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EFFECT OF METAL STRESS OF IRON, COPPER AND ZINC ON AMYLASE, ACID PHOSPHATASE AND ALKALINE PHOSPHATASE ACTIVITY IN *Phaseolus vulgaris*

MITTAL N[1], KAUR A[2] AND KAUR A[*2]

1: Dept. of Biochemistry, Goswami Ganesh Dutt Sanatan Dharma College, Sector-32C, Chandigarh (UT) India – 160030

2: Dept. of Biotechnology, Goswami Ganesh Dutt Sanatan Dharma College, Sector-32 C, Chandigarh (UT) India - 160030

***Corresponding Author: Dr. Avneet Kaur: E Mail: avneet.kaur@ggdsd.ac.in**

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ABSTRACT

Acid and alkaline phosphatases in plants play a major role in the supply and metabolism of inorganic phosphate for the maintenance of cellular metabolism. Phosphate plays an extremely important role in a variety of reactions in germinating seed including energy metabolism and synthesis of nucleic acids and membranes. In the present study, the effect of different concentrations of iron, copper and zinc (50mM, 100mM, 150mM, 200mM) on hydrolysis enzymes activity e.g., amylase, acid and alkaline phosphatase was studied in *Phaseolus vulgaris*. In the present study, with the increasing concentration of iron (Fe), zinc (Zn) and copper (Cu) toxicity there was decrease in phosphate levels. There was significant decrease in acid and alkaline phosphatase levels in seeds of *Phaseolus vulgaris* treated with Zn and Cu heavy metal with increasing metal concentration ($p \leq 0.05$). No significant change on acid phosphatase activity was observed with Fe metal toxicity.

Keywords: *Phaseolus vulgaris*, amylase, acid and alkaline phosphatase

INTRODUCTION

In agriculture lands, soil is a valuable and non-renewable resource essential for germination of seeds, survival and growth of plants. The soil fertility and growth of plants are dependent on various soil enzyme activities e.g., arylsulfatase, alkaline phosphatase, β-glucosidase, cellulase, dehydrogenase, invertase, protease, and urease. These enzymes take part in degradation of plant residues and also inthe transformation of nitrogen, phosphorus and sulphur **[1]**.

Soil enzyme activity is involved in nutrient cycling and availability to plants and can be used as an index of soil functioning **[2]**. Anthropogenic activities, ongoing technological advancements in industrialization and urbanization process and abiotic stress factors including salinity, drought, extreme temperatures, chemical toxicity, affect the amount and activities of soil enzymes at different functional levels, reduce the growth and the yield of plants and cause excessive pollutant concentrations in plants. Heavy metals (HM) are one of the major groups of pollutants in soil environment, arising from repeated applications of sewage sludge, municipal wastes and animal slurries, the activity of smelting industries, impurities in fertilizers and deposition of air pollutants from burning of fossil fuels and various industrial activities.

Heavy metals such as Cadmium (Cd), Zinc (Zn) and Cupper (Cu) has been found to inhibit root growth and cause increase in lipid peroxidation and modulated antioxidative enzyme activity in root **[3]**. Cd reduces elongation of roots due to inhibited mitosis, decreased synthesis of cell wall components, damaged Golgi apparatus or changes in the metabolism of polysaccharides in the root cap.

Alkaline and acid phosphatases are hydrolytic enzymes that catalyze the hydrolysis of esters of phosphoric acid and also carry out transphosphorylation in the presence of large concentrations of phosphate acceptors in a thermodynamically favourable process, which occurs in acidic and alkaline medium, respectively **[4]**. Acid phosphatases are believed to increase orthophosphate (Pi) availability under phosphorous deficient conditions Various study have reported use of phosphatases for enhanced bioremediation of heavy metals as enzymatic precipitation produces less toxic or biodegradable end products **[5]**. Alkaline phosphatases have a potential role in utilization of phosphomonoesters as the source of Pi required for maintenance of cellular metabolism **[6]**.

Phaseolus vulgaris is an annual plant which is a major source of protein for people in developing countries. In the present study, the effect of different concentrations of iron, copper and zinc (50mM, 100mM, 150mM, 200mM) was studied on various hydrolysis enzymes activity (amylase, acid and alkaline phosphatase).

MATERIALS AND METHODS

The seeds of *Phaseolus vulgaris* were purchased from local markets and were sterilized in 2% bevastin to avoid fungal infection. Seed germination was carried out in glass petri plates. Each dish contained 10mL of different concentration (10mM, 50mM, 100mM, 150mM, 200mM) of metal ($ZnSO_4.7H_2O$, $CuSO_4.5H_2O$ and $FeSO_4$) and 10mL of distilled water (control). *In vitro* germination was designed for three replicates and all growth parameters (no. of seeds germinated, root length, shoot length, root/shoot length) were recorded at different time intervals. The germinated seeds were homogenized in distilled water, pH 7. The homogenates were centrifuged at 5000g for 10minutes at 4^{O}C and the supernatant was used as enzyme sample. The protein estimation was done and total protein was expressed as mg of protein hydrolysed per mg protein **[7]**. Acid and alkaline phosphatase activity was measured **[8]**. The activity of α-Amylases enzyme was measured colorimetrically by estimating the amount of reducing sugar formed (maltose) by 3,5 – dinitrosalicyclic acid (DNS) method **[9]**.

Statistical analysis: Statistical analysis was based on one-way analysis of variance (ANOVA). The effects of heavy metal treatment were considered statistically significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

There was significant increase in the protein content with increase in Cu concentration ($p \leq 0.05$). The increase in protein content could be due to enhanced accumulation of total soluble protein owing to the synthesis of metal-chelating polypeptides **[8]**. These metals induced proteins have a role in the maintenance of heavy metal homeostasis or detoxification **[10]**. The Cu metal toxicity also increase the synthesis of heat shock proteins (HSPs) which helps in the protection and repair of protein under stress conditions **[11]**. However, with increasing concentration of Fe or Zn metal toxicity there was no significant effect on protein content ($p \leq 0.05$).

There was significant decrease in the carbohydrate metabolizing enzyme i.e., amylase with increasing concentration of Fe ($p \leq 0.05$) as reported in literature **[11, 12]**. The decrease in activity with increase in the concentration of Fe is due to fact that Fe forms complex with carbohydrate **[13]**. The decrease in amylase activity was more significant with Cu toxicity as compared to Fe and Zn ($p \leq 0.05$) **[14]**. The proteomics studies have revealed that the decrease in amylase activity due to Cu toxicity inhibits seed germination which affects overall metabolism, water uptake and failure to mobilize reserve food in plants **[15]**. The inhibition in the activities of alpha-amylase which maintains active respiratory metabolism results in the decrease in growth parameters as observed in present study resulting in biomass mobilization by release of glucose and fructose **[16]**.

In the present study, with the increasing concentration of Fe, Zn and Cu toxicity there was decrease in phosphate **(Figure 1)**. The decrease in phosphate content seems to be correlated with acid phosphatase activity because decrease in acid phosphatase activity will decrease the rate of release of inorganic phosphate from reserves. It was observed that heavy metal ions are known to serve as inhibitors for acid phosphatase activity **[17]**. The decrease in acid phosphatase activity could also be due to loss of one or more isozymes **[18]**. The various other factors that contribute to decrease in enzyme activity could be modulation of enzyme activity by divalent cations through delayed enzyme solubilisation and activation or inhibition of enzyme activity by heavy metals due to interference with the PO^{3-} binding sites **[17]**.

The acid and alkaline phosphatases are involved in hydrolysis of variety of phosphate esters. The acid and alkaline phosphatases levels in treated *Phaseolus vulgaris* with Zn and Cu heavy metal was significantly affected with progressive decrease in enzyme levels with increasing metal concentration. There was simultaneous decrease in the phosphate levels with increasing concentration of Zn and Cu metal treated *Phaseolus vulgaris* seeds. The Cu and Zn at high concentrations might interfere with the inorganic phosphate (Pi) binding site, which impair the mobilization of phosphate and thus effect the growth of the plants **[19].** The heavy metals have the ability to replace Zn^{2+} from the active site phosphatase resulting in changes in enzyme conformation and consequently inhibition of activity. The effect on acid phosphatase activity was significantly more pronounced with increasing concentration of Cu than Zn. The inhibition of soil phosphatases by Cu ions is due to Cu interaction with –SH groups of aminoacids of the active site of the enzyme **[20]**. The decrease in the enzyme activity could also be due to binding of Cu not only to the enzyme molecule but also the enzyme-substrate complex **[21]**. The increased concentration of Zn toxicity also decreased acid phosphatases levels but to a lesser extent than Cu which can be explained by the fact that microbial phosphatases are generally metallo-enzymes in which the metal is Zn or Mg **[22]**. There was no change on acid phosphatase activity with Fe metal toxicity **[23]**. It could due to the fact that the Fe could be the component involved in acid phophatase synthesis. The active form of the mammalian purple acid phosphatase contains a binuclear Fe^{3+} - Fe^{2+} metal center **[24]**. The inhibition of acid phosphatase due to heavy metal contamination, results in the slower nitrogen and phosphorus cycling. The lower N/P ratio in the soil cause stronger N limitation for plants and microorganisms compounds **[19]**. The heavy metals have strong effect on intracellular enzymes of active microorganisms fixers and heterotrophic bacteria, fungi biomass **[25]**, taxonomic diversity of microbes compounds **[26]**, and bacteria transcriptional activity in metal (loid) contaminated soils.

This study showed toxic impact on germination and seedling growth of *Phaseolus vulgaris* as the metals (Cu, Fe, Zn) decreased the enzyme activity of amylase, acid and alkaline phosphatase). The inhibitory effect on *Phaseolus vulgaris* seedlings was more pronounced in copper than zinc and iron.

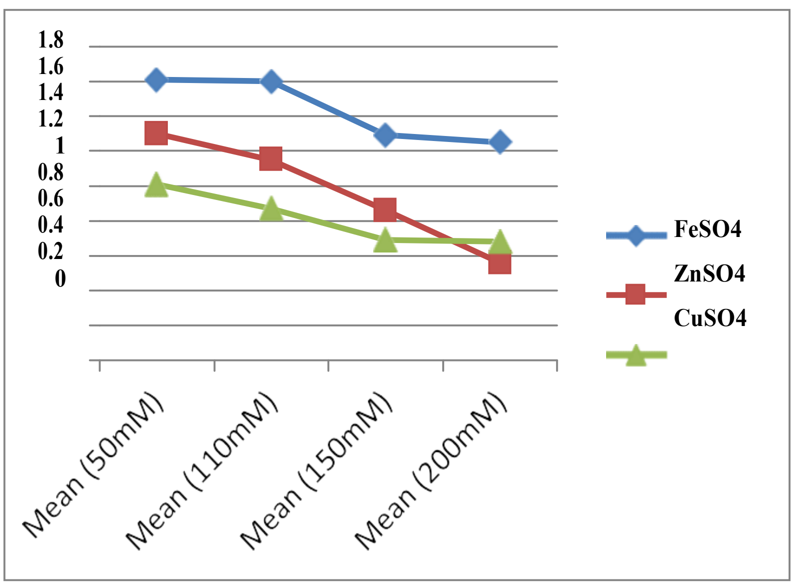

Figure 1: Figure showing acid inorganic phosphorous in *Phaseolus vulgaris* treated with different concentration of iron, zinc and copper

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