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A Physiological Approach to Study the Interaction of Cadmium and Zinc in Groundnut (*Arachis hypogaea* L.) Seedlings

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ABSTRACT

Background: Heavy metal toxicity affects plant growth and alters physiological processes. Soils in many areas are often contaminated by cadmium and zinc which show varied response on plants by their interactive effects. The experiment was done to study the effect of cadmium and zinc as sole presence and in combination in groundnut seedlings.

Methods: The laboratory experiment was conducted on groundnut cultivar TG 51 in sand culture using modified Hoagland solution. After initial screening, three concentrations of cadmium (Cd 100, Cd 300 and Cd 500 µM) and two concentrations of zinc (Zn 50 and Zn 150 µM) were selected for studying their effects individually and in combination on physiological and biochemical parameters.

Result: The reduction in root length increased over control as the concentration of cadmium in the medium increased. Cadmium or zinc alone led to a decrease in chlorophyll a, b and relative water content of the leaf. Zinc supplement at 150 µM not only mitigated the negative effect of Cd 100 µM and 300 µM, but also increased the chlorophyll content above control level. Zinc supplement not only increased the protein content over the control but also mitigated to some extent the adverse effects of cadmium in protein content when applied in combination. Under both cadmium and zinc treatment, the inhibition of nitrate reductase (NR) activity over unstressed control was found. Different treatment combinations, however, reduced the negative effects of cadmium, although zinc could not completely override such damage, change the level of toxicity. Treatment with Cd 100 µM and 300 µM induced an increase in phenol content over the control, while higher concentration (500 µM) of the metal led to a decrease in this potent antioxidant compound. Presence of Zinc in the growing medium significantly enhanced the accumulation of phenolic compounds highlighting its protective role against oxidative damage.

Key words: Cadmium, Chlorophyll, Groundnut, Nitrate reductase, Phenol.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) accounts for approximately 50% of the total oilseed production in India (Mulgir *et al.,* 2014). The 70% of area and 75% of groundnut production has been concentrated in Gujrat, Andhra Pradesh, Tamil Nadu and Orissa (Madhusudhana, 2013). Bianucci *et al*. (2012) reported considerable metal tolerance in groundnut. Cadmium is a group IIB transition element and also a non-essential heavy metal. Globally, cadmium content in soil is about 0.01-2 mg Kg^{-1}, with an average of 0.35 mg Kg^{-1} of soil (Kebata-Pendias and Pendias, 2001). Cadmium toxicity may interfere in many processes, such as carbohydrate metabolism (Sanita di Toppi and Gabrielli, 1999), nitrate absorption and reduction (Hernandez *et al.,* 1996), the uptake and distribution of macro and micro-nutrients (Van Assche and Clijsters, 1990) and photosynthesis (Pietrini *et al.,* 2003). Zinc is an essential micronutrient and a member of group IIB transition element. It is an important component of many vital enzymes like Cu/Zn-SOD and structural stabilizer for many proteins, membrane and DNA-binding proteins (Vallee and Falchuk, 1993). However, like any other element, Zn at high concentration is toxic to the plant and inhibits several metabolic processes. Wallace (1982) noted that more than one metal in metalliferous environments in potentially toxic concentrations may have synergistic, additive or antagonistic effects on plants.

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Cadmium and zinc have similar valance state and electronic configuration, cadmium is non-essential, while zinc is an essential heavy metal for plant growth (Narwal *et al*., 1993). Sometimes this two metals are showing antagonistic interactions (Balen *et al*., 2011), while in some other cases synergistic interactions have been noted (Nan *et al*., 2002). A number of research works have been conducted in groundnut on the effect of cadmium stress (Gao *et al*., 2012; Nagaraju *et al*., 2015) and the effect of application of zinc (Saha *et al*., 2015; Rahevar *et al*., 2015;

Nadaf and Chidanandappa, 2015). But the information on the interaction of cadmium and zinc in groundnut is still lacking. In the present study, the effect of Cd-Zn interaction on growth and different physiological and biochemical parameters in groundnut seedlings was investigated.

MATERIALS AND METHODS

Plant culture

The experiment was conducted with TG-51 genotype of groundnut (*Arachis hypogaea* L.) and seeds were collected from AICRP on groundnut, Kalyani in the year 2017. The entire experiment was done in research laboratory of Department of Plant Physiology, Bidhan Chandra Krishi Viswavidyalaya. Cadmium was supplemented in the form of $CdCl_2.H_2O$, while zinc was applied in the form of $ZnCl_2$. On the basis of results obtained from a preliminary study on growth of embryonic axis and cotyledon biomass using different concentrations of cadmium and zinc, three concentrations of cadmium (Cd 100, Cd 300 and Cd 500 µM) and two concentrations of zinc (Zn 50 and Zn 150 µM) were chosen for further studies. All the possible combinations of cadmium and zinc concentrations (total six) were used to study their interaction effect on groundnut.

The seeds were surface sterilized with 0.1% $HgCl_2$ (w/v) solution for two minutes then washed with distilled water. In each petri dish twenty seeds were arranged on Whatman No.1 filter paper, moistened with solutions of different cadmium and zinc concentrations and allowed to germinate at 28±1°C temperature and around 80±1% relative humidity. After 72 hours of germination, the observations of fresh and dry weight of cotyledon and embryonic axis were recorded.

The selected concentrations and their every possible combinations were used for further study. After 72 hours, the germinated seeds were transferred to plastic beakers containing neutral sand. Five seedlings were raised in each beaker. Full strength of Hoagland solutions were prepared as per Epstein (1972) containing the metals and their combinations and were applied as the nutrient supplement after transplanting. The nutrient solutions were repeated on every 4th day. A control set was also grown without containing any of the two metals in the Hoagland solution. The seedlings were raised under laboratory condition of diffused light. All the growth and physiological parameters were recorded on 21-day old seedlings.

Estimation of biochemical and physiological parameters

Chlorophyll a and b content in the leaf sample was estimated as per the method of Arnon (1949). These following formulae were used to calculate the chlorophyll content.

Milligram of chlorophyll a per gram of fresh tissue =

$$= [(12.7 \times A_{663}) - (2.69 \times A_{645})] \times \frac{V}{1000 \times W}$$

Milligram of chlorophyll b per gram fresh tissue =

$$= [(22.9 \times A_{645}) - (4.68 \times A_{663})] \times \frac{V}{1000 \times W}$$

Where,

V = Volume of the extract (mL).

W = Fresh weight of tissue (g).

A = Absorbance.

Relative leaf water content (RLWC) was estimated as per Perez *et al.* (2002). It was expressed as follows:

$$RLWC\ (\%) = \frac{FW - DW}{TW - DW} \times 100$$

Where,

FW= Fresh weight of tissue (g).

TW= Turgid weight of tissue (g).

DW= Dry weight of tissue (g).

The soluble protein content in the leaf of the seedlings was estimated following the method of Lowry *et al*. (1951). BSA standard curve was used to calculate the protein content.

Extraction of nitrate reductase and the estimation of enzyme activities were done as per the method of Jaworski (1971).

Total phenol content in the leaves of seedlings was estimated following the method of McDonald *et al.* (2001). Total phenol content was expressed in terms of gallic acid equivalent.

Statistical design and analysis

The experiment was conducted in completely randomized design (CRD) with three replications. All the mean data were statistically analyzed by INDOSTAT version 7.1 software.

RESULTS AND DISCUSSION

The data were recorded (Table 1, Plate I) on fresh and dry weight of cotyledon and embryonic axis of seeds after 72 hours of germination and analysis of variance indicated highly significant ($P \leq 0.05$) variation among all the treatments in respect of all the parameters studied. Perusal of means revealed that the fresh weight of both cotyledon and embryonic axis decreased concurrently along with the increase in concentration of the metals in germinating nutrient medium. The range of decrease in case of fresh weight of cotyledon varied from 5.41% at 100 µM to 61.63% at 1000 µM Cd and from 8.88% at 50 µM to 35.78% at 300 µM Zn over control. The corresponding ranges for fresh weight reduction of embryonic axis were from 3.81% at 100 µM to 74.03% at 1000 µM Cd and from 7.37% at 50 µM to 76.70% at 300 µM Zn over control. The dry weight of cotyledon and embryonic axis also followed the same pattern. The ranges of dry weight reduction varied from 3.48% at 100 µM of cadmium to 47.26% at 1000 µM of cadmium, while the corresponding values for zinc varied from 0.71% at 50 µM of zinc to 47.26% at 300 µM. Dry weight of embryonic axis also reduced under the metal treatments having the range from 7.12% at 100 µM of cadmium to 47.46% at 1000 µM and from 3.05% at 50 µM of zinc to 60.68% at 300 µM of zinc. Such inhibitory effects of cadmium and zinc on growth of embryonic axis might be a consequence of disarray in mitotic cycle as well as

Table 1: Effect of different concentrations of cadmium and zinc on fresh weight and dry weight of cotyledon and embryonic axis of germinating seed of groundnut cv. TG-51.

Treatments	Fresh weight (g/seed)		Dry weight (g/seed)	
	Cotyledon	Embryo	Cotyledon	Embryo
Control	0.795	0.383	0.448	0.059
Cd 100 µM	0.752 (-5.41)	0.368 (-3.81)	0.433 (-3.48)	0.055 (7.12)
Cd 200 µM	0.729 (-8.37)	0.331 (-13.58)	0.401 (-10.58)	0.053 (-10.85)
Cd 300 µM	0.705 (-11.34)	0.256 (-33.23)	0.397 (-11.51)	0.051 (-13.90)
Cd 400 µM	0.690 (-13.23)	0.231 (-39.76)	0.394 (-12.00)	0.050 (-15.25)
Cd 500 µM	0.669 (-15.89)	0.212 (-44.72)	0.375(-16.38)	0.049 (-16.27)
Cd 600 µM	0.627 (-21.15)	0.205 (-46.45)	0.353 (-21.24)	0.047 (-19.66)
Cd 700 µM	0.621 (-21.98)	0.188 (-50.78)	0.333 (-25.79)	0.045 (-23.05)
Cd 800 µM	0.416 (-47.65)	0.149 (-61.08)	0.318 (-28.96)	0.039 (-33.90)
Cd 900 µM	0.405 (-49.08)	0.135 (-64.79)	0.293 (-34.63)	0.037 (-37.29)
Cd 1000 µM	0.305 (-61.63)	0.099 (-74.03)	0.236 (-47.26)	0.031 (-47.29)
Zn 50 µM	0.725 (-8.88)	0.355 (-7.37)	0.445 (-0.71)	0.057 (-3.05)
Zn 100 µM	0.705 (-11.34)	0.228 (-40.54)	0.436 (-2.63)	0.045 (-23.05)
Zn 150 µM	0.672 (-15.49)	0.173 (54.70)	0.369 (-17.63)	0.037 (-36.95)
Zn 200 µM	0.614 (-22.76)	0.139 (-63.79)	0.300 (-32.98)	0.031 (-47.46)
Zn 250 µM	0.561 (-29.52)	0.097 (-74.71)	0.268 (-40.12)	0.026 (-56.27)
Zn 300 µM	0.511 (-35.78)	0.089 (-76.70)	0.236 (-47.26)	0.023 (-60.68)
CD (P = 0.05)	0.024	0.009	0.014	0.002

Data in parentheses indicate percentage increase (+) or decrease (-) over control.

Table 2: Effect of cadmium-zinc interactions on root length of groundnut cv. TG-51.

Treatments	Root length (cm)
Control	12.82
Cd 100 µM	9.70 (-24.34)
Cd 300 µM	9.58 (-25.27)
Cd 500 µM	8.32 (-35.10)
Zn 50 µM	11.16 (-12.95)
Zn 150 µM	11.14 (-13.10)
Cd 100 µM + Zn 50 µM	10.92 (-14.82)
Cd 300 µM + Zn 50 µM	10.56 (-17.63)
Cd 500 µM + Zn 50 µM	8.70 (-32.14)
Cd 100 µM + Zn 150 µM	11.26 (-12.17)
Cd 300 µM + Zn 150 µM	10.95 (-14.59)
Cd 500 µM + Zn 150 µM	9.30 (-27.46)
CD (P = 0.05)	0.394

Data in parentheses indicate percentage increase (+) or decrease (-) over control.

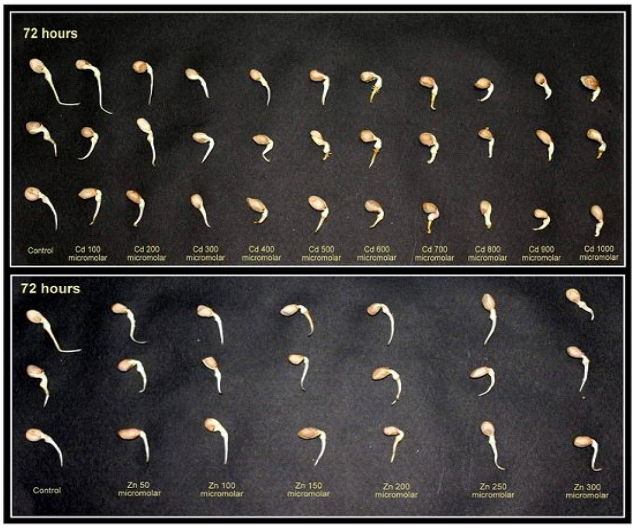


Plate I: Effect of different concentrations of cadmium and zinc on growth of embryonic axis in groundnut cv. TG-51 at 72 hours of germination.

chromosomal aberration caused by heavy metals resulting in disturbance of cell division (Rajput *et al*., 2019). However, from the overall data on growth of embryonic axis as well as fresh and dry weight of cotyledons under different concentrations of cadmium and zinc, three concentrations of cadmium (*viz,* 100, 300 and 500 µM) and two concentrations of zinc (*viz,* 50 and 150 µM) and their combinations were finally selected for cadmium-zinc interaction studies in the present experiment because these concentrations of two metals were found to be not too severe nor too mild for embryonic growth of groundnut.

Perusal of data indicated that all the treatments caused significant ($P \leq 0.05$) reduction in root length of groundnut seedling. The percent reduction in root length increased from 24.34% to 35.10% over control as the concentration of cadmium in the medium increased from 100 to 500 µM (Table 2). The two concentrations of zinc used in the experiment also produced negative effects on root length, the severity been increased under higher concentration. The adverse effects

of both these metals on root length might be attributed to reduction in mitotic index affecting cell division and elongation as was also noted earlier by Borboa and Delatorre (1996). Zinc supplements reduced the severity of cadmium stress on root length to some extent in all treatment combinations, although the inhibitory effect could not be completely rectified.

Perusal of data revealed that treatment with cadmium or zinc alone led to a decrease in chlorophyll a and chlorophyll b content of the leaf and the effect became more drastic along with an increase in the concentration of metal in medium (Table 3, Plate II). The result corroborated the early finding of Sivakumar *et al*. (2001). However, the reduced biosynthesis and enhanced chlorophyll degradation along with disorganized chloroplasts (Rascio *et al.,* 2008) might be attributed for the observed inhibitory effects of cadmium on leaf chlorophyll, whereas possible obstruction of Fe transport to chloroplast under excess Zn might be the underlying factor for decreased pigment content (Symeonidis and Karataglis, 1992). However, the Zn supplementation (150 µM) of cadmium-treated seedlings not only mitigated the negative effect of Cd 100 µM and 300 µM, but also increased the chlorophyll content above control level. The result was consistent with the finding of Aravind and Prasad (2005).

The data on relative leaf water content (RLWC) indicated non-significant differences among the treatments. In all the cases cadmium and zinc alone led to a reduction in RLWC and the highest reduction was noted down in case of Cd 500 µM treatment (Table 4). Supplementation of highest concentration of zinc was able to reduce the Cd-induced negative effects to some extent.

The leaf protein content of the seedlings significantly ($P \leq 0.05$) decreased under cadmium treatment with the effect being more adverse along with an increase in cadmium concentration (Table 4). The observed decrease in protein content under cadmium stress might be a consequence of reduction in protein synthesis (Blaestrasse *et al*., 2003). In contrast, the protein content increased in the Zn-treated seedlings and the corresponding values were 26.09 and 1.31% over control at 50 and 150 µM, respectively. The observation was consistent with the earlier suggestions regarding the essentiality of zinc in many metabolic processes including its role as protein stabilizer (Vallee and Falchuk, 1993). However, the combination of Zn 150 µM with different concentrations of cadmium somewhat lowered

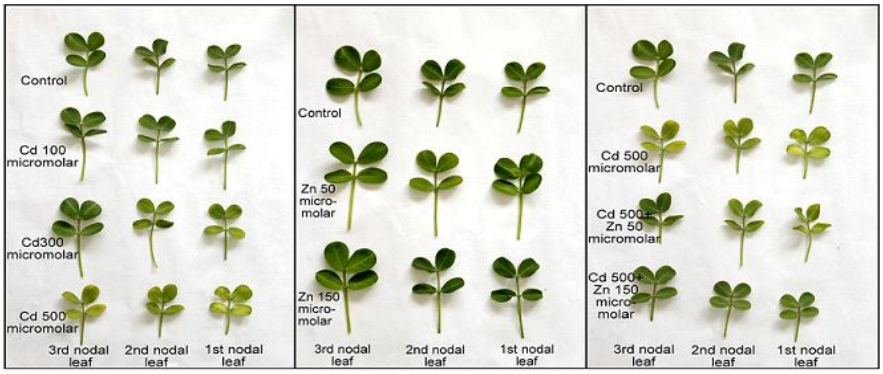


Plate II: Effect of cadmium and its interaction with zinc on leaf growth at different nodal position of groundnut cv. TG-51.

Table 3: Effect of cadmium-zinc interactions on chlorophyll content in leaves of groundnut cv. TG-51.

Treatments	Chlorophyll a (mg g^{-1} fresh weight)	Chlorophyll b (mg g^{-1} fresh weight)	Chlorophyll a/ chlorophyll b
Control	0.84	0.38	2.23
Cd 100 µM	0.69 (-17.37)	0.32 (-14.14)	2.15 (-3.76)
Cd 300 µM	0.70 (-16.50)	0.30 (-20.16)	2.33 (4.59)
Cd 500 µM	0.70 (-16.84)	0.27 (-27.70)	2.57 (15.02)
Zn 50 µM	0.80 (-4.85)	0.35 (-7.67)	2.30 (3.06)
Zn 150 µM	0.39 (-53.48)	0.27 (-28.14)	1.44 (-35.26)
Cd 100 µM + Zn 50 µM	0.81 (-3.09)	0.33 (-13.49)	2.50 (12.02)
Cd 300 µM + Zn 50 µM	0.74 (-11.23)	0.34 (-10.45)	2.21 (-0.87)
Cd 500 µM + Zn 50 µM	0.68 (-19.06)	0.30 (-20.05)	2.26 (1.24)
Cd 100 µM + Zn 150 µM	0.96 (14.92)	0.38 (2.31)	2.51 (12.32)
Cd 300 µM + Zn 150 µM	0.86 (2.56)	0.39 (3.00)	2.22 (-0.42)
Cd 500 µM + Zn 150 µM	0.74 (-11.13)	0.36 (-5.43)	2.10 (-6.03)
CD (P = 0.05)	0.030	0.013	0.085

Data in parentheses indicate percentage increase (+) or decrease (-) over control.

Table 4: Effect of cadmium-zinc interactions on relative leaf water content (RLWC), soluble protein content, nitrate reductase (NR) activity and phenol content in leaves of groundnut cv. TG-51.

Treatments	RLWC	Protein (mg g^{-1} fw)	NR activity (mM hr^{-1} g^{-1} fw)	Phenol (mM of gallic acid g^{-1} fresh weight)
Control	91.37	168.42	1.76	4.36
Cd 100 µM	88.42 (-3.23)	134.27 (-20.28)	0.81 (-53.70)	4.76 (9.11)
Cd 300 µM	87.94 (-3.75)	118.39 (-29.71)	0.76 (-56.83)	4.56 (4.52)
Cd 500 µM	86.74 (-5.07)	117.10 (-30.47)	0.68 (-61.02)	3.58 (-17.94)
Zn 50 µM	89.50 (-2.04)	212.36 (26.09)	0.79 (-54.74)	4.66 (6.81)
Zn 150 µM	87.19 (-4.57)	170.63 (1.31)	0.83 (-53.00)	5.15 (17.97)
Cd 100 µM + Zn 50 µM	88.62 (-3.00)	136.12 (-19.18)	0.93 (-47.07)	5.19 (18.95)
Cd 300 µM + Zn 50 µM	87.65 (-4.07)	115.62 (-31.35)	0.80 (-54.39)	5.02 (15.14)
Cd 500 µM + Zn 50 µM	87.00 (-4.78)	108.42 (-35.62)	0.74 (-57.88)	4.93 (13.04)
Cd 100 µM + Zn 150 µM	90.94 (-0.47)	156.98 (-6.80)	1.47 (-16.04)	5.30 (21.41)
Cd 300 µM + Zn 150 µM	89.19 (-2.38)	154.76 (-8.11)	0.90 (-48.81)	4.70 (7.73)
Cd 500 µM + Zn 150 µM	89.01 (-2.58)	140.21 (-16.75)	0.89 (-49.51)	4.41 (1.08)
CD (P = 0.05)	NS	5.520	0.041	0.178

Data in parentheses indicate percentage increase (+) or decrease (-) over control. NS = Non-significant.

the adverse effect of cadmium stress alone, thus, indicating antagonistic effects of this metal combination.

The activity of nitrate reductase (NR) in the leaves of groundnut seedlings decreased significantly ($P \leq 0.05$) under all concentrations of cadmium and zinc as well as their interactions (Table 4). In case of cadmium, the percent inhibition of the enzyme activity over unstressed control was found to be 53.70, 56.83 and 61.02% for Cd 100, 300 and 500 µM, respectively, thus, indicating increases in toxic effect of the metal concomitant with an increase in concentration in the medium. However, there was a little difference between two concentrations of zinc used in the experiment in respect of negative effect on the enzymatic activity. The different treatment combinations of cadmium and zinc could not remarkably change the level of metal toxicity except Cd 100 µM + Zn 150 µM which significantly lowered the negative effect of sole treatment of Cd 100 µM on NR.

The mean data on leaf phenol content revealed highly significant ($P \leq 0.05$) variations among the treatments. Treatment with Cd 100 and 300 µM induced 9.11 and 4.52% increase in phenol content (Table 4), respectively, over control. This observation was corroborative of the early observation by Garcia *et al.* (2012) and might be interpreted as a consequence of Cd-induced accumulation of cinnamic acid derivatives (Kovacik *et al*., 2009). The leaf phenol content also increased under zinc treatment at 50 and 150 µM and the corresponding values were 6.81 and 17.97% over control, respectively. Moreover, the supplements of zinc with cadmium also induced positive effect in respect of phenol accumulation in leaf but the level varied under different combinations. The influence of zinc on phenol accumulation in leaf was reported earlier by Chamani *et al*. (2016). Plant phenolic compounds are potent non-enzymatic antioxidant and thus, increase in their contents in the leaf might lead to scavenging of reactive oxygen species (ROS) that are responsible to create oxidative stress.

CONCLUSION

The results of the present experiment indicated the adverse effects of cadmium on the growth of embryonic axis as well as the content of chlorophyll, protein and nitrate reductase activity in the groundnut seedlings. On the other hand, zinc, in spite of being an essential element, exerted negative effects on most of these characters at high concentrations. The groundnut seedlings showed varied responses towards mixed metal treatment in respect of growth parameters and biochemical traits. The presence of zinc in the growing medium in combination with cadmium lowered the damaging effect of cadmium to some extent but could not completely override it. Zinc significantly enhanced the accumulation of phenolic compounds highlighting its protective role against oxidative damage.

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Conflict of interest

All authors declared that there is no conflict of interest.

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