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Toxic effect of cadmium on seed germination, growth and biochemical contents of cowpea (*Vigna unguiculata* l.) plants

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Abstract:
Treatment of cowpea (*Vigna unguiculata* L.) with different concentrations of cadmium decreased the germination percentage, growth parameters and biochemical contents. The experiment was conducted at Botanical Garden, Department of Botany, Government Arts College, Thiruvannamalai, Tamil Nadu, during the period of January to March 2011. In the pot culture experiment, cowpea plants were analysed on three different sampling (viz., 15th, 30th and 45th) days, in soil amended with various levels of cadmium (viz, 10, 30, and 50 mg kg^{-1}). The inner surfaces of pots were lined with a polythene sheet. Each pot containing 3kg of air dried soil. Six seeds were sown in each pot. All pots were watered to field capacity daily. Plants were thinned to a maximum of three per pots, after a week of germination. Cadmium at all levels (10,30 and 50mg kg^{-1}) tested, decreased the growth parameters such as, root and shoot length, fresh and dry weight of root and shoot, total leaf area and biochemical constituents such as, chlorophyll-*a*, chlorophyll-*b*, total chlorophyll and carotenoid contents of cowpea plants compared to untreated plants.

Keywords: Cadmium, Seed Germination, Growth, Biochemical Contents, Cowpea

INTRODUCTION

The problem of heavy metal pollution is increasing throughout the world. Their presence in the atmosphere, soil and water can cause serious problems to all organisms (6). Heavy metals bio-accumulation in plants can be highly dangerous (22), since plant is a member of the food chain and may create a risk for man and animals, through the contamination of their food supplies (7). Cowpea (*Vigna unguiculata* L.) is a staple food for the majority of world population (8). Germination and early seedling growth have been regarded as critical phases, which are greatly influenced by stressful conditions (24). Growth changes are the first most obvious reactions of plants under stress. Heavy metals uptake and accumulation in plants have been shown to result in negative effects on plant growth (4). Stunted growth, chlorosis and necrosis, leaf epinasty and red-brown discoloration are visible symptoms of severe metal phytotoxicity (28). In addition, the effect of heavy metals on growth depends on the type of metal ion, plant species and growth stage at which the metal is applied (25). The present investigations extent of changes in growth parameters such as, root and shoot length, fresh and dry weight of root and shoot and total leaf area, and biochemical constituents such as, chlorophyll a chlorophyll b total chlorophyll and carotinoids contents in cowpea plants due to cadmium toxicity.

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MATERIALS AND METHODS

Seed materials

The certified seeds of *Vigna unguiculata* (L.) were purchased from Tamil Nadu Agricultural University, Agricultural Research Station, Paramakudi, Ramanathapuram district. Seeds with uniform size, colour and weight were chosen for the experimental purpose.

Seed Germination

The cowpea (*Vigna unguiculata* L.) seeds were purchased from Tamil Nadu Agricultural University, Agricultural Research Station, Paramakudi. Twenty healthy seeds were surface sterilized with O.1% mercuric chloride solution (21), and were spread uniformly in Petri dishes lined with filter paper. The Petri dishes were treated with an equal volume of the different concentrations of $CdCl_2$ solutions (0, 10, 30 and 50 ppm). The seeds were allowed to germinate in the dark in an incubator at 25°C for 5 days. Percentage (%) germination was recorded when the radicle reached 2 mm in length. The germinated seeds were counted and removed every day until the end of the test period. Five replicates were used for each treatment.

Experimental soil

The soil used in the experiment was sandy loam in nature and the pH of the soil was 7.2. It contains 126 kg available N, 76 kg available P and 98 kg available K/ha, and micro nutrients of 18.32mg available Cu, 190.28mg Fe, 172mg Mn and 20.44mg Zn/kg, cadmium was not available in this experimental soil. The cadmium chloride ($Cd\ Cl_2$ ½ H_2O) was used as cadmium source.

Pot culture experiment

The pot culture experiment was conducted at Botanical Garden, Department of Botany, Government Arts College, Thiruvannamalai, Tamil Nadu, during the period of January to March-2011. Surface sterilized cowpea seeds were sown in pots (15 cm in diameter) containing mixture of sandy loam soil in nature, cowpea plants were grown in pots containing untreated soil (Control) and soil mixed with various levels of cadmium (viz., 10, 30 and 50 mg kg^{-1}). The inner surfaces of pots were lined with a polythene sheet. Each pot contained 3kg of air dried soil. Six seeds were sown in each pot. All pots were watered to field capacity daily. Plants were thinned to a maximum of three per pots, after a week of germination. Each treatment including the control was replicated five times. Data points in the tables and figures represent the means, with all deviation bars shown (±1 standard deviations of mean). Both the mean and standard deviation were performed where appropriate using the statistical package on Microsoft_ Excel Version-2007.

Growth Measurements

The plant samples were collected on 15th 30th and 45th days after sowing. Three plants from each replicates of pot were analyzed for the various growth parameters. The following growth parameters: length and fresh weight of shoot and root were measured using standardized procedures; dry weight was determined after drying of plant material in an oven 70^0C, and total leaf area was calculated by measuring the length and width and multiplied by a correlation factor (0.69), derived from the method of (14).

Biochemical estimations

Leaves as treated and control plants were used for the estimation of chlorophyll-a, chlorophyll-b and total chlorophyll as per (1), Carotenoids as per (15) method.

RESULTS AND DISCUSSION

Physio-chemical properties of the soil

The pot culture experiments were conducted in Botaniocal Garden, Department of Botany, Government Arts College, Thiruvannamalai. The soil condition was sandy loam in nature and pH, EC, organic carbon and available macro and micro nutrients are given in table -1.

Table-1 Physio-chemical properties of the experimental soil

Soil type	pH	EC	Moisture content	Organic carbon	Available(kg/h^{-1})			DTPA-TEA extractable (mg kg^{-1})				
					N	P	K	Cu	Fe	Mn	Zn	Cd
Sandy loam	7.2	0.4	22.10	0.58	126	76	96	18.32	190.28	172	20.44	-

Seed Germination

The results of the present study revealed that cadmium adversely influenced the germination process of cowpea seeds (fig-1). These observations are in accordance with the findings of (13) who determined the inhibitory effects of some heavy metals ($CdCl_2$, $CuS0_4$, $PbCl_2$, $HgCl_2$) on Miscanthus species. During this study, the decrease in the germination percentage of cowpea seeds may be related to the negative effects of cadmium on water uptake and water movement (19). In addition, Barcelo, *et al.* (3) indicated that cadmium affected water relations not only by decreasing water absorption and transport, but also by lowering water stress tolerance. Hence, the higher cadmium concentration in the germination medium of cowpea seeds seems to reduce the availability of water in the embryo axis, and this may be the reason for the low seedling establishment.

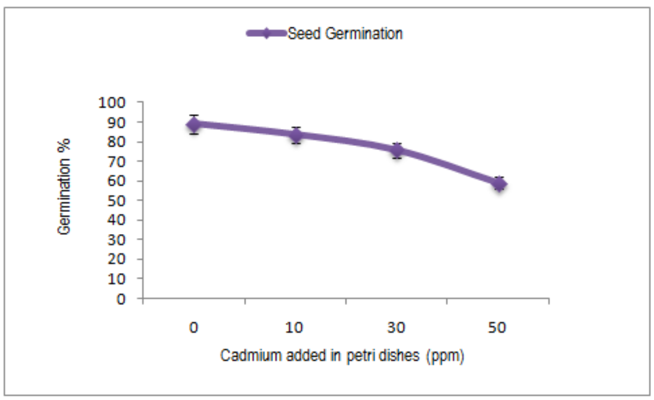
Growth

The root and shoot length and elongation rate are essential for plants exploring for water and mineral nutrients. The length, fresh and dry weight of root and shoot of cowpea plants has been adversely affected due to cadmium treatment, when compared to the control (fig-2-10). There was a gradual decrease in the root and shoot length and fresh and dry weight with an increase in cadmium level 10, 30 and 50mg kg^{-1} in the soil in all the sampling days. These results are also in consonance with the observations of (5) in soybean, (20) in *Phyllanthus amarus* and (29) in garlic. The inhibitory action of excess of cadmium in root and shoot length might be due to reduction in cell division, toxic effect of heavy metals on photosynthesis, respiration and protein synthesis. These obviously contributed to the retardation of normal growth (16). Hagemeyer *et al.* (12) and Marcnano *et al.* (17) also suggested that the morphological and structural effects caused by metal toxicity in plants was due to decrease in root elongation, root tip damage, decrease in root formation, suppression of elongation growth rate of cells, affecting the ultracellular structure of meristematic cells and inhibition of the size of plant cells and inter cellular spaces. Taken up in excess by plants, this non-essential element directly or indirectly inhibits physiological processes such as respiration, photosynthesis, plant–water relationships, loss of cellular turgor, inhibiting the activity of the cell and its enlargement, nitrogen metabolism and mineral nutrition, resulting in poor growth and low biomass (2, 11, 22).

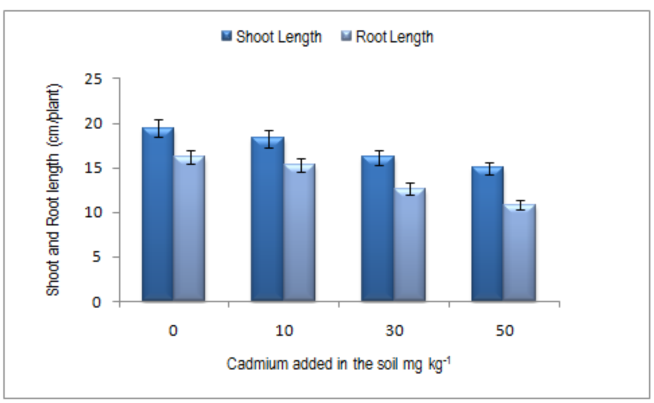
The total leaf area of cowpea was maximum at control plants, in all the sampling days. They are decreased with increase in cadmium level in the soil (fig-11). Similar observations were made by (23) in *Populus canescens*, (30) in *Sedum alfredii*. Our results are in agreement with the findings of (9, 10, 26) also reported that the decrease in leaf area of plant at higher concentrations of cadmium may be due to decreased activities of many enzymes involved in the fixation of CO_2, changes in the thylakoid organization, reduction of chlorophyll contents and inhibition of photosynthesis activities and disturbing the interaction of chlorophylls molecules into the stable complex.

Biochemical

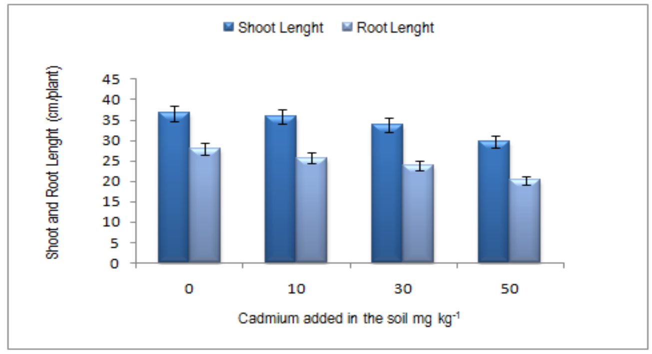
Results on the effect of cadmium on photosynthetic pigments of leaves of cowpea plants were recorded on 15th 30th and 45th days are presented in (fig-12-14). The photosynthetic pigments such as chlorophyll-*a*, chlorophyll-*b*, total chlorophyll and carotinoid content of cowpea leaves decreased with increase in cadmium level (10, 30 and 50mg kg^{-1}) in the soil, in all the sampling days. Higher the cadmium contents lesser the values. The above results were in agreement with the findings of (18) also suggested that the cadmium caused significant reduction in chlorophyll contents in pea which could be due to inhibition of chlorophyll biosynthesis by inhibiting δ-aminolevulinic acid dehydrogenase and breakdown of pigments or their precursors. The decline in the levels of chlorophyll and carotenoids may be due to the inhibition of cadmium at the protochlorophyllide stage interferes with the enzyme protechlorophyllide reductase in barley leaves observed by (27).



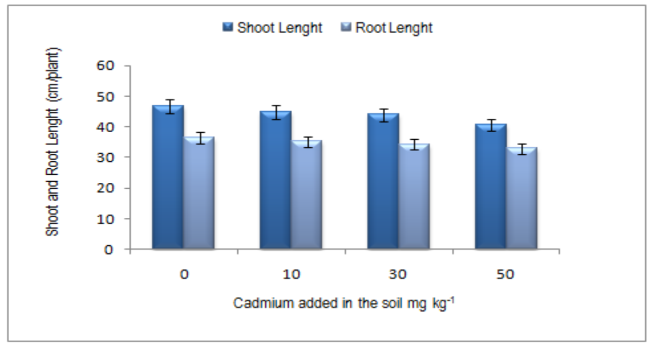
Values are means ± Standard deviations
Fig-1 Effect of cadmium on seed germination of cowpea on 5th Days



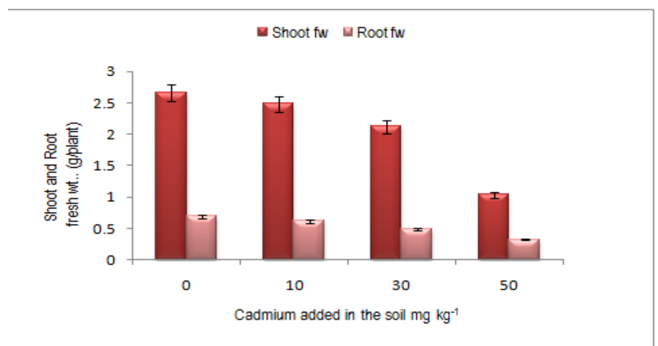
Values are means ± Standard deviations
Fig-2 Effect of cadmium on shoots and root length of cowpea on 15th Days



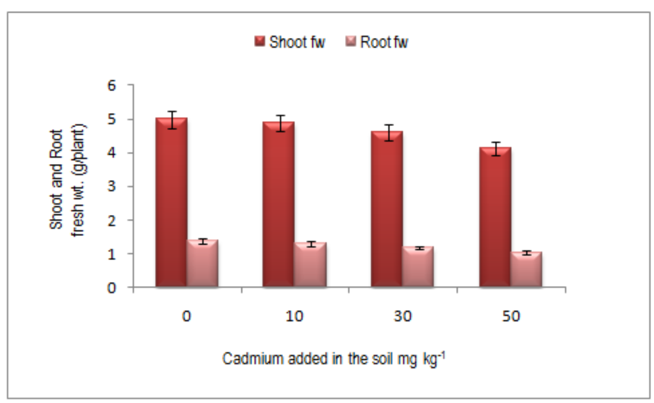
Values are means ± Standard deviations
Fig-3 Effect of cadmium on shoot and root length of cowpea on 30th Days.



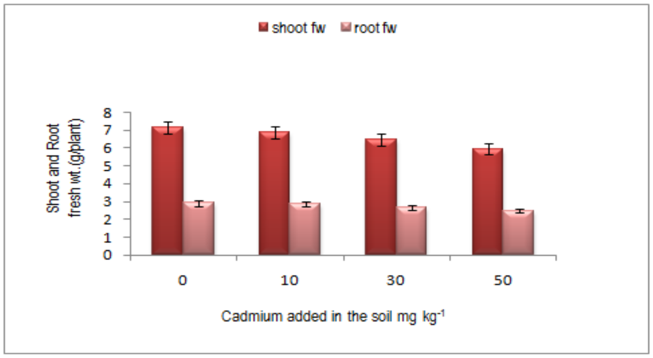
Values are means ± Standard deviations
Fig-4 Effect of cadmium on shoot and root length of cowpea on 45th Days.



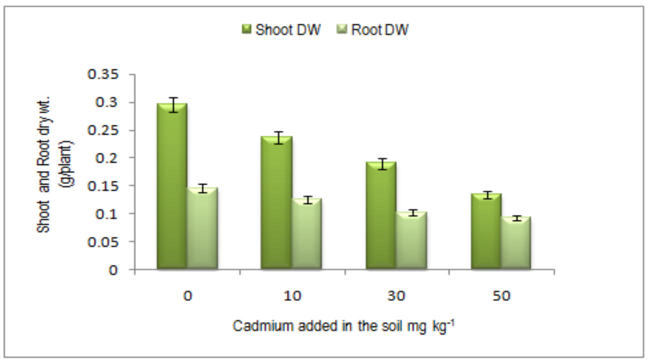
Values are means ± Standard deviations
Fig-5 Effect of cadmium on fresh weight of cowpea on 15th Days.



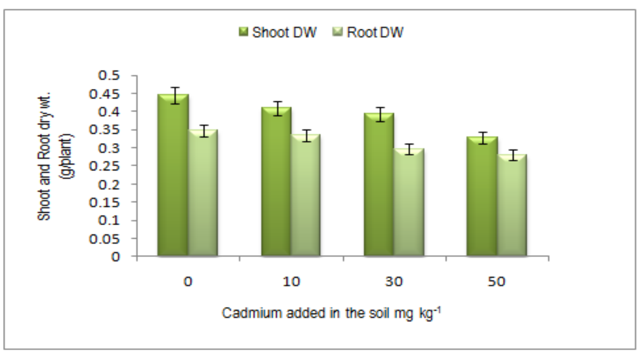
Values are means ± Standard deviations
Fig-6 Effect of cadmium on fresh weight of cowpea on 30th Days



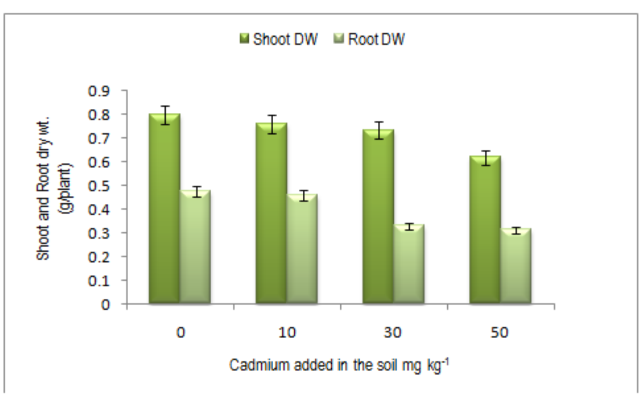
Values are means ± Standard deviations
Fig-7 Effect of cadmium on fresh weight of cowpea on 45th Days.



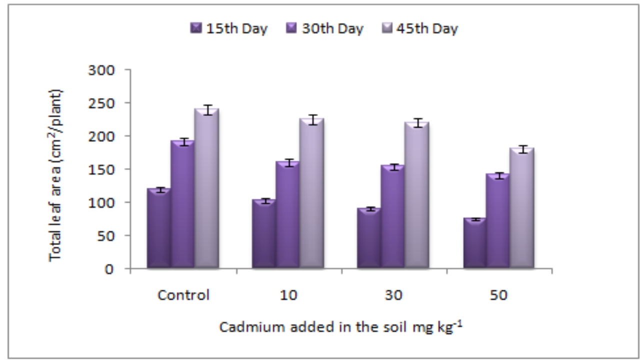
Values are means ± Standard deviations
Fig-8 Effect of cadmium on dry weight of cowpea on 15th Days.



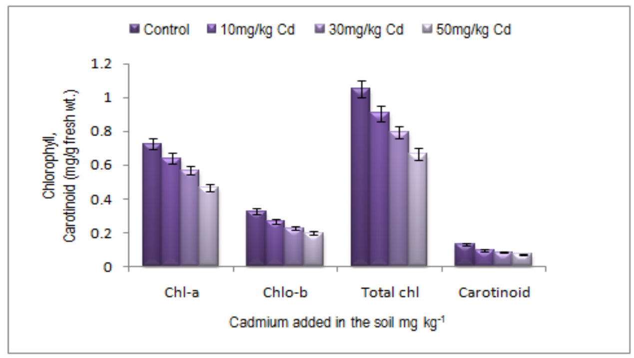
Values are means ± Standard deviations
Figure-9 Effect of cadmium on dry weight of cowpea on 30th Days.



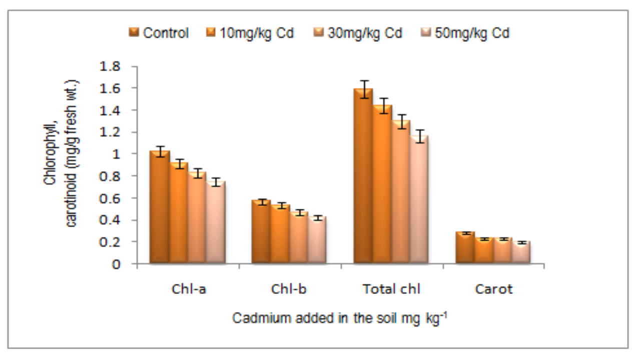
Values are means ± Standard deviations
Fig-10 Effect of cadmium on dry weight of cowpea on 45th Days.



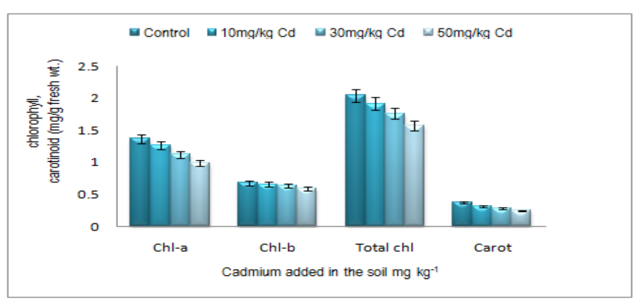
Values are means ± Standard deviations
Figure-11 Effect of cadmium on leaf area of cowpea.



Values are means ± Standard deviations
Fig-12 Effect of cadmium on photosynthetic pigments of cowpea on 15th Days.



Values are means ± Standard deviation
Fig-13 Effect of cadmium on photosynthetic pigments of cowpea on 30th Days.



Values are means ± Standard deviations

Fig-14 Effect of cadmium on photosynthetic pigments of cowpea on 45th Days.

CONCLUSION

The results of the present study have shown that cadmium treatment was inhibitory to seed germination, plant growth and biochemical constituents of cowpea plants, when compared to control plants. The loss of these may be due to inhibition of cell division, impairment of PSII activity, directly or indirectly inhibits physiological processes such as respiration, photosynthesis, plant–water relationships, loss of cellular turgor, inhibiting the activity of the cell and its enlargement, changes in the thylakoid organization, resulting in poor growth and low biomass. The decreased chlorophyll contents of cowpea might be due to the active involvement of cadmium in iron uptake and chlorophyll biosynthesis. So there was a consequents reduction in the growth of root and shoot length, fresh and dry weight, leaf area, chlorophyll and carotinoids of plants. The shoot length of cadmium treated cowpea plants was higher than the root length.

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