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Effect of cadmium chloride ($CdCl_2$) on the growth and biochemical content of black gram (*vigna mungo* L.)

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Abstract: Heavy metal of soils due to intensive industrial activities and agricultural development is usually a source of environmental problems. Heavy metal phytotoxicity is considered to be main factor limiting plant growth when cultivate in acid soils. Moreover, these metals have strong impact on human health through the food chain. So the present investigations were carried out to explore the effect of Cadmium Chloride ($CdCl_2$) on the growth of black gram (*Vigna mungo* L.). The experiment was conducted in the laboratory condition. The heavy metal solutions were freshly prepared at the time of experiment in different concentrations *viz.,* (Control, 5, 10, 15, 20 and 25 ppm) of ($CdCl_2$). All results, when compared to control show ($CdCl_2$) adversely affecting the normal growth of plants by reducing the morphological parameters such as germination percentage, root length, shoot length, fresh weight, dry weight, root nodules, total leaf area and number of leaves were analysed in 7th day seedlings. The pigment content like *viz.,* chlorophyll a, b, total chlorophyll, carotinoid, and the biochemical parameters such as protein and sugars of black gram (*Vigna mungo* L.) seedlings was investigated in same day. The ($CdCl_2$) treated plants caused reduction in all parameters of experimental plants. The sugar content increased in the ($CdCl_2$) treated plants when compared to control plants.

Keywords*:* Cadmium Chloride, Black gram, Germination percentage, morphological parameters, pigment content, biochemical analysis

INTRODUCTION

In recent years, heavy metal contamination has become a serious problem in all over the world as these metals persevere in the soil for longer period due to their non biodegradability[1]. Excessive accumulations of heavy metals in agricultural systems through traffic emissions may results in soil contamination and elevated heavy metal uptake by crops and thus affect food quality and safety[2].

A major amount of metals has been on the indict into the environment by anthropogenic actions in exacting by industrial processes like electroplating, chrome plating, leather tanning, textile dyeing, batteries, paints and waste *etc.*[3]. Heavy metal pollution is guilty for many negative consequences both for human health and the environment. During the past decades the annual widespread release of heavy metals[4] reached 22000 t (metric ton) for Cd, 939000 t for Cu, 1350000 t for Zn, and 738000 t for Pb. Soil contamination with toxic metals such as Cd, Pb, Cr, Zn, Ni and Cu, as a result of worldwide industrialization has increased noticeably[5]. The former group includes Mn, Fe, Cd, As, Pb and Hg. The metals most damaging[3] to crops are Cd, Cu, Mo, Ni, Pb and Zn. Cadmium is well known as a highly toxic environmental element due to its vast toxicity and higher mobility from soil to plants and further down to food chain. Although some metals are regarded essential nutrients, excess concentrations of all metals lead to various toxic effects such as oxidative stress and inhibition of enzyme activities[6].

Among the heavy metals can be found usually at trace levels in soil and vegetation and living organisms. However, these have a toxic effect on organisms at high concentrations. Heavy metal affected by plants to change inhibitory effect on plant growth, enzymatic activity, stoma function, photosynthesis activity and accumulation of other nutrient elements and also damages the root system. In addition embarrassment the nature heavy metals cause a severe damage on agricultural production. Contaminating agricultural lands with toxic heavy metals such as Cd cause significant product losses in terms of agricultural plants which have important functions in food chains and endanger human health[7,8]. So the present investigation has been carried out the effect of different concentrations of cadmium chloride on seed germination and biochemical content of blackgram (*Vigna mungo* L.)

MATERIALS AND METHODS

The blackgram (*Vigna mungo* L.) verities IPU-941 seeds were obtained from Agricultural Department (Seeds), Dharmapuri District, Tamil Nadu. The uniform seeds are selected for the experimental purpose. Cadmium chloride stock solution prepared by dissolving the molecular weight of ($CdCl_2$) and different concentrations *viz.*, (Control, 1.0, 2.0, 3.0, 4.0 and 5.0 ppm) of ($CdCl_2$) the solution were prepared freshly at the time of experiments. The plastic cups were filed with 1 Kg of garden soil, selected black gram seeds were sown in the plastic cup and one set of plastic cup irrigated with normal tap water was maintained as the control.

Shoot length and root length: Five plants from each plastic cup were randomly selected for recorded the shoot length and root length of experimental plants. They were measured by using centimetre scale.

Root nodules: Five plants from each plastic cup with intact roots were removed with the help of digging fork. The root nodules were carefully separated from the soil by gently pinching and washing the soil particles. The root nodules were counted and recorded.

Total leaf area[9]

Five plant samples were collected at 7th day sampling plants and the length and breadth of the leaf samples were measured and recorded. The total leaf area was calculated by using the Kemp's constant.

Total leaf area = L × B × K

Where, L - length, B - breadth and K - Kemp's constant (for dicot - 0.66).

Fresh weight and dry weight: Five plant samples were randomly selected at 7th day plants. Their fresh weight was taken by using an electrical single pan balance. The fresh plant materials were kept in a hot air over at 80°C for 24 hr and then their dry weight were also determined.

Biochemical Analyses: The photosynthetic pigments such as chlorophyll a, b, total chlorophyll and carotenoid and the biochemical contents such as protein, and sugars (reducing, non-reducing and total sugars) were analysed in the treatment plants. The test plants were randomly collected at 7th day of plants

Chlorophyll (Arnon, 1949): Five hundred mg of fresh leaf material was ground with a mortar and pestle with 10 mL of 80 per cent acetone. The homogenate was centrifuged at 800 rpm for 15 min. The supernatant was saved and the residue was re-extracted with 10 mL of 80 per cent acetone. The supernatant was saved and the absorbance values were read at 645 and 663 nm in a UV-spectrophotometer. The chlorophyll a, chlorophyll b and total chlorophyll contents were estimated and expressed in mg g-1 fresh weight basis.

Chlorophyll 'a' = (0.0127) × (O.D 663) - (0.00269) × (O.D 645)

Chlorophyll 'b' = (0.0229) × (O.D 645) - (0.00488) × (O.D 663)

Total chlorophyll = (0.0202) × (O.D 645) + (0.00802) × (O.D 663)

Carotenoid[10]: The same plant extract used for chlorophyll estimation was used for carotenoid estimation. The acetone extract was read at 480 nm in a UV-spectrophotometer. The carotenoid content was calculated by using the following formula and it is also expressed in mg g-1 fresh weight basis.

Carotenoid = (O.D 480) – (0.114) × (O.D 663) – (0.638) × (O.D 645)

Estimation of protein[2]

Extraction: Five hundred mg of plant materials (root, stem and leaf) were weighed and macerated in a pestle and mortar with 10 mL of 20 per cent trichloroacetic acid. The homogenate was centrifuged for 15 min at 600 g. The supernatant was discarded. To the pellet, 5 mL of 0.1 N NaOH was added and centrifuged for 5 min. The supernatant was saved and made up to 10 mL of 0.1 N NaOH. This extract was used for protein estimation.

Estimation: One mL of the extract was taken in a 10 mL test tube and 5 mL of reagent 'C' was added. The solution was mixed and kept in darkness for 10 min. Later, 0.5 mL of Folin-phenol reagent was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm in a UV-spectrophotometer.

Preparation of reagents

Reagent A: 0.4 g of sodium hydroxide was dissolved in 100 mL of distilled water. To this solution, 2 g of sodium carbonate was added.

Reagent B: One per cent of copper sulphate was mixed with equal volume of 2 per cent sodium potassium tartarate.

Reagent C: Fifty mL of reagent A and one mL of reagent B were taken and mixed freshly at the time of experiment.

Folin-phenol reagent: One mL of Folin-phenol reagent was diluted with 2 mL of distilled water.

Estimation of sugars[11]

Extraction: Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 mL of 80 per cent ethanol. The homogenate was centrifuged for 10 min at 800 g. The supernatant was saved. Then, the ethanol was evaporated in water both at 50°C. The net content was made up to 20 mL with distilled water and the extract was used for the estimation of reducing sugar.

Estimation: One mL of extract was taken in a 25 mL marked test tube. One mL of reagent 'C' was added. Then, the mixture was heated for 20 min at 100°C in a boiling water bath, cooled and 1 mL of arseno-molybdate reagents was added. The solution was thoroughly mixed and diluted to 25 mL with distilled water. The sample was read at 520 nm in a UV-spectrophotometer.

Preparation of reagents

Reagent A: Twenty five gram of anhydrous sodium carbonate, 25 g of sodium potassium tartarate, 20 g of sodium bicarbonate and 200 g of anhydrous sodium sulphate were dissolved in 800 mL of distilled water and made up to 1000 mL. Then, it was filtered and stored in a glass stoppered brown bottle.

Reagent B: Fifteen per cent copper sulphate containing 1 or 2 drops of concentrated sulphuric acid.

Reagent C: Fifty mL of reagent A and one mL of reagent B were mixed and it was prepared freshly at the time of experiment.

Arsenomolybdate reagent: To 450 mL of distilled water, 25 g of ammonium molybdate, 21 mL of concentrated sulphuric acid were added and 3 g of sodium arsenate was dissolved in 25 mL of distilled water. The mixture was kept in water both at 37°C for 24 to 48 hr. The reagent was stored in a glass stoppered brown bottle.

RESULTS AND DISCUSSION

Hasty growth of urban population and industrialization results in generation of enormous quantities of waste materials (heavy metals or effluents) perennially. Heavy metals are metallic elements which have atomic weight from 63.546 to 200.590 and specific weight gravity higher than 4. The heavy metal stress have an unpleasant effect on growth and development of the plant showing some physiological and biochemical characteristics of damages[12].

The decrease of germination percentage and root length, shoot length, fresh weight, dry weight of black gram seedlings with increase of $CdCl_2$ concentrations are given in (Table 1). The highest seed germination percentage (95.00 %), root length (5.70 cm/seedling), shoot length (13.55 cm/seedling), fresh weight (5.87 g/seedling), dry weight (2.20 g/seedling), were observed in the control treatments seedlings. Similarly, the lower germination percentage (35.00 %), root length, (0.90 cm/seedling) shoot length (2.10 cm/seedling), fresh weight (0.46 g/seedlings), dry weight (0.18 g/seedlings) were observed at 25ppm $CdCl_2$ concentrations. The main reasons maybe cadmium causes reduction in the seed germination root and shoots growth. The quick inhibition of root function was evident in terms of reductions in both ion and water uptake[13]. This might be in total surprising since roots are the first to come in contact with the injurious cadmium. These results are in accordance with reports indicating inhibition of water conductance in roots by toxic metals[14] the other reasons various types of stress, including $CdCl_2$, induce the assimilation of lignin into the cell walls of maize roots with the result that

cell-wall strictness increases and cell wall expansion is reduced[15,16,17]. The fresh weights of shoot as well as root length were used as useful indicators of $CdCl_2$, toxicity in plants. In our study, $CdCl_2$ stress showed a higher decline in these parameters as compared to control.

Table 1: Effect of different concentrations of Cd Cl_2 (ppm) on growth parameters of Black gram on 7^{th} day plants.

$CdCl_2$ Concentration (ppm)	Germination percentage	Shoot length (cm/plant)	Root length (cm/plant)	Fresh weight (mg/g fr. wt.)	Dry weight (mg/g dry wt.)
Control	95.00 ± 4.75	13.55 ± 0.675	5.70 ± 0.285	5.87 ± 0.293	2.20 ± 0.11
5	78.00 ± 3.90	9.13 ± 0.456	4.32 ± 0.216	4.18 ± 0.209	1.80 ± 0.09
10	62.00 ± 3.10	7.10 ± 0.355	3.54 ± 0.177	2.27 ± 0.114	1.02 ± 0.05
15	51.00 ± 2.55	6.36 ± 0.318	3.02 ± 0.151	1.63 ± 0.081	0.70 ± 0.03
20	42.00 ± 2.10	2.82 ± 0.141	1.02 ± 0.051	0.80 ± 0.041	0.49 ± 0.02
25	23.00 ± 1.15	2.10 ± 0.105	0.90 ± 0.045	0.36 ± 0.018	0.15 ± 0.007

± Standard deviation

The pigment content of blackgram seedlings the maximum value of chlorophyll a, b, total chlorophyll and carotinoid content were observed at control plants (Table 2) (2.86, 1.81, 4.67 and 2.03 mg/g fr. wt.) at 7^{th} day seedlings While the minimum values was recorded at 25 ppm (0.29, 0.15, 0.44 and 0.54 mg/g fr. wt.) of $CdCl_2$ concentration. The experiential tendency to decrease in the photo- synthetic rate of cadmium chloride treated of black gram plants related to reduce plastid pigment concentration. Cadmium affects the free photoconvertible reductase complex due to its interference with sulphydryl site on the reductase protein which explains the reducing of pigments concentration[18].

Table 2: Effect of different concentrations of Cd Cl_2 (ppm) on growth parameters and pigment content of Black gram on 7^{th} day plants.

$CdCl_2$ Concentration (ppm)	Root nodules	Total Leaf area (cm^2/leaves)	Chl 'a' (mg/g fr. wt.)	Chl 'b' (mg/g fr. wt.)	Total Chlorophyll
Control	43.30 ± 2.16	6.02 ± 0.301	2.86 ± 0.143	1.81 ± 0.090	4.67± 0.23
5	32.21 ±1.61	5.65 ± 0.282	2.02 ± 0.101	1.28 ± 0.064	3.30± 0.165
10	26.73 ± 1.33	5.02 ± 0.251	1.27 ± 0.063	0.97 ± 0.048	2.24± 0.112
15	19.31 ± 0.968	3.85 ± 0.192	0.91 ± 0.045	0.44 ± 0.022	1.35± 0.067
20	9.38 ± 0.469	2.46 ± 0.123	0.58 ± 0.029	0.38 ± 0.019	0.96± 0.048
25	-	0.52 ± 0.026	0.29 ± 0.014	0.15 ± 0.007	0.44±0.022

± Standard deviation

Heavy metals inhibit metabolic processes by inhibiting the action of enzymes and this may be the most important cause of inhibition[9]. Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis[20]. The heavy metal of cadmium chloride was reported to affect chlorophyll biosynthesis and inhibit the chlorophyll reductase and synthesis [21,22]. Mukherji and Maitra[23] 1976 reported that the loss in chlorophyll content can consequently lead to disruption of photosynthetic machinery and the decline in the levels of these

pigments clearly shown the metal interference with pigment metabolism. The loss in chlorophyll content can consequently lead to disruption of photosynthetic machinery.

The protein, content (0.53) was decreased in 25ppm concentration of Cadmium chloride on the 7th day seedling samples (Table 3). The protein content decreased with high concentration of cadmium chloride. Protein content under $CdCl_2$ influence may be affected due to enhanced protein hydrolysis resulting in decreased the protein content and protein synthesis becoming reduced under metals stress condition[24].The reducing sugar, non-reducing sugar and total sugar were increased in increasing the $CdCl_2$ concentration (Table 3) when compared to control plants. The sugar content however, higher concentrations of 25 ppm $CdCl_2$ showed an increase the sugar content at 7th day seedlings respectively. The increase in sugar content in the black gram seedlings under different treatment of $CdCl_2$ solutions might be to overcome the Cd stress on plants by increasing carbohydrate synthesis.

Table 3: Effect of different concentrations of Cd Cl_2 (ppm) on biochemical content of Black gram on 7th day plants.

$CdCl_2$ Concentration (ppm)	Carotinoid (mg/g fr. wt.)	Protein (mg/g fr. wt.)	Reducing Sugar (mg/g fr. wt.)	Non reducing sugar (mg/g fr. wt.)	Total sugar (mg/g fr. wt.)
Control	2.03 ± 0.101	2.91 ± 0.145	0.24 ± 0.012	0.19 ± 0.009	0.43± 0.021
5	1.86 ±0.093	2.34 ± 0.117	0.37 ± 0.018	0.24 ± 0.012	0.61± 0.030
10	1.50 ± 0.075	1.63 ± 0.081	0.56 ± 0.028	0.38 ± 0.019	0.94± 0.047
15	0.91 ± 0.045	1.08 ± 0.054	0.72 ± 0.036	0.63 ± 0.031	1.35± 0.067
20	0.66 ± 0.033	0.92 ± 0.046	0.93 ± 0.046	0.86 ± 0.043	1.79± 0.089
25	0.54 ± 0.027	0.53 ± 0.026	1.51 ± 0.075	1.20 ± 0.06	2.71± 0.135

± Standard deviation

It conclusion the Cd is one of the most highly discrete metals by anthropogenic behavior. The agricultural soils are polluted by fertilizer impurities (Cd_2^+), use of refuge derived compost and sewage sludge. Cadmium is easily taken up by plants because geochemically, it is quite mobile element in water and soil ecosystems. Cadmium has a shocking reputation for living being highly toxic and threatening to plant growth as well as all ecosystems.

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