Journal of Phytology 2011, 3(8): 43-46
ISSN: 2075-6240
www.scholarjournals.org

www.journal-phytology.com

Zinc Alleviates Cadmium Induced Toxicity in *Vigna radiata* (L.) Wilczek

Manoj Kumari[1*], V.K. Sinhal[2], Alok Srivastava[1], and V. P. Singh[1]

[1]*Department of Plant Science, M.J.P. Rohilkhand University, Bareilly -243006*
[2]*Department of Botany Maharaja Agrasen Mahavidyalaya, Bareilly, UP*

Article Info

Article History

Received : 11-04-2011
Revised : 02-07-2011
Accepted : 07-07-2011

*Corresponding Author

Tel : +91-9219894002, 9358660040

Email: mrs.manojsingh@rediffmail.com

Summary

Cadmium is a non-essential toxic element without any metabolic significance whereas Zn is an essential element but turns toxic at higher concentrations. The analysis of waste water of Bareilly city shows the presence of Cd (0.2 mgL^{-1}) and Zn (7.3 mgL^{-1}). Therefore, the present investigation was designed to evaluate the interactive effect of various concentrations of Zn (6.3, 7.3 and 8.3 mgL^{-1}) with Cd (0.2 mgL^{-1}) in *Vigna radiata*. Plants were treated with Cd (0.2 mgL^{-1}) and Zn (6.3, 7.3 and 8.3 mgL^{-1}) alone and in combination with each other. The growth parameters like plant height, fresh weight of plants, number of root nodules, chlorophyll \`a\` and \`b\` content and biochemical parameters like nitrate reductase (NR) activity, glutamine oxo glutarate aminotransferase (GOGAT) activity, and protein content were significantly reduced under the influence of Cd (0.2 mgL^{-1}) and Zn (6.3, 7.3 and 8.3 mg L^{-1}) as compared to their respective controls while glutamate dehydrogenase activity was increased under these conditions. However, growth and biochemical parameters were increased with combined treatment of Zn and Cd as compaired to Cd treatment alone while GDH activity was decreased under these conditions. The Zn treatment did not show significant reduction at lower concentration but at higher concentration (8.3 mgL^{-1}) inhibitory effect on groth parameters were observed. The variety K-851 was found to be more susceptible to metal induced toxicity than variety PDM-139.

Key Words: Antagonism, Cadmium, Enzyme activity, Toxicity, Zinc

Introduction

Heavy metal pollution is of considerable importance and relevant to present scenario due to the increasing levels of pollution and its obvious impact on human health through the food chain [1]. Heavy metals at extremely micro concentrations affect different cellular components, thereby interfering with the normal metabolic function. The coexistence of essential and non-essential elements in the ecosystem leads to interactions that may be additive, antagonistic or synergistic [2]. Cadmium is highly toxic causing lipid peroxidation [3, 4], inactivation of DNA mismatch repair (MMR) pathways [5] and inhibition of chlorophyll biosynthesis [6]. On the other hand Zn is an important component of many vital enzymes having catalytic, cocatalytic and structural role as structural stabilizer for proteins, membranes and DNA binding proteins (Zn-fingers). Zn is found to be involved in many cellular functions such as protein metabolism, gene expression, chromatin structure, photosynthetic carbon metabolism and indole acetic acid metabolism [7] yet its higher concentrations cause toxicity [8]. Alleviation of Cd induced toxicity by Zn has been observed by Hassan [9]. Zinc is known to have a stabilizing and protective effect on the biomembranes against Cd induced oxidative and peroxidative damage, loss of plasma membrane integrity [10] and also alteration of the permeability of the membrane with the help of enzymatic and non-enzymatic antioxidant.

In Bareilly region, *Vigna radiata* (L.) Wilzeck is an important cultivated pulse crop. It has been analysed that waste water of Bareilly city contains toxic amount of Cd^{2+} in association with Zn. Thus present investigation has been undertaken to evaluate the anagonistic effect of Zn on different concentrations of Cd in *Vigna radiata*, by taking the parameters of biomass accumulation and nitrate assimilation.

Material and Methods

The seeds of two cultivars of *Vigna radiata* (L.) Wilzeck, ie., PDM-139 and K-851 were washed thoroughly with distilled water and allowed to imbibe the water for 6 hours. The seeds were sown in field on ridges separated by deep furrows. The raised plants were irrigated 3 times with Cd (0.2 mg L^{-1}) and Zn (6.3, 7.3, and 8.3 mgL^{-1}) alone and in combination with each other at the intervals of 10 days. For individual metal treatments untreated plants were taken as control. While for combination treatments individual metal treated plants (Cd 0.2mgl^{-1}) were considered as control. The effects of Cd and Zn alone and in combinations were studied on plant height, fresh weight of plants, number of root nodules, chlorophyll a and b contents, protein content, NR (nitrate reductase), GOGAT (Glutamine Oxo Glutarate amino Transferase) and GDH (Glutamate dehydrogenase) activity. Chlorophyll a and b contents were determined by the method of Arnon [11], nitrogen content by Lang and Martin [12], protein by Lowry *et al.* [13], NR Activity by Srivastava [14], GDH activity by Wolf and Williams [15] and GOGAT activity was determined by the method of Meers et al. [16].

One way ANOVA was carried out to compare the means of different treatments at 5% level of significance.

Results

As compared to their respective control, a marked significant reduction was noticed in plant height and fresh weight of plants in both the cultivars i.e., PDM-139 and K-851 of *Vigna radiata* under the influence of Cd (0.2 mgL^{-1}) and Zn (8.3 mgL^{-1}). Among both the heavy metals maximum reduction in these parameters was noticed by Cd (0.2 mgL^{-1}) in both the cultivars. In combined treatments of Cd and Zn, all the concentrations of Zn showed promotory effect on plant height and fresh weight of plant and number of root nodules as compared to control (0.2 mgL^{-1} of concentration of Cd was treated as control, in both the cultivars) protein content and Chlorophyll a and b content were also reduced significantly under the influence of higher concentrations of Zn (8.3 mgL^{-1}) and Cd (0.2 mgL^{-1}). In combined treatment, all the concentrations of Zn showed antagonistic effects on Cd (0.2 mgL^{-1}) induced toxicity but maximum antagonistic effect was noticed with 6.3 mgL^{-1} concentration of Zn in both the cultivars (Table-1&2).

Table 1. Antagonistic effects of Zinc on Cadmium induced toxicity on growth parameters of *Vigna radiata* (L.) Wilczek.

Heavy metal concentration (mgL^{-1})	Plant height (c.m)	Fresh weight of plants (gm)	No. of root Nodules	Chlorophyll 'a' content(mg g^{-1} fresh wt.)	Chlorophyll 'b' content(mg g^{-1}fresh wt.)
Variety PDM-139	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Untreated (Control)	32.08±0.44	5.66±0.29	16±0.33	4.78±0.17	2.64±0.18
Cd 0.2	19.70±0.35	3.14±0.12	9.0±0.49	3.06±0.49	1.53±0.09
Zn 6.3	31.4±0.63	5.5±0.20	16.4±0.33	4.64±0.15	2.46±0.08
Zn 7.3	30.8±0.68	5.2±0.11	15.8±0.52	4.51±0.19	2.30±0.12
Zn 8.3	24.70±0.43	4.8±0.24	13.1±0.33	3.92±0.18	2.16±0.04
CD at 5%	2.29	0.36	1.47	0.35	0.29
Cd 0.2 (Control)	19.70±0.35	3.14±0.12	9.0±0.49	9.0±0.49	1.53±0.09
Zn 6.3+Cd 0.2	30.90±0.49	5.0±0.28	15.7±0.28	4.24±0.15	2.31±0.13
Zn 7.3+Cd 0.2	29.84±0.65	4.81±0.23	15.15±0.33	3.80±0.19	2.13±0.06
Zn 8.3+Cd 0.2	26.66±0.49	3.91±0.11	12.85±0.33	3.44±0.18	1.97±0.12
CD at 5%	1.41	0.39	1.30	0.24	0.21
Variety K-851					
Untreated (Control)	31.00±0.64	5.48±0.20	14.4±0.46	4.68±0.14	2.48±0.09
Cd 0.2	18.90±0.18	2.76±0.11	7.0±0.40	3.00±0.07	1.41±0.02
Zn 6.3	30.52±0.57	5.30±0.28	13.80±0.46	4.30±0.23	2.36±0.18
Zn 7.3	28.86±0.30	4.78±0.18	12.95±0.33	4.11±0.16	2.03±0.10
Zn 8.3	23.70±0.68	3.95±0,14	10.11±0.36	3.37±0.10	1.98±0.10
CD at 5%	2.02	0.30	1.24	0.42	0.19
Cd 0.2 (Control)	18.90±0.18	2.76±0.11	7.0±0.40	3.00±0.07	1.41±0.02
Zn 6.3+Cd 0.2	30.10±0.38	4.96±0.28	13.20±0.22	4.18±0.18	2.19±0.12
Zn 7.3+Cd 0.2	26.68±0.52	4.56±0.18	11.88±0.28	3.95±0.16	1.94±0.10
Zn 8.3+Cd 0.2	23.30±0.71	3.70±0.20	9.54±0.52	3.07±0.17	1.79±0.14
CD at 5%	2.34	0.28	0.74	0.26	0.31

±Values indicates standard error,*Significant at 5% level.

Table 2. Antagonistic effects of Zinc on Cadmium induced toxicity on biochemical parameters of *Vigna radiata* (L.) Wilczek.

Heavy metal concentration (mgL^{-1})	NR activity (µmol NO_2hr^{-1}g^{-1})	GOGAT activity(µmolNADPH oxi min^{-1}g^{-1}fr.wt) (gm)	GDH activity(µmolNADPH oxi min^{-1}g^{-1}fr.wt) (gm)	Protein content (mg g^{-1}dry wt)
Variety PDM-139	Mean±SE	Mean±SE	Mean±SE	Mean±SE
Untreated (Control)	2.58±0.11	418.14±1.18	260.15±1.89	43.81±0.44
Cd 0.2	1.55±0.16	215.49±1.57	480.19±1.01	26.05±0.19
Zn 6.3	2.45±0.15	400.16±1.39	300.32±2.17	41.77±0.81
Zn 7.3	2.18±0.11	380.70±2.14	390.10±2.81	38.81±0.38
Zn 8.3	1.91±0.04	295.15±1.98	460.32±1.62	33.79±0.38
CD at 5%	0.19	23.20	21.85	2.70
Cd 0.2 (Control)	1.55±0.16	215.49±1.57	480.19±1.01	26.05±0.19
Zn 6.3+Cd 0.2	2.21±0.10	395.90±2.05	295.51±0.98	40.57±0.57
Zn 7.3+Cd 0.2	2.08±0.07	366.79±1.03	380.50±1.80	35.65±0.78
Zn 8.3+Cd 0.2	1.75±0.09	280.50±1.81	441.42±2.10	31.88±0.29
CD at 5%	0.14	15.72	46.85	2.07

Variety K-851				
Untreated (Control)	2.51±0.19	416.42±1.57	266.19±1.79	42.07±0.69
Cd 0.2	1.44±0.09	205.38±1.18	490.13±0.99	23.85±0.22
Zn 6.3	2.30±0.08	395.15±2.01	315.25±1.75	40.98±0.94
Zn 7.3	2.08±0.11	360.78±1.57	395.35±2.09	36.29±0.38
Zn 8.3	1.81±0.09	272.19±1.81	451.36±1.32	30.20±0.44
CD at 5%	0.24	26.66	50.92	2.69
Cd 0.2 (Control)	1.44±0.09	205.38±1.19	490.13±2.29	23.85±0.22
Zn 6.3+Cd 0.2	2.19±0.11	375.99±2.05	305.50±1.68	36.22±0.63
Zn 7.3+Cd 0.2	1.94±1.50	351.41±1.32	387.13±1.81	29.88±0.29
Zn 8.3+Cd 0.2	1.56±0.08	260.20±2.19	448.85±2.10	28.88±0.75
CD at 5%	0.34	20.28	50.74	2.26

±Values indicates standard error, *Significant at 5% level.

Enzyme like NR, and GOGAT activities were also decreased by individual treatments of Cd (0.2 mgL^{-1}) and Zn (8.3 mgL^{-1}) as compared to their respective controls while GDH activity was increased with Cd and Zn in both the cultivars. In combined treatments the lowest dose of Zn (6.3mgL^{-1}) was found to be more effective to alleviate cadmium induced toxicity resulted in increased NR and GOGAT and decreased GDH activity. In combined treatments, the maximum recovery of Cd induced toxicity was noticed in GOGAT activity with the lowest dose of Zn (6.3mgL^{-1}) in cultivar PDM-139 (Table 2). Further, among both the cultivars, the reduction in these parameters was more pronounced in K-851 then PDM-139.

Discussion

In the present investigation Cd and Zn both were found to be phytotoxic in nature and significantly reduced the plant height and fresh weight of plant at higher concentration but in combined treatments, Zn antagonized the toxic effects of Cd. Cadmium is a nonessential element and exert hazardous effects on plant height and fresh weight of plants [6] while Zn is an essential element for plant growth but its excess amount exert toxic effects on plant height and fresh weight of plants [17, 18] and number of root nodules. Cd induced toxicity and its alleviation by Zn was also reported by some earlier workers like Arunachalam et al.[19]. The higher concentration of heavy metals has been reported to retard the cell division and differentiation, reduce their elongation and effect plant growth and development [20].

Protein and chlorophyll 'a' and 'b' content were also inhibited by Cd and Zn treatments. Cd is known to specifically inhibit chlorophyll biosynthesis through binding with functional sulphydryl group (-SH) of the enzymes δ-amino laevulinic acid dehydratase and protochlorophyllide reductase. The protein content under heavy metal stress may be affected due to (i) enhanced protein hydrolysis resulting in decrease of soluble proteins [21], (ii) catalytic activity of heavy metals [22] and (iii) protein synthesis reduced under all stress conditions. In combined treatments, due to antagonistic effect of Zn over Cd, the reduction in chlorophyll 'a' and 'b' and protein contents was recovered by Zn supplementation. Similar results were also noticed by Kevrson et al. [23] and [24].

The results of present investigation clearly indicate that activity of enzymes (NR, and GOGAT) involved in nitrogen metabolism drastically reduced by different concentrations of Zn and Cd alone as compared to their respective controls. In combined treatments the reductions in NR and GOGAT were significantly recovered. The similar results were also earlier reported by Bharti et al. [25] . The inhibition of nitrate reductase activity under the influence of heavy metal supply may be due to reduction in nitrate supply to the site of the enzyme synthesis [26], inhibition of NADH binding to sulphydryl (-SH) group of nitrate reductase [18], reduced stability of the enzyme, its thiomodulation, phosphomodulation and impaired electron transport function [27]. In plants GOGAT is localized in chloroplast in leaves and plastid in roots. Heavy metals have been found to disorganize the chloroplastic structure. Thus GOGAT activity significantly decreased by Zn and Cd. The activity of GDH enzyme was increased under the influence of Cd and Zn as compared to their respective control because under stress condition in GS-GOGAT pathway of nitrogen metabolism enzyme GDH works more efficiently than GOGAT. Thus increase in GDH activity is an indication of stress condition [18].

Thus the present investigation showed that Zn and Cd at higher concentration exert toxic effects on growth and biochemical parameters in both cultivars of *Vigna radiata* but in combined treatments the lower doses of Zn alleviate the toxic effects of Cd.

References

[1] Athar, R. and Masood, A. (2002).Heavy metal toxicity in legume microsymbiont system. *J. Plant Nutr.*25: (2) 369-386.

[2] Siedlecka A 1995 Some aspects of intractions between heavy metals and plant minral nutrients. *Acta Soc Bot Pol* 3 265-272.

[3] Carpena, R Vazquez S Esteban E Fernandez PM Resario DFM (2003). Cadmium stress in white lupin: effect on nodule structure and functioning. *Plant Physiol Biochem* 41 911-919.

[4] Unyayer, S Celik A Celik FO & Gozel A 2006 Cadmium induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium stivum* and *Vicia faba. Mutagenesis* 21 77-81

[5] Banerjee, S & Rozas HF (2005). Cadmium inhibit mismatch repair by blocking the ATPase activity of the MSH2-MSH6 complex. *Nucleic Acid Research* 33 1410-1419.

[6] Garg, R (2005). Phytotoxic cytogenetic and biochemical effects of heavy metals (Cu^{2+} and Cd^{2+}) in *Vigna mungo* (L.). Ph.D. Thesis, M.J.P.Rohilkhand University, Bareilly.

[7] Cakmak, & Braum H J (2001). Genotypic variation for zinc efficiency. In: Application of physiology in wheat breeding, eds. Renolds MP Ortiz-monasterio JI & MacNab A D.F. CIMMYT, Mexico Pp183-199.

[8] Sinhal, V.K. (2007). Phytotoxic and cytogenetic effects of Zn^{2+} and Pb^{2+} in *Vicia faba. Poll. Res.* 26: 417-420.

[9] Hassan, MJ Zhang G Wright FWu & Chen Z (2005). Zinc alleviates growth inhibition and oxidation and oxidative stress caused by Cadmium in Rice. *Biores Technol* 168 255-261.

[10] Cakmak. (2000). Possible roles of zinc in protecting plant cells damage by reactive oxygen species. *New Phytol* 146 185-205.

[11] Arnon, D I 1949 Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris. Plant Physiol* 24 1-15.

[12] Kumari M. (2008). Combined effects of Cu^{2+} and Zn^{2+} on Cd^{2+} induced toxicity in Vigna radiate L. Wilczek. Ph.D., thesis, M.J.P. Rohilkhand University, Bareilly, India.

[13] Lowery, OH Roserbrough NJ Farr AL & Randall RJ (1951). Protein measurement with Folin-Phenol reagent. *J Biol Chem* 193 265-275.

[14] Srivastava, H S 1974 *In vitro* activity of enzyme nitrate reductase in maize seedlings. *Indian J Biochem* 11 230-232.

[15] Wolf, PL & Williams D 1973 Practical clinical enzymology: In Techniques and interpretation. Wiley Interscience Publication, New York.

[16] Meers, LJ Tempest DW & Brown CM 1970 Glutamine (amide):2-Oxoglutarate amino transferase, oxido-reductase (NADP) and enzyme involve synthesis of glutamade by some bacteria. *J Gen Microbiol* 64 187-194.

[17] Sinhal, VK Singh VP & Srivastava A (2006). The effects of Zn^{2+} and Pb^{2+} on the nitrogen metabolism in *Vigna mungo* (L.) Hepper. *J Indian Bot Soc* 85 56-60.

[18] Sinhal, VK Gupta SK Srivastava A Singh UB & Singh VP (2007). Effect of zinc on growth and NR activity in Black gram antagonized by magnesium and sucrose, *Indian J Plant Physiol* 12 95-99.

[19] Arunachalam K, Maithani AK & Pandiaraj S 1996 Effect of zinc and cadmium on primary productivity of *Hydrilla. Indian J Plant Physiol* 1 45-51.

[20] Soares, CRFS Grazziotti PH Siquaira JO & Carvalno JHDe 2001 Zinc toxicity on growth and nutrition of *Eucalyptus muculata* and *Eucalyptus arophylla. Pesquisa Agropecuaria Brasileira* 36 339-348.

[21] Melinchuk, Y Lishko AK & Kalinin FI 1982 Cadmium effect on free amino acid cotent in germs of pea seeds at early germination stages. *Fiziologia Biokhimiya Kuliurnyh Rastnii* 14 385-395.

[22] Bhattacharya, M & Choudhry MA (1995). Heavy metal (Pb^{2+} and Cd^{2+}) stress induced damages in Vigna seedling and possible involvement of phytochelatin like substances in mitigation of heavy metal stress. *Indian J Exp Biol* 33 236-238.

[23] Kevreson, S Papovic N & Kandrac J (2001). Nitrigen and protein metabolism in young pea plants as effected by different concentrations of nickel, cadmium, lead and molybdenum. *J. Plant Nutr* 6 281-297.

[24] Shukla, UC Singh J Joshi PC & Kakkar P 2003 Effect of bioaccumulation of cadmium on biomass productivity, essential trace elements, chlorophyll biosynthesis and micromolecules of wheat seedling. *Biol Trace Elements Res* 92 257-273.

[25] Bharti, N Singh RP & Sinha SK (1996). Effect of cadmium chloride on heavy metal induced alteration in growth and nitrate assimilation of *Sesamum indicum* seedling. *Phytochem.* 41 105-109.

[26] Burzynski, M & Grabowski A (1984). Influence of lead on nitrate uptake and reduction in Cucumber seedling. *Acta Soc Bot Pol* 53 77-86.

[27] Vyas, J& Gadre R 1995 Inhibition of nitrate reductase activity of sunflower plants developed on N and Mo different fertilization levels. *Analele-Institutului-de-Ceretari-Pentru-Pedologie si-Agrochimic.* 43 589-591.