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EFFECT OF LEAD ON GROWTH AND NITRATE REDUCTASE ACTIVITY IN *VIGNA MUNGO* (L.) AND ITS ALLEVIATION BY SUCROSE TREATMENT

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In the present investigation, the effect of different concentrations of Pb^{2+} (2.7, 3.7 & 4.7 mg/lit) on growth and NR (nitrate reductase) activity in two varieties of *Vigna mungo* (L.) (CV. PU-35 and CV. T-9) was examined. An attempt was also made to study the effect of different doses of sucrose (1,5,10,15&20 mg/lit) on inhibition /alleviation of Pb^{2+} toxicity. A concentration dependent decrease in growth like seedling height, total chlorophyll, nitrogen, protein content and NR activity was observed under the influence of different doses of Pb^{2+}. In the present investigation sucrose showed inhibitory effect on Pb^{2+} toxicity and the most pronounced inhibition or alleviation of Pb^{2+} toxicity was noticed by 20 mg/lit dose of sucrose in variety PU-35 than the variety T-9.

INTRODUCTION :

Pollution of agricultural land by Pb^{2+} imposes a serious risk to environment and human health. Pb^{2+} stress causes direct and indirect effects on plant growth and metabolism (Singh *et.al.* 2003, Alroud 2003, and Sinhal *et.al.* 2006). There is an urgent need to alleviate the toxic effects of Pb^{2+} from plants. Sugars may act as one of the agent to neutralize the adverse effects of Pb^{2+} on plants because they may provide carbon skeleton for amino acid biosynthesis, hence they may increase the activities of the enzymes involved in primary amination (Vyas and Puranik 1993, Sinhal *et.al.*, 2007).Thus the present investigation has been undertaken to understand the individual and combined effects of Pb^{2+} individually and in combination with sucrose in terms of growth and NR activity in *Vigna mungo* (L.), grown as major pulse crop in North-West India.

MATERIALS AND METHODS:

Two cultivated varieties of *Vigna mungo* (L.) Hepper, PU-35 & T-9 were presoaked in distilled water for 8 hours and were sown in two separate field plots(first plot is of 6x6m size having 6 rows and second is of 30x10m size having 30 rows). In first part, the irrigation was done with different doses of Pb^{2+} (2.7, 3.7 & 4.7 mg/lit) and in second part the combined treatments of different doses of Pb^{2+} (2.7, 3.7 & 4.7 mg/lit) and sucrose (1, 5, 10, 15 & 20 mg/lit) was given at the interval of 15 days. The effect of this irrigation was observed on seedling height, total chlorophyll, total nitrogen, protein content & NR activity of leaves. The chlorophyll content was determined by the method of Arnon (1949), Nitrogen content was determined by Micro-Kjeldahl method of Lang (1958), Protein content was measured by the method of Lowry *et al.*(1951) and NR activity was determined by the method of Srivastava (1974).

RESULTS AND DICUSSION

The present investigation clearly indicated that the seedling height decreased with increase in concentration of Pb^{2+} in both the varieties as compared to their respective controls. The maximum reduction of seedling height was noticed with the higher dose of Pb^{2+} in both the varieties (Table -1). Higher concentration of Pb^{2+} was reported to retard cell division and differentiation and reduce their elongation and effect plant growth and development (Lane *et al.* 1978, Kastori *et al.* 1998). Tomar *et al* (2000) studied the effect of Pb^{2+} on *Vigna radiata* and concluded that higher concentration of Pb^{2+} reduces plant height which may be due to decrease in mitotic frequency and accumulation of Pb^{2+} in the cell wall components especially, pectic substances and hemi celluloses.

Table 1: The individual effect of lead (different concentrations) on seedling height, total chlorophyll, total nitrogen, protein content and Nitrate reductase activity in two cultivars of *Vigna mungo* (L.)

(Results are mean of five determinants)

Concentration of treatment (mg/lit)	Seedling height (in cm)	Chlorophyll Content (mg g^{-1} fr wt.)	Nitrogen Content (mg g^{-1} dry wt.)	Protein Content (mg g^{-1} dry wt.)	NR Activity (µM NO_2^- $hr^{-1}g^{-1}$ fr wt)
Variety PU-35 (Control)	**18.36±0.35**	**1.33±0.12**	**17.99±0.70**	**50.96±1.54**	**20.24±0.35**
Pb^{2+} +2.7	16.56*±1.25	1.22*±0.85	15.10*±1.45	42.35*±2.21	18.25*±1.30
Pb^{2+} +3.7	14.25*±1.20	1.02*±1.28	13.25*±2.15	35.30*±1.95	16.20*±1.38
Pb^{2+} + 4.7	13.25*±0.80	0.95*±1.15	11.05*±2.45	31.95*±1.24	14.25*±1.45
Variety T-9 (Control)	**17.76±0.21**	**1.32±0.90**	**17.77±0.66**	**49.55±0.61**	**20.10±0.85**
Pb^{2+} + 2.7	16.00*±1.15	1.20*±0.95	14.50*±2.12	40.25*±1.95	16.80*±1.25
Pb^{2+} + 3.7	13.80*±1.25	1.00*±0.85	12.80*±2.00	34.15*±1.85	15.02*±2.15
Pb^{2+} +4.7	12.50*±1.45	0.89*±0.92	10.25*±1.25	30.15*±1.35	13.20*±2.95

±Values indicates standard error.
*Significant at 5% level.

Similarly dose dependent decrease in chlorophyll, nitrogen and protein content was also noticed under the influence of different concentrations of Pb^{2+} (Table-1). Similar findings were also reported by Baszinsky *et al.* (1980) and Prasad & Prasad (1987) and they concluded that there are two enzymes i e. d-aminolaevulinic acid (ALA) dehydratase and protochlorophyllide reductase which are involved in chlorophyll biosynthesis in higher plants and are inhibited by heavy metals (Pb^{2+} and Hg^{2+}) because these metals bind with functional sulphydryl (-SH) group of the enzymes. According to Chhetri *et al*., (2004) when heavy metals (Pb^{2+} Zn^{2+} and Cd^{2+}) toxicity crosses the threshold (1mM), the protein level decreases and this might be due to the breakdown of protein synthesis mechanism at toxic concentration level of heavy metals or due to reduced incorporation of free amino acids into protein.

The supply of 2.7, 3.7 and 4.7 mg/lit of Pb^{2+} substantially inhibited *in vivo* NR activity in leaves of *Vigna mungo*. The higher dose of Pb^{2+} reduced more NR activity in both the varieties (Table - 1). The enzyme nitrate reductase is substrate inducible enzyme which is found in cytosol and it's activity often regulates the rate of protein synthesis in plants (Sinhal *et. al*, 2006). According to Singh *et al.* (1998), the inhibition of NR activity under the influence of Pb^{2+} may be multifacial, e.g., due to reduced supply of NADPH, disorganization of chloroplast, less NO_3^- supply to the site of synthesis caused by water stress and direct effect of Pb^{2+} on protein synthesis because it has a strong affinity for functional –SH group of the enzyme.

The reduction in seedling height, chlorophyll, nitrogen and protein content & NR activity by Pb^{2+} treatment was ameliorated significantly by different doses of sucrose (1,5, 10, 15 & 20 mg/lit) in combination with each dose of Pb^{2+}(2.7, 3.7 & 4.7 mg/lit). The more pronounced significant reduction in Pb^{2+} toxicity was observed with 2.7 mg/lit of Pb^{2+} + 20 mg/lit of sucrose treatment (Table -2). Puranik and Srivastava (1983) and Vyas & Puranik (1993) observed that sucrose enhances the stability of NR activity and mobilize endogenous nitrate pool for the enzyme activity. Sugars may provide carbon skeleton for amino acid biosynthesis, hence they should increase the activities of enzymes involved in primary amination. Sinhal *et.al.*, (2007) concluded that sucrose and magnesium antagonized the toxic effects of heavy metal(Zn^{2+}) on growth and NR activity in *Vigna mungo*

In conclusion, Pb^{2+} reduces the growth parameters like seedling height, total chlorophyll, total nitrogen, protein content and NR activity of *Vigna mungo* and sucrose is able to alleviate Pb^{2+} toxicity. The alleviation of Pb^{2+} toxicity by sucrose

Table 2: Influence of sucrose in combination with lead (different concentrations) on seedling height, total chlorophyll, total nitrogen, protein content and Nitrate reductase activity in the leaves of *Vigna mungo*(L.)

(Results are mean of five determinants)

Concentration of treatment (mg/lit)	Seedling height (in cm)	Chlorophyll Content (mg g^{-1} fr wt.)	Nitrogen Content (mg g^{-1} dry wt.)	Protein Content (mg g^{-1} dry wt.)	NR Activity (μM NO_2- $hr^{-1}g^{-1}$ fr wt)
Variety PU-35 (Control)	**18.36±0.35**	**1.33±0.12**	**17.99±0.70**	**50.96±1.54**	**20.24±0.35**
Pb^{2+} 2.7+ sucrose 1	16.65*±0.30	1.24*±0.85	15.00*±1.45	42.25*±2.15	18.20*±1.62
Pb^{2+} 2.7+ sucrose 5	16.90*±1.25	1.26*±0.90	15.15*±1.22	42.42*±2.19	18.35*±0.98
Pb^{2+} 2.7 + sucrose 10	17.25*±1.10	1.29*±1.20	15.26*±1.24	43.56*±1.95	18.55*±1.25
Pb^{2+} 2.7 + sucrose 15	17.95*±1.30	1.30*±1.45	15.80*±0.95	45.50*±1.05	18.92*±1.35
Pb^{2+} 2.7+ sucrose 20	18.25*±1.45	1.32*±1.42	17.59*±0.65	49.95*±1.65	19.98*±1.24
Pb^{2+} 3.7+ sucrose 1	14.30*±0.95	1.02*±1.55	13.05*±1.15	35.00*±1.70	16.15*±0.65
Pb^{2+} 3.7+ sucrose 5	14.80*±0.99	1.08*±0.95	13.25*±1.05	35.98*±1.95	16.39*±0.85
Pb^{2+} 3.7 + sucrose 10	15.10*±0.75	1.14*±1.25	13.45*±1.35	36.45*±2.15	16.95*±1.20
Pb^{2+} 3.7 + sucrose 15	15.65*±1.20	1.20*±1.05	14.00*±2.25	37.85*±1.65	17.02*±1.62
Pb^{2+} 3.7+ sucrose 20	16.25*±1.35	1.22*±0.85	14.20*±1.22	40.65*±1.74	18.26*±1.45
Pb^{2+} 4.7+ sucrose 1	13.28*±1.15	0.80*±1.20	11.00*±1.88	31.35*±0.98	14.26*±0.96
Pb^{2+} 4.7+ sucrose 5	13.45*±1.29	0.84*±0.96	11.25*±1.42	32.42*±1.91	14.51*±0.85
Pb^{2+} 4.7 + sucrose 10	14.20*±1.75	0.92*±0.85	11.95*±0.99	32.92*±1.45	14.69*±1.15
Pb^{2+} 4.7 + sucrose 15	14.45*±1.05	0.96*±0.86	12.10*±1.48	33.50*±1.23	15.00*±1.65
Pb^{2+} 4.7+ sucrose 20	14.88*±1.80	0.98*±0.95	12.26*±1.45	34.62*±1.45	15.45*±1.82
Variety T-9 (Control)	**17.76±0.21**	**1.32±0.90**	**17.77±0.66**	**49.55±0.61**	**20.10±0.85**
Pb^{2+} 2.7+ sucrose 1	16.10*±1.15	1.14*±1.25	14.50*±1.43	41.32*±1.09	16.75*±1.30
Pb^{2+} 2.7+ sucrose 5	16.38*±1.25	1.19*±1.20	14.91*±1.34	42.30*±1.54	16.90*±0.92
Pb^{2+} 2.7 + sucrose 10	16.95*±1.45	1.22*±0.98	15.20*±1.46	42.90*±1.62	17.10*±1.43
Pb^{2+} 2.7 + sucrose 15	17.00*±0.95	1.24*±0.96	15.96*±1.59	44.60*±1.35	17.58*±1.61
Pb^{2+} 2.7+ sucrose 20	17.30*±0.80	1.25*±0.75	16.98*±1.60	45.00*±1.49	18.52*±1.89
Pb^{2+} 3.7+ sucrose 1	14.00*±1.05	0.98*±0.65	12.50*±3.52	34.65*±1.62	15.00*±2.35
Pb^{2+} 3.7+ sucrose 5	14.28*±1.75	0.99*±0.98	12.78*±2.50	34.99*±1.25	15.65*±6.45
Pb^{2+} 3.7 + sucrose 10	14.75*±1.95	1.01*±0.69	13.05*±1.50	35.10*±1.32	16.10*±1.65
Pb^{2+} 3.7 + sucrose 15	15.00*±2.05	1.05*±0.91	13.59*±1.52	36.25*±1.46	16.50*±1.78
Pb^{2+} 3.7+ sucrose 20	15.50*±1.65	1.08*±0.55	14.00*±1.46	38.95*±1.62	17.00*±1.15
Pb^{2+} 4.7+ sucrose 1	13.05*±1.25	0.70*±0.75	10.50*±1.67	30.55*±2.12	13.35*±3.10
Pb^{2+} 4.7+ sucrose 5	13.56*±1.85	0.78*±1.22	10.92*±0.96	31.05*±1.65	13.85*±1.69
Pb^{2+} 4.7 + sucrose 10	13.98*±1.92	0.81*±1.05	11.10*±1.27	31.52*±1.69	14.00*±1.62
Pb^{2+} 4.7 + sucrose 15	14.49*±1.85	0.90*±1.15	11.75*±2.10	32.01*±1.82	14.33*±1.58
Pb^{2+} 4.7+ sucrose 20	14.99*±2.15	0.93*±1.24	12.05*±1.65	33.10*±1.80	14.98*±1.62

±Values indicates standard error.
*Significant at 5% level

was more pronounced in variety PU-35 than the variety T-9 because variety PU-35 is less susceptible to Pb^{2+}as compared to variety T-9.

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