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Effect on Amylase Activity and Growth Paramaters Due to Metal Toxicity of Iron, Copper and Zinc

KEYWORDS: Amylase activity, Seed germination, metal toxicity

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ABSTRACT *Increasing contamination and widespread accumulation of heavy metals in soil has become a major concern due to their adverse effect on plant growth and metabolism. Taking this account, an attempt was made to study the effect of 50 mM concentration of CuSO4, FeSO4 and ZnSO4 on the root/shoot length, protein content, enzyme activity (α-Amylase) in Vigna Radiata. There was significant decrease in germination and root/shoot length in seeds treated with CuSO4, FeSO4 and ZnSO4 as compared to controls. The root/shoot length decreased more significantly in the presence of CuSO4 ($p<0.0001$), ZnSO4 ($p<0.0003$) as compared to FeSO4 ($p<0.003$). Significant decrease in protein content has been found in Vigna Radiata when treated with CuSO4 and ZnSO4 ($p<0.0001$). However, there was no significant decrease in protein content when treated with FeSO4. There was significant decrease in α-amylase enzyme activity in seeds treated with of CuSO4, FeSO4 and ZnSO4 as compared to controls ($p<0.001$).*

INTRODUCTION

Heavy metals contamination of soil due to rapid industrialization, urbanization, intensive agriculture, land application of sewage sludges, mining, etc pose serious risks to humans, plant and animals [1]. Heavy metals such as copper (Cu), iron (Fe), zinc (Zn), lead (Pb), mercury (Hg), etc are major environmental pollutants which enter plants mainly through root system [2]. However, these metals are also essential for plant growth and development. Cu participates in numerous physiological processes such as photosynthesis and respiration and is an essential co-factor for key enzymes involved in various metabolic pathways such as ATP synthesis, elimination of superoxide radicals, etc [3] Zn is also needed for the formation of auxin, chlorophyll and cytochrome pigments. Zn is also an integral part of various enymes such as carbonic anhydrase, alcohol dehydrogenase, superooxide dismutase and RNA polymerase. Fe is a major constituent of redox systems such as heme protein (cytochromes, peroxidases) and iron sulfur proteins (ferroxidin, acontiase) and also plays an important role in biological process such as photosynthesis and chlorophyll biosynthesis.

However, these metals may exert toxic effects to plants at high concentrations resulting in toxicity symptoms, growth inhibition, lower biomass production and disruption of membrane structure in different plant species [4]. The bioaccumulation of heavy metals in excessive concentration may replace essential metals in pigments or enzymes disrupting their function and causing oxidative stress. The heavy metal toxicity is due to high affinity to sulfydryl groups and disulfide bonds which cause damage to secondary structure of proteins and alter the enzymatic activities [5]. Higher concentration of Zn results in decreased growth and development, metabolism, alternation in catalytic efficiency of enzymes and induction of oxidative stress [6]. Heavy metal stress also results in production of reactive oxygen species which can be allayed by several antioxidant enzymes such as peroxidases, catalase and superoxide (SOD) [7]. Excess of copper and iron concentration generates oxidative stress in intact roots due to the formation of reactive oxygen species owing to decrease in peroxidase activity [8]. The Fe toxicity also results in impaired cellular structures, damaged membranes, DNA and protein.

India has been the largest producer, consumer and importer of pulses because pulses dominantly constitute the sample diet of the people in India. Since metal pollution in India is a multielement problem there is a need to study the effects of various metals (Cu, Zn and Fe) on *Vigna Radiata*. Taking this into account, the present study was conducted to investigate the effect of Cu, Zn, and Fe on plant growth and physiology which will help to understand the biological mechanism taking by seedlings in response to physiologically toxic concentration of metals.

MATERIALS AND METHOD

The present study was carried at Dept. of biotechnology at GGDSD College, Chandigarh. The seeds of Vigna Radiata used for petri plate experiments were washed with distilled water and then were surfaced sterilized with 0.1% HgCl2 and 70% ethyl alcohol are treated with to avoid fungal/infection infection. After that the seeds were washed with autoclaved water thrice to remove excess of fungicide. The 50mM concentration of Zn, Cu and Fe were prepared in autoclaved distilled water by using Zinc sulphate (ZnSO4), copper sulphate (CuSO4), and iron sulphate (FeSO4), respectively. Pure distilled water was used as a control. The seeds were soaked in 50mM concentration of ZnSO4, CuSO4, FeSO4 and distilled water (control) for 3-4 hours. The seeds were then transferred to filter paper placed on petri plates having 10 ml solution of 50mM concentrations of ZnSO4, CuSO4 and FeSO4, distilled water as control and the petri plates were labelled accordingly. The petri plates were incubated in dark for 24 hrs and then transferred to light conditions for 72 hrs. The growth parameters like germination, root and shoot length were observed after 24 hrs and 72 hrs respectively. The experiment was conducted in triplicate. The germination percentage was calculated on the basis of seed germination as compared to control. The germinated seeds were homogenized in autoclaved distilled water. The crude homogenate

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was then centrifuged at 5000x g for 10 minutes. The supernatant was collected and stored at 4oC till further use.

Protein Estimation by Lowry method:

The Lowry protein assay method was used for total protein concentration determination [9].

alpha-amylase assay:

The activity of α-Amylases enzyme is measured calorimetrically by estimating the amount of reducing sugar formed (maltose) by 3,5 – dinitrosalicyclic acid (DNS) method [10].

Statistical analysis:

The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD). The statistical differences represented by letters were obtained through one-way analysis of variance (ANOVA) followed by students t-test ($p < 0.05$).

RESULTS AND DISCUSSION

Metal toxicity is an important factor governing germination and growth of plants [11]. Since germination and seedling growth is considered as an indicator of metal stress on plant ability to survive, the present study focused on seed germination, plant growth protein content in *Vigna Radiata* seeds at initial stage.

The germination percentage in *Vigna Radiata* seeds were adversely affected due to the application of heavy metals. The maximum germination percentage was present in seeds treated with 50mM $FeSO_4$ (70%) followed by $CuSO_4$ (65%) and then $ZnSO_4$ (55%) as shown in Table 1. There was significant reduction in seed germination and seedling growth of *Vigna Radiata* seeds as reported in earlier studies [12,8] This could be due to accumulation of metal in their cell sap or because of the failure of sub-cellular organelles to adjust to high metal conc. Shafiq et al [13] reported that decrease in seed germination of plant can be attributed to the accelerated breakdown of stored food materials in seed resulting in reduction of meristematic cells. The increased germination percentage in the presence of 50mM concentration of Fe as compared to Cu and Zn could be due to the fact that free Fe ions are released by hydrolyzing enzyme from its organic component. [14]. The pronounced effect of Cu and Zn on seed germination is in accordance with other studies [15, 16]. The reduction of seed germination can also be attributed to alterations of selection permeability properties of cell membrane [17] or due to osmotic effect [18]. There was significant decrease in alpha amylase activity in presence of Fe, Zn and Cu as reported in literature [19]. Decrease in amylase activity is an important factor for germination inhibition as it impairs the supply of sugar to developing embryo axes and induces biomass mobilization by release of glucose and fructose which inhibits the breakdown of starch and sucrose in primary root and shoot, thereby effecting the germination [20, 15].

With the addition of heavy metal concentration of Fe, Zn and Cu, there was significant decrease in root/shoot length similar to finding by other studies [15] (Table 1). The reduced seedling length during metal stress could be due to low water potential, hampered nutrient uptake and secondary stress [21]. The inhibitory action of excess of copper and zinc in root and shoot length might be due to reduction in cell division, inducement of chromosomal aberrations and abnormal mitosis [22]. Fe toxicity causes reduction in root/shoot length due to toxic effect on photosynthesis, respiration and protein synthesis [23].

Significant decrease in the protein content has also been found in *Vigna Radiata* when treated with the copper and zinc as observed by Singh and Sinha (2005)[24] (Table 1). However, there was no significant decrease in the protein content due to Fe toxicity. The decrease in protein content as observed at higher concentrations of copper and zinc in *Vigna Radiata may* be because of enhanced protein degradation process [25]. Enhanced protease activity under stress conditions could also be reason for hydrolysis of proteins into amino acids for storage and for osmotic adjustments during metal stress. John et al 2009 [21] reported that that these heavy metals may have induced lipid peroxidation and fragmentation of proteins due to toxic effects of reactive oxygen species which led to reduced protein content.

CONCLUSION

Presently, increased industrialization in developing countries like India has resulted into enormous deterioration of air, water and soil. Liquid effluents which are the main source of metal toxicity are frequently released in water bodies resulting in bioaccumulation of metals. The present study concludes that, heavy metal treatment produced toxic impact on germination and seedling growth of *Vigna Radiata*. However, there is a need of understanding various underlying mechanisms to overcome stress which will lead to better agricultural produce despite heavy metal toxicity.

Table 1. Table showing the effect of 50 mM concentration of CuSO4, FeSO4 and ZnSO4 on the germination percentage, root/shoot length, protein content and enzyme activity (α-Amylase) in *Vigna Radiata*. Significant differences from control (p<0.05) are marked with *

S. NO	TEST	CONTROL	$FeSO_4$	$CuSO_4$	$ZnSO_4$
1.	GERMINATION %	100%	70%±11.55 (p=0.0020)*	65%±23.80 (p=0.025)*	55%±19.1 (p=0.003)*
2.	ROOT/ SHOOT LENGTH (cm)	7.888±1.843	1.15±0.90 (p=0.003)*	0.8525±0.395 (p=0.0001)*	1.55±0.4322 (p=0.0003)*
3.	PROTEIN CONTENT (mg/ml)	1.36±0.1905	1.1700±0.304 p=(0.4106)	0.6760±0.750 (p=0.0001)*	0.64720±0.106 (p=0.0001)*
4.	α-Amylase activity (µm/ml/ min)	6.90±0.425	0.970±0.49 p=(0.0001)*	0.65-±0.277 (p=0.0001)*	1.78±0.774 (p=0.0005)*

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