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Mercury induced oxidative stress of antioxidants in *Clitoria ternatea* L.

M. Priya[1], V. Balakrishnan[1,*], A. Kiruthika Lakshmi[1], R. Aruna[2], K. C. Ravindran[3]

[1]Department of Biotechnology, K.S. Rangasamy College of Technology, Tiruchengode - 637 215, Tamil Nadu, India

[2]Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai - 600 106, Tamil Nadu, India

[3]Department of Botany, Faculty of Science, Annamalai University, Annamalainagar - 608 002, Tamil Nadu, India

*E-mail address: palanivbalu@gmail.com

ABSTRACT

Biofertilizers are the special formulation of specific beneficial microorganisms that promote the growth of plant crops by converting the unavailable form of nutrients into available form. Here, the effect of heavy metal stress on antioxidant enzymes were studied in *Clitoria ternatea* L. leaves. *Clitoria ternatea* L. plant was grown for 30 days and the heavy metal mercuric chloride was sprayed after 10 days from the date of planting. Effect of mercuric chloride was observed in treated plants. The selected plant *Clitoria ternatea* L. was grown under mercuric chloride treatment in a specified concentration 1 µg/10 ml. The control plant maintained without the treatment of mercuric chloride. Antioxidant effect of mercuric chloride was measured under controlled and treated conditions. The selected plant *Clitoria ternatea* L. was grown under mercuric chloride in treatment. Further it increases H_2O_2 content and the antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were observed in mercuric chloride treated plants when compared with control. Here mercuric chloride was accumulated more in matured leaves. The results are discussed with the literature.

***Keywords*:** Biofertilizer; Antioxidant; Enzymes; Phosphorus; Stress

1. INTRODUCTION

Phosphorus is a major nutrient for plants inducing vigorous growth and also contributing to their disease resistance. Phosphorous helps in root formation and plant growth. The plants utilize only 10-15 % of phosphate applied. The balance 85-90 % remains in insoluble form in the soil. The bio promoter has highly efficient phosphate solubilising bacteria (*Bacillus megaterium*) that grow and secrete organic acids, which dissolve this unavailable phosphate into soluble form and make it available to the plants. Thus, the residual phosphate fertilizers in the soil can be well utilized and external application can be optimized.

For the protection from the oxidative stress plant cells contain both oxygen radical detoxifying (antioxidant) enzymes such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), (Del Rio *et al.,* 1998). SOD, the first enzyme in the detoxifying process,

catalyzes the dismutation of O_2^- to H_2O_2 and O_2. CAT mediates the cleavage of H_2O_2 evolving O_2 (Scandalios 1993), and POX reduces H_2O_2 to H_2O using several reductants available to the cells (Foyer *et al.,* 1994).

Altered activities of these antioxidant enzymes and antioxidants commonly have been reported in plants and are used frequently as indicators of stress (Koricheva *et al.,* 1997) In parallel to metal induced tissue damage or cell death alteration of antioxidant enzyme activities (Somashekaraiah *et al*., 1992) and antioxidant levels (Sinha *et al*., 1997) as well as enhancement of both lipid peroxidation (Ouariti *et al.,* 1997) and phytochelatin synthesis (Gupta and Goldbrough 1991) have been observed. Therefore, the metal induced phytotoxicity may be mediated by oxidative stress.

However, the changes in AOS metabolism and the enzymes activities involved in scavenging AOS in response of exposing plants to metal have not been investigated in detail. The objective of present study is to investigate whether Hg-induced phytotoxicity expressed as growth inhibition and antioxidant activity destruction in *Clitoria ternatea* L. plants is mediated by oxidative stress. The data show that *Clitoria ternatea* L. plants exposed to toxic dose of mercury produce H_2O_2 and the activities of related antioxidant enzymes are altered indicating that Hg-induced phytotoxicity can be mediated by oxidative stress (Fig. 1).

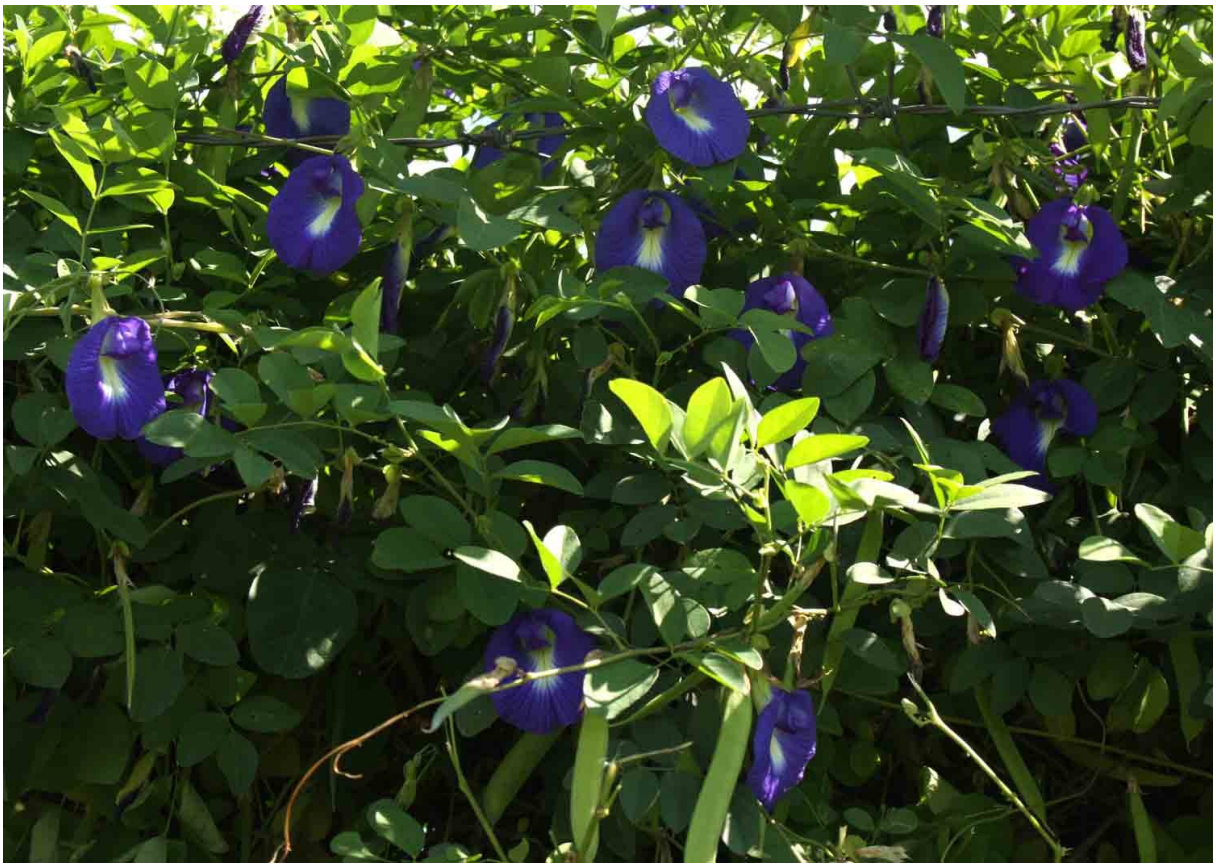

Figure 1. *Clitoria ternatea* L.

Many types of environmental stresses both biotic and abiotic produce characteristic changes in physiological and metabolic processes of higher plants. Thus, peroxidase activity

usually increases in plant tissues under various stress conditions such as the influence of toxic elements mechanical injuries or attack by parasitic organisms.

Due to the heavy metal treatment an increase of peroxidase activity was observed in the early phenophases of green bean and tomato plants and in maize roots. The rise of peroxidase activity was used for rapid testing of strawberry plants infected by viruses. A significant decline of the chlorophyll concentration was observed in heavy metal stressed wheat plants by Chiscato *et al.,* (1998).

High soil levels of arsenic provoke some changes in pigment concentration in green bean and tomatoes and they correspond to an alteration of the chloroplasts in cells.

2. MATERIALS AND METHODS

2. 1. Seed germination and seedlings growth in Heavy metal treatment

Seeds of *Clitoria ternatea* L. were collected from Kerala Agricultural University, Kerala. Seeds were surface sterilized with 70 % ethanol for 2 minutes, followed by 20 % commercial bleach containing 0.02 % of Tween-20 for 2 minutes and washed several times with sterilized double distilled water.

Seeds were placed in sterile petridishes (diameter 10 cm) lined with two sterile filter papers each with 50 % Hoagland solution. Seeds were maintained at 4 ºC under dark conditions for 3-4 days (stratification) and was transformed to 24 ºC for another 2 days and this seeds were used for germination.

Clitoria ternatea L. seeds were germinated in mercuric chloride sprayed soil (T1) in the concentration of 1 µg/ml and biofertilizer mixed with water was sprayed in the soil (T2) in concentration of 1 µg/ml in pots and the control plant was also maintained without sprayed with mercuric chloride and biofertilizer. Shoot and root length was measured after 10 days of germination.

2. 2. Analysis of Antioxidant enzymes

2. 2. 1. Superoxide dismutase (SOD) (E.C. 1.15.1.1) activity

Superoxide dismutase activity of *Clitoria ternatea* L. leaves was determined by the method of Beauchamp and Fridovich (1971) by following the photoreduction of nitroblue tetrazolium. The reaction mixture such as control, T1 and T2 contained 50 mmol/l phosphate buffer (pH 7.8), 0.1 mmol/l EDTA, 13 mmol/l methionine, 75 µmol/l NBT, 2 µmol/l riboflavin and 100 µl of the supernatant.

Riboflavin was added as the last component and the reaction was initiated by placing the tubes under two 15 W fluorescent lamps. The reaction was terminated after 10 minutes by removing the reaction tubes from the light source. Non-illuminated and illuminated reactions without supernatant served as calibration standards.

The photoreduction of NBT (production of blue formazan) was measured at A560. One unit of SOD was defined as the enzyme activity that inhibited the photoreduction of NBT to blue formazan by 50 % and SOD activity of the extracts was expressed as SOD units per mg of protein.

2. 2. 2. Peroxidase (POD) (E.C.1.11.1.7) activity

Peroxidase activity of *Clitoria ternatea* L. leaves was determined at 25 ºC with guaiacol Hemeda and Klein (1990). In the presence of H_2O_2, POD catalyses the transformation of guaiacol to tetraguaiacol (brown product). The oxidation of guaiacol was measured by the increase in absorbance at 470 nm for 1 min. The reaction mixture control, T1 and T2 contained of 20 mmol/l guaiacol, 2.8 ml of 10 mmol/l phosphate buffer (pH 7.0) and 0.1 ml enzyme extract. The reaction was started with 20 µl of 40 mmol/l H_2O_2. One unit of POD activity was expressed as POD units per min and mg of protein.

2. 2. 3. Catalase (CAT) (E.C.1.11.1.6) activity

Catalase activity of *Clitoria ternatea* L. leaves was measured according to the method of Beer and Sizer (1952), with minor modifications. The reaction mixture control, T1 and T2 contained 1.5 ml of 100 mmol/l phosphate buffer (pH 7.0), 0.1 mmol/l EDTA, 20 mmol/l H_2O_2 and 50 µl of enzyme extract. The reaction was initiated by addition of the extract. The decrease of H_2O_2 was monitored at 240 nm and quantified by its molar extinction coefficient (36 mol/l cm) and the results expressed as CAT units per min and mg of protein.

3. RESULTS

3. 1. Antioxidant enzymes against heavy metal stress

3. 1. 1. Superoxide dismutase (SOD) (E.C. 1.15.1.1) activity

SOD can eliminate O_2^-, decreases peroxidation of membrane lipids and maintain cell membrane stability. SOD activity for control plant was 2.04, T1 plant was 2.14 and T2 plant was 2.23. The percentage of SOD activity in *Clitoria ternatea* L. leaves slightly decreased for both T1 and T2. As compared with T1 with T2 the percentage of SOD activity decreased in T1 and increased in T2. The percentage of SOD activity in T1 was 91.47 and T2 was 95.32 (Figure 1).

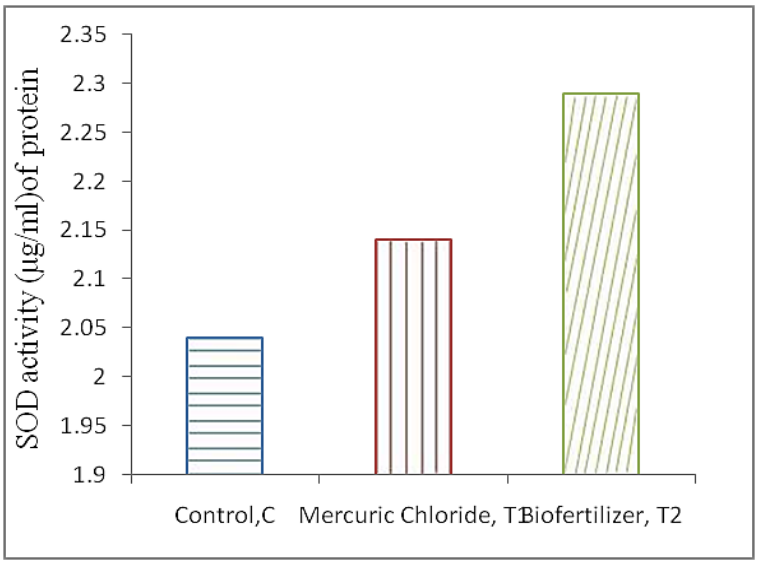

Fig. 1. Effect of heavy metal stress on superoxide dismutase activity in leaves of *Clitoria ternatea* L. plants.

3. 1. 2. Peroxidase (POD) (E.C.1.11.1.7) activity

POD plays a role in decreasing H_2O_2 accumulation eliminating MDA resisting cell peroxidation of lipids and maintaining cell membrane integrity. Heavy metal stress resulted in POD activity increased in *Clitoria ternatea* L. leaves. POD activity for control plant was 2.06, T1 plant was 2.19 and T2 plant was 2.29. The percentage of POD activity in *Clitoria ternatea* L. leaves slightly decreased for both T1 and T2. The percentage of POD activity in T1 was 89.95 and T2 was 94.06 (Figure 2).

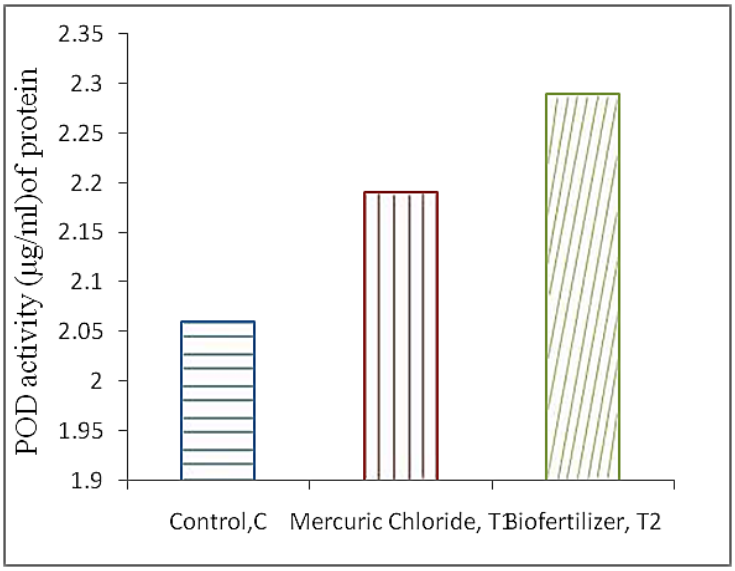

Fig. 2. Effect of heavy metal stress on peroxidise dismutase activity in leaves of *Clitoria ternatea* L. plants.

3. 1. 3. Catalase (CAT) (E.C.1.11.1.6) activity

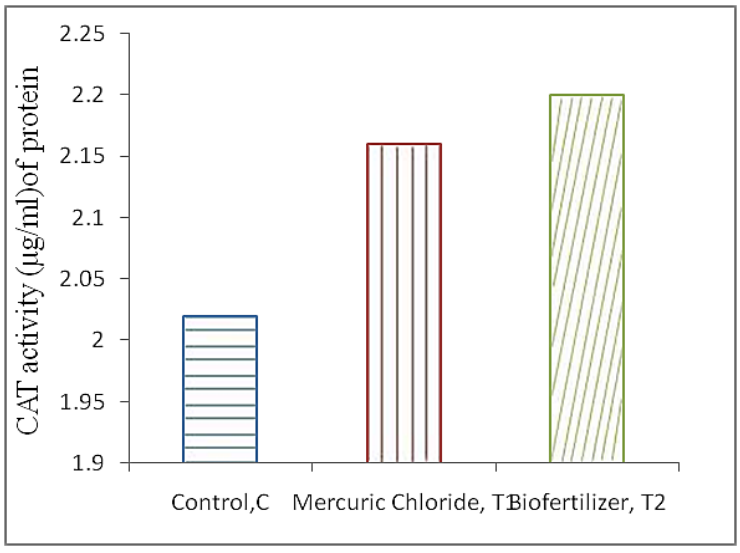

Fig. 3. Effect of heavy metal stress on catalase activity in leaves of *Clitoria ternatea* L. leaves.

CAT can eliminate H_2O_2 and play a key role in elimination of O_2^-. CAT activity in leaves of *Clitoria ternatea* L. was nearly same at all level of stress. In *Clitoria ternatea* L. CAT activity of leaves increased with increasing heavy metal stress. CAT activity for control plant was 2.02, T1 plant was 2.16 and T2 plant was 2.20. The percentage of CAT activity in *Clitoria ternatea* L. leaves slightly decreased for both T1 and T2. The percentage of CAT activity in T1 was 91.81 and T2 was 93.51 (Figure 3).

4. DISCUSSION

4. 1. Antioxidant enzymes against heavy metal stress

Microorganisms offer a biological rescue system capable of solubilising the insoluble inorganic phosphate of soil and make it available to plants. Phosphate solubilising bacteria (Phosphobacteria) may be important for plant nutrition by increase phosphate uptake by the plants and playing a significant role as PGPR in the bio fertilization of crops. Mercury induced reactive oxygen species in plants (Vajpayee *et al*., 2002). Further, to mitigate and repair the damage initiated by reactive oxygen species, plants have evolved a complex system involving of antioxidant enzymes. Ascorbate peroxidase (APX) is the member of the ascorbic acid glutathione cycle and plays a crucial role in eliminating poisonous H_2O_2 from plant cells.

In this study, mercury used in the experiment inhibited APX activity in *Clitoria ternatea* leaves at all the treatment durations. This is contrast to the results earlier described in different plants growing under heavy metal stress (Weckx and Clijsters 1996). This might be due to the harmful effects of the over production of H_2O_2 or its poisonous active oxygen derivatives, because of manifold increase in SOD activity of the mercury treated *Clitoria ternatea.* Amongst various enzymes involved in quenching of reactive oxygen species, guaiacol peroxidase (GPX) and catalase have their importance in elimination of H_2O_2. The stimulated activities of these enzymes (GPX and catalase) and reduced APX activity found in this study led to the conclusion that elimination of H_2O_2 in *Clitoria ternatea*, was achieved by GPX and catalase. While APX took a little part in detoxification of H_2O_2 due to its sensitivity to mercury. Furthermore, GPX participates in the lignin biosynthesis and might build up a physical barrier against poisoning of the heavy metals. Therefore, hyperactivities of GPX, catalase and SOD in *Clitoria ternatea* L. might be attributed to the strategies adopted by the *Clitoria ternatea* L. to overcome the toxicity of the mercury in treated plants.

It may be concluded from the present study that *Clitoria ternatea* could grow in mercury sprayed soil and accumulate high amount of mercury in leaves. Mercury accumulation by *Clitoria ternatea* affects various physiological processes. Mercury induced oxidative stress was tolerated by this plant through the hyperactivity of antioxidant defense system. The H_2O_2 formed by the superoxidation of active oxygen species was quenched by catalase and POD. However, CAT took a little part in quenching of H_2O_2 due to its sensitivity to the mercury. Therefore, reduced CAT activity was recorded in mercury treated *Clitoria ternatea*.

The activities of SOD, CAT and POD were investigated to determine whether Hg exposure influenced these antioxidant enzymes. All enzyme activities, estimated on a fresh weight basis, were substantially increased by Hg exposure. Examination of enzymes, which decompose the H_2O_2 generated by SOD, indicated that the activities of CAT and POD also increased in response to Hg exposure. The levels of H_2O_2 formed in response to Hg exposure might be comparable to the activities of CAT. This experiment was highly contaminated with heavy metals in T1 ($HgCl_2$) and T2 (Biofertilizer) sprayed plants. This however, did not

impose any significant effect in the development and growth of the (*Clitoria ternatea*) plants. There was a constant increase in the metals concentration in the plants tissue in leaves, which was sufficiently correlated with the metals in the watering solutions and not with the metals in the substrate. However, our results are laboratory based and before exploiting the results in field, a pilot field study is recommended.

Summing up, it was proposed that the reduced growth of *Clitoria ternatea* leaves, the use of mercury containing leaves in medicinal preparations is not advised due to its toxic effect and health risk.

5. CONCLUSIONS

It may be concluded from the present study that *Clitoria ternatea* could grow in mercury sprayed soil and accumulate high amount of mercury in leaves. Mercury accumulation by *Clitoria ternatea* affects various physiological processes. Mercury induced oxidative stress was tolerated by this plant through the hyperactivity of antioxidant defense system. The H_2O_2 formed by the superoxidation of active oxygen species was quenched by catalase and POD. However, CAT took a little part in quenching of H_2O_2 due to its sensitivity to the mercury. Therefore, reduced CAT activity was recorded in mercury treated *Clitoria ternatea*.

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