Current Botany 2011, 2(8): 08-13
ISSN: 2220-4822
Available Online: http://currentbotany.org/

Effect of cadmium on seed germination, growth and pigments content of Zinnia plant

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Abstract

The present work made to the effect of cadmium on seed germination, growth and pigments content of *Zinnia elegans* (L.) plant. The experiment was conducted at Botanical Garden, Department of Botany, Annamalai University, Tamil Nadu, during the period of January to March 2011. In the pot culture experiment, Zinnia plants were analysed on three different sampling (viz., 15th, 30th and 45th) days, in soil amended with various levels of cadmium (10, 20, 30, 40, and 50 mg kg^{-1} soil). 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 (10,20,30,40 and 50mg kg^{-1}) tested, decreased the seed germination, growth parameters such as, root and shoot length, number of leaves, total leaf area and biochemical constituents such as, chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid contents of zinnia plants compared to untreated plants.

Keywords: Zinnia, Cadmium, seed germination, growth and biochemical

INTRODUCTION

Environment consists of both biotic and abiotic components. It creates favorable conditions for existence and development of living organisms nowadays. All organisms are mainly affected directly or indirectly because of the environment pollution or contamination. The pollution problem has become a global one. Pollution is an undesirable change in the physical, chemical or biological characteristics of out air, water and land or soil that will harmful to human and other life, industrial processes, living conditions and cultural assets (Spilhaus, 1966 and Odum 1975). Environmental pollution is the result of rapid industrialization, technological advancement and geometrically increases in human population.

Due to industrial emissions and the application of both sewage sludge and phosphate fertilizers containing cadmium (Cd), heavy metal pollution is now a serious environmental problem in the world (Davis, 1984). Cadmium is one of the most toxic heavy metals causing serious problems in crops (Prasad, 1995). It is widely recognized that cadmium taken up by plants is the main source of cadmium accumulation in food (Lopez-Millan et al., 2009). Cadmium can be easily absorbed by plant roots and transported to shoots (Sanita di Toppi and Gabbrielli, 1999), results in disorders in biochemical and physiological processes, and then affects plant growth and morphology (Sgherri et al., 2002). Roots are likely to be firstly affected by heavy metals since much more metal ions are accumulated in roots than shoots (Sanita di Toppi and Gabbrielli, 1999). Thus, Cd toxicity obviously inhibits plant root growth (Liu et al., 2003) and affects root morphology (Daud et al., 2009). In shoots, cadmium is reported to reduce chlorophyll content and inhibit leaf photosynthesis via suppressing biosynthesis of chlorophyll and function of the photochemical reaction centers (Chugh and Sawhney, 1999). Photosystem II (PSII) is known to be most affected by cadmium toxicity (Baker, 1991), and its damage or malfunction under stress can be easily detected by the changes of the chlorophyll a fluorescence parameters (Maxwell and Johnson, 2000). Cadmium enters into the environment through weathering of rocks, forest fires and volcanic eruptions. It may be naturally present in air, water, soil and foodstuffs. Rapid industrialization has increased the natural limit of cadmium to a toxic level. The present investigations extent of changes in growth parameters such as, root and shoot length, number of leaves 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.

MATERIALS AND METHODS

Seed materials

The certified seeds of *Zinnia elegans* (L.) were purchased from Tamil Nadu Agricultural University, (TNAU) Coimbatore, Seeds with uniform size, colour and weight were chosen for the experimental purpose.

Seed Germination

The Zinnia (*Zinnia elegans* L.) seeds were purchased from Tamil Nadu Agricultural University, (TNAU) Coimbatore. Twenty healthy seeds were surface sterilized with O.1% mercuric chloride solution (Ramasubramanian, et al.1993), 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, 10,20,30,40 and 50 ppm). The seeds were allowed to

Received: May 11, 2011; Revised August 23, 2011; Accepted August 23, 2011.

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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, Annamalai University, Tamil Nadu, during the period of January to March-2011. Surface sterilized Zinnia seeds were sown in pots (15 cm in diameter) containing mixture of sandy loam soil in nature, zinnia plants were grown in pots containing untreated soil (Control) and soil mixed with various levels of cadmium (viz., 10,20,30,40 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.

Sampling

The plant samples were collected on 15th 30th and 45th days after sowing. Three plants from each replicates of a 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, number of leaves 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 Kalra and Dhiman, (1977). Leaves as treated and control plants were used for the estimation of chlorophyll-a, chlorophyll-b and total chlorophyll as per Arnon, (1949), Carotenoids as per Kirk and Allen, (1965) method.

EXPERIMENTAL RESULTS

Physio-chemical properties of the soil

The pot culture experiments were conducted in Botaniocal Garden, Department of Botany, Annamalai University, Tamil Nadu, India. The soil condition was sandy loam in nature and pH, EC, organic carbon and available macro and micro nutrients are given in table -1.

Germination

Germination percentage values of zinnia cultivars under cadmium treatments of different concentrations were presented in (Fig- 1). The highest germination percentage of zinnia (*Zinnia elegans*) was recorded at control. The lowest germination percentage of zinnia due to cadmium treatment at 50 mg/l concentration. These observations are in accordance with the findings of (Hsu, F.R. and C. Hung Chou, 1992.) who determined the inhibitory effects of some heavy metals ($CdCl_2$, $CuS0_4$, $PbCl_2$, $HgCl_2$) on Miscanthus species. The same pattern of response was noticed in the case of *Raphanus sativus* L. due to cadmium treatment by Vijayaragavan et al. (2007). 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 (Poschenreider, et al, 1989.). In addition, Barcelo, et al. (1986) 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 zinnia plants has been adversely affected due to cadmium treatment, when compared to the control (Table -2, 3, 4). 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 50 mg kg^{-1} in the soil in all the sampling days. The above results were in agreement with the findings of Chen, et al. (2003) in soybean, Rai, et al. (2005) in *Phyllanthus amarus* and Xu, et al. (2008) 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 Kupper, et al. (1996). Hagemeyer et al. (2002.) and Marcnano et al. (2002) 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 Barcelo and Poschenrieder (1990, Gabbrielli, et al. (1990) and Sanita di Toppi and Gabbrielli (1999).

Total leaf area showed a decreasing trend with progressive increase in cadmium level in zinnia in all the sampling days. They are decreased with increase in cadmium level in the soil. Similar observations were made by Schutzendubel and Polle (2002) in *Populus canescens*, Zhou and Qiu (2005) in *Sedum alfredii*. Our results are in agreement with the findings of Filippis and Ziegler, (1993), Fodor, et al. (1996) and Skorzynska-Polit and Baszynski (1997) 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.

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	-

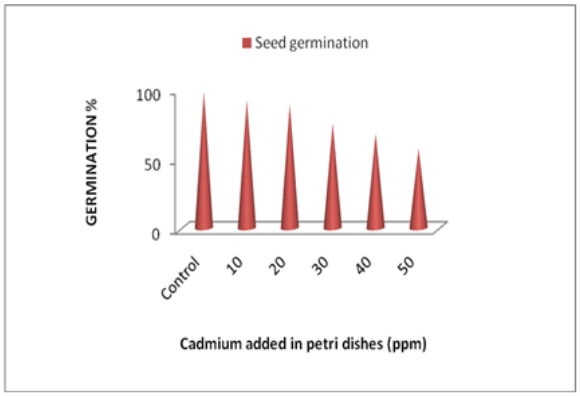

Fig-1 Effect of cadmium on seed germination of cowpea on 15th Days

Table 2. Effect of cadmium on morphological parameters of zinnia on 15 days

Cadmium treatment (mg kg^{-1})	Root length (cm)	Shoot length (cm)	Total No of Leaves	Leaf area (cm^2)
Control	13.04	21.08	6.00	74.69
10	12.03 ±0.03	20.51 ±0.05	5.00 ±0.003	66.74 ±0.19
20	11.05 ±0.03	19.55 ±0.05	4.60 ±0.003	62.78 ±0.17
30	10.08 ±0.02	16.72 ±0.04	4.40 ±0.002	60.14 ±0.17
40	9.64 ±0.27	15.76 ±0.04	4.00 ±0.002	57.83 ±0.16
50	8.75 ±0.25	13.62 ±0.03	3.50 ±0.001	51.58 ±0.14

Table 3. Effect of cadmium on morphological parameters of zinnia on 30 days

Cadmium Treatment(mg kg^{-1})	Root length (cm)	Shoot length (cm)	Total No of Leaves	Leaf area (cm^2)
Control	17.05	26.03	9.62	135.78
10	16.02 ±0.04	25.07 ±0.07	8.75 ±0.25	122.69 ±0.03
20	15.50 ±0.04	24.05 ±0.06	8.61 ±0.24	108.75 ±0.03
30	13.75 ±0.03	22.74 ±0.06	7.45 ±0.21	100.26 ±0.02
10	10.93 ±0.03	21.65 ±0.06	7.05 ±0.20	96.78 ±0.27
50	9.85 ±0.28	18.27 ±0.05	6.55 ±0.18	86.46 ±0.24

Table 4. Effect of cadmium on morphological parameters of zinnia on 45 days

Cadmium treatment (mg kg^{-1})	Root length (cm)	Shoot length (cm)	Total No of Leaves	Leaf area (cm^2)
Control	22.75	37.90	20.32	173.74
10	20.81 ±0.059	35.76 ±0.102	19.24 ±0.055	165.81 ±0.047
20	18.68 ±0.053	34.63 ±0.098	17.25 ±0.049	159.4 ±0.045
30	16.55 0.047	32.71 ±0.093	16.84 ±0.048	134.79 ±0.038
40	14.61 ±0.042	30.55 ±0.087	15.76 ±0.045	127.83 ±0.036
50	13.87 ±0.039	28.31 ±0.081	12.62 ±0.036	115.55 ±0.033

Table 5. Effect of cadmium on photosynthetic pigment contents (mg kg^{-1} fr. Wt) of Zinnia on 15 days

Cadmium treatment(mg kg^{1})	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll	Carotenoid
Control	0.640	0.563	1.203	0.573
10	0.607 ±0.018	0.510 ±0.014	1.117 ±0.031	0.508 ±0.014
20	0.596 ±0.017	0.476 ±0.013	1.072 ±0.030	0.485 ±0.013
30	0.568 ±0.016	0.435 ±0.012	1.003 ±0.029	0.407 ±0.012
40	0.532 ±0.015	0.398 ±0.011	0.930 ±0.026	0.391 ±0.011
50	0.481 ±0.014	0.362 ±0.010	0.843 ±0.024	0.374 ±0.010

Table 6. Effect of cadmium on photosynthetic pigment contents (mg kg^{-1} fr. Wt) of Zinnia on 30 days

Cadmium treatment(mg kg^{-1})	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll	Carotenoid
Control	0.781	0.653	1.434	0.654
10	0.765 ±0.022	0.641 ±0.018	1.406 ±0.040	0.558 ±0.016
20	0.761 ±0.021	0.635 ±0.018	1.396 ±0.039	0.516 ±0.015
30	0.750 ±0.021	0.624 ±0.017	1.374 ±0.039	0.494 ±0.014
40	0.722 ±0.020	0.615 ±0.017	1.358 ±0.038	0.416 ±0.012
50	0.698 ±0.019	0.501 ±0.014	1.259 ±0.035	0.396 ±0.011

Table 7. Effect of cadmium on photosynthetic pigment contents (mg kg^{-1} fr. Wt) of Zinnia on 45 days.

Cadmium treatment(mg kg^{-1})	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll	Carotenoid
Control	0.889	0.765	1.654	0.781
10	0.871 ±0.025	0.749 ±0.021	1.620 ±0.046	0.763 ±0.021
20	0.852 ±0.243	0.735 ±0.021	1.587 ±0.045	0.691 ±0.019
30	0.841 ±0.024	0.729 ±0.020	1.570 ±0.044	0.553 ±0.016
40	0.798 ±0.023	0.697 ±0.019	1.495 ±0.042	0.502 ±0.014
50	0.696 ±0.019	0.545 ±0.015	1.240 ±0.035	0.486 ±0.014

Pigment content

The photosynthetic pigments chlorophyll '*a*' chlorophyll '*b*' total chlorophyll and Carotenoids content of zinnia under cadmium stress is represented in (Table 5, 6, 7). The highest photosynthetic pigments of zinnia chlorophyll a, chlorophyll b, total chlorophyll and Carotenoids content were recorded at control plants in all the sampling days. The lowest photosynthetic pigments of zinnia due to cadmium at 50 mg kg^{-1} concentration. The results have been in conformity with the findings of Sharavanan et al. (1997) due to cadmium on *Vigna unguiculata*. Our results are in consonance with the findings of Padmaja, et al. (1990) 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 Stobart, et al. (1985).

CONCLUSION

The results of the present study have shown that cadmium treatment was inhibitory to seed germination, plant growth and biochemical constituents of Zinnia plants, when compared to control plants. The loss of these may be due to 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 Zinnia 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 Zinnia plants was higher than the root length.

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