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Effects of cadmium on growth and metabolism of *Phaseolus mungo*

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Abstract

An attempt has been made to assess the response of *Phaseolus mungo* L. under influence of cadmium chloride ($Cd\,Cl_2$) with special reference to growth, morphology, yield and biochemical aspects. Surface sterilized seeds of *Phaseolus mungo* L. cv. T-9 were exposed to various concentrations of $Cd\,Cl_2$ solution (10^{-2} M, 10^{-4} M, 10^{-5} M, 10^{-8} M and control) for 12 hr at room temperature and these seeds were transferred to petriplates and polythene bags in triplicate. 10^{-2} M conconcentration was found to have deleterious effects on seed germination, germination relative index, length and dry weight of root and shoot, shoot root ratio and seedling vigour index, plant height, phytomass, number of leaves and branches, leaf area and chlorophyll contents while 10^{-8} M revealed slightly promotory effects. Phytotoxicity percentage and chlorophyll stability index were maximum in (10^{-2} M) concentration, while minimum in 10^{-8} M conconcentration of $Cd\,Cl_2$. Nitrate and nitrite reductase activity was markedly inhibited at higher conconcentration. Low dose of Cd (10^{-8} M) did not affect soluble sugar contents of seeds but it induced a significant increase at higher conconcentration (10^{-2} M). It however, did not affect protein contents of seeds accept at higher concentration.

Key words

Phaseolus mungo, Cadmium, Nitrate reductase activity, Sugar and protein contents

Introduction

Heavy metals are known to cause membrane damage, structural disorganization of organelles impairment in the physiological functioning of the plants and ultimately growth retardation (Chien and Kao, 2000; Long *et al*., 2003). Industrial and agricultural activities have led to a considerable increase in heavy metals in the different environmental compartments, especially in soil over the course of recent decade. There are some reports which indicate that heavy metals pollution including cadmium (Cd) in soil arises as a result of various anthropogenic activities such as continuous use of sewage water (Brar *et al*., 2002), sewage sludge (Singh and Sakal, 2001) and fertilizers (Indra and Sivaji, 2006). Heavy metals pollution in the environment is quite relevant in the present scenario due to other delterious effect on human health via food chain because they cause damage at DNA level and their carcinogenic effects in animals and humans are probably caused by their mutagenic ability (Knasmuller *et al*., 1998). Among an array of heavy metals, Cd is a hazardous metal causes 'Hormesis' in plants (Calabrese and Baldwin, 1997) and hypertension, anemia and liver damage in human beings. Cadmium also reduced the absorption of nitrate and its transport from roots to shoots by inhibiting nitrate reductase activity in the shoots (Hernandez *et al.,* 1996). The inhibition of root Fe (III) reductase induced by Cd led to Fe (II) deficiency and it seriously affected photosynthesis (Alcantara *et al.,* 1994).

Adid and Okamato (1992) observed that decrease in growth of roots and shoots was due to the suppression of the elongation growth rate of cells, specially in the stem, because of an irreversible inhibition exerted by Cd on the proton pump responsible for the process. Inhibiting effects of Cd stress on seed germination and seedling growth of *Phaseolus mungo* L. have been observed by Kalyanraman and Sivagurnathan (1993). Cadmium most severely inhibits plant growth and even cause plant death by disturbing the uptake of nutrients (Gussarson *et al.*, 1996; Sandalio *et al.*, 2001) and inhibiting photosynthesis via degradation of chlorophyll (Somashekaraiah *et al.*, 1992). Cadmium also acts as inhibitor of nitrate reductase enzyme (Keshan and Mukherjee, 1994) and also induce oxidative damage characterized by an accumulation of lipid peroxides and oxidized proteins as a result of the inhibition of antioxidant systems in plants (Sandalio *et al.*, 2001; Vitoria *et al.*, 2001).

Present study was conducted to compare the physiological, biochemical, growth and yield response of perishable, legume *Phaseolus* mungo L. cv T-9 grown in Cd contaminated soil.

Materials and Methods

Uniform healthy seeds of *Phaseolus mungo* L. cv.T-9 were obtained from Indian Agricultural Research Institute, New Delhi-12 and were made surface sterilized with 0.1% $HgCl_2$ solution. Cadmium solution was prepared by dissolving the molecular weight of cadmium chloride (Cd Cl_2) in 1 l of Hoagland's nutrient solution. This solution was known as 1 M solution of Cd and served as a stock solution (S.S.), other molar concentration (10^{-1} to 10^{-8} M) were prepared from this 1 M (one molar) solution.

In the present investigation, there were two methods of Cd treatment employed *i.e.* presoaking and irrigation. For presoaking treatment, sterilized seeds of *Phaseolus mungo* L. were imbibed upto 12 hr in the different molar concentration of Cd *viz.* (10^{-2}, 10^{-4}, 10^{-5}, 10^{-8} M and control) and for irrigation process metal was also given to soil in the form of above molar concentration before sowing. The surface sterilized seeds were sown in polythene bags in triplicate containing 10 kg of sandy loam soil (pH 7.45). The experiment was conducted at Environmental Science Laboratory during the year 2004, 2005 and 2006. Observations on seedling growth, morphological and biochemical attributes were recorded. Data were analysed statistically and significant value critical difference at 5% level were calculated. Seedling vigour index was calculated according to Abdulbaki and Anderson (1973) and phytotoxicity percentage of root and shoot was measured by the formula derived by Chou and Muller (1972). Chlorophyll contents were estimated by adopting the method of Smith and Benetiez (1955). Chlorophyll stability index was determined according to Meena *et al.* (2004). Nitrate and nitrite reductase activity, soluble sugar and protein contents of seeds were estimated following the method of Sadasivum and Manickam (1992). The morphological attributes such as plant height, phytomass, number of leaves, branches and leaf area were recorded at 30, 45, 60 and 90 DAS (days after sowing) while post harvest data *i.e.* days to first flower bud initiation, days to 50% flowering, total number of flowers, number of pod, seed yield, biological yield, net primary productivity and harvest index/plant were also observed at harvest.

Results and Discussion

Seeds of *Phaseolus mungo* L. were germinated in four concentrations of Cd *viz.* (10^{-2}, 10^{-4}, 10^{-5}, 10^{-8} M) and control in petriplates and polythene bags. Results revealed that germination percentage was inversely proportional to the concentration of Cd. Based on parameters like germination relative index, seedling vigour index and number of lateral roots *Phaseolus mungo* L. was categorized into three groups *viz.* Cd tolerant (10^{-8} M), moderatively sensitive (10^{-4} M and 10^{-5} M) and highly sensitive (10^{-2}M) (Table 1). These observations are in the confirmation with Tandon (1993, 1994) and Siddhu *et al.* (2008). There was significant reduction in the length, dry weight and shoot root ratio in 10^{-2}M concentration while statistically significant increase observed in these parameters in 10^{-8} M concentration. Our observations are in the consonance with those of Ali Khan and Siddhu (2006) and Siddhu *et al.* (2008). (Table 1). This inhibition in germination and seedling growth may be due to the high affinity of Cd to sulfahydryl groups of proteins.

Present investigation revealed that lower concentration showed increase in plant height and phytomass. However, higher concentration 10^{-2} M hampered the plant height and phytomass significantly. Phytotoxicity percentage was observed in the following order - 10^{-2} M>10^{-4} M>10^{-5} M>10^{-8} M concentration These observations are in the agreement with those of Mehindirata *et al.* (2000) and Ali Khan and Siddhu (2006). Leaves became curled and tended to abscise easily in the higher concentration 10^{-2} M of Cd. Number of leaves, branches and leaf area decreased as the concentration of metal increased. Strong decrease in leaf area was correlated to accumulation of chlorophyll pigments as disturb integration of chlorophyll molecules into stable complex (Skkorzynska Polit and Baszynski, 1997). Similar findings have been reported by Mehindirata *et al.* (2000) (Table 2).

Cadmium significantly reduced the pigment contents (chlorophyll a, b, proto chlorophyll and total chlorophyll) in a concentration-duration manner in both presoaking and irrigation process (Table 3). Padmaja *et al.* (1990), Mehindirata *et al.* (1999), Hasan *et al.* (2009) and Pandey *et al.* (2007) reported the similar results while working with Cd . Heavy metals induced changes in chlorophyll contents may be ascribed to the decreased Fe contents in leaves or an impairment of the ability of roots for transport of Fe. These could be a sequence of Cd induced changes in Mg and Fe contents in leaf. Bhattacharyya and Choudhari (1994) reported the inhibition of chlorophyll biosynthesis at protochlorophyllide stage by interference with the enzyme protochlorophyllide reductase. In view of the data obtained in present investigation it seems reasonable to conclude that chlorophyll stability index play significant role in assessing Cd toxicity. Chlorophyll stability index could be a reliable index for determining the degree of Cd stress tolerance of crop plants. Chlorophyll stability index of chl *a* and chl *b* was reported maximum in 10^{-2} M and minimum in 10^{-8} M Cd concentration (Table 4).

Perusal of data revealed that nitrate and nitrite reductase activity in the leaves of *Phaseolus mungo* L. cv. T-9 has been observed to be inversely proportional to the concentration(10^{-2}, 10^{-4}, 10^{-5} and 10^{-8}M) of Cd respectively. 10^{-2} M concentration of Cd significantly reduced the nitrate and nitrite reductase activity (Table 5). This inhibition could be due to inhibition of reductant supply. However, 10^{-8} M concentration of Cd found to augment the activity of both the enzyme. Our observation are in agreement with that of Mishra *et al.* (1994) Keshan and Mukherji (1994) and Dinkar *et al.* (2009). *In vivo* production of NADH can be inhibited due to reduced rates of photosynthesis. Moreover, Cd may stimulate reduced nicotinamide adenine dinucleotide (NADH) oxidation and subsequent decline in NADH pool available to the enzyme. Reduction in nitrate reductase activity due to stress might be a result of a reduced uptake of nitrogen (Bhandal and Kaur, 1992). Low dose of Cd (10^{-8} M) did not affect soluble sugar contents but higher concentration induced a significant increase at 10^{-2} M, which remained nearly unchanged

Table - 1: Effect of cadmiumon seeding growth of *Phaseolus mungo*

Concentration / DAS	Germination (%)	Germination relative index (G.R.I.)	Seedling vigour index (S.V.I.)	Number of lateral roots	Radicle length	Plumule length	Phytotoxicity percentage of radicle length	Phytotoxicity percentage of plumule	Shoot root ratio (SRR)	Dry weight of radicle	Dry weight of plumule
Control											
3	75.00	855.00	505.950	12.050	4.930	1.816	-	-	3.240	4.500	14.583
5	91.66	1008.15	1045.040	17.000	5.480	5.954	-	-	3.803	7.361	28.000
7	95.00	1007.00	1866.180	17.855	7.420	12.224	-	-	3.699	8.130	30.077
10	95.00	950.00	2051.810	18.000	8.344	13.254	-	-	3.569	8.756	31.255
10^{-2} M											
3	66.66*	758.10*	136.053*	7.500*	1.345*	0.696*	72.718*	61.674*	3.638*	1.924*	7.000*
5	73.33*	806.30*	390.262*	11.888*	2.322*	3.000*	57.627*	49.613*	2.109*	4.012*	8.464*
7	85.00*	901.00*	688.840*	12.112*	3.550*	4.554*	52.156*	62.745*	2.426*	4.130*	10.022*
10	85.00*	850.00*	923.780*	12.244*	4.654*	6.214*	44.223*	53.116*	2.261*	4.865*	11.000*
10^{-4} M											
3	70.00*	798.00*	241.640*	8.400*	2.344*	1.108*	52.454*	38.986*	2.880*	3.000*	8.642*
5	75.00*	825.00*	543.225*	15.125*	3.022*	4.221*	44.854*	29.106*	3.217*	5.110*	16.442*
7	86.00*	918.49*	877.519*	15.465*	4.562*	5.564*	38.517*	54.482*	3.129*	5.989*	18.742*
10	86.00*	866.50*	1061.065*	15.632*	5.244*	7.000*	37.152*	47.185*	2.878*	6.756*	19.445*
10^{-5} M											
3	73.33*	835.62*	320.892*	12.375*	3.220*	1.156*	34.685*	36.343*	2.517*	3.800*	9.566*
5	80.00*	880.00*	758.160*	16.400*	4.245*	5.232*	22.536*	12.126*	2.917*	6.242*	18.212*
7	91.00*	971.49*	1127.234*	16.750*	4.955*	7.343*	33.221*	39.929*	3.024*	7.454*	22.546*
10	91.00*	916.50*	1356.568*	17.223*	5.845*	8.955*	29.949*	32.435*	2.831*	8.244*	23.345*
10^{-8} M											
3	78.33*	930.81*	582.775*	13.888*	4.995	2.445*	-1.318*	-34.636*	3.160	4.820	15.233*
5	93.33*	1045.00*	1121.453*	17.285*	5.624	6.392*	-2.628*	-7.356*	3.832*	7.567	29.000*
7	98.00*	1024.49*	1957.982*	18.455*	7.654	12.885*	-3.153*	-5.407*	3.623	8.450	30.623*
10	98.00*	966.50*	2174.862*	18.742*	8.552	13.566*	-2.493*	-2.354*	3.354	9.345*	31.344

DAS = Days after sowing, * = Critical difference significant at 5%

Table - 2: Effect of cadmium on number of leaves, leaf area, branches, plant height, phytotoxicity and phytomass plant^{-1} of *Phaseolus mungo*

Particulars	Concentrations	Plant age (days)					
		30	45	60	75	90	105
Number of leaves plant^{-1}	Control	14.60	28.00	40.333	15.230	-	-
	10^{-2} M	11.42*	18.00*	26.333*	9.218*	-	-
	10^{-4} M	12.72*	19.90*	29.800*	12.450*	-	-
	10^{-5} M	14.20*	25.00*	39.666*	14.320*	-	-
	10^{-8} M	16.00*	30.00*	46.666*	17.800*	-	-
Leaf area (cm^2 plant^{-1})	Control	348.356	9011.520	14907.000	5668.758	-	-
	10^{-2} M	75.372*	3090.600*	8114.250*	2913.348*	-	-
	10^{-4} M	143.863*	5393.890*	9590.832*	4070.415*	-	-
	10^{-5} M	312.400*	7468.750*	14026.109*	5156.918*	-	-
	10^{-8} M	415.040*	9810.000*	18993.06*	7357.113*	-	-
Number of branches plant^{-1}	Control	-	-	4.000	4.333	-	-
	10^{-2} M	-	-	3.000*	3.500*	-	-
	10^{-4} M	-	-	3.200*	3.800*	-	-
	10^{-5} M	-	-	3.666*	4.250*	-	-
	10^{-8} M	-	-	5.000*	6.400*	-	-
Plant height (cm plant^{-1})	Control	24.750	28.212	29.950	30.083	31.460	-
	10^{-2} M	20.900*	23.375*	25.925*	26.125*	28.582*	-
	10^{-4} M	22.875*	25.330*	26.200*	27.125*	30.870*	-
	10^{-5} M	23.900*	26.200*	28.950*	29.135*	31.251	-
	10^{-8} M	25.460*	28.582	30.570*	31.250*	32.782*	-
Phytotoxicity of stem plant^{-1}	Control	-	-	-	-	-	-
	10^{-2} M	15.555*	17.145*	13.439*	13.156*	9.148*	-
	10^{-4} M	7.575*	10.215*	12.520*	9.832*	1.875*	-
	10^{-5} M	3.434*	7.131*	3.338*	3.151*	0.6643*	-
	10^{-8} M	-2.868*	-1.311*	-2.070*	-3.879*	-4.2021*	-
Phytomass (gm plant^{-1})	Control	0.495	0.6720	1.555	3.855	5.425	-
	10^{-2} M	0.2241*	0.3364*	1.038*	1.1016*	1.845*	-
	10^{-4} M	0.3425*	0.5214*	1.3591*	2.628*	3.562*	-
	10^{-5} M	0.4214*	0.5620*	1.4870*	2.857*	3.876*	-
	10^{-8} M	0.5892*	0.7370*	1.6520*	4.215*	6.427*	-

* = Critical difference significant at 5%

with further increase in dose of Cd (Table 5). Manisha and Dhingra (2004) reported similar observations while working on pea. Inhibition in protein contents at higher concentration (10^{-2} M) were observed while lower concentration (10^{-8} M) was found to elevate the protein contents. Cadmium treatment decreased the protein contents of seeds at higher concentration, which was further confirmed by Steffens (1997) and Rolli *et al.*, (2010) (Table 5).

As far the photoperiod concern flower bud was initiated earlier in plants exposed to lower concentration (10^{-8} M) of Cd while higher concentration (10^{-2} M) resulted into delay intiation. Significant decline in total number of flowers per plant due to Cd stress was observed even in the lowest dose. Similar inhibitory effects of Cd on flower production have been reported by Siddhu *et al.*, (2008). Data on pod setting revealed that Cd decreased the number of pods significantly in 10^{-2} M concentration. Such a reduction in pod setting may be ascribed to formation of non-functional flowers possibly at the fag end of flowering ,which abscised without being converted into pods (Dhingra, 2002). A similar observation has been reported by Kumar and Dhingra (2005) in mungbean (Table 6).

Number of seeds per pod and harvest index decreased with the increasing concentration of Cd. Delterious effects of Cd on these parameters have also been reported in mungbean by Kumar and Dhingra (2005), Ali Khan and Siddhu (2006). Results obtained by Manisha and Dhingra (2003) indicated that the reduction in seed yield in pea has been found to be associated with decline in number of flowers, number of seeds and seed size. Reduction in accumulation in biomass lead to a decrease in net primary productivity of *Phaseolus mungo* L. at higher concentration (10^{-2} M) (Table 6). Biological yield and harvest index/plant was decreased at 10^{-2} M concentration in terms of reduction in number of pod and leaf, root and stem growth while lower conc. Promoted the growth of these attributes. Siddhu *et al.* (2008) reported the same findings in case of *Solanum melongena L.* It can be concluded from present investigation that higher concentration of Cd is toxic for *Phaseolus*

Table - 3: Effect of cadmium on chlorophyll contents (mg g^{-1} f.wt.) of *Phaseolus mungo*

Particulars	DAS	Concentrations	Protochlorophyll	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll
Preasoaking treatment	30^{th}	Control	1.1350	0.2591	0.0754	0.3345
		10^{-2}M	0.2417*	0.0711*	0.0356*	0.1068*
		10^{-4}M	0.3925*	0.0904*	0.0455*	0.1359*
		10^{-5}M	0.6569*	0.2221*	0.0652*	0.2873*
		10^{-8}M	0.7930*	0.2488*	0.0742*	0.3231*
	60^{th}	Control	0.7030	0.2223	0.0588	0.2811
		10^{-2}M	0.1240*	0.0140*	0.0136*	0.0276*
		10^{-4}M	0.2065*	0.0619*	0.0181*	0.0801*
		10^{-5}M	0.2420*	0.0629*	0.0237*	0.0867*
		10^{-8}M	0.2775*	0.0646*	0.0283*	0.0929*
	90^{th}	Control	0.5893	0.1697	0.0483	0.2180
		10^{-2}M	0.1683*	0.0152*	0.0084*	0.0236*
		10^{-4}M	0.1985*	0.0336*	0.0145*	0.0481*
		10^{-5}M	0.2111*	0.0379*	0.0189*	0.0569*
		10^{-8}M	0.2330*	0.0454*	0.0219*	0.0673*
Irrigation treatment	30^{th}	Control	1.827	0.4532	0.1770	0.6300
		10^{-2}M	0.9304*	0.2099*	0.0923*	0.3023*
		10^{-4}M	1.6910*	0.2937*	0.1080*	0.4018*
		10^{-5}M	1.7080*	0.3088*	0.1127*	0.4215*
		10^{-8}M	1.7180*	0.3562*	0.1151*	0.4718*
	60^{th}	Control	1.3880	0.2921	0.0995	0.3917
		10^{-2}M	0.7410*	0.0989*	0.0367*	0.1356*
		10^{-4}M	0.8870*	0.1066*	0.0409*	0.1476*
		10^{-5}M	0.9280*	0.1177*	0.0461*	0.1638*
		10^{-8}M	1.0810*	0.1347*	0.0562*	0.1909*

DAS = Days after sowing, * = Critical difference significant at 5% level

Table - 4: Effect of cadmium on chlorophyll stability index of *Phaselous mungo*

Particulars	DAS	Concentrations	Chlorophyll stability index of Chl *a*	Chlorophyll stability index of Chl *b*
Presoaking treatment	30^{th}	10^{-2}M	0.7255*	0.5269*
		10^{-4}M	0.6510*	0.3959*
		10^{-5}M	0.1428*	0.1348*
		10^{-8}M	0.0397*	0.0218*
	60^{th}	10^{-2}M	0.9370*	0.7610*
		10^{-4}M	0.7212*	0.6915*
		10^{-5}M	0.7166*	0.5960*
		10^{-8}M	0.7092*	0.5190*
	90^{th}	10^{-2}M	0.9100*	0.8257*
		10^{-4}M	0.8018*	0.6993*
		10^{-5}M	0.7766*	0.6070*
		10^{-8}M	0.7321*	0.5463*
Irrigation treatment	30^{th}	10^{-2}M	0.5368*	0.4785*
		10^{-4}M	1.3519*	0.3898*
		10^{-5}M	1.3186*	0.3293*
		10^{-8}M	1.2144*	0.3497*
	60^{th}	10^{-2}M	0.6611*	0.6330*
		10^{-4}M	0.6350*	0.5889*
		10^{-5}M	0.5970*	0.5366*
		10^{-8}M	1.5388*	0.4351*

DAS = Days after sowing, Chl.= Chlorophyll, * = Critical difference significant at 5% level

Table - 5: Effect of cadmium on nitrate (μg NO_2^{2-} prod min^{-1} g^{-1} f.wt.) and nitrite (μg/NO_2^- red. min^{-1}g^{-1}f.wt.) reductase activity of leaves at 45th and 60th day (in parentheses), soluble sugar and protein contents (mg gm^{-1} f.wt.) of seeds of *Phaseolus mungo*

Concentrations	Nitrate reductase activity	Nitrite reductase activity	Soluble sugar contents	Protein contents
Control	4.342 (7.920)	3.50 (5.627)	36.00	222.55
10^{-2}M	2.00 (4.747)*	1.33 (2.895)*	63.45*	162.32*
10^{-4}M	3.332 (4.933)*	1.822 (3.983)*	57.88*	194.45*
10^{-5}M	3.885 (5.287)*	2.00 (5.164)*	41.00*	213.44*
10^{-8}M	4.544 (5.600)*	2.892 (5.373)*	35.33	239.45*

* = Critical difference significant at 5%

Table - 6: Effect of cadmium on days to first flower bud initiation, days to 50% flowering, total number of flower plant^{-1}, number of pod plant^{-1}, net primary productivity, seed yield (gm), biological yield (gm) and harvest index plant^{-1} of *Phaseolus mung*

Concentrations	Days to first flower bud initiation	Days to 50% flowering	Total no of flowers plant^{-1}	Number of pods plant^{-1}	Net primary productivity	Seed yield plant^{-1} (gm)	Biological yield plant^{-1} (gm)	Harvest index plant^{-1}
Control	48.00	50.33	15.2	3.444	0.3250	1.356	2.925	46.363
10^{-2} M	49.666*	52.33*	5.7271*	2.454*	0.01903*	0.666*	1.713*	38.884
10^{-4} M	49.000	51.33	7.3629*	2.666*	0.02054**	0.769*	1.849*	41.6290
10^{-5} M	48.330*	50.66	8.999*	3.000*	0.0234*	0.907*	2.108*	43.029
10^{-8} M	44.000*	49.66	11.1*	3.222*	0.02837*	1.116*	2.553*	43.713

* = Critical difference significant at 5%

mungo L. while lower concentration is promotory (tolerance level) to all plant attributes which might be used as phytoremediation in 'green cure' technology in anthropogenic ecosystem.

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