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CHANGES IN ANTIOXIDANT LEVEL IN *MACROTYLOMA UNIFLORUM* L. GROWN UNDER LEAD TOXICITY

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Abstract:

Lead (Pb) is one of the hazardous heavy metal pollutants of the environment that originates from various sources like mining and smelting of lead-ores, burning of coal, effluents from storage battery industries, automobile exhausts, metal plating and finishing operations, fertilizers and pesticides. Ten days old plant of *Macrotyloma uniflorum* L (Horse Gram) was exposed to different concentrations of lead [0, 50, 100 and 150 ppm Pb (NO3)2•4H2O] for 14 days in earthen pots. Exposure of *Macrotyloma uniflorum* L to excess Pb resulted in a significant root growth inhibition though shoot growth remained less affected. Pb-treated plant showed decreased level of Protein, Aminoacid, Lipids, Starch, Sugar and increased antioxidant activity such as Super Oxide Dismutase (SOD), Glutathione peroxidase, catalase and Glutathione Reductase (GR) when compared to controls. Results of the current study revealed that, Pb induces oxidative stress in growing plants and increased quantity of SOD, Glutathione Peroxidases, catalase and GR indicated that these enzymes could serve as inevitable components in the antioxidative defense mechanism of *Macrotyloma uniflorum* L. against Pb induced oxidative injury.

Key words: *Macrotyloma uniflorum,* Super Oxide Dismutase and Glutathione peroxidase.

Introduction

Lead (Pb) is one of the hazardous heavy metal pollutants of the environment that originates from various sources like mining and smelting of lead-ores, burning of coal, effluents from storage battery industries, automobile exhausts, metal plating and finishing operations, fertilizers, pesticides and from additives in pigments and gasoline (Eick *et al.*, 1999). Its

increasing level in soil environment inhibits the germination of seeds and exert a wide range of adverse effects on growth and metabolism of plants (Godbold *et al.,* 1991; Moustakas *et al.,* 1994 and Kastori *et al.,* 1992). Hence, the present study is aimed to assess a general picture of the antioxidant metabolism of *Macrotyloma uniflorum* L. (Horse Gram) with differential sensitivity to Pb stress. Current approach might provides a better understanding about the role of this plant in the reclamation of Pb contaminated soils. This information could be useful for selecting plants to grow on Pb-contaminated soil. Horse Gram grows as an annual plant with an attractive appearance. Here is a general description of this climbing plant:

Macrotyloma uniflorum Lam

Kingdom : Plantae

Family : Fabaceae

Genus : Macrotyloma

Species : *M. uniflorum*

It is widely used in Ayurvedic medicine for treating numerous health disorders including rheumatism, worm infestations, conjunctivitis and piles. They have astringent and diuretic properties. They are believed to be helpful in keeping the body warmth during winter. Controlling fever is another health benefit of Horse Gram.

Materials and Methods

The plant extracts were screened for the presence of biologically active phytochemical compounds such as sugars, aminoacids, proteins, phenols, terpenoids, etc., using standard protocols as described by Brindha *et al*., (1995).

Results and Discussion

Table 1: Preliminary phytochemical analysis of *M. uniflorum.*

Phytochemical constituents	**Normal plant**	**Lead toxicity induced plant**		
		T1(50ppm)	**T2(100ppm)**	**T3(T3150ppm)**
Alkaloids	+	-	-	+
Terpenoids	-	-	-	-
Coumarins	-	-	-	-
Tannins	+	+	-	-
Flavonoids	+	-	-	-
Phenolic compound	+	-	-	+

Volatile oil	-	-	-	-
Quinones	+	+	+	-
Steroids	+	-	-	-
Saponine	+	-	-	-

Table-2: The level of antioxidant enzyme in Normal and lead toxicity plants.

Conc of Lead Nitrate in ppm	**PEROXIDASE**		**CATALASE**	
	ROOT	**LEAVES**	**ROOT**	**LEAVES**
Control	0.05±1.00	0.08±1.89	7.4±2.7	10±1.3
50	0.03± 1.87	0.09±1.90	13±1.7	16±1.5
100	0.04±1.98	0.90±0.09	32±1.4	28±1.2
150	0.06±1.78	0.08±0.09	66±1.9	56±1.9

Table-3: Quantitative assay of antioxidant enzymes in Normal and lead toxicity induced test plants.

Con of Lead nitrate in ppm	**GLUTATHIONE REDUCTASE**		**GLUTATHIONE TRANSFERASE**	
	ROOT	**LEAVES**	**ROOT**	**LEAVES**
Control	1.9±1-0	2.0±3.0	15.89±3.0	14.09±0.09
50	2.0±3.0	3.7±1.0	16.98±9.0	12.0±3.0
100	3.7±1.0	2.0±3.0	14.09±0.09	12.0±3.0
150	2.3±1.9	4.02.0±3.03	13.09±1.90	12.7±3.0

Table-4: The level of antioxidant enzyme in Normal and lead toxicity plants.

Con of lead in ppm	**SOD**		**LPO**	
	ROOT	**LEAVES**	**ROOT**	**LEAVES**
Control	1.05±1.00	2.08±1.89	44±2.7	20±1.3
50	2.03± 1.87	3.09±1.90	43±1.7	36±1.5
100	304±1.98	4.90±0.09	52±1.4	48±1.2
150	4.06±1.78	5.08±0.09	76±1.9	56±1.9

In the present investigation, normal as well as lead acetate treated plants were taken and extracted with water for phytochemical analysis. It is observed that, the normal plant extract showed a marked presence of alkaloid, quinone, flavanoids, tannins, glycoside, phenolic compound and steroids. But the lead acetate treated plants have limited number of phytoconstituents such as tannin, quinone, phenolic compound and alkaloid (Table 1).

Catalase and peroxidase

Catalase and peroxidase activities (Table 2) are elevated in both parts of the plant i.e roots and leaves under all regimes of lead stress. Catalase is an important oxidoreductase enzyme that involves in the removal of toxic peroxides by breaking the toxic H_2O_2 into water and molecular oxygen. A decline in catalase activity under Pb toxicity was observed in the current studty, which suggested a possible delay in removal of catalase mediated H_2O_2 and toxic peroxides and in turn an enhancement in the free radical mediated lipid peroxidation under Pb toxicity. With increasing levels of Pb treatment, a concomitant decline in catalase activity was observed in roots while in leaves a higher Pb treatment level at 1000 mM led to marked inhibition in enzyme activity. At 800 ppm of Pb, when compared to control, two times more elevated activities of both the enzymes were noted which was indicated by their quantification. The activity of the enzymes is gradually increasing as the severity and duration of stress was increasing in Horse gram. Further, highest percentage of enzyme activity was recorded in roots than in leaves of the plant.

Our reults are supported by the findings of Feieraband *et al.*, (1986). They reported the declined activity of catalase under salinity, chilling, drought and hypoxia conditions. However, unlike our studies, in sub cellular compartments of pea root cells, increased catalase activity was observed when plants were grown in nutrient medium containing 0.5 or 1 mM $Pb(NO_3)_2$. A reduction in catalase activity under stressful conditions has been attributed to the inactivation of enzyme protein due to ROS, decrease in enzyme synthesis or change in assembly of enzyme subunits

Glutathione reductase and glutathione S-transferase

Glutathione reductase catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). GSH is involved in the redox regulation of the cell cycle and has often been considered to play an important role in defense of plants and other organisms against oxidative stress. Being a major water soluble antioxidant in plant cells, GSH directly reduces most active oxygen species. The activities of glutathione reductase and glutathione S-transferase increased in Pb stressed plants. This result is well evidenced by the stress intensity dependent elevation of peroxidase

activity in both the plants in the present study. In plant cells also, GST may have a similar role. In the present study, elevated activity of GST in horse gram under Pb stress may be attributed that it could catalyze the conjugation of Pb ions to glutathione (GSH) or directly act as binding protein to accommodate Pb ions. Higher increase in the activity of GST under Pb stress conditions may detoxifies the Pb ions at high degree in horsegram further support its tolerant nature.

Superoxide dismutase

SOD and catalase have been identified as enzymatic protectors against peroxidation reactions. SOD is an essential component of antioxidative defense system in plants and it dismutates two superoxide radicals (O_2^{+}-) to water and O_2. Superoxide dismutase activity is increased due to lead (Pb) in roots and leaves of both the plants with increase in stress intensity. However, the enzyme activity increased 2–3 folds in roots and leaves of both the plants on day 12 at 800 ppm Pb treatment compared to controls. The magnitude of elevation of superoxide dismutase activities are found to be relatively more in root tissue than in leaf tissue of both the plants under Pb stress conditions.

Lipid peroxidation

Malondialdehyde content in roots and leaves of the plants was elevated due to Pb toxicity and the magnitude of elevation ranged from 2.3 to 3.5 folds over controls at 800 ppm Pb in the Horse gram. The root malondialdehyde content is increased more than leaf malondialdehyde content in the plants under Pb stress conditions.

Lead is one of the most abundant heavy metals polluting the soil environment Eick *et al*. (1999), It is readily absorbed by plants mainly through the root system and thereafter exerts its toxicity symptoms. Metal phytotoxicity occurs when metals move from soil to plant roots and are further transported to various sites in the shoots. The effect of Pb hytotoxicity include stunted growth, chlorosis, blackening of the root systems, alteration in water and nutritional status of plants as well as various plant processes (Kastori *et al*., 1992). Contamination of soil by heavy metals is of widespread occurrence as a result of human, agricultural and industrial activity. Lead (Pb) is one of the most abundant heavy metals polluting the soil environment (Eick *et al.,* 1999; Godbold *et al.* 1991; Moustakas *et al.,* 1994 and Kastori *et al.,* 1992). It is readily absorbed by plants mainly through the root system and thereafter exerts its toxicity symptoms. Metal phytotoxicity occurs when metals move from soil to plant roots and are further transported to various sites in the shoots. The effects of Pb phytotoxicity include stunted growth, chlorosis, blackening of the root systems (Godbold *et al.* 1991), alteration in water and nutritional status of plants (Kastori *et al.,* 1992) as well as various plant processes (Godbold *et al.* 1991 and Kastori

et al., 1992). Lead stress in the present study results a significant increase in the SOD activity in both plant species studied, but with higher degree in horsegram. This increase in superoxide dismutase activity could possibly be the result of both a direct effect of heavy metal ions and an indirect effect mediated via an increase in levels of them (Chongpraditnum *et al*., 1992). The effect of Pb stress on SOD expression is likely to be governed by the tissue and sub cellular sites at which oxidative stress is generated as supported by the higher activity of SOD in roots than in leaves of Pb stressed plants. Present study indicated that, the involvement of free radicals in membrane lipid peroxidation in the plants subjected to Pb stress could be a possible reason for the increase in malondialdehyde content. Generally, free radical generation and membrane damage would be low in tolerant plants and thereby formation of lower levels of malondialdehyde content. Hence, the present study revealed that , relatively lower degree of increase in malondialdehyde content in horse gram due to Pb stress may support its tolerant nature. The Pb content increases in the plants with the intensity of stress. Plants absorb Pb in its soluble form from soil through roots and there may be a limited translocation to the shoots. Based on comparative studies of metal content in plant parts Baker and Walker (1990) suggested that uptake, translocation and accumulation mechanisms differed for various heavy metals and for the species. It is known that the root system partially defends the above ground parts from Pb (Broyer *et al*., 1972).

Summary and Conclussion

One-month old Horse Gram (*Macrotyloma uniflorum)* was exposed to different regimes of Pb stress as $Pb(NO_3)_2$ at 0, 50,100 and 150 ppm concentrations. The extent of oxidative damage as the rate of lipid peroxidation, antioxidative response and the accumulation of Pb in roots and shoots of plant was evaluated after 12 days of Pb stress. Pb treated plant showed increased levels of lipid peroxidation as evidenced from the increased malondialdehyde content coupled with the increase in the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), glutathione S-transferase (GST) compared to control (untreated) plants. Pb stress caused significant changes in the activity of antioxidative enzymes. The effect of Pb was found to be concentration dependent. Higher concentration of Pb (150 ppm) resulted 2- to 3-fold increase in SOD, catalase and peroxidase activities, 3- to 5-fold increase in GR activity and 3- to 4-fold increase in GST activity in roots and leaves of green gram .Pb stress caused a significant increase in the rate of peroxidation as showed in the levels of malondialdehyde content in roots and leaves of both plant species. However localization of Pb was greater in roots than leaves in both plants. Lipid peroxide levels and antioxidative

enzyme activities was higher in green gram and also more in roots than leaves. These results suggest that Pb toxicity causes oxidative stress in plants and the antioxidative enzymes SOD, CAT, POD, GR, and GST could play a vital role against oxidative injury. The present study concludes that the Pb stress triggered a defense mechanism against oxidative stress in crop species. However horse gram concertedly showed regulation to ensure proper protection against reactive oxygen species generated during Pb stress and has better tolerance to Pb stress and could be considered for reclamation of Pb contaminated soils.

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