

CALCIUM INTERACTION WITH CdCl₂ INDUCED EFFECTS ON SEEDLING GROWTH AND METABOLISM OF VIGNA MUNGO L. AND SOLANUM MELONGENA L.

Geeta Siddhu* and Gulfam Ali

Department of Botany, Kisan(P.G) College, Simbhaoli-245207, India

Email: dr.geetasiddhu@yahoo.in

Received-31.05.2015, Revised-08.06.2015

Abstract: In the present research work surface sterilized seeds of *Vigna mungo* L. and *Solanum melongena* L. were raised to analyzed changes in germination, seedling growth, chlorophyll contents, nitrate and nitrite reductase activity under CdCl₂, CaCO₃ stress singly and in combination 10⁻² M+200 ppm, 10⁻⁴ M +100 ppm 10⁻⁵ M+50 ppm, 10⁻⁸ M+25 ppm and control were investigated. Observations were recorded at 3, 5, 7, 10, 30, 45 and 60th day of sowing displayed significant decrease in all the attributes of both crop plants on CdCl₂ application. However, activity of nitrate and nitrite reductase, seedling growth, and chlorophyll contents were enhanced in lower Cd stress 10⁻⁸ M. Application of CaCO₃ shows the more elevations than CdCl₂ singly while combined effect of Cd+Ca is more pronounced in comparison to their individual effects.

Keywords: CaCO₃, NR activity, Chlorophyll contents, *Vigna mungo* L. and *Solanum melongena* L.

INTRODUCTION

A mong the heavy metals cadmium (Cd) is a highly toxic and persistent environmental contaminant introduced into the soil, through anthropogenic activity phosphate fertilizers, sewage sludge and atmospheric fallout from industrial and municipal activity (Wagner *et al.*, 1993). Although Cd is not essential for plants but because of its mobility in soil and solubility in water it is readily absorbed by plants, where it adversely affects the growth and metabolism and in high concentration it can lead to cell death and destruction of whole plant (Benavides *et al.*, 2005). Cadmium enters into the cells due to its similar chemical and physical characteristics to plant nutrients, using Ca channels or Fe, Mn or Zn transporters and reduces the uptake of iron (Fe), nitrogen(N), phosphorus (P), potassium (K), Zinc (Zn), copper (Cu) and sodium (Na) (Wojcik *et al.*, 2006). Cadmium is one of the most toxic metals in plants because it is active at concentrations much lower than other heavy metals and it also irreversibly replaces other metal ions in essential metalloenzymes (Jackson *et al.*, 1990). Cadmium accumulation in plants causes disturbance in membrane function (Hernandez and Cooke, 1997), Enzyme activity (Tamas *et al.*, 2006).

Excessive level of HMs in the soil environment adversely affect the germination of seeds, plant height, interfere with the activities of many key enzymes (Ashan *et al.*, 2007 and kuriakosa and

Prasad 2008), Nitrate reductase activity (Hernandez *et al.*, 1996), Chlorophyll contents and phytomass (Siddhu *et al.*, 2012). Seed is a stage in life cycle of plant that is well protected against various stresses. However, after imbibition and subsequent vegetative developmental processes, they become stress sensitive (Li *et al.*, 2005). Heavy metals have high affinity to sulfhydryl groups and disulfide bond which causes damage to secondary structure of proteins (Siediecka and Kurpa, 2002). The mechanism of action lies in their ability to form strong bonds with base and phosphate of nucleic acids (Tabaldi *et al.*, 2007). Cadmium accumulation in plants causes disturbance in membrane function (Hernandez and Cooke, 1997). Tyler and McBride (1981) and Brown *et al.*, (1983) investigated cadmium to be more mobile in soil than other common trace metals. McBride (1980) found that Cd was sorbed from dilute solution by CaCO₃ by chemisorption. Mechanism of Cd immobilization was discussed by Zahan (1986). The effect of combined metal Cd and Ca on *Vigna mungo* L. and *Solanum melongena* L. may be quite different from those of individual metals due to their mitigation to encounter the toxic effect of Cd through cation exchange chemisorption. Therefore, in the present study, an attempt has been made to investigate combined effect of Cadmium and calcium on seed germination, seedling growth, Chlorophyll contents & NR activity of *Vigna mungo* L. and *Solanum melongena* L. to encounter toxic effects of cadmium.

*Corresponding Author

MATERIAL AND METHOD

Uniform healthy seeds of *Phaseolus mungo* L. cv.T-9 and *Solanum melongena* L. cv.Pusa uttam 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 (228.35) in one liter of Hoagland's nutrient solution. This solution was known as 1M solution of cadmium and served as a stock solution (S.S.), other molar conc. were prepared from this 1M (one molar) solution.

In the present investigation, there were two methods of cadmium treatment employed i.e. presoaking and irrigation. For presoaking treatment sterilized seeds of *Phaseolus mungo* L. and *Solanum melongena* L. were imbibed upto 12 hrs in the different molar conc. of cadmium viz. (10^{-2}M , 10^{-4}M , 10^{-5}M , 10^{-8}M , CaCO_3 as 25,50,100,200 ppm and in combination as $10^{-2}\text{M}+200$ ppm, $10^{-4}\text{M}+100$ ppm, $10^{-5}\text{M}+50$ ppm, $10^{-8}\text{M}+25$ ppm, & control) and for irrigation process metal was also given to soil in the form of above molar conc. before sowing. The surface sterilized seeds were sown in polythene bags in triplicate containing 10 kgs. 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 (Abdulkali 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). Nitrate and nitrite reductase activity, soluble sugar and protein contents of seeds were estimated the method given by Sadasivum and Manickam (1992).

RESULT AND DISCUSSION

Seeds of *Phaseolus mungo* L. and *Solanum melongena* L. were germinated in different. conc. of cadmium singly(10^{-2}M , 10^{-4}M , 10^{-5}M , 10^{-8}M , CaCO_3 as 25,50,100,200 ppm and is combination as $10^{-2}\text{M}+200$ ppm, $10^{-4}\text{M}+100$ ppm, $10^{-5}\text{M}+50$ ppm, $10^{-8}\text{M}+25$ ppm, & control) in petriplates polythene bags

in triplicate. Results on Germination percentage, seedling vigour index (S.V.I) and number of lateral roots shows -10.52%, -54.97% and -31.97% reduction in 10^{-2}M CdCl_2 singly while interaction of both metal shows a decrease in percent of reduction (-7.36%, -47.33%, and -16.57%). However CaCO_3 and $10^{-8}+25$ ppm show significant stimulations in these parameters. These observations are in the conformation with that of Siddhu *et al.* (2008). (Table -1 & 2.)

It is obvious from the results of present investigation that seed germination under the different treatments were reduced significantly in 10^{-2}M of CdCl_2 while application CaCO_3 along with CdCl_2 shows a remarkable increase. Peusal of data on seedling growth the *Vigna mungo* L. and *Solanum melongena* L. in CdCl_2 shows (-44.22%, -53.11%, -44.438%, -64.805%, -36.64% and -14.93%, -43.87%, -46.11%, -30.65% & -28.7%) reduction oven control in radical & plumule length & dry wt. and shoot root ratio respectively in 10^{-2}M at 10^{th} day however percentage of reduction decrease (-37.39%, -47.27%, -35.35%, -60.93%, -39.61% and -8.14%, .36-57%, -45.57%, -22.96%, -41.51%) due to interaction of CdCl_2 and CaCO_3 in ($10^{-2}\text{M} + 200$ ppm) (Table-1 & 2). The inhibition was none in root than shoot because plant roots are finest point of contact with heavy metals in nutrient media (Zhang *et al.*, 2009). The reason for reduced seedling growth under metal treatment could be the reduction in meristematic cells present in the region and some enzymes present in cotyledons and in endosperm.

When Cd-Ca on Cd-Zn interactions are considered, the absorption of Cd decreased in vegetative parts of the plants. Such interactions can be accounted by antagonism process. It is presumed that Ca^{+2} compete with Cd ions or exchange sites on the roots because the radius of Calcium is similar to that of cadmium. Increase significance has been observed in radical & plumule length & dry wt. and shoot root ratio in $10^{-8}\text{M} + 25$ ppm. The observed significance is (7.22%, 3.15%, 10.14%, 10.75%, -1.23% & 1.58%, 2.80%, 5.93%, 11.17% & 5.01%) respectively. However, Ca Singly increased the length and dry wt. of radical and plumule. Similar trend of observation have been reported with CaCO_3 by Salim and Nair (1982). Cadmium reduced the root growth by decreasing Ca in root, calcium being a requirement for root growth development is affected (Greger and Bertell, 1992) (Table-1 & 2).

In our experiment on *Vigna mungo* L. it has been observed that Cadmium alone decreased the germination and seedling growth but along with Ca. the toxic effect of cadmium are reduced to some extent. The combine application of both the elements increases the germination and seedling growth while Ca alone increased the germination and growth of seedlings. Similar observations have been reported by Mohan and Saran (1999). He reported that soaking of CaCl_2 and KNO_3 alleviated the effects of Cd toxicity to some extent.

The synergistic effects of cadmium and calcium on the chlorophyll contents at the age of 30th, 60th days of *Vigna mungo* L. and *Solanum melongena* L. in 10^{-2}M +200 ppm of Ca + Cd and in 25 ppm of CaCO_3 have been observed. Cadmium treatment caused a significant reduction in the leaf chlorophyll contents of both the crop plants compared with their controls. The maximum decline -34.88%, -44.54%, -51.48% & -46.24% in proto. Chlorophyll. Chl a. chl b. and total chlorophyll contents of *Solanum melongena* L. over control were observed (Table). Destruction of chlorophyll pigments reported due to the instability of pigment-protein complex which may be correlated to the indirect effects of Na and Cl^- ions and also due to impaired uptake of Mg, Fe and Ca by plants (Jaleel *et al.*, 2008). On the contrary, combined treatments of 10^{-2}M + 200 ppm could reduces proto. Chlorophyll. Chl a. chl b. and total chlorophyll contents only by -64.05%, -58.49%, -45.74%, -62.52% & -45.74%, -33.36%, 38-49% and -45.74% in *Solanum melongena* L. and *Vigna mungo* L. respectively. The extend of percent of reduction was less in 10^{-8}M +25ppm of Cd + Ca treatment, thus showing a remedial effects CdCl_2 treatment. Observation on CaCO_3 singly shows the same trend. Calcium might prevent the damage from cellular dehydration by balancing osmotic strength of cytoplasm (Arshi, *et. al.*, 2006 ab). CSI result shows the stimulation in 10^{-2}M + 200 ppm and 200 ppm of CaCO_3 however, 10^{-8}M +25 ppm, 25ppm & 50ppm revealed significant reduction (Table-3).

Nitrate and nitrite reductase activity of *Vigna mungo* L. and *Solanum melongena* L. revealed its inversely

proportional to the conc. of cadmium (10^{-2}M , 10^{-4}M , 10^{-5}M , 10^{-8}M). These observations are in the conummeration with that of Mehindirata *et al.* (1999) and Ali khan and Siddhu (2006). Combined result on effect of CaCO_3 and CdCl_2 levels in nitrate and nitrite reductase activities showed increase in 10^{-8}M +25 ppm at 45th and 60th day. Observed promotion is 3.81%, -15.60% & -33.75%, -9.23% in nitrate reductase enzyme activity and -8.57%, -8.35% & -89.56%, -65.45% in nitrite reductase enzyme activity however, significant reduction -42.85%, -43.13%, -51.74%, & -39.46% in 10^{-2}M +200ppm of Cd + Ca at 45th & 60th day. Activity of nitrate reductase enzyme is increased with increasing CaCO_3 concentration. Nishi *et al.* (1996) reported that CdCl_2 could recover the loss of in vivo NRA in roots caused by either of the metal combination whereas the salt could recover the loss in leaf NRA caused only by Pb^{2+} , Cd^{2+} (1.0 mM each) and organic N contents of root and leaf however, increased significantly with CaCl_2 alone and with the metals supplied in various combinations (Table-4).

Concluding thoughts

In present is study combined effect of Cd+Ca on *Vigna mungo* L. and *Solanum melongena* L. have been observed quite different from those of individual metal pollutant due to their mitigation to encounter the toxic effects of cadmium through cation exchange chemisorptions. Results due to application of both metals revealed more pronounced than CdCl_2 singly, however CaCO_3 results shows the slight alleviations in all plants attributes in comparison to cadmium.

ACKNOWLEDGEMENT

Authors extend sincere thanks to Professor Y. Vimla, Department of Botany, C.C.S. University, Meerut, Dr. Attar Singh and Dr. N.K. Parsad (Plant Physiology Division , I.A.R.I., New Delhi) and providing laboratory facilities and valuable suggestions during this work.

Table 1. Effect of cadmium chloride on seedling growth of *Vigna mungo* L.

CdCl ₂ treatment	DAS	Germination percentage	Germination on relative index (G.R.I.)	Seedling vigour index (S.V.I.)	Shoot: Root ratio	Dry weight of root	Dry Weight of shoot	Number of lateral roots	Root length	Root length	Phytotoxicity percentage of root length	Phytotoxicity percentage of root length
Control	3	75.00	855.00	505.950	3.240	4.500	14.583	12.050	4.930	1.816	0.000	0.000
	5	91.66	1008.15	1048.040	3.803	7.361	28.000	17.000	5.480	5.954	0.000	0.000
	7	95.00	1007.00	1866.180	3.699	8.130	30.077	17.855	7.420	12.224	0.000	0.000
	10	95.00	950.00	2051.810	3.569	8.756	31.255	18.000	8.344	13.254	0.000	0.000
CdCl₂+CaCO₃												
10 ⁻² M + 200ppm	3	70.00*	798.00*	213.360*	4.126*	2.100*	8.666*	8.655*	1.847*	1.201*	62.535*	33.865*
	5	83.00*	916.30*	599.059*	2.272*	4.201*	9.545*	12.055*	3.244*	3.945*	40.802*	33.742*
	7	88.00*	935.98*	902.202*	2.208*	4.980*	11.000*	12.428*	4.345*	5.869*	41.442*	51.987*
	10	88.00*	883.00*	1080.540*	2.155*	5.666*	12.211*	12.631*	5.245*	6.988*	37.140*	47.276*
10 ⁻⁴ M+ 100ppm	3	73.00*	835.62*	322.514*	3.381*	3.121*	10.555*	9.000*	2.954*	1.464*	40.811*	19.383*
	5	88.33*	971.30*	827.387*	3.464*	5.323*	18.442*	15.625*	4.222*	5.145*	22.956*	13.587*
	7	91.66*	971.49*	1075.721*	3.802	6.124*	20.242*	15.798*	5.212*	6.524*	29.757*	46.629*
	10	91.66*	916.50*	1325.036*	3.000*	7.000*	21.000*	15.912*	6.232*	8.224*	25.311*	37.950*
10 ⁻⁵ M+ 50ppm	3	74.00	850.62*	427.760*	3.263	4.245	13.852*	12.445	3.885*	1.576	21.196*	13.215*
	5	90.00	990.00*	951.120*	3.848	6.898*	26.545*	16.675	5.022*	5.546*	8.357*	6.852*
	7	93.33*	998.98*	1293.180*	3.679	7.844	28.864*	16.875	5.644*	8.212*	23.935*	32.820*
	10	93.33*	933.00*	1529.678*	3.527	8.345*	29.440*	17.033*	6.545*	9.845*	21.560*	25.720*
10 ⁻⁸ M+ 25ppm	3	81.66*	930.81*	661.364*	3.023	5.622*	17.000*	14.000*	5.245	2.854*	-6.389*	-57.158*
	5	95.00*	1026.30*	1230.630*	4.062	7.876*	32.000*	18.122*	6.000	6.954*	-9.489*	-16.795*
	7	98.33*	1041.98*	2112.128*	3.844	8.643*	33.224*	18.724*	8.245	13.235*	-11.118*	-8.270*
	10	98.33*	983.00*	2307.500*	3.525*	9.644*	34.000*	19.011*	9.255	14.212*	-10.918*	-7.228*
CaCO₃												
25 ppm	3	61.66*	702.81*	213.096*	4.357*	2.300*	10.022*	9.000*	2.000*	1.456*	59.432*	19.823*
	5	68.33*	751.30*	464.029*	3.263*	4.545*	14.834*	12.345*	2.559*	4.232*	53.302*	28.921*
	7	81.66*	865.49*	884.622*	3.143*	5.700*	17.916*	12.745*	4.500*	6.333*	39.353*	48.192*
	10	81.66*	816.50*	1058.598*	3.023*	6.200*	18.745*	13.142*	5.684*	7.255*	31.879*	45.261*
50ppm	3	66.66*	759.81*	317.634*	3.921*	3.400*	13.333*	9.445*	3.221*	1.544	34.665*	14.977*
	5	75.00*	825.00*	735.750*	4.027*	5.555*	22.375*	15.755*	4.566*	5.244*	16.678*	11.924*
	7	83.33*	882.98*	1063.624*	4.270	6.666*	28.466*	15.862*	5.765*	6.999*	22.304*	42.743*
	10	83.33*	833.00*	1286.615*	3.906*	7.455*	29.122*	16.054*	6.666*	8.774*	20.109*	33.801*
100ppm	3	68.33*	778.62*	391.804*	3.140	4.342*	13.636*	12.454*	4.122*	1.612	16.389*	11.233*

200ppm	5	78.33*	861.30*	854.580*	3.757	6.984	26.245*	16.855*	5.244	5.666	4.306*	4.833*
	7	88.33*	935.98*	1295.447*	3.641	7.844	28.567*	16.925*	5.955*	8.711*	19.740*	28.738*
	10	88.33*	883.00*	1538.885*	3.448	8.656*	29.854*	17.062*	7.000*	10.422*	16.107*	21.367*
	3	70.00*	798.00*	589.190*	3.014	5.712*	17.220*	14.455*	5.433*	2.984*	-10.202*	-64.317*
	5	81.00*	898.15*	1110.410*	4.097	7.942*	32.545*	18.322*	6.500*	7.000*	-18.613*	-17.568*
	7	90.00*	954.00*	1997.280*	3.885	8.754*	34.011*	19.000*	8.550*	13.642*	-15.229*	-11.600*
	10	90.00*	900.00*	2158.920*	3.416	10.211*	34.885*	--	9.422*	14.566*	-12.919*	-9.898*

* Significant at 5% level.

DAS = Days after sowing

Table 2. Effect of cadmium chloride on seedling growth of *Solanum melongena* L.

CdCl ₂ treatment	DAS	Germination percentage	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 length	Shoot root ratio (SRR)	Dry weight of radicle	Dry weight of plumule
Control	3	23.33	265.62	-	-	-	-	-	-	-	-	-
	5	85.00	935.00	444.805	-	5.233	-	-	-	-	0.538	-
	7	96.66	1024.49	1123.189	5.482	7.676	3.945	-	-	3.378	0.740	2.500
	10	96.66	966.50	1320.955	7.524	7.886	5.780	-	-	3.129	0.926	2.900
CdCl₂+CaCO₃												
10 ⁻² M + 200ppm	3	6.66*	37.62*	0.0*	-	-	-	-	-	-	-	-
	5	70.0*	421.3*	267.68*	-	3.824*	-	36.955*	-	-	0.363*	-
	7	88.33*	901.0*	746.39*	2.445*	5.453*	3.00*	28.951*	23.954*	3.985*	0.426*	1.7*
	10	88.33*	850.0*	963.68*	2.622*	7.244*	3.66*	8.141*	36.564*	4.428*	0.504*	2.234*
10 ⁻⁴ M+ 100ppm	3	11.66*	57.0*	0.0*	-	-	-	-	-	-	-	-
	5	73.33*	476.3*	281.88*	-	3.844*	-	26.543*	-	-	0.454*	-
	7	91.66*	935.98*	905.32*	3.256*	6.666*	3.211*	13.138*	18.60*	3.910*	0.514*	2.012*
	10	91.66*	883.0*	1066.55*	3.578*	7.324*	4.312*	7.126*	25.397*	3.467*	0.676*	2.346*
10 ⁻⁵ M+ 50ppm	3	13.33*	75.81*	0.0*	-	-	-	-	-	-	-	-
	5	83.3	495.0*	404.33*	-	4.854*	-	7.242*	-	-	0.489*	-
	7	93.33*	954.0*	988.36*	3.624*	6.945*	3.645*	9.394*	7.604*	3.875*	0.598*	2.320
	10	93.33*	900.0*	1152.90*	4.625*	7.585*	4.768*	3.816*	17.508*	2.958	0.892*	2.640*

10 ⁻⁸ M+ 25ppm	3	26.66*	301.81*	0.0*	-	-	-	-	-	-	-	-
	5	86.66	981.75*	518.66*	-	5.985*	-	-14.37*	-	-	0.567*	-
	7	98.33	1052.58*	1220.17*	6.885*	7.989*	4.42*	-4.091*	-12.040*	3.795*	0.761	2.888*
	10	98.33	993.0*	1371.99*	8.678*	8.011	5.942	-1.833*	-2.802*	3.286	0.981*	3.224*
CaCO₃												
25 ppm	3	0.0	0.0*	0.0*	-	-	-	-	-	-	-	-
	5	30.0*	330.0*	120.75*	-	4.025*	-	23.084*	-	-	0.384*	-
	7	85.0*	901.0*	740.94*	2.566*	5.68*	3.037*	25.993*	23.016*	4.789*	0.454*	2.176*
	10	85.0*	850.0*	924.8*	2.8*	6.898*	3.982*	11.488*	31.107*	4.571*	0.538*	2.461*
50ppm	3	0.0*	0.0*	0.0*	-	-	-	-	-	-	-	-
	5	50.0*	550.0*	241.4*	-	4.828*	-	7.739*	-	-	0.477*	-
	7	86.66*	918.49*	892.16*	3.455*	6.875*	342*	10.423*	13.307*	4.00*	0.555*	2.222*
	10	86.66*	866.5*	1094.42*	3.756*	7.754	4.765*	1.673*	17.560	3.538*	0.769*	2.722*
100ppm	3	1.66*	18.81*	0.0*	-	-	-	-	-	-	-	-
	5	58.33*	641.3*	298.70*	-	5.121	-	2.140*	-	-	0.512	-
	7	88.33*	935.98*	1070.11*	3.757*	7.35*	3.588*	4.234*	9.049*	3.678*	0.666*	2.452
	10	88.33*	883.0*	1115.51*	4.8*	7.765	4.875*	1.534*	15.657*	3.16	0.900	2.844
200ppm	3	3.33*	37.62*	0.0*	-	-	-	-	-	-	-	-
	5	65.00*	715.0*	392.88*	-	6.044*	-	-15.505*	-	-	0.600*	-
	7	96.66	1024.49*	1266.43*	7.00*	8.137*	4.965*	-6.026*	-25.855*	3.471	0.777*	2.7*
	10	96.66	966.5*	1392.19*	8.898*	8.414*	5.898*	-6.695*	-3.615*	3.260*	1.012*	3.4*

* Significant at 5% level.

DAS = Days after sowing

Table 3. Effect of CdCl₂ + CaCO₃ on chlorophyll contents (mg/g. f. wt.) of *Solanum melongena* L. and *Vigna mungo* L.

		<i>Solanum melongena</i>				<i>Vigna mungo</i>			
Treatment	DAS	P. chl.	Chl. a	Chl. b	Total chl.	P. chl.	Chl. a	Chl. b	Total chl.
CdCl ₂									
Control	30 th	1.735	0.5788	0.168	0.7468	1.827	0.453	0.1770	0.630
10 ⁻² M		1.258*	0.242*	0.0145*	0.256*	0.930*	0.209*	0.092*	0.302*
10 ⁻⁴ M		1.380	0.325	0.056*	0.409	1.691	0.293	0.108	0.401*
10 ⁻⁵ M		1.627*	0.381*	0.068*	0.448*	1.708*	0.308*	0.112	0.421
10 ⁻⁸ M		1.702*	0.422*	0.072*	0.494*	1.718*	0.356*	0.115*	0.471*
Control	60 th	1.1797	0.36038	0.0986	0.459	1.388	0.292	0.099	0.391
10 ⁻² M		0.638*	0.0701*	0.0281*	0.099*	0.741*	0.098*	0.036*	0.135*
10 ⁻⁴ M		0.733*	0.213*	0.0290	0.242	0.887	0.106	0.040	0.147

10 ⁻⁵ M		0.784	0.218	0.0364*	0.254	0.928*	0.117	0.046	0.163*
10 ⁻⁸ M		0.928*	0.226*	0.0423*	0.268*	1.081*	0.134*	0.056*	0.190*
CdCl₂+CaCO₃									
10 ⁻² M + 200ppm	30 th	1.2582*	0.2424*	0.1450*	0.2569*	0.956*	0.216*	0.096*	0.312*
10 ⁻⁴ M+ 100ppm		1.3805	0.3254	0.0564	0.4096	1.709	0.303	0.102	0.406
10 ⁻⁵ M+ 50ppm		1.627*	0.3801*	0.0682	0.4483	1.765	0.318	0.108	0.427*
10 ⁻⁸ M+ 25ppm		1.702*	0.4223	0.0723*	0.4946*	1.780*	0.386*	0.117*	0.504*
CdCl₂+CaCO₃									
10 ⁻² M + 200ppm	60 th	0.6386*	0.07018*	0.0290*	0.0992*	0.753*	0.105*	0.041*	0.146*
10 ⁻⁴ M+ 100ppm		0.7335	0.2133	0.2956	0.2428	0.895	0.112	0.045	0.160*
10 ⁻⁵ M+ 50ppm		0.7842*	0.2185*	0.0364	0.2549*	1.078*	0.128	0.048	0.176
10 ⁻⁸ M+ 25ppm		0.9285*	0.2265*	0.0423*	0.2688*	1.182*	0.141*	0.067*	0.209*
CaCO₃									
25 ppm	30 th	1.338*	0.392*	0.033*	0.426*	0.967*	0.231*	0.099*	0.330*
50 ppm		1.459*	0.421	0.086	0.507	1.721	0.312	0.112	0.425
100 ppm		1.752	0.484*	0.088	0.573*	1.769	0.325*	0.114	0.440*
200 ppm		1.775*	0.512*	0.090*	0.602*	1.789*	0.406*	0.119*	0.525*
CaCO₃									
25 ppm	60 th	0.659*	0.188	0.0335*	0.221*	0.762*	0.112*	0.044*	0.156*
50 ppm		0.749	0.223*	0.0338	0.257	0.905*	0.120	0.049	0.169
100 ppm		0.807*	0.235	0.0428*	0.278*	0.930	0.135*	0.053*	0.187*
200 ppm		0.946*	0.253*	0.0495*	0.302*	1.087*	0.149*	0.069*	0.218*

DAS=Days after sowing

*Significant at 5% level

Table 4. Effect of cadmium chloride on Nitrate ($\mu\text{g. NO}_2^-$ prod/min/gm f.w.t) and nitrite ($\mu\text{g. NO}_2^-$ red/min/gm f.w.t) reductase activity of leave at 45th and 60th day (in parentheses), of *Vigna mungo L.* and *Solanum melongena L.*

Treatment	Nitrate reductase activity		Nitrite reductase activity	
	<i>Vigna mungo</i>	<i>Solanum melongena</i>	<i>Vigna mungo</i>	<i>Solanum melongena</i>
CdCl ₂				
Control	4.642 (7.920)	2.45 (5.35)	3.50 (5.627)	1.677 (2.446)
10 ⁻² M	2.00* (4.747)*	0.324* (1.343)*	1.33* (2.895)	0.125* (0.326)*
10 ⁻⁴ M	3.332* (4.933)*	1.122 (2.23)	1.882 (3.983)	0.135 (0.456)
10 ⁻⁵ M	3.885 (5.287)	1.253 (2.501)	2.00 (5.164)	0.148 (0.624)
10 ⁻⁸ M	4.544* (5.600)*	1.376* (4.770)*	2.892* (5.373)*	0.165* (0.695)*
CdCl₂+CaCO₃				
10 ⁻² M + 200ppm	2.245* (4.794)*	0.565* (1.435)*	1.55* (3.22)*	0.133* (0.563)*
10 ⁻⁴ M+ 100ppm	3.55 (5.122)	1.340 (2.356)	2.00 (4.176)	0.140 (0.622)
10 ⁻⁵ M+ 50ppm	4.00 (5.395)	1.476 (2.621)	2.35 (5.265)	0.160 (0.752)
10 ⁻⁸ M+ 25ppm	4.465* (6.684)*	1.623* (4.856)*	3.200* (5.558)*	0.175* (0.845)*
CaCO₃				
25 ppm	2.125* (4.765)*	0.600* (1.564)*	1.25* (3.00)*	1.019* (1.465)*
50 ppm	3.245 (4.954)	1.455 (2.466)	1.88 (3.55)	1.342 (1.636)
100 ppm	3.955 (5.255)	1.562 (2.855)	1.22 (5.18)	1.421 (1.784)
200 ppm	4.352* (6.255)*	1.755* (5.245)*	3.00* (5.412)*	1.485* (1.944)*

* Significant at 5% level

REFERENCES

- Abdulkaki, A. and Anderson, J.D.** (1973). Physiological and biological determination of seeds in "Seed biology" edited by TT Kozlowski, Academic Press, New York, pp. 283-310.
- Ahsan, N., Lee, D.G., Lee, S.H., Kang, K.Y., Lee J.J. and Kim, P.J.** (2007) Excess copper induced physiological and proteomic changes in germinating rice seeds. *chemosphere*, **67**, 1182-1193.
- Ali Khan, M.A. and Siddhu, G.** (2006). Phytotoxic effect of cadmium (Cd) on physiology of Urd bean [*Vigna mungo* (L.) Hepper]. *Ad. Plant Sci.*, **19** (11): 439-444.
- Arshi, A., Abdin, M.Z. and Iqbal, M.** (2006b). Sennoside content and yield attributes of *Cassia angustifolia* Vahi. As affected by NaCl and CaCl₂. *Sci. Hort.*, **11**, 84-90.
- Arshi, A., Abdin, M.Z. and qbal, M.I** (2006a). Effects of CaCl₂ on growth performance, photosynthetic efficiency and nitrogen assimilation of *Cichorium intybus* L. grown under NaCl stress. *Acta Physiol. Plant.*, **28**, 137-147.
- Arshi, A., Ahmad, A., Aref, I.M. and Iqbal, M.** (2010). Calcium interaction with salinity-induced effects on growth and metabolism of soybean (*Glycine max* L.) cultivars. *J. Environ. Biol.* **31**, 795-801.
- Benavides, M.P., Gallego, S.M. and Tomar, M.L.** (2005). Cadmium toxicity in plants. *Braz. J. Plant Physiol.*, **17**, 21-34.
- Chou, C.H. and Muller, C.H.** (1972). Allelopathic mechanism of *Archtoostaphyous glandulosa* var. *Zazaensis*. *Am. Mid. Nat.*, **88** : 324-347.
- Greger, M. and Bertell, B.G.** (1992). Effects of Ca and Cd²⁺ on carbohydrate metabolism in sugarbeet (*Beta vulgaris*). *J. Exp. Bot.*, **43** : 167-173.
- Hernandez, L.E. and Cooke, D.T.** (1997). Modification of the root plasma membrane, lipid composition of cadmium treated *Pisum sativum*. *J. Exp. Bot.*, **48**, 1375-1381.
- Hernandez, L.E., Carpena-Ruiz, R., Garate, A.** (1996). Alteration in the mineral nutrition of pea seedling exposed to cadmium. *J. Plant Nutr.* **19**; 1581-1598.

- Jackson, P.J., Delhaize, E. and Robinson, N.J.** (1990). Mechanism of trace metal tolerance in plants. In: Environment at injury to plants (Ed.: F. Katterman). Academic Press, New York.
- Kuriakosa, S.V., Prasad, M.N.V.** (2008). Cadmium stress affects seed germination and seedling growth in *sorghum bicolor* (L.) moench by changing the activities of hydrolyzing enzymes. *Plant growth regul.* **54**, 143-156.
- Li, W., Khan, M.A., Yamaguchi, S. and Kamiya, Y.** (2000). Effects of heavy metals on seed germination and early seedling growth of *Arabidopsis thaliana*. *Plant Growth Regul.*, **46**, 257-262.
- Mehindirata, S., Mahmooduzzafar, T.O. and Muhamad, I.** (1999). Cadmium induced changes in foliar responses of *Solanum melongena* L. *Phytomorphology*, **49** (3) : 295-302.
- Mohan, R. and Saran, B.** (1999). Effect of pre-sowing seed treatment on germination and seedling growth in black gram under cadmium stress. *Neo Botanica*, **7** (2) : 113-115.
- Nishi, B., Singh, R.P. and Sinha, S.K.** (1996). Effect of calcium chloride on heavy metal induced alteration in growth and nitrate assimilation of *Sesamum indicum* seedlings. *Phytochemistry*, **41** (1): 105-109.
- Sadasivum, S and Manickam, A., Amino acids and proteins in** (1992). Biochemical methods for agricultural science. Wiley Eastern Limited, New Delhi (India), PP 56-58.
- Sadasivum, S. and Manickam, A., Enzymes in** (1992). Biochemical methods for agricultural science. Wiley Eastern Limited, New Delhi (India), 100-103.
- Sadasivum, S. and Manickam, A., Enzymes in** (1992). Biochemical methods for agricultural science. Wiley Eastern Limited, New Delhi (India), 100-106 & 122.
- Sadasivum, S. and Manikam, A.** (1992). Carbohydrates in: Biochemical methods for agricultural science. Wiley Eastern Limited, New Delhi (India), PP 5-6.
- Salim, M.A. and Nair, R.V.** (1982) : Note on the effect of graded doses of lime and phosphorus on the growth, yield and quality of Urd bean (*Vigna mungo*) in the sandy clay loam soils of Kerala. *Legume Research*, **5** (2): 115-118.
- Schutzendubel, A., Schwanz, P., Teichmann, T., Gross, K., Lagenfeld-Heyser, R., Godbold, D.L. and Polle, A.** (2001). Cadmium induced changes in antioxidative systems, hydrogen-peroxide content and differentiation in scots pine roots. *Plant Physiol.*, **127** : 887-898.
- Siddhu, G. and Ali Khan, M.A.** (2012). Effect of cadmium on growth and metabolism of *Phaseolus mungo* L. *J. Environ. Biol.* **33**, 173-179.
- Siddhu, G., Sirohi, D.S., Kashyap, K., Khan, I.A. and Khan, M.A.A.** (2008). Toxicity of cadmium on the growth and yield of *Solanum melongena* L. *J. Environ. Biol.*, **29**, 853-857.
- Siedlecka, A. and Krupa** (2004). Functions of enzymes in heavy metal treated plants. In: Physiology and biochemistry of metal toxicity and tolerance in plants (Eds.: M.N.V. Prasad and K. Strazalka). Dordrecht, Hingham, HA: Kluwer Academic Publishers. PP. 303-324.
- Smith, J.H.C. and Benitez, A.** (1995). Modern methods of plant analysis. K. Peach and M.V. Iracey Vol. III. Springer Heidelberg., 142-192.
- Solanki, R., Anuj, Poonam, and Dhankhar, R.** (2011). Zinc and copper induced changes in physiological characteristics of *Vigna mungo* (L.). *J. Environ. Biol.*, **32**, 747-751.
- Tabaldi, L.A., Ruppenthal, R., Cargnelutti, D., Morsh, V.M., Pereira, L.B. and Schetinger, M.R.C.** (2007). Effects of metal elements on acid phosphatase activity in cucumber (*Cucumis Sativus* L.) Seedling. *Environ. Exp. Bot.*, **59**, 43-48.
- Tamas, L., Bocova, B., Huttova, J., Mistrik, I. and Olle, M.** (2006). Cadmium induced inhibition of apoplastic ascorbate oxidase in barley roots. *Plant Growth Regul.*, **48**, 41-49.
- Wagner, G.J.** (1993) Accumulation of Cadmium in crop plants and its consequences to human health. *Adv. Agron.*, **51**, 173-212.
- Wojcik, M., Skorzynska Polit, E. and Tukiendorf, A.** (2006). Organic acids accumulation and antioxidant enzyme activities in *Thlaspi caerulescens* under zinc and cadmium stress. *Plant Growth Regul.*, **48**, 145-155 (2006).
- Zahan, T.** (1986). A laboratory study of the immobilisation of cadmium in soils. *Environ. Pollut. (Series B)*, **12** : 265-280.
- Zhang, H., Lian, C. and Shen, Z.** (2009). Proteomic identification of small, copper-response proteins in germinating embryos of *Oryza sativa*. *Ann. Bot.*, **103**, 923-930.

