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Responses of *Beta vulgaris* exposed to cadmium and zinc through soil drenching

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Abstract

The present study investigates the responses of Indian palak (*Beta vulgaris* L. cv. All Green H1) exposed to cadmium (Cd) and zinc (Zn) at growth, biochemical and physiological levels. The results revealed that Cd and Zn accumulation was higher in shoots as compared to the roots of *B. vulgaris* plants. The increased application rates of Zn in combination with Cd significantly reduced the accumulation of Cd in below and above ground parts of *B. vulgaris*, whereas it increased Zn accumulation. Treatments of *B. vulgaris* with Cd and Zn individually or in combination significantly reduced the leaf area, biomass, photosynthetic pigments, photosynthetic rate, Fv/Fm ratio and protein contents at $p<0.05$. Contrary to this, lipid peroxidation, ascorbic acid, proline and thiol contents and peroxidase activity increased significantly as compared to control at $p<0.05$. The results also revealed that the combined effect of Cd and Zn were more pronounced at higher concentrations as compared to other treatments. Thus, the present study suggests that Zn may be applied to Cd contaminated field to reduce Cd accumulation in plants. However, finding of a suitable dose and toxicity level of Zn must be worked out further its application.

Key words

Accumulation, *Beta vulgaris,* Cadmium, Response, Zinc

Introduction

Heavy metals contaminated soil has significant and negative effects on various growth, physiological and biochemical characteristics of plants (Singh and Agrawal, 2007; Siddhu *et al.,* 2008; Dinkar *et al.,* 2009; Rolli *et al.,* 2010; Sbartai *et al.,* 2011; Srinivas *et al.,* 2013; Fernandez *et al.,* 2013). It has been reported that accumulation of heavy metals in tissue influences the enzymatic and non-enzymatic antioxidant systems which ultimately affect the yield of the plants (Singh and Agrawal, 2007; Sharma *et al.,* 2010; Li *et al.,* 2013; Feigl *et al.,* 2013). Singh and Agrawal (2007) have shown that Indian palak, when grown on soil amended with 20% and 40% sewage sludge, a source of heavy metals (Indra and Shivaji, 2006) could accumulate Cd up to 20 mg kg^{-1} in shoots and 30 mg kg^{-1} in roots and Zn up to 70 mg kg^{-1} in shoots and 220 mg kg^{-1} in roots.

An interaction between Cd and Zn in root uptake and transport or accumulation in edible portion of plants has been reported by several workers (McKenna *et al.,* 1993; Chaoui *et al.,* 1997; Hart *et al.,* 2002; Sharma *et al.,* 2008; Garg and Kaur, 2013). A significant impact of Cd and Zn interaction on dry matter yields and their accumulation and distribution in different parts of *Cajanus cajan* L. Millsp (Garg and Kaur, 2013), *Daucus carota* L. cv. Pusa Kesar (Sharma *et al.,* 2008), young leaf of *Lactuva sativa* L (McKenna *et al.,* 1993) and roots and shoots of *Phaseolus vulgaris* L (Chaoui *et al.,* 1997) has been reported. The interaction between Cd and Zn is also governed by various environmental factors, such as nutritional status and concentrations of heavy metals in soil. Application of Cd to the soil deficient in Zn resulted in reduced concentrations of Zn in wheat, whereas an application of Cd to soil with adequate Zn either stimulated or had no effect on the uptake of Zn (Koleli *et al.,* 2004). Several studies have shown that addition of Zn to soil can decrease Cd accumulation (Moraghan, 1993, Oliver *et al.,* 1994; Choudhary *et al.,* 1995)

Beta vulgaris L. cv. All green H1, also known as Indian palak, is a leafy vegetable used by the people all over India to

overcome iron deficiency. Palak is commonly grown in peri-urban areas of India where they are exposed to Cd and Zn through contaminated irrigation water (Sharma *et al.,* 2007). Other sources like phosphate fertilizers, sewage sludge, etc., also add Cd and Zn to the soil. Since the interactive effect of Cd and Zn is also influenced by other environmental conditions, the present study was undertaken to study the interactive effect of Cd and Zn on Indian palak, at growth, biochemical and physiological levels.

Materials and Methods

A field experiment was conducted for two consecutive years at the Agriculture Farm of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (lat. 25° 18' N, long. 83° 01' E, alt. 76.19 m). Twenty one plots of 1.5 m × 1.5 m in size with margin of 0.25 m were prepared as per the local agriculture practices and recommended doses of farm yard manure, nitrogen as urea, phosphorus as single superphosphate and potassium as potash @ 30 ton ha^{-1}, 120, 80 and 80 kg ha^{-1}, respectively were incorporated to a depth of 0.25 m before sowing and mixed properly. The seeds of tested palak variety (All green H1) were procured from the Institute of Vegetable Research, Varanasi. Ten healthy seeds were hand sown at a 2 cm depth in rows of each plot. After emergence, the plants were thinned to one per 15 cm × 15 cm area. Uniform moisture content was maintained through irrigation with defined volume of water to the respective plots. Manual weeding was performed, depending upon weed intensity during the course of experiment.

There were seven treatments, each with three replicates along with control, designed in randomized blocks. Cd and Zn were supplied as $CdCl_2 \cdot H_2O$ and $ZnSO_4 \cdot 7H_2O$, respectively. After 7 days of germination and followed by initial irrigation, plants were supplied with 10 ml of water, Cd (10 and 100 mg l^{-1}), Zn (100 and 300 mg l^{-1}) alone and 10 ml each of Cd and Zn through soil drench from 08:00 to 09:00 hrs. The treatments were repeated at 10 day intervals for up to 45 days after germination (DAG).

For analysis of growth characteristics, i.e. leaf area, dry weight, plants were harvested in triplicates from each treatment at 60 DAG. Leaf area was measured using a portable leaf area meter (LICOR-3000, LICOR, INC, Lincon, NE). Dry weights of plants were measured for roots and shoots, separately and added for the total plant biomass. Root to shoot ratio was calculated by dividing the dry weights of root with that of shoot.

Lipid peroxidation in 30-day-old harvested leaves was measured in terms of malondialdehyde by thiobarbituric acid reaction as described by Heath and Packer (1968). Total phenolics and ascorbic acid contents in leaf tissue were determined following the methods of Bray and Thrope (1954) and Keller and Schwager (1977), respectively. Total peroxidase, thiols, protein and proline content in leaf extracts were measured following the methods of Britton and Mehley (1955), Fahey *et al*. (1978), Lowry *et al*. (1951) and Bates *et al*. (1973), respectively. Chlorophyll and carotenoid contents in extracts of leaf tissues were quantified using the formulae of Machlachlan and Zalik (1963) and Duxbury and Yentsch (1956), respectively. OD was measured using an UV-VIS Spectrophotometer (Systronic 119, India). The net photosynthetic rate and Fv/Fm ratio were measured at 45 DAG using a portable photosynthetic system (LI-6200, LI-COR, INC, Lincon, NE) and Plant Efficiency Analyzer (PEA, MK2 9414, Hansatech Instruments, Ltd., England, U.K.) at ambient conditions when photosynthetically active radiation and CO_2 concentration varied from 1000-1200 µmole m^{-2} s^{-1} and 320-342 g m^{-2}, respectively.

For Cd and Zn analyses, plants along with soil were sampled from the replicate plots of the respective treatments at 60 DAG and were oven/air dried. Samples were homogenized by grinding in a stainless steel blender, passed through a 2 mm sieve and kept at room temperature for further analyses. One gram of each dried soil and vegetable sample were digested in 15 ml of tri-acid mixture (70% high purity HNO_3, 65% $HClO_4$ and 70% H_2SO_4 in 5:1:1 ratio) at 80 °C until a transparent solution was obtained (Allen *et al.,* 1986). The resulting solution was filtered and diluted to 50 ml using double distilled water. Concentrations of Cd and Zn in filtrates of samples were determined by atomic absorption spectrophotometer (Model 2380 Perkin-Elmer, Inc., Norwalk, CT).

The instrument was calibrated using standard solutions of the respective heavy metals. Standard stock solutions of Cd and Zn obtained from Sissco Research Laboratories Pvt. Ltd., India, were diluted to the desired concentrations to calibrate the instrument. Procedural blanks without vegetable and soil samples, each representing 25% of the sample populations, were randomly inserted in the analytical programme. The elemental concentrations of procedural blanks were generally found to be <5% of the mean analyte concentrations for all the tested heavy metals. Blanks (5% tri-acid mixture) or standards for quality control were measured in every five samples to detect contamination and drift. Precision and accuracy of analysis was also ensured through analysis of samples against standard reference material (SRM 1570) of the National Institute of Standards and Technology for Cd and Zn. Results were within 100±2% of the certified values. All the chemicals used were of Merck, Suprapure, analytical grade.

The data were subjected to one way ANOVA and then to Duncan's multiple range tests to separate the means. Statistical analyses were performed using of SPSS (version 16.0) software.

Results and Discussion

Data of two experimental years were not significantly different and were pooled. The treatments, which affected Cd and Zn accumulation in roots and shoots of *B. vulgaris* are shown in

Table 1. Concentrations of Cd and Zn in both the roots and shoots of *B. vulgaris* were observed to be higher than that of controls. Accumulation of Cd and Zn in shoots were slightly higher than that of roots in the test plant. This may be due to more transpiration, accumulation of water and more biomass production of test plant (Moreno *et al*., 2002). Further, accumulation of both the metals were higher when treated with Cd and Zn in combination as compared to control as well as in their individual treatments, but Cd concentration decreased with increasing concentrations of Cd and Zn used in combination. Zn, when present in soil together with Cd, acts as a competitive inhibitor of Cd due to their similar chemical property. This may cause reduced uptake of Cd in plant parts. The concentrations of Cd in roots and shoots of *B. vulgaris* exceeded the safe limit (1.5 mg kg^{-1}) of Indian standards in all the treatments (Sharma *et al.,* 2007). However, Zn accumulation was above the safe limits (50 mg kg^{-1}) of Indian standard only in those plants treated with Zn and Zn+Cd (Table 1). Accumulation of Cd and Zn in roots and shoots of *B. vulgaris* was higher than the safe limit; this may be ascribed to their more bioavailability in soil. Accumulation of Cd and Zn in edible portion of test vegetable above the safe limit may pose serious threats to the health of consumers. Increasing Cd/Zn ratios resulted in decreased Cd concentration in roots and shoots of *B. vulgaris*. However, Zn concentration in plant parts increased with increased Cd/Zn ratio. This result was also in agreement with Smilde *et al.* (1992) and Sharma and Agrawal (2006). The combined applications of Cd and Zn at both low and high concentrations resulted in higher accumulation of Cd and Zn in roots and shoots as compared to controls and their single treatments. These results indicated a synergistic interaction of Cd and Zn and consequently their accumulation in different parts of *B. vulgaris* when applied in combination.

Interactive effects of Cd and Zn on tissue concentrations of heavy metals in *Lactuca sativa*, *Spinacea oleracea*, *Triticum aestivum* and *Zea mays* have been reported by Smilde *et al.* (1992), in *Docus carota* by Sharma and Agrawal (2006) and *Ablemoschus esculentus* by Sharma and Agrawal (2010). Interaction between Cd and Zn was found antagonistic at low Cd:Zn ratio. The synergistic interaction between Cd and Zn applied to loamy soil was also observed, where Zn uptake increased with Cd application (Smilde *et al.,* 1992; Moustakas *et al.,* 2011). In a hydroponics study with *L. sativa* and *S. oleracea*, McKenna *et al.* (1993) found that Cd increased Zn uptake in young leaves of *L. sativa*, while Moraghan (1993) reported that Zn reduced Cd concentration by 50% in *Linum usitatissimum and Triticum aestivum, respectively*. Zhao *et al.* (2011) reported that addition of Zn alleviated cadmium toxicity in *Triticum aestivum* if zn/Cd ration was >10/1. Reports on the interactive effects of Cd and Zn and their tissue concentrations

Table 1: Cd and Zn concentration in roots and shoots of *B. vulgaris* grown under different metal treatments

Treatment (mg l^{-1})	Cadmium (mg kg^{-1} d.wt.)		Zinc (mg kg^{-1} d.wt.)	
	Root	Shoot	Root	Shoot
0	4.40 ± 0.10^{e}	5.88 ± 0.04^{d}	41.40 ± 1.85^{d}	63.93 ± 0.75^{d}
Cd - 10	14.77 ± 0.60^{d}	16.97 ± 0.47^{c}	43.68 ± 1.23^{d}	66.47 ± 1.17^{d}
Zn - 100	4.79 ± 0.06^{e}	4.95 ± 0.09^{d}	62.98 ± 1.08^{c}	96.67 ± 1.17^{c}
Cd+Zn (10; 100)	24.26 ± 0.72^{a}	23.41 ± 0.96^{a}	86.32 ± 2.28^{b}	136.37 ± 5.69^{b}
Cd - 100	18.20 ± 0.60^{c}	20.30 ± 0.25^{b}	42.72 ± 1.18^{d}	67.22 ± 1.23^{d}
Zn - 300	4.07 ± 0.12^{e}	4.97 ± 0.04^{d}	86.33 ± 1.70^{b}	136.70 ± 3.56^{b}
Cd+Zn (100; 300)	20.17 ± 0.76^{b}	21.27 ± 0.96^{b}	110.23 ± 3.25^{a}	194.73 ± 5.85^{a}

Values are mean ± SE of six replicates in a column followed by the different letters are significantly different, pd"0.05 (Ducan's Multiple Range Test)

Table 2 : Effects of Cd and Zn, singly and in combination on the biochemical responses of *B. vulgaris* plant

Treatments (mg l^{-1})	Ascorbic acid (mg g^{-1} f.wt.)	Total phenols (mg g^{-1} f.wt.)	Proline (mg g^{-1} f.wt.)	Total peroxidases (μM pur. form min^{-1} mg^{-1} f.wt.)	Thiols contents (μM g^{-1} d.wt.)
Control	1.45 ± 0.14^{e}	4.43 ± 0.53^{d}	49.25 ± 1.80^{d}	6.89 ± 0.68^{e}	2.13 ± 0.05^{d}
Low					
Cd – 10	2.45 ± 0.28^{d}	14.69 ± 1.17^{c}	64.69 ± 2.50^{c}	11.67 ± 0.86^{c}	2.37 ± 0.11^{c}
Zn – 100	1.86 ± 0.09^{e}	7.64 ± 0.52^{d}	60.68 ± 1.07^{c}	9.41 ± 0.37^{e}	2.47 ± 0.10^{c}
Cd+Zn(10; 100)	4.35 ± 0.58^{b}	18.69 ± 1.19^{b}	100.48 ± 3.39^{a}	18.97 ± 0.92^{b}	2.73 ± 0.22^{b}
High					
Cd – 100	3.37 ± 0.20^{c}	20.41 ± 1.05^{b}	82.52 ± 1.75^{b}	15.82 ± 0.63^{b}	2.76 ± 0.10^{b}
Zn – 300	2.68 ± 0.20^{d}	14.41 ± 1.16^{c}	69.84 ± 1.05^{c}	16.26 ± 1.42^{b}	2.58 ± 0.01^{bc}
Cd+Zn(100; 300)	6.43 ± 0.56^{a}	26.86 ± 1.35^{a}	98.81 ± 3.95^{a}	25.56 ± 0.47^{a}	3.45 ± 0.24^{a}

Values (mean ± SE of six replicates) in a column followed by the different letters are significantly different, p≤0.05 (Ducan's Multiple Range Test)

are not consistent (Nan *et al.,* 2002; Peralta-Videa *et al.,* 2003; Sharma and Agrawal, 2006).

In the present study, shoots of *B. vulgaris* accumulated slightly more Cd and Zn as compared to roots (Table 1). In contrast and Zn in pigeon pea (Paivoke, 2003). Zn concentration in different plant parts was not influenced by Cd treatments. This might be due to lower affinity of Cd for membrane transporter proteins in root cells as compared to Zn. Both Cd and Zn enter into root cells via a common membrane transporter protein. Zn also showed competitive inhibition of Cd uptake in *Glycine max* and in seedlings of *T. aestivum* (Hart *et al.,* 2002).

Effects of single and combined treatments of Cd and Zn at low and high doses on biochemical and physiological characteristics of *B. vulgaris* plant are shown in Tables 2, 3 and Figs. 1, 2. High dose of Cd+Zn produced more free radicals, resulting in more lipid peroxidation, increased ascorbic acid, total phenolics, thiols, proline and total peroxidase as compared to other treatments. Zn is an essential heavy metal for plants but becomes toxic in excess amount. Excessive amount of Zn in soil produces stress conditions in plants and lead to increased amount of lipid peroxidation, ascorbic acid, proline and thiol. The threshold level of Zn in leaf tissue (300-500 mg kg^{-1} d.wt.) produces toxicity in plants as reported by Mohammad and Moheman (2009). In the present study, low concentration of Zn (100 mg l^{-1}) did not produce toxic effect to the plant, however high level (300 mg l^{-1}) produced toxic effect. The maximum decrease in photosynthetic pigments revealed that Cd+Zn at higher concentration were more toxic than lower concentration. Low dose of Cd+Zn caused a maximum reduction in photosynthetic rate, followed by the high dose of Cd+Zn, and Cd alone; the least was found for Zn as compared to control (Fig. 1). The Fv/Fm ratio was significantly influenced by the combined treatments of both Cd and Zn (Fig. 2).

High accumulation of Cd and Zn in leaves enhanced production of free radicals that caused membrane damage resulting in lipid peroxidation. Results of the present study showed increased lipid peroxidation and higher production of antioxidants and lower production of protein and photosynthetic pigment contents which suggests that exposure of *B. vulgaris* to both the heavy metals caused greater production of free radicals in foliage (Tables 2 and 3). *B. vulgaris* and *Abelmoschus esculents* grown on sewage sludge amended soil and Cd contaminated soil, respectively also showed increased levels of all the above parameters (Singh and Agrawal 2007; Sharma *et al.,* 2010; Siddhu and Ali-Khan 2012). Net photosynthetic rate was significantly affected by the combined applications of Cd and Zn both at low and high concentrations and showed greater negative effect as compared to individual treatments (Fig.1). Earlier studies have reported a marked reduction in photosynthesis rate due to Cd exposure (Sharma *et al.,* 2010) and other heavy metals through sewage sludge amendment (Singh and Agrawal, 2007). Cd and Zn showed negative effects on leaf area, dry matter production and biomass allocation (Table 4). The reduction in leaf area and total dry matter of *B. vulgaris* by 50 and 73%, respectively occurred at higher dose of Cd+Zn (Table 4).

The reduction of biomass due to Cd toxicity was ascribed to inhibition of chlorophyll biosynthesis and photosynthesis. The reduction in leaf area of *B. vulgaris* due to transfer of heavy metal from sewage sludge amended soil have also been reported by Singh and Agrawal (2007) and due to interaction between Cd and Zn in *Daucus carota* (Sharma *et al.,* 2008). A decrease in leaf area and dry weight of *Phaseolus mungo* due to increased Cd

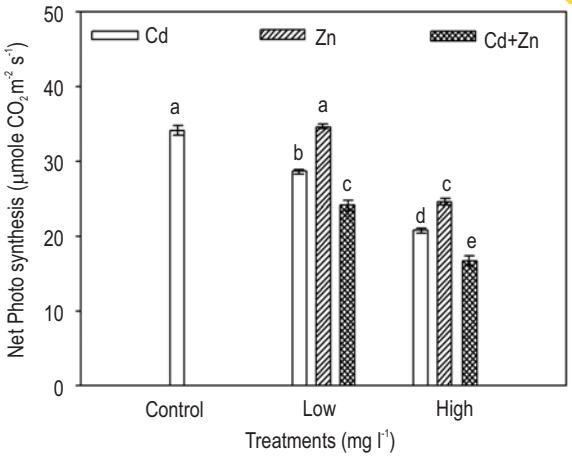

Fig. 1 : Effect of low and high concentrations of Cd and Zn on net photosynthesis in 45 day old leaves of *B. vulgaris* plants. Bars (mean ± SE of six replicates) bearing different letters are significantly different from other at $p \leq 0.05$ (Ducan's Multiple Range Test). Low : Cd 10 mg l^{-1}, Zn 100 mg l^{-1}; High: Cd 100 mg l^{-1}, Zn 300 mg l^{-1}

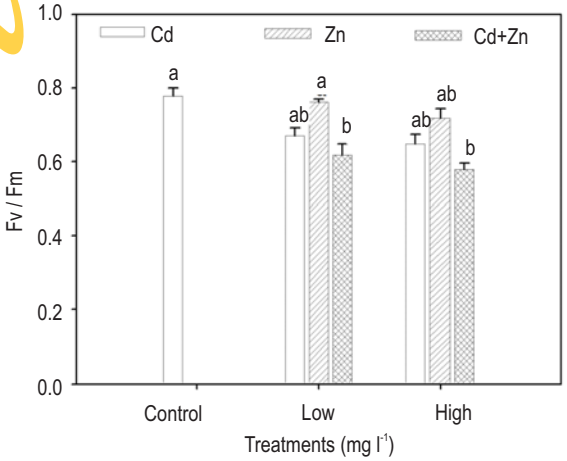

Fig. 2 : Effect of low and high concentrations of Cd and Zn on the Fv/Fm ration of 45 days old leaves of *B. vulgaris*. Bars (mean ± SE of six replicates) bearing different letters are significantly different from other at $p \leq 0.05$ (Ducan's Multiple Range Test). Low: Cd 10 mg l^{-1}, Zn 100 mg l^{-1}; High: Cd 100 mg l^{-1}, Zn 300 mg l^{-1}

Table 3 : Effect of Cd and Zn on the protein content, lipid peroxidation and photosynthetic pigments in leaves of *B. vulgaris* plant

Treatments	Protein content	Lipid peroxidation	Photosynthetic pigments (mg g^{-1} d.wt.)			
(mg l^{-1})	(mg g^{-1} DW)	(ng ml^{-1})	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid
Control	16.14 ± 0.99[a]	4.98 ± 0.47[c]	0.83 ± 0.02[a]	0.71 ± 0.01[a]	1.54 ± 0.03[a]	0.51 ± 0.01[a]
Low						
Cd – 10	12.72 ± 0.81[b]	6.53 ± 0.53[bc]	0.70 ± 0.02[b]	0.63 ± 0.01[b]	1.33 ± 0.01[b]	0.36 ± 0.01[c]
Zn – 100	13.91 ± 0.32[a]	5.34 ± 0.43[c]	0.78 ± 0.02[a]	0.76 ± 0.01[a]	1.55 ± 0.03[a]	0.44 ± 0.01[b]
Cd+Zn(10; 100)	10.29 ± 1.26[c]	6.77 ± 0.29[b]	0.56 ± 0.01[d]	0.53 ± 0.03[d]	1.09 ± 0.03[d]	0.28 ± 0.02[d]
High						
Cd – 100	9.43 ± 0.98[c]	8.57 ± 0.70[a]	0.63 ± 0.01[c]	0.60 ± 0.02[bc]	1.23 ± 0.03[c]	0.39 ± 0.02[c]
Zn – 300	11.51 ± 0.79[b]	8.41 ± 0.32[a]	0.71 ± 0.02[b]	0.63 ± 0.02[b]	1.34 ± 0.02[b]	0.46 ± 0.01[b]
Cd+Zn(100; 300)	8.98 ± 0.95[d]	9.53 ± 0.94[a]	0.61 ± 0.01[cd]	0.57 ± 0.02[cd]	1.17 ± 0.01[c]	0.36 ± 0.01[c]

Values (mean ± SE of six replicates) in a column followed by the different letter are significantly different, p>0.05 (Ducan's Multiple Range Test)

Table 4 : Effect of Cd and Zn on the leaf area, total plant dry matter production and biomass allocation pattern in *B. vulgaris* plant

Treatments (mg l^{-1})	Leaf area (cm^2 $plant^{-1}$)	Total dry wt (g $plant^{-1}$)	Root : Shoot ratio (g g^{-1})
Control	433.54 ± 8.81[a]	3.57 ± 0.08[a]	0.26 ± 0.005[d]
Low			
Cd – 10	363.28 ± 5.20[b]	1.48 ± 0.03[b]	0.57 ± 0.023[b]
Zn – 100	404.07 ± 10.32[a]	3.47 ± 0.04[a]	0.26 ± 0.005[d]
Cd+Zn(10; 100)	210.43 ± 6.06[c]	1.18 ± 0.04[c]	0.57 ± 0.013[b]
High			
Cd – 100	250.61 ± 8.56[c]	1.05 ± 0.03[d]	0.53 ± 0.007[c]
Zn – 300	367.77 ± 8.90b	1.50 ± 0.03[b]	0.57 ± 0.017[b]
Cd+Zn(100; 300)	280.48 ± 11.41[c]	0.74 ± 0.01[e]	0.60 ± 0.032[a]

Values (mean ± SE of six replicates) in a column followed by the different letter are significantly different, p>0.05 (Ducan's Multiple Range Test)

concentrations was also reported by Siddhu and Ali-Khan (2012). Siddhu *et al.* (2008) have further reported the toxic effect of Cd on the growth and yield of *Solanum melongena* plants. Recently, Garg and Kaur (2013) reported that combined treatment of Cd and Zn (25+1000) was effective in increasing the yield of *C. cajan* plant.

It can be concluded from the findings of the present study that combined treatment of Cd and Zn was more toxic than single treatment of Cd or Zn. Zn may be applied to Cd contaminated soil to reduce uptake and accumulation of Cd in plants, however, the suitable dose and toxicity of Zn needs to be worked out for its use under field conditions.

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