

CADMIUM INDUCED SEEDLING MORTALITY, FOLIAR TOXICITY SYMPTOMS AND PLANT GROWTH IN MUSTARD (*Brassica campestris* L.)

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ABSTRACT

Cadmium toxicity is one of the major abiotic stresses that adversely affects growth, development, productivity of crop plants. It is highly soluble in water and soil and therefore is an extremely powerful pollutant. Cadmium disturbs the function of chloroplasts by accumulating to higher levels in aerial parts. It inhibits the enzymes needed for chlorophyll biosynthesis as well as enzymes for carbon dioxide fixation i.e., Ribulose-1, 5-biphosphate carboxylase (RUBPCase) and phosphoenol pyruvate carboxylase (PEPCase). *Brassica campestris* were grown in the soil supplemented with different concentrations of Cadmium (0, 50 and 100 mg kg⁻¹ soil). Seedling mortality, foliar toxicity symptoms and plant growth in mustard (*Brassica campestris* Linn.), were studied. A significant decline in the shoot length was observed in the plants when treated with Cadmium at various concentrations i.e., 0, 50 and 100 mg kg⁻¹ soil in the form of Cadmium chloride. Root development observed at different concentrations of Cadmium showed constant decline. A significant decline in the number of nodes and internodes, number of leaves, branches, was observed when the plants were treated with Cadmium at different concentrations. Thus it was observed that Cadmium treatment resulted in the decreased number of leaves, branches, nodes and internodes.

Keywords: *Brassica campestris*, Cadmium, Ribulose-1, 5-biphosphate carboxylase (RUBPCase)

1. INTRODUCTION

Cadmium is highly toxic metal released into the environment by mining industrial activities, sewage sludge, use of fertilizers and atmospheric deposition and due to the rapid increase in concentration of Cd in environment has become toxic to agricultural soil causing effects on plants [1]. Cadmium is considered as the major environmental concern to the agricultural system with long biological half-life and even at low concentration is highly toxic [2]. Cadmium is a phototoxic non-essential heavy metal commonly added to the agricultural soils through anthropogenic activities. It is an extremely powerful pollutant and due to its high toxicity and greater solubility in soil and water it has high potential to affect plant growth adversely continuously by increasing overtime [3]. Once Cd enters into the plant it produces deleterious effects on both plants and animals. Cadmium

can affect the plants from very beginning of their life cycle i.e., seed germination to production of grains. Cadmium can be accumulated to higher levels in the aerial organs [4], receiving in the chloroplast and disturbs the functions of chloroplast by inhibiting the activity of enzymes needed for the chlorophyll biosynthesis and CO₂ fixation [5][6] or the aggregation of pigment protein complexes of the photosystems [7]. Excess concentration of cadmium leads to the production of reactive oxygen species (ROS), such as superoxide anion (O₂⁻) hydroxyl (OH⁻) radicles and hydrogen peroxide (H₂O₂) and cause damage to the plants metabolic reactions[8][3]. Cadmium toxicity in plants causes inhibition of photosynthesis electron chain PS II, alters enzyme structure by interaction with sulfhydryl groups, lipid peroxidation inhibition of ATPase activity, disruption of channels and transporters [9]. At the molecular level, Cadmium has been attributed to (1) blocking of essential functional groups in biomolecules [10], (2) displacement of essential metal ions from biomolecules [11] and (3) production of ROS by autoxidation and Fenton reaction [12][13][14]. Cd²⁺ accumulation also affects stomatal opening by interacting with the balance of the water, disturbs the Calvin cycle enzymes, photosynthesis and carbohydrate metabolism, changes the antioxidant metabolism, and lowers the crop productivity. Cadmium at high concentration leads to the formation of (ROS) which includes superoxide anion (O₂⁻), hydroxyl (OH⁻) free radicles and H₂O₂[15] and thiobarbituric acid reactive substances(TBARS) and electrolyte leakage causing oxidative stress [16] that leads to the oxidative damage to the membrane, photosynthetic pigments, biomolecules such as lipids, proteins, and nucleic acids resulting in dramatic reduction of growth and productivity and eventually causing death of plants. It has been reported that Cd produces DNA strand breaks, DNA-protein cross links, oxidative DNA damage, chromosomal aberrations, dysregulation of gene expression resulting in the increase in proliferation, depressed apoptosis, and changed DNA repair [3].

Brassica campestris L. flowers contain *flavonol glycoside*. Seeds possess a glycoside sinigrin or potassium myronate, 35 to 50% fatty oil and about 20% protein. Seeds are used in exacerbations, cancer and tumours. Roots are emollient and diuretic and its juice is used in chronic cough and bronchial catarrh. The essential oil of mustard is an extremely powerful irritant, causing severe blisters on the skin and therefore is used as a counter irritant in highly dilute concentrations. Cd is not essential for plants, in several species Cd is easily taken up by the roots and readily translocated and accumulated in shoots [17][18]. Elevated levels of Cd have been shown to inhibit seed germination, cell growth, as well as plant growth, nutrient uptake and distribution lead to inhibit photosynthesis ultimately resulting in reduced growth and productivity. After exposure to Cd reduction of root and shoot elongation, browning and decomposing of roots, rolling of leaves and chlorosis can occur. It was found that Cd inhibit lateral root formation while the main root becomes rigid, brown and twisted [19][20][21]. The aim of this work was to characterize the response of *Brassica campestris* L. seedlings grown in presence of two different concentrations of cadmium and to obtain more information about the effect of cadmium on growth parameters.

2. MATERIALS AND METHODS

Experimental site and design:

The study was conducted at the botanical garden of Jamia Hamdard University which is located in New Delhi, North India. The experiment was carried out between the months of October and January. 20 pots filled with 2.5 kg of soil were used.

Seeds of *Brassica campestris* L. variety PT-303 and TL-15 were sown in pots filled with 2.5 kg of soil. On the same day basal treatment was given to each pot which contains urea 15 gms, phosphorous 6.80 gms, potassium 4.54 gms and ZnSO₄ 3.14 gms. The treatment of Cadmium in the form of Cadmium Chloride was given after the seeds were fully established. Two different concentrations of cadmium was used along with the control. The treatment of Cadmium was given to both varieties PT-303 and TL-15 of *Brassica campestris* L. in the form of Cadmium Chloride at various concentrations i.e. 0, 50 and 100 mg kg⁻¹ soil.

All parameters were studied at the pre flowering stage of the plants [22]. The parameters studied were seedling survival, leaf toxicity (chlorosis), shoot length, root length, number of nodes, number of branches. At the pre flowering stage symptoms of cadmium toxicity were studied and compared with control. Number of seedlings survived were noted in both varieties at both concentrations of Cadmium and were compared with that of control. Leaf toxicity was observed and compared with that of control. Plants were then harvested and washed carefully to remove the dust particles and then they were blotted with blotting paper. Data were recorded on the root and shoot length, number of nodes and number of branches. Root and shoot length were measured in centimetres and were compared with that of control.

3. RESULTS

Seedling survival:

Plants were grown in 100 and 50 mg of cadmium per kilogram of soil showed less survival rate in both varieties as compared to control as shown in fig 1. The seedling survival rate was better in V1 at 50 mg concentration of cadmium per kilogram of soil than that of V2. The seedling survival rate was same in both varieties at 100 mg of cadmium per kg of soil. This clearly indicates the toxic effect of cadmium on seedling survival rate.

Growth parameters:

A significant decline in the shoot length was observed in the plants when treated with cadmium at various concentrations i.e. 0, 50 and 100 mg kg⁻¹ soil in the form of cadmium chloride. Under control conditions maximum shoot length were produced however at 100 mg kg⁻¹ cadmium level these attributes were reduced in both varieties as shown in fig 2.

When plants were treated with cadmium a decline in the root growth, number of nodes and number of internodes was observed at different concentrations of cadmium as shown in fig 3, 4 and 5 respectively. Plants grown in cadmium showed foliar toxicity such as chlorosis in addition other growth damages. The percentage of chlorosis in cadmium treated plants was higher as compared to control. At 100 mg Cadmium per kg soil V2 showed high percentage of chlorosis than that of V1 as shown in figure 6.

4. DISCUSSION

It has long been recognized that the environmental pollutants have directed impact on the quality and production of various plants. Cadmium is one of the environmental pollutant which directly or indirectly affects the mineral uptake because of which almost every morpho-physiological and chemical processes in the plant were affected and thus leads to an influence on growth, development and yield of the plants however the degree of impact is greatly governed by the nutritional status of the plant and thus if the plants are provided with the appropriate amount of nutrient elements, the damage caused by the pollutants may be reduced. A retardation in the growth was found in the plants treated with the cadmium as it may cause many metabolic aberrations and imbalances in essential nutrients [23][24] and has been found to be toxic at concentration above 0.1 μM . It has been observed to alter the plasma membrane permeability and affecting element transport process across the membrane it interferes with the nutrient uptake [25][26][27]. It also reduces the availability of growth material to the plant by limiting the root access to water and nutrients [28]. It has been reported that it may interfere with cell division [29].

Chlorosis of leaf tissue may be due to Cadmium ion mobilization and its transport to the above ground parts of the plant decrease in iron uptake by roots resulting in inhibition of the formation of chloroplast through the inhibition of protein synthesis reported by [30][31] as well as may interfere with the normal absorption and translocation of nutrients [32].

5. FIGURES

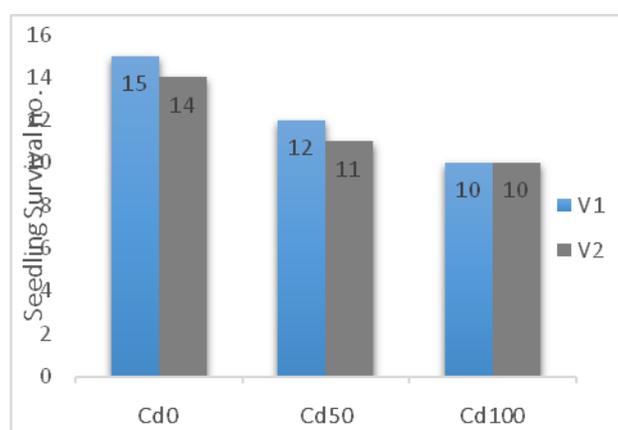


Fig 1. Effect of Cadmium on seedling survival rate.

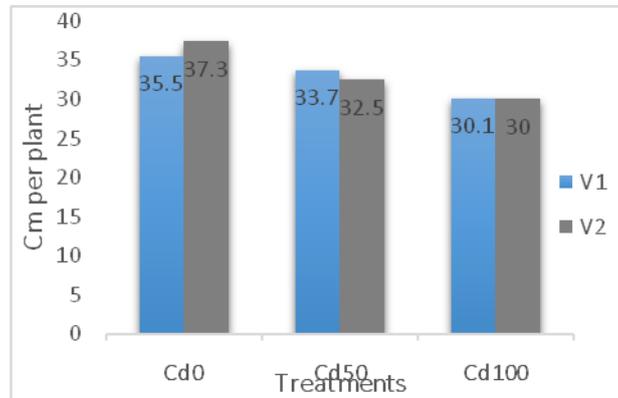


Fig 2. Effect of Cadmium on shoot length.

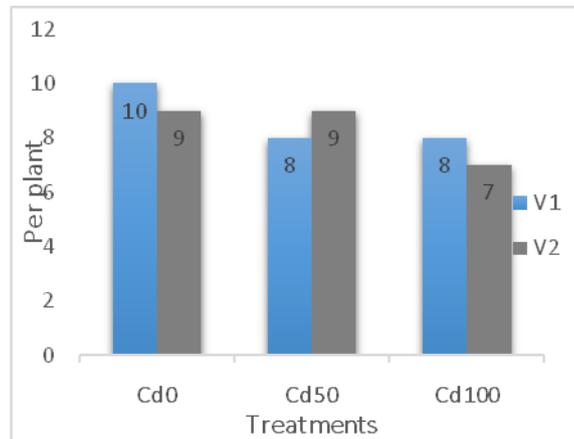
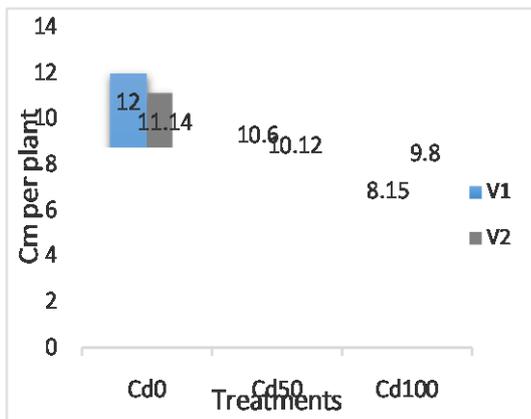


Fig 3. Effect of Cadmium on root length. Fig 4. Effect of Cadmium on number of nodes.

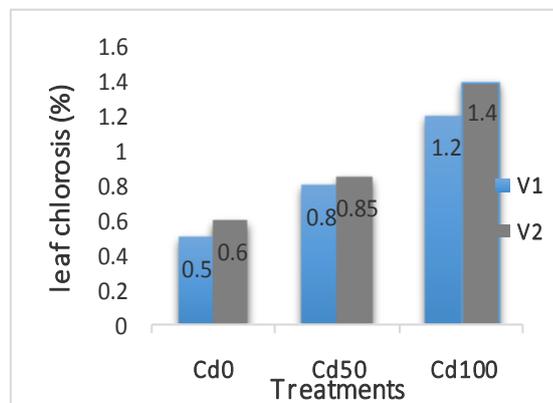
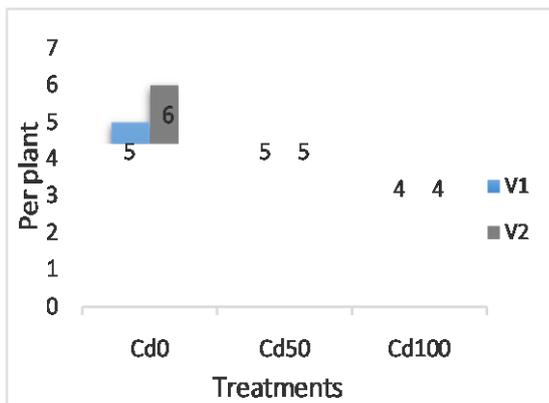


Fig 5. Effect of Cadmium on number of internodes.

Fig 6. Leaf chlorosis in response to Cadmium treatment.

6. CONCLUSION

Studies on cadmium induced seedling mortality, foliar toxicity symptoms, plant growth (*Brassica campestris* Linn.), was considered and various parameters were studied. The study showed differential response under different levels of cadmium as it enhanced the seedling mortality more notably at higher levels but decreased number of leaves, branches, nodes and internodes were observed and signs of chlorosis were recorded.

REFERENCES

- [1] Di Toppi, L.S. and R. Gabbrielli: Response to cadmium in higher plants, *Environ.Exp. Bot.*, 41, (1999), 105-130.
- [2] Wagner GJ Accumulation of cadmium in crop plants and its consequences to human health, *Adv Agron* 51, (1993), 173- 213.
- [3] Nazar R., Iqbal N., Syeed S., Khan N. A., Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. *J. Plant Physiol.*, 168, (2012), 807–815.
- [4] Pence, N. S., Larsen, P. B., Ebbs, S. D., Letham, D. L. D., Lasat, M. M., Garvin, D. F., Eide, D., and Kochian, V., The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*, *Proc Natl Acad Sci USA* 97, (2000), 4956–4960.
- [5] Böddi B., Oravec A.R., Lehoczki E. Effect of cadmium on organization and photoreduction of protochlorophyllide in dark-grown leaves and etioplast inner membrane preparations of wheat. *Photosynthetica* (Prague) 31(3), (1995), 411-420.
- [6] Krupa Z., Baszynski T., Some aspects of heavy metals toxicity towards photosynthetic apparatus – direct and indirect effects on light and dark reactions, a review, *Acta Physiol. Plantarum*, 17, (1995), 177-190.
- [7] Horvath, G., M. Droppa, A. Oravec, V. I. Raskin and J. B. Marder, Formation of the photosynthetic apparatus during greening of Cadmium poisoned barley leaves, *Planta*. 199, (1996), 238-243.
- [8] Takeda T, Yokota A, Shigeoka S, Resistance of photosynthesis to Hydrogen peroxide in algae. *Plant physiol* 36, (1995), 1089-1095.
- [9] Van Asshe, F., and H. Clijsters, Effects of metal on enzyme activity in plants. *Plant Cell Environment*, 13, (1990), 195-206.
- [10] Schutzendubel A., Polle A., Cadmium and H₂O₂ induced oxidative stress in *Populus x canescens* roots, *Plant Physiol. Biochem.*, 40, (2002), 577-584.
- [11] Rivetta A., N. Negrini, M. Cocucci, Involvement of Ca²⁺-calmodulin in Cd²⁺ toxicity during the early phases of radish (*Raphanus sativus* L.) seed germination, *Plant, Cell and Environment*, 20, (1997), 600-608.
- [12] Van Asshe, F. and H. Clijsters, Effects of metal on enzyme activity in plants. *Plant Cell Environment*, 13, (1990), 195-206.
- [13] Gallego S.M., Benavides M. P. & Tomaro M. L., Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress, *Plant Science*, 121, (1996), 151–159.

- [14] Chaoui A, Mazhoudi S., Ghorbal M. H., El Ferjani E., Cadmium and Zinc induction of lipid peroxidation and effect on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.), *Plant sci.* 127, (1997), 139-147.
- [15] Dixit P., Mukherjee P. K., Ramachandran V. & Eapen S., Glutathione transferase from *Trichoderma virens* enhances cadmium tolerance without enhancing its accumulation in transgenic *Nicotiana tabacum*, *PLoS One* 6(1), (2001), e16360. doi:10.137.
- [16] Mobin, M., and N. A. Khan, Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress, *J Plant Physiol*, 164, (2007), 601-61.
- [17] Hardiman R. T. & Jacoby B., Absorption and translocation of Cd²⁺ in bush beans (*Phaseolus vulgaris*), *Physiologia Plantarum* 61, (1984), 670–674.
- [18] Wagner G, Accumulation of cadmium in crop plants and its consequences to human health. In: Sparks DL (ed.). *Advances in Agronomy*, Newark, USA: Academic Press, (1994), pp. 173–212.
- [19] Krantev A., Yordanova R., Janda, T., Szalai G., Popova L., Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. - *J. Plant Physiol.*, 165, (2008), 920-931.
- [20] Yadav S. K., Heavy metals toxicity in plants, An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants, *South African Journal of Botany* 76, (2010), 167– 179.
- [21] Rascio N. & Navari-Izzo F. Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Science*, 180, (2011), 169–181.
- [22] Rama Rao, N. and G.S.Sekhon, Critical potassium levels in selected crops. *Gurgaon, Haryana Potash Research Institute of India*, (1989), p. 28-33.
- [23] Pahlsson A. M. B., Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants, *Water Air Soil Pollut*, 47, (1989), 287-319.
- [24] Van Bruwaene R., R. Kirchmann and R. Impens, Cadmium contamination in agriculture and zootechnology, *Experientia*. 40, (1984), 43-52.
- [25] De Filippis, L. F., The effect of heavy metal compounds on the permeability of *Chlorella* cells, *Z. Pflanzenphysiol.* 92, (1979), pp. 39-49.
- [26] Lindberg S. and G. Wingstrand, Mechanism for Cadmium inhibition of (K⁺, Mg²⁺) ATPase activity and K⁺ (⁸⁶Rb) uptake in roots of sugarbeet (*Beta vulgaris*). *Physiol. Plant*, 63, (1985), 181-186.
- [27] Gussarsson M. and P. Jensen, Effects of Copper and Cadmium on uptake and leakage of potassium ions in birch (*Bet-ula pendula*) roots. *Tree Physiol.*, 11, (1992), 905-313.
- [28] Godbold D. L. and A. Hutterman, Effect of Zinc, Cadmium and mercury on root elongation of *Piceaabies* (Karst.) seedlings, and the significance of these metals to forest die-back, *Environ.Pollut.*, 38, (1985), 375-381.
- [29] Vázquez M. D., Barcelo J., Poschenrieder C., Madico J., Hatton P., Baker A. J. M., Cope G. H., Localization of zinc and cadmium in *Thlaspi caerulescens* (Brassicaceae), a metallaphyte that can hyperaccumulate both metals, *J Plant Physiol*, 140, (1992), 350-355.
- [30] Naguib M. I., A. A. Hamed and S.A. A 1-Wakeel, Effect of Cadmium on growth criteria of some crop plants, *Egyptian J. Bot.*, 25 (1986), 1-12.

[31] Keshan T. J. and S. M-ukherji, Effect of Cadmium toxicity on chlorophyllase content, hill activity and chlorophyllase activity in *Vigna radiate* L. leaves, *Indian J. Plant Physio.*, Vol 35, No.3, (1992), 225-230.

[32] Moral R., I. G-omez, J. N. Pedreno, and J. Matax, Effects of Cadmium on nutrient distribution, yield and growth of tomato grown in soilless culture, *J.Plant Nutr.*, 17, (1994), 953-962.