5, 1.0 mM) on the morpho-physiological and biochemical parameters in Mustard seedlings, a germinating tray experiment was conducted in November. All the parameters were observed in seven-day-old seedlings. The results showed that various concentrations of Cd significantly affected plant growth. There was a higher accumulation of the Cd in roots than in shoots. Cd toxicity in plants also produces effects on chlorophyll biosynthesis, and carotenoid content thereby reducing photosynthesis. The protein content was also affected by Cd. Increasing concentrations of Cd correlated positively with increased antioxidant catalase activity. The highest effect in all the parameters was observed at 1.0mM Cd concentration.

Keywords: Mustard, Heavy metal, Cadmium, Seedlings.

INTRODUCTION

Plants are sessile organisms susceptible to various environmental stresses, such as heavy metals, drought, and salinity stresses, which can ultimately affect crop yield. Heavy metals are a major soil contaminant due to uncontrolled human activities. The increasing release of industrial and vehicular effluents, and improper disposal of wastewater, fertilizers, and pesticides are primarily responsible for soil contamination by heavy metals[1]. Among heavy metals, Cd is a non-essential and most deleterious heavy metal pollutant commonly released into the arable soil from various industrial, mining, and farming practices[2], and has been ranked No. 7 among the top 20 toxins that affect human health by entering the food chain[3,4]. Although Cd is a highly phytotoxic metal, it is easily taken up by plant roots growing on Cd-contaminated soils and transported to above-ground plant parts[5,6,7]. Some cities like Ranipet, Kanpur, and Vadodara in India are polluted by heavy metals[8]. The Hindon River, a tributary of the Yamuna River in India, has been contaminated by HMs. This is due to polluted wastewater from industries in Ghaziabad city that drains into the river without proper treatment. As a result, the contaminated water is used for irrigating nearby agricultural land, which affects the quality of crops such as cereals and vegetables. This issue poses a threat to the health and safety of the people who consume these crops. *Brassica juncea* L. (Indian mustard) is widely used as a model plant for phytoremediation, belongs to the mustard family (Brassicaceae or Cruciferae), and has numerous common names used, e.g., brown mustard, Chinese mustard, or oriental mustard as it has large biomass and can accumulate a maximum of 400 µg/g DW Cd in shoots[9]. *B. juncea* has 10 times higher biomass production than other HM accumulators, a fast growth rate, and accumulates other toxic heavy metals present in the soil; hence it has been considered an ideal system for phytoremediation[10]. Mustard plants have the property of being able to accumulate Cd in their leaves, which in turn causes stunted growth[11]. Green strategies (i.e., phytoremediation) also use mustard plants to bioremediate Cd-contaminated soils. Unfortunately, the green strategy also has some limitations, as most hyperaccumulating plants show growth retardation, low yield, and minimal economic value. We selected One high-yielding medium-maturity hybrid of *Brassica juncea* L. var. pioneer 45s46 for the present experiment. It is a hybrid variety containing bold grain and better oil percentage with 110-125 days life span and is tolerant to some diseases and viruses. Cd toxicity negatively affects hyperaccumulator plants and causes inhibition of growth. Plants growing on Cd-contaminated soil result in Cd accumulation in all plant parts, which inhibits plant growth, affects nutrient uptake, alters the chloroplast ultrastructure, inactivates enzymes of CO_2 fixation, inhibits photosynthesis and induces lipid peroxidation and antioxidant machinery [4,12,13,14]. Cadmium toxicity causes the over-production of reactive oxygen species (ROS) and results in damage to plant membranes and the destruction of cell biomolecules and organelles[15]. Cadmium also reduces plant uptake of Fe and Zn, resulting in leaf chlorosis. Generally, Cd interferes with the transport and uptake of Ca, P, Mg, K, and Mn[16]. Some plant species or cultivars have developed tolerance to Cd. It has been found that cultivars differ in their ability to detoxify Cd in between and within the plant species, which plays a significant role in the expression of high tolerance in crop plants to Cd toxicity[17]. Therefore, smart selection of plant cultivars with the ability to tolerate Cd in the soil could be

the best strategy to counteract the inhibitory effects of Cd in crop plants. The present study investigated the effects of different concentrations of Cd on morpho-physiological and biochemical attributes in the seedling stage of mustard grown in a germinating tray.

MATERIALS AND METHODS

Plant material:

Seeds of Mustard (*Brassica juncea* L.) were collected from the seed section of the Division of Genetics, ICAR-Indian Agricultural Research Institute, (PUSA) New Delhi, India. Seeds of the same size were selected for the present study.

Figure 1: Seeds of *B. juncea* L.

EXPERIMENTAL DESIGN

Seed viability test:

The tetrazolium test was used to determine the viability of the seeds. Mercuric chloride (HgCl2) was used in an aqueous solution of 0.2 percent to sterilize the seeds' surface for 10 minutes. They were then washed with running tap water carefully. After sterilization and washing seeds were immersed in running tap water for 24 hours at 25±2^{0}C. The embryos were collected and placed in $TzCl_2$ for 24 hours in the dark. The embryo that was dyed red or pink was considered viable. The data were typically expressed as a percentage of viability.

Sterilization and sowing of seeds:

Collected seeds of Mustard were surface sterilized with 0.2% mercuric chloride ($HgCl_2$) solution for 10 min, washed with distilled water, and kept for soaking. After soaking for 24 hours, seeds were sown in a germinating tray to evaluate their germination. Using ($CdCl_2$) as the source of Cd. Three treatments of different concentrations (0.1, 0.5, and 1mM Cd) were used to see their germination; tap water was used as the control. Each germinating tray containing 10 seeds was kept in field-like (incubation at 25 ±2 ^{0}C) conditions. Each treatment had three replicates.

Germination assay:

The percentages of seed germination were recorded after 72 h. For germination assay, we mainly considered a seed as a 'germinated seed', where a prominent radical emergence occurred after proper incubation. Until the germination was done, it was monitored every day. The percentage of germination for each seed was presented and was calculated as follows- Germination % = (Total no. of germinated seeds / Total no. of seeds) × 100

PHYSICAL PARAMETERS

Plants growing in different treatment conditions were used to examine physical characteristics such as root length, shoot length, Total length, fresh weight, dry weight, moisture percentage, etc.

Fresh weight, dry weight, and moisture content:

After 7 days of shoot emergence, during the seedlings stage, fresh weight, dry weight, moisture percentage, and content of air-dried seedlings were assessed on a digital weighing balance. Before keeping them in the oven they were placed into little, clearly labeled paper envelopes. The material was dried at 60^0 for 48 hours, then the dried seedlings were weighed. The following formula was used to calculate moisture percentage is shown below-

% Moisture content = Fresh weight – Dry weight/ Fresh weight×100

Vigour -index (V.I.):[26]

To calculate the vigour -index we used the formula given by Abdul Baki and Anderson (1973). Vigour index (V.I.) = Root length + Shoot length × germination %

Germination speed (G.S): [27]

Germination speed was calculated by using the formula given by ISTA (1976).

Germination speed (G.S) = Germination percentage (%) / Day of completion of germination × 100

Tolerance index:

Tolerance index (T.I) = Mean root length of longest root in treated – Mean root length of control *100

Phytotoxicity:

% Phytotoxicity = Root or Shoot length of control- Root or shoot length of treated/Root or shoot length of control *100

Relative growth index:

RGI= Average dry wt. of root or shoot treated/Average dry wt. of root or shoot control*100

BIOCHEMICAL ANALYSIS

We performed biochemical parameters such as Chlorophyll A, Chl B, total Chl carotenoid, protein content, and catalase activity.

Total chlorophyll content[23]:

10 ml of 80% acetone was used to grind fifty milligrams of finely crushed fresh leaves. Then it was centrifuged for 5 minutes at 5000-10,000 rpm. The same process was repeated until the residue was colorless after the supernatant was transferred. The solution absorbance was read compared to a blank (80% acetone) at 645 and 663 nm. The following equation was used to estimate the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll:

Chl. a (mg/g. f. wt.) = 12.7 (A_{663}) – 2.69(A_{645}) × V/ 1000×W

Chl. b (mg/g. f. wt.) = 22.9(A_{645}) – 4.89(A_{663}) × V/ 1000×W

Total Chl. (mg/g. f. wt.) = 20.2 (A_{645}) + 8.02(A_{663}) × V/ 1000×W

Carotenoid content[23]:

10 ml of 80% acetone was used to grind fifty milligrams of finely crushed fresh leaves. Then it was centrifuged for 5 minutes at 5000-10,000 rpm. The same process was repeated until the residue was colorless after the supernatant was transferred. The solution absorbance was read compared to blank (80% acetone) at 480-510.

Determination of total protein[24]:

Coomassie® Brilliant Blue G-250 (100 mg) is dissolved in 50 ml of 95% ethanol to create 1 L of protein reagent. 100 ml of this solution was mixed with (w/v) 85% ortho-phosphoric acid. Then this solution was diluted to make a final volume of 1 L. Protein was extracted by homogenizing the 100 mg fresh plant sample in the chilled 0.05M tris buffer (PH–7.0) (Arulsekar and Parfitt, 1986). Homogenate was transferred to 10 ml centrifuged tubes and centrifuged at 10,000 – 12,000 rpm for 20 minutes at 4^0C. To avoid oxidation sample tubes were kept in ice cubes and closed until centrifugation was done. After that, clear supernatant was used for the protein assay following centrifugation. 1 ml of each sample supernatant and working standard solutions were transferred to test tubes. Then 5ml of the dye containing Bradford was added to each test tube. Then at 595 wavelength absorbance was recorded against a blank solution using a spectrophotometer after 5 minutes and before 1 hour.

Assay of catalase activity (CAT)[25]:

The enzyme was extracted using 250 mg of fresh plant tissue and homogenized in 5 ml of 1M tris buffer (PH-6.8). After that, it was centrifuged at 12,100 rpm for 20 minutes at 4^0C. Then 0.2 ml (200 µl) Clear supernatant was taken and 2.5 ml of 50 mM sodium phosphate buffer (PH-7.0) was added. The addition of 0.3 ml (300 µl) Hydrogen peroxide (H_2O_2) of (3%). Then at 240 nm absorbance was recorded against blank solution spectrophotometrically after 20 seconds to 1 minute. Catalase activity was calculated using a molar absorptivity of 43.6 M cm-1, where one unit is equivalent to the moles of hydrogen peroxide oxidized each minute per mg of protein.

RESULTS

In the present study, Brassica plants were exposed to different concentrations of Cd. Different morpho-physiological and biochemical attributes, including changes in plant growth, plant weight, Chlorophyll content, oxidative stress, antioxidant enzymes, and metabolite activities were deduced. The results are presented below.

Seed germination under the different Cd concentrations:

Exposure to Cd caused a significant reduction in the germination percentage. In the 1mM treatment, seed germination was significantly affected compared to the control (Fig.2) Vigour index was found to decrease with increasing Cd concentration. The highest reduction in vigor index was found in 1.0mM Cd concentration.

Figure 2: (a) Germination after 48 hours (b) 7 Days old seedlings

Relative growth rate index:

The relative growth rate of 7-day-old seedlings was measured and found to be highest in the control. When treated with 0.1mM Cd and 0.5mM Cd concentration, there was only a slight change in RGI. However, a significant reduction in RGI was observed in the 1.0mM Cd concentration. (Figure 3).

Effects of exogenous Cadmium on growth parameters:

Various concentrations of Cd significantly impacted the height of Brassica cultivar plants. Without Cd treatment, the highest plant height (5.1 cm.) is recorded, while the lowest plant height (3.1 cm.) was observed in Brassica under 1.0mM Cd treatment. As Cd concentration increased, plant height decreased, indicating an antagonistic interactive effect of Cd and plant growth. Phytotoxicity was also observed in the root and shoot, with the highest phytotoxicity occurring in both root and shoot under 1.0mM Cd treatment. The root was found to be more sensitive to Cd, as it decreased significantly at 1.0mM Cd concentration. Moreover, Cd accumulation was higher in the root than in the shoot. (Figure 4).

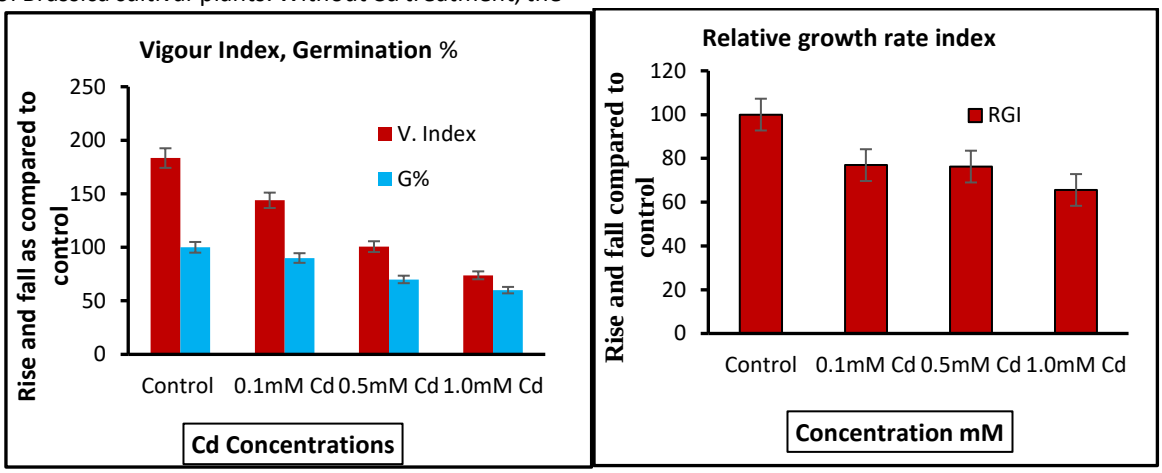
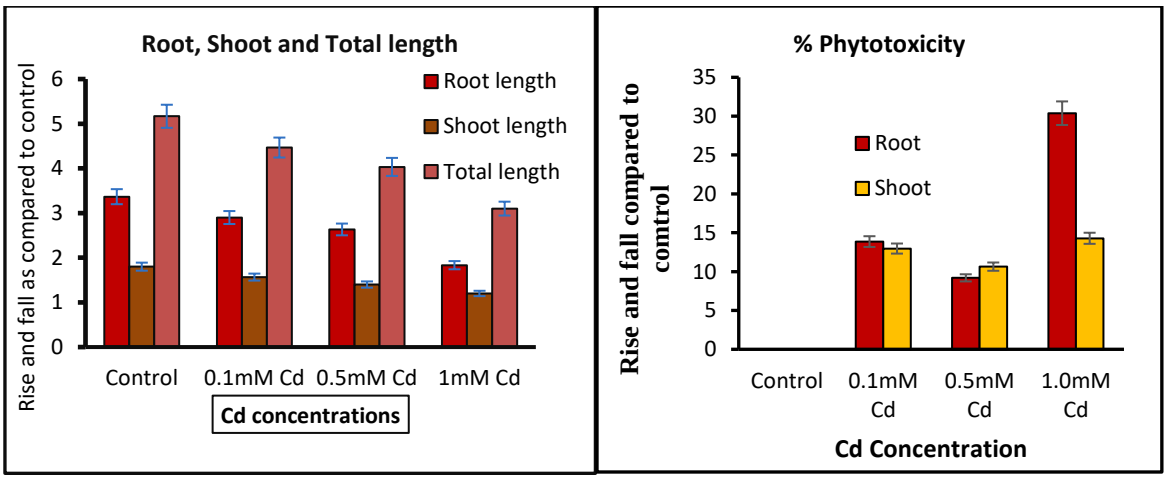


Figure 3: Effect of various concentrations of Cd on Mustard (a) effect on vigor index and germination percentage. (b) effect on relative growth rate Index).



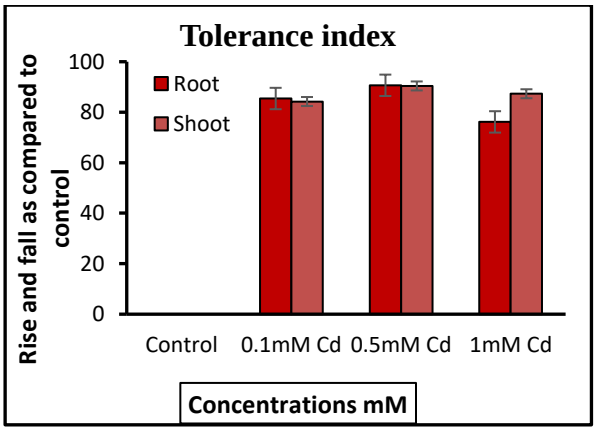


Figure 4: Effect of various concentrations of (Cd). (a) Root shoot length and total length. (b) percentage phytotoxicity in root and shoot. (c) Tolerance index).

Effect on Plant fresh weight and Dry weight:

The presence of cadmium hurt plant biomass, resulting in a decrease in both fresh and dry weight. As the concentration of Cd increased, the fresh weight of plants decreased. The control group, which was not exposed to any cadmium, had the highest fresh weight, while the plants treated with a 1.0 mM Cd concentration had the lowest fresh weight. The effect of Cd on the dry weight of plants was also recorded, which decreased as the Cd concentration increased. Additionally, the moisture content of the plants increased with increasing Cd concentrations. (Figure 5).

Effect on Photosynthetic pigments:

Photosynthetic pigments including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid in leaves of Brassica seedlings were found to decrease with increasing concentration of Cd as compared to control. A higher reduction in these pigments was found when treated with a 1.0 mM concentration of Cd.

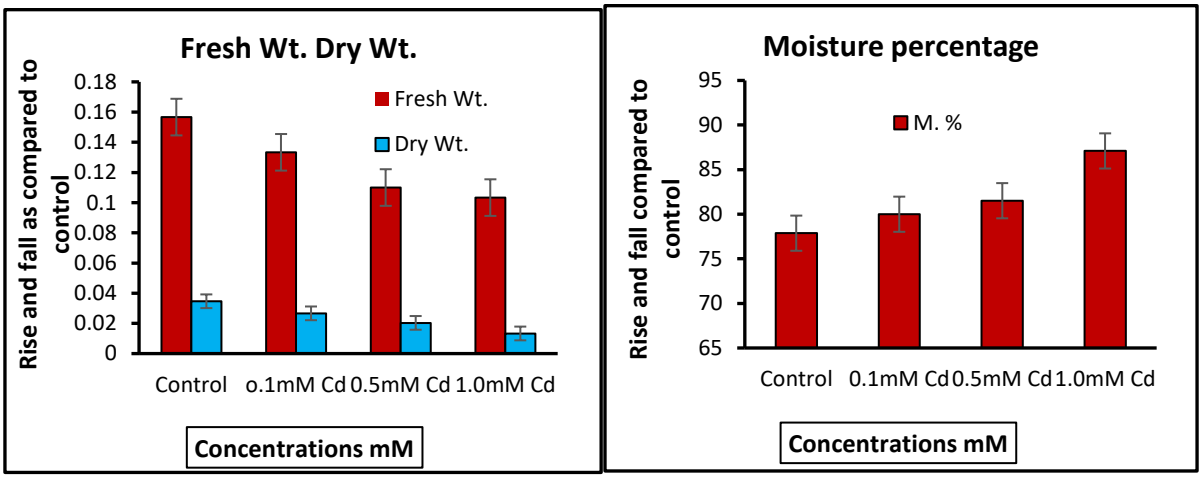


Figure 5: Effect of various concentrations of Cd on Mustard. (a) Effect on plant fresh weight and dry weight (b) effect on moisture percentage).

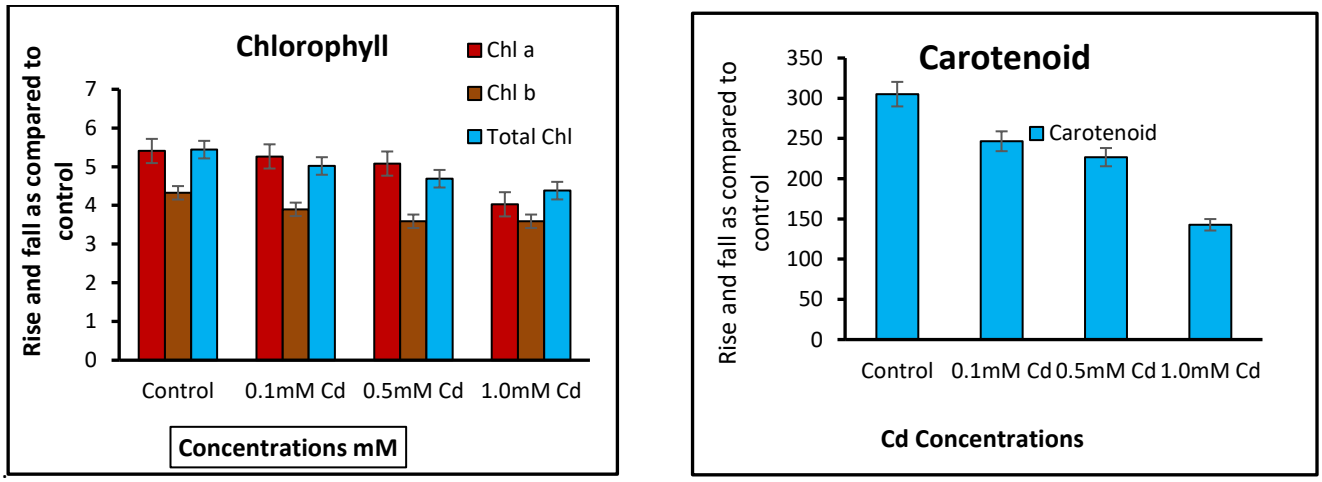


Figure 6: Effect of various concentrations of Cd on Mustard. (a) Effect on Chlorophyll content. (b) Effect on carotenoid content

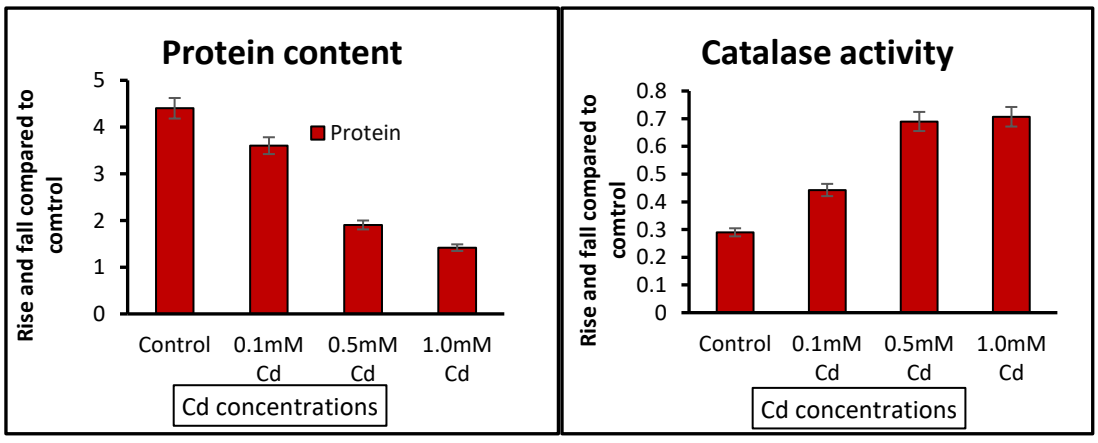


Figure 7: Effect of various concentrations of Cd on Mustard. (a) Effect on protein content. (b) Effect on catalase activity).

Chlorophyll A content was recorded maximum in control plants (5.40 mg g^{-1} FW) whereas 0.1 mM and 0.5 Mm, showed a very slight variation in Chlorophyll A level, where 0.1 mM Cd contained more Chlorophyll a (5.2 mg g^{-1} FW) compared to 0.5 mM Cd contained (5.02 mg g^{-1} FW) Cd treatments caused more changes in Chlorophyll A when treated with 1.0mM concentration which contained (4.02 mg g^{-1} FW), whereas it caused fewer changes in Chlorophyll B content where Chlorophyll B content was found similar (3.58 mg g^{-1} FW) when treated with 0.5mM and 1.0mM Cd. The total Chlorophyll content was recorded as the highest in control as compared to all treated. (Figure 6).

Effect on protein content and Catalase (CAT) activity:

Cadmium exposure to mustard seedlings resulted in a decline in protein content in all treatments, in contrast to the control. Interestingly, the most significant reduction in protein levels was observed when treated with 0.5 mM and 1.0 mM Cd. Additionally, catalase activity was found to increase with the increase in concentration of Cd, indicating that the plant was attempting to mitigate the oxidative stress caused by the heavy metal exposure. (Figure 7).

DISCUSSION

The current study shows that when mustard plants are exposed to high levels of Cd in their growth medium, excessive Cd affects all the growth parameters of mustard plants that were examined, including plant height, root length, and biomass per plant (Fig. 4,5) This growth inhibition seems to be caused by a decrease in the plant's net photosynthesis and water-use efficiency, which were both affected by Cd exposure. In this regard,[18] also observed that growth attributes such as shoot length, root length, leaf area, and plant dry mass were maximally and significantly reduced by 150 mg Cd kg^{-1} soil. The inhibition in growth could be due to a reduction in the rate of cell division and elongation, which is caused by an irreversible inhibition of the proton pump that is responsible for these processes[19]. Similar to our study, Cd application reduced fresh weights of shoots and roots by close to 50%, and dry weights of shoots and roots by about 35% in barley[20]. Biochemical parameters including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid in leaves of brassica seedlings were found to decrease with increasing concentration of $CdCl_2$ as compared to the control (Fig. 6). This can also be attributed to the disruptive action of metals on chlorophyll synthesis as observed by[21], on photosystem efficiency[22], on the activity of photosynthetic enzymes[18]. Our study also found a decrease in protein content upon exposure to various concentrations of Cd (Fig. 7). A decrease in protein content could be due to the inactivation of protein by ROS. It could be due to the degradation of protein by protease. It is also observed by[15], Cadmium-related DNA damage involves the destruction of cell membranes and nucleic acids, photosynthetic protein damage, and decreased synthesis of protein, which influence the growth of the entire Plant.

CONCLUSION

The current study sheds light on how Indian mustard responds differently to cadmium (Cd) stress during the seedling stage when exposed for a short period. Seeds were exposed to a range of Cd concentrations from 0.1mM to 1mM. As Cd concentration in the medium increased, the accumulation of Cd in seeds increased as well, resulting in decreased plant growth such as root shoot, fresh weight and dry weight, chlorophyll content, and protein content. However, catalase activity increased. These changes indicate that Cd is highly toxic to *B. juncea*. The study shows that plant growth inhibition due to Cd exposure interferes with water uptake by seeds and is also disrupted by oxidative stress, which may be interrupted by other physiological and metabolic processes. A reduction in root-shoot length, chlorophyll, protein content, and greater accumulation of antioxidant and other stress-related regulatory proteins in the germination and seedlings of *B. juncea* upon exposure to Cd stress suggest that they might work together to establish a new homeostasis in response to Cd.

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