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Studies on the Effect of Lead Stress on the Growth and Biochemical Characteristics of *Vigna radiata* (L.)Wilczek

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ABSTRACT

Contamination of soil by heavy metals is of widespread occurrence as a result of human, agricultural and industrial activities. Among the heavy metals, lead(Pb) is a potential pollutant that readily accumulates in soils and sediments. Pb toxicity causes a range of damages to plants from germination to yield formation; however, its toxicity is both time and concentration dependent. Its exposure at high rates disturbs the plant water and nutritional relations and causes oxidative damages to plants. The present study is aimed to study the effect of different concentrations of lead on the growth, biochemical and enzymatic characters of ***Vigna radiata** (Green gram)*. The results indicated that the growth characters (shoot length, root length, plant fresh weight and dry weight) as well as biochemical characters (chlorophyll, total soluble sugar and total soluble protein) of green gram gradually decreased with increasing concentration of lead. The *in vivo* nitrate reductase activity was found decreased with the increasing concentration of lead treated plants. On the contrary, the enzymatic characters like the activity of peroxidase and catalase were significantly increased when treated with 2mM to 10mM concentration of lead.

Key words: Lead toxicity, *Vigna radiata*, Growth, Biochemical, Enzymes characteristics

INTRODUCTION

In recent years, the contamination of natural ecosystems by heavy metals represents a worldwide environmental concern, endangering agricultural systems (Mica *et al.*, 2006). In developing countries, this problem has arisen from long-term use of untreated wastewater for irrigation, leading to increased concentrations of heavy metals in soil (Lu *et al.*, 2015). This prevent plants from reaching their maximum genetic potential for growth, development and reproduction. Once deposited on the ground, plants are able to take up these elements from the soil, and introduce them into the food chain, raising the risk of metal toxicity concerns for humans and animals (Roy and McDonald, 2015).

Lead(Pb) is one of the major heavy metal of the antiquity and has gained considerable importance as a potent environmental pollutant.Apart from the natural weathering processes,Pb contamination of the environment has resulted from mining and smelting activities,lead containing paints,gasoline and explosives as well as from the disposal of municipal sewage sludges enriched in Pb(Chaney and Ryan,1994)

Plants grow on heavy metal polluted soils, resulted in the reduction in growth, due to changes in their physiological and biochemical activities especially true when the heavy metals does not play any beneficial role towards the growth and development of plants. Thus, it is evident from several research findings that judicious use and presence of heavy metals causes toxic effect on plants, animals and many living organisms after certain limit.Reduced rate of seed germination and plant growth under stress is mainly due to Pb with potassium in plants.Lead induced structural changes in photosynthetic apparatus and reduced biosynthesis of chlorophyll pigments causes retardation of carbon metabolism(Usman et al.,2019). Therefore, indeed to intensify the research for better understanding of heavy metal toxicity on plants and allied areas to maintain the ecological harmony of our planet, there are two aspects on the interaction of plants and heavy metals, one hand, heavy metals show negative effects on plant sand other hand, plants have their own resistance mechanisms against toxic effects and for detoxifying heavy metal pollution (Asati *et al.*, 2016).

MATERIAL AND METHODS

A nursery experiment was conducted to study the impact of lead on the growth, biochemical and certain enzymatic characters of green gram (*Vigna radiata*). The seeds with uniform size, colour and weight were chosen for the experimental purpose and surface sterilized with 0.1% $HgCl_2$ for 1 minute and thoroughly washed with distilled water 3 to 5 times. Seeds were presoaked for 12 hours in distilled water and were sown in sterilized soil mixture. The soil mixture was prepared by mixing black soil, red soil and sand in the ratio of 1:1:1.

The lead stress was given to plants as lead acetate. 1 Molar lead acetate solution was prepared as stock and then the respective concentrations were prepared from the stock solution. The heavy metal lead was treated separately in the experimental plants with different concentration as 2 mM, 4 mM, 6 mM, 8 mM and 10 mM (w/v) in five replicates. The aqueous solutions of heavy metals were applied in the soil at the time of sowing the seed. Then the plants were watered with the respective concentration of metals on every alternate days. A set of plants without heavy metal treatment were also maintained as control. Growth and biochemical parameters were analysed on the 30^{th} day after planting (DAP).

In the treated and control plants, the growth characters such as shoot length, root length, fresh and dry weight of plant were analyzed. The biochemical characters such as chlorophyll (Wellburn and Lichtenthaler, 1984), total soluble sugar (Jayaraman, 1981) and total soluble protein (Lowry *et al.*, 1951) were analyzed in treated and untreated control plants. Further, some enzymatic characters such as nitrate reductase activity (Jaworski, 1971), peroxidase (Addy and Goodman, 1972) and catalase (Kar and Mishra, 1976) were analyzed. The data were reported as mean ± SE and in the figure parentheses represent the per cent activity. Values are expressed as means ± standard deviation of three independent data.

RESULTS AND DISCUSSION

Effects of heavy metal on the growth characters of *Vigna radiata*

The heavy metal application reduced the shoot length of ***Vigna radiata***. The effect was varied from 6 % to 49%. The maximum reduction of 49 % was found in the plants treated with 10 mM concentration of lead. Plants treated with lead also reduced the root length. The results showed that, decreased root length ranging from 5 % to 31 % with a maximum reduction of 31% at the highest concentration of heavy metal treatment. The reduction of shoot and root length directly affected the fresh weight of green gram and the reduction was higher in 10 mM concentration of (82 %) lead treated plants. The same trend was also observed in the dry weight of green gram plants. The reduction of plant dry weight was 84% at 10 mM concentration of lead to that of the control set of plants (Table 1). The lead inhibits germination of seeds and retards growth of seedlings, decreases germination percentage, germination index, root/shoot length, tolerance index and dry mass of roots and shoots. The growth ,development, fresh biomass and growth tolerance index of root, shoot and leaves were negatively affected by increasing levels of lead concentrations in tomato seedlings. Similar results were obtained by some other studies at the calculated lead concentrations: root, shoot and leaf growth; fresh and dry biomass is greatly reduced in *Pisum sativum* and *Zea mays* (Cimrin, 2007). Same results were obtained by some other studies with reference to root, shoot and leaf growth, fresh and dry biomass were critically reduced in *Pisum Sativum*, *Zea mays*,*Paspalum distichum* and *Cynodon dactylon*, *Lycopersicon esculentum*, *Ipomoea aquatic*, *Phaseolus vulgaris* and *Lens culinaris* (Kastori *et al.*, 1992; Kevresan *et al.*, 2001; Shua *et al.*, 2002; Jaja and Odomena, 2004; Gothberg *et al.*, 2004).

Effects of heavy metal on the biochemical characters of *Vigna radiata*

In the lead treated plants, the total chlorophyll content was found reduced (5% - 81%) gradually with increasing concentrations of metal solutions. It was observed that 47% reduction at 8 mM lead treatment. Also observed that a significant reduction of total soluble sugar content over control was noticed from 6 mM concentration of lead to 10mM treated plants. Further, the plants treated with lead reduced the protein content of green gram plants. The reduction was ranged from 2% to 33% at 6mM to 10mM lead (Table 2). It was also found that the substitution of the central magnesium in chlorophyll by heavy metals was an important type of damage in plants growing in a metal contaminated environment. This substitution prevents photosynthetic light harvested in the affected chlorophyll molecules and results in a breakdown of photosynthesis (Kupper *et al.*, 1998; Vajpayee *et al.*, 1999). Shankar *et al*. (2005) stated that, the decrease in the chlorophyll *a* / *b* ratio brought about by heavy metals indicated that, the metal toxicity possibly reduces the size of the peripheral part of antenna complex of the pigment molecule. The decrease in chlorophyll *b* on heavy metal stress might be due to the destabilization and degradation of the proteins of the peripheral part. The inactivation of enzymes involved in the chlorophyll biosynthetic pathway could be attributed to the general reduction in chlorophyll content in most plants under stress. Reduction in the chlorophyll content of heavy metal treated leaves may be due to the accelerated ageing of photosynthetic apparatus (Polit *et al.*, 1998). The reduction in soluble protein level with associated increase in free amino acids under stress may be attributed both to disruption in protein synthesis as well as enhanced proteolysis (Sha and Dubey, 1998).

Satyakala and Jamil (1992) observed a reduction in the protein contents in the roots, leaves and petioles of water hyacinth and lettuce plants after chromium treatment and suggested that, the metal ions present in the effluents seems to interfere with protein synthesis, which is one of the major components for biochemical activities. The leaf protein level accounts for the major portion of RUBP carboxylase enzyme which is essential for primary carboxylating activity in photosynthesis. A decrease in leaf protein indicates the reduction in RUBPase activity. The reduction in the level of pigment content and leaf protein, directly accounts for the reduction in the photosynthetic activity, which in turn, influences the soluble sugar content of the treated plant leaf, as it is a photosynthetic product. Hence it is inferred that, the heavy metals have a significant impact on protein, which in turn affect the other metabolisms of plants. Similar results was reported in the effluents treated *Phaseolus mungo* (Ramasubramanian *et al.*, 1993).

Effects of heavy metal on the enzymatic characters of *Vigna radiata*

Nitrate Reductase is a key enzyme that catalyses the nitrate into nitrite. In the present study, the *in vivo* nitrate reductase activity of the leaves was significantly inhibited to about 24 % (at 6 mM concentration of lead)compared to the control. To fight the reactive oxygen intermediate species (ROIs) which is formed through oxidative damage produced by heavy metal stress, plants naturally adopt defense mechanisms, called antioxidant system, comprising of enzymes such as catalase and peroxidase which are capable of removing ROIs. Both enzymes are acting on the same substrate, *i.e.*, H_2O_2 and hence, their activities were also analyzed. The catalase activity was found to be increased in plants treated with all the concentrations. The increase was 35 % in plants treated with 4 mM solution of lead. Further, the peroxidase activity was increased with increasing the concentration of lead. The maximum activity was observed with plants treated with lead at the concentration of 10mM (Table 3). Lead can also alter the activity and quantity of the key enzyme of various metabolic pathways such as those of the photosynthetic Calvin cycle, nitrogen metabolism, and sugar metabolism. Like different heavy metals, lead treatment also affects the activity of a wide range of enzymes of different metabolic pathways (Stevens *et al*., 1997; Kumar and Dubey, 1999; Verma and Dubey, 2003). Lead caused a significant decrease on germination in two cultivars of *Brassica napus*. By increasing lead concentration more decreasing in germination has been shown. Growth parameters on both cultivars have been decreased by lead effect. The enzyme activity of catalase and peroxidase in root and shoot on both cultivars by increasing lead concentration has been increased. The activity of root peroxidase has shown significant decrease only in high lead concentration. Catalase is an antioxidant enzyme that catalyses H_2O_2 into H_2O and O_2. Catalase is a key enzyme which removes toxic peroxide radicals during stress (Lin and Kao, 2000). The effect of long stress of lead on pulses has been reported by Malecka *et al*. (2001) that,the catalase activity on the plant root has been increased. Increasing catalase activity was observed on cytosol and peroxisome. It seems that the activity of antioxidant enzymes like catalase in plants at environmental stress is a reason during protein synthesis of de novo enzyme (Lozano *et al*., 1996).

CONCLUSION

In the present study, the heavy metal lead as lead acetate affected the growth characters of green gram compared to the control plants. The lead stress significantly reduced the growth characters in the treated green gram seedlings irrespective of all the concentrations. The reduction was gradually increased from 2mM to 10Mm concentration of lead treated plants. Similar declining trend was noticed on the biochemical characters of heavy metal treated green gram plants . The activity of antioxidant scavenging enzymes such as catalase and peroxidase was increased in green gram seedling in different concentrations of lead. The treatment of heavy metal also affected the enzymatic characters. The activity of peroxidases and catalase were significantly increased with increasing concentration of lead. From the present study, it is clear that the excess amount of heavy metal, especially lead when absorbed by plants become toxic to the plants. The toxic effect was reflected in the plant growth, biomass production, biochemical characters and enzymatic characters of green gram.

Further studies were needed in this regard for the remediation and cleaning up of lead contaminated soils. This may be potentially solved through phytoextraction and rhizofiltration which are environmentally sound and economically viable.

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Table 1: Impact of various concentration of lead on the growth characteristics of *Vigna radiata*

Growth characters	Control	2mM	4Mm	6mM	8mM	10mM
Root Length (cm)	11.4 ± 0.050 (100)	10.8 ± 0.02 (95)	10.1 ± 0.251 (89)	9.6 ± 0.052 (84)	8.5 ± 0.015 (75)	7.9 ± 0.010 (69)
Shoot Length (cm)	34.3 ± 0.015 (100)	32.1 ± 0.057 (94)	29.6 ± 0.010 (86)	26.4 ± 0.020 (77)	22.5 ± 0.100 (66)	17.6 ± 0.251 (51)
Fresh Weight (gm)	2.33 ± 0.01 (100)	1.54 ± 0.003 (66)	1.38 ± 0.025 (59)	1.09 ± 0.015 (47)	0.74 ± 0.001 (32)	0.43 ± 0.005 (18)
Dry Weight (gm)	0.44 ± 0.004 (100)	0.36 ± 0.30 (82)	0.31 ± 0.005 (70)	0.25 ± 0.015 (57)	0.14 ± 0.003 (32)	0.07 ± 0.015 (16)

Table 2: Impact of various concentration of lead on the biochemical characteristics of *Vigna radiata*

Biochemical characters	Control	2mM	4mM	6mM	8mM	10mM
Total Chlorophyll (mg/gLFW)	3.18 ± 0.02 (100)	3.02 ± 0.005 (95)	2.83 ± 0.035 (89)	2.54 ± 0.050 (80)	1.68 ± 0.04 (53)	0.59± 0.01 (19)
Total Soluble Sugar (mg/gLFW)	4.46 ± 0.025 (100)	4.04 ± 0.030 (91)	3.52 ± 0.040 (79)	3.13 ± 0.03 (70)	2.84 ± 0.025 (64)	2.42± 0.026 (54)
Total Soluble Protein (mg/gLFW)	6.21 ± 0.005 (100)	6.11 ± 0.025 (98)	5.62 ± 0.020 (90)	5.06 ± 0.030 (81)	4.53 ± 0.02 (73)	4.14 ± 0.04 (67)

Table 3: Impact of various concentration of lead on the Enzyme activity of *Vigna radiata*

Enzymatic characters	Control	2mM	4mM	6mM	8mM	10mM
Nitrate Reductase activity (µ mole/g LFW)	8.18 ± 0.035 (100)	7.84 ± 0.040 (96)	7.53 ± 0.040 (93)	6.22 ± 0.055 (76)	5.46 ± 0.035 (67)	5.07 ± 0.02 (62)
Catalase activity (µ mole/g LFW)	4.63 ± 0.055 (100)	5.41 ± 0.045 (117)	6.24 ± 0.050 (135)	7.06 ± 0.002 (152)	8.09 ± 0.055 (175)	9.27 ± 0.025 (200)
Peroxidase activity (µ mole/g LFW)	2.63 ± 0.02 (100)	3.42 ± 0.025 (130)	4.73 ± 0.060 (179)	5.65 ± 0.05 (214)	6.48 ± 0.132 (246)	7.34 ± 0.055 (279)