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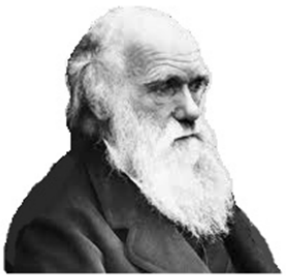

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Antioxidant Enzyme Mechanism of Cluster Bean (*Cyamopsis tetragonaloba* (L.) Taub). Under Cobalt Stress

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ABSTRACT

The research work has been carried out to estimate the low level cobalt application enhancing antioxidant enzyme activities of cluster bean plants. The experiments were conducted in earthen pots containing 3 kg of air dried soil. The inner surface of pots was lined with polythene sheet. Cluster bean plants were raised in soil amended with different concentrations of cobalt (0, 50, 100, 150, 200, and 250 mg/kg). The antioxidant enzyme activities were analysed at 30, 60, and 90 days after sowing (DAS). The significant enhance antioxidant enzyme activities were found at 50 mg/kg cobalt application in the soil in all the sampling days when compared to control. Further increase in the cobalt level (100-250 mg/kg) in the soil decreased antioxidant enzyme activities of cluster bean plants in all the sampling days.

***Keywords*:** Cobalt, *Cyamopsis tetragonaloba,* Catalase, Peroxidasa, Polyphenol oxidase

1. INTRODUCTION

The presence of heavy metals in the environment is of major concern because of their toxicity and threat to plant and animal life. Heavy metal availability in soil, which depends on natural processes, particularly lithogenic and pedogenic, as well as anthropogenic factors, such as industrial activity and mining, sewage disposal, traffic, etc., are mainly responsible for the increase in the concentration of heavy metals in air, water, and soil. The presence of heavy metals in residues from city and sewage sludge, as well as some inorganic fertilizers applied to agricultural lands is beginning to be the cause as a new source of pollution in soils and plants.

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The heavy metal concentrations are increasing as environmental pollutants, particularly in densely industrialized and heavy road traffic areas. The heavy metals can create a major ecological crisis since they are non-degradable and often accumulate through trophic level causing a deleterious biological effect. Some of the heavy metals are considered as essential trace elements for the normal growth of plants.

The biological importance of cobalt was first recognized by the discovery that small amounts of the elements would caus certain deficiency symptoms in plants. Even though cobalt has been recognized as a micronutrient for animals and certain microorganisms, until recently when there has been no conclusive evidence of its essentiality for higher plants. The cobalt as a heavy metal pollutant in plants has been studied previously.

In abiotic stress, metal response will result in the production of reactive oxygen species (ROS) which lead to the activation of defense mechanisms in terms of antioxidant enzymes. Generation of ROS, such as superoxide, H_2O_2 and hydroxyl molecules cause rapid cell damage by triggering off a chain reaction. Plants under stress produce some defence mechanisms to protect themselves from the harmful effect of oxidative stress. ROS scavenging is one among the common defense response against abiotic stresses. ROS scavenging depends on the detoxification mechanism provided by an integrated system of non-enzymatic reduced molecules like ascorbate and glutathione and enzymatic anti-oxidants. The major ROS scavenging activities include complex non-enzymatic (ascorbate, glutathione, α-tocopherol) and enzymatic anti-oxidants, like catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD), polyphenol oxidase (PPO), peroxidase (POX), etc. The pathways include the water-water cycle in chloroplasts and the ascorbate-glutathione cycle. Antioxidant mechanisms may provide a strategy to enhance metal tolerance in plants.

Cluster bean (*Cyamopsis tetragonaloba* (L.) Taub.) is one of the world's main sources of edible vegetable oils and high-protein livestock feed. There are many reports on the effect of heavy metals up on the morphological and biochemical characteristics of Cluster bean.

The effect of cobalt on nodulation, leg-haemoglobin content and antioxidant enzyme activities of crop plants attracted little attention. So, the present investigation was carried out to evaluate the effect of this heavy metal, cobalt on the root nodule formation, leg-haemoglobin content antioxidant enzyme activities of *Cyamopsis tetragonaloba.*

2. MATERIALS AND METHODS

The seeds of Cluster bean (*Cyamopsis tetragonaloba)* were obtained from Tamilnadu Agricultural University, Tamilnadu, India. Plants were grown in untreated soil (control) and in soil to which cobalt had been applied (50, 100, 150, 200, and 250 mg/kg soil). The cobalt as finely powdered cobalt chloride ($CoCl_2$) was applied to the surface soil and thoroughly mixed with soil. Ten seeds were sown in each pot. All the pots were watered to field capacity daily. Plants were thinned to a maximum of five per pot, after a week of germination. The total number of root nodules and leg-haemoglobin content were analysed at 15 days intervals, namely 15, 30, 45, 60, and 75 days after sowing (DAS).

The leg-haemoglobin contents of root nodules were analysed by following method. Catalase (CAT) (EC 1.11.1.6) activity was measured according to the method of Chandlee and Scandalios, 1963, with a small modification. 0.5 g of frozen plant material was homogenized

in a pre-chilled pestle and mortar with 5 ml of ice cold 50 mM sodium phosphate buffer (pH 7.5), containing 1 mM phenylmethylsulfonyl fluoride (PMSF).

The extract was centrifuged at 4 °C for 20 min at 12,500 rpm. The supernatant was used for enzyme assay. The assay mixture contained 2.6 mL of 50 mM potassium phosphate buffer (pH 7.0), 400 µL of 15 mM H_2O_2 and 40 µL of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm.

Peroxidase (POX; EC 1.11.1.7) was assayed by the method of Kumar and Khan, 1982. Assay mixture of POX contained 2 mL of 0.1 M phosphate buffer (pH 6.8), 1 mL of 0.01 M pyrogallol, 1 mL of 0.005 M H_2O_2, and 0.5 mL of enzyme extract. The solution was incubated for 5 min at 25 °C after which the reaction was terminated by adding 1 mL of 2.5 N H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank, prepared by adding the extract after the addition of 2.5 N H_2SO_4 at zero time. The activity was expressed in unit mg^{-1} protein. One unit (U) is defined as the change in the absorbance by 0.1 $min^{-1} \cdot mg^{-1}$ protein. Polyphenol oxidase (PPO; EC 1.10.3.1) activity was assayed by the method of Kumar and Khan, 1982. Assay mixture for PPO contained 2 mL of 0.1 M phosphate buffer (pH 6.0), 1 mL of 0.1 M catechol, and 0.5 mL of enzyme extract. This was incubated for 5 min at 25 °C, after which the reaction was stopped by adding 1 mL of 2.5 N H_2SO_4. The absorbancy of the purpurogallin formed was read at 495 nm. To the blank 2.5 N H_2SO_4 was added of the zero time of the same assay mixture. PPO activity is expressed in U mg^{-1} protein (U = Change in 0.1 absorbance $min^{-1} \cdot mg^{-1}$ protein). The enzyme protein was estimated by the method of Bradford ,1976, for expressing all the enzyme activities.

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± SD for six samples in each group. *P* values ≤ 0.05 were considered as significant.

3. RESULTS

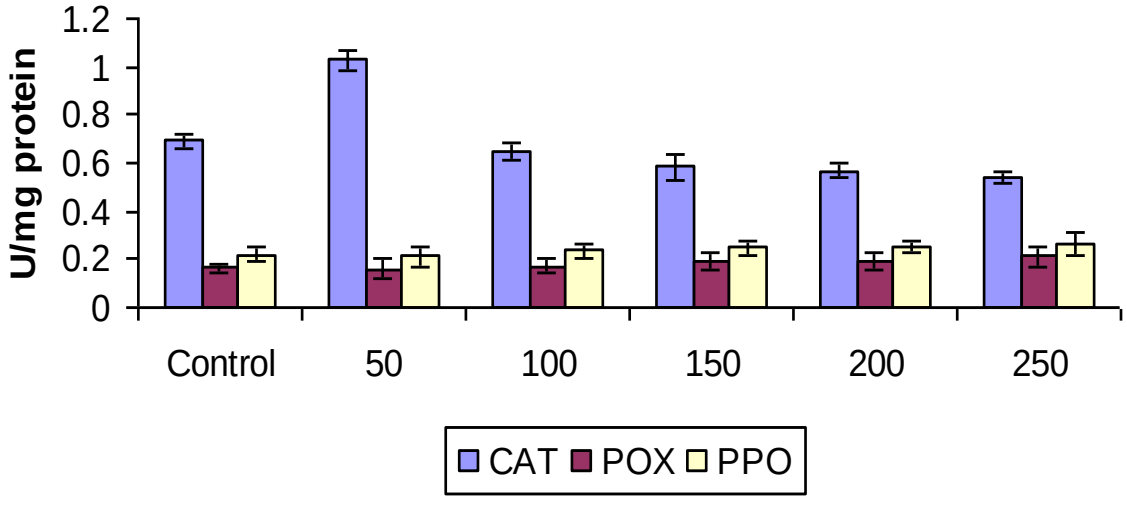

Figure 1. The effect of different concentrations of cobalt on catalase, peroxidase and polyphenol oxidase activity (units mg^{-1} protein) of Cluster bean

4. DISCUSSION

The maximum number of nodule formation occurred in 50 mg/kg on 75 DAS and minimum number of nodule formation was observed at 250 mg/kg on 15 DAS. The number of nodule formation of Cluster bean decreased (except 50 mg/kg) with an increase in the cobalt concentration of soil. Similar reduction in the number of nodule formation was observed under different heavy metal treatments in Cluster bean. The leg-heamoglobin contents of nodule of Cluster bean plants increased at 50 mg/kg soil level and decreased further with an increase in cobalt level in the soil. Minimum leg-haemoglobin content was recorded at 250 mg/kg cobalt level. This can be compared with earlier report. CAT activity decreased with increasing concentration of Co (100-250 mg·kg^{-1}) than the control and low level of Co (50 mg·kg^{-1}) treated Cluster bean plants. POX and PPO activities increased (except 50 mg·kg^{-1}) with an increase in Co level in the soil. To be able to endure oxidative damage under conditions which favours increased oxidative stress, such as high/low temperatures, water deficit, salinity, etc., plants must possess efficient antioxidant system. Plants possess antioxidant systems in the form of enzymes, such as SOD, APX, CAT, and metabolites viz., ascorbic acid, glutathione, α-tocopherol, carotenoid, flavonoids, etc. These antioxidant enzymes and metabolites are reported to increase under various environmental stresses, as well as comparatively higher activity has been reported in fungicide, triadimefon in medicinal plants, suggesting that higher antioxidant enzymes activity has a role in imparting tolerance against any type of environmental stresses. From the results of this investigation it can be concluded that, the application of cobalt at 50 mg/kg may be proved to be a useful tool in increasing the root nodule formation and leg haemoglobin contents in Cluster bean plants. This study is of high significance, because it was previously reported that using isotopic nitrogen, roots and nodules grown in the presence of the added cobalt possess a greater capacity for nitrogen fixation than those without the added cobalt.

5. CONCLUSION

Cobalt treatment at all levels tested (except 50 mg/ kg) decreased the nodules, leg-haemoglobin contents of plants; antioxidant enzyme (CAT) activity of cluster bean plants. However, the antioxidant enzymes (POX and PPO) increased with an increase in cobalt level in the soil. From the present investigation it can be concluded that the 50 mg /kg level of cobalt in the soil is beneficial for the growth of cluster bean plants.

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