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NICKEL TOXICITY EFFECT ON ANTIOXIDANT ACTIVITY OF GROUND NUT (*Arachis hypogea* L.)

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Abstract

This study pertains to the effects of heavy metal salts Nickel on the ground nut (*Arachis hypogea*) at various concentrations viz 5, 10, 25, 50, 75,100, 200 and 300 mg/L. The salts were given as treatments to the ground nut seeds at various ascending levels of doses till proving toxic. Effect of Ni concentrations as well as the antioxidant activities of Ascorbic acid content, superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and polyphenoloxidase was studied at various stages 30th, 60th and 90th DAS in leaf, stem and root of ground nut plants. Similarly the antioxidant activities were increased with increase in 10 mg/L concentration of nickel in 90th day after treatment. So, it could be concluded that Nickel at lower concentration had an inducing effect on plant growth and inhibited the same at higher concentrations. The data's are followed by maximum readings were observed in the concentration of 10 mg/L of Nickel for all the antioxidant activity.

Keywords: Nickel, Ground nut, antioxidant, SOD, CAT, POD and Polyphenoloxidase.

1. Introduction

Trace metals contamination of agricultural soils has become one of the most significant environmental problems today. Metal contamination of agricultural soils by atmospheric deposition or by disposal of sewage sludge constitutes a risk of either leaching of metals into the ground water or excessive accumulation in top soil. Metal uptake by crop plants can have strong adverse impact on human health through the food chain. There are 35 metals that concern us because of occupational and residential exposure; 23 of these are the heavy elements or "heavy metals". Heavy metal can include elements lighter than carbon and can exclude some of the heaviest metals[3]. Nickel is just one of a variety of ubiquitous trace metal emitted into the environment from both natural and anthropogenic sources. The compounds such as nickel acetate, nickel carbonate, nickel hydroxide and nickel oxide are used in a variety of industrial process. These compounds ultimately accumulate in the soil and environment, and can be easily taken up by plants. Thus they can enter into the food chain and cause deleterious effects to animals and human.

Nickel (Ni) is a micronutrient required at very low concentration by plants. It is one of the toxic heavy metals, and is recognized for its negative effects on the environment where it bio-accumulates and poses a serious threat to human and environmental health. All nickel compounds, except for the metallic nickel, have been classified as human carcinogens by International Agency for Research on Cancer. As Nickel is an important heavy metal pollutant, it is of interest to study its effect on the germination and seedling growth of groundnut (*Arachis hypogea* L.), an important major oil seed crop of Odisha.

The present investigation assesses the extent of Ni antioxidant activity in groundnut plant (*Arachis hypogea* L.). Groundnut is the major oilseeds crop accounting for 45% of oilseed area and 55% of oilseeds production of the country. The selected variety of the groundnut has a lifespan of about 100 days. The experiment was carried out in field experiments under nickel stress at variable concentrations. The objective of the study was mainly to assess the phytotoxic impacts of different concentration of nickel in growing groundnut plant (*Arachis hypogea* L.) with periodical investigation at its developing stages (30, 60 and 90days) and at matured stage (100days) of Ni treated plants. The phytotoxic effects of varying concentrations of nickel on the growth, development and biomolecular changes in groundnut plants supplemented to increasing concentrations of nickel was assessed.

METERIALS AND METHODS

The present investigation was carried out to the effect of different concentrations of chromium on germination and growth of ground nut (*Arachis hypogea* L.). The research work comprises of the following aspects, antioxidant parameters such as ascorbic acid, SOD, Catalase, Peroxidase and Polyphenoloxidse of *Arachis hypogea* L.seeds grown under different concentrations of Nickel.

Seed materials

Ground nut (*Arachis hypogea* L.) seeds of variety TMV-14 were obtained from Farm Oilseeds Research Station, TNAU, Tindivanam, Villupuram District, Tamil Nadu, India. The healthy seeds of ground nut were chosen and used for both laboratory and field experiments.

Preparation of Nickel solution:

Nickel Chloride ($Nicl_2$) salt was used as Ni source for the present study. A known weight (129.5994 g/mol) of $Nicl_2$ salt was dissolved in 1000 ml of distilled water to get a concentration of 1000 ppm as Ni. From this standard solution, the various concentrations (5, 10, 25, 50, 75, 100, 200 and 300 mg/l) of nickel solution were prepared and used both for laboratory and field experiments. Effect of Ni concentrations as well as the activities of Ascorbic acid content, superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione S-transferase (GST) was studied in leaf, stem and root of ground nut plants.

RESULTS

Catalase

The enzyme catalase activity in the leaf, stem and root of the grondnut grown under different concentrations of nickel are presented in fig.1. The highest catalase activity was recorded in leaf (2.26, 3.37 and 2.96 min^{-1} mg^{-1} protein), stem (2.02, 3.18 and 2.66 min^{-1} mg^{-1} protein) and root (1.57, 2.72 and 2.16 min^{-1} mg^{-1} protein) were recorded in 10 mg/l concentration of Nickel treated plants at 30, 60 and 90 DAS respectively. The lowest catalase content of leaf (0.46, 0.32 and 0.25 min^{-1} mg^{-1} protein), stem

(0.52, 0.84 and 0.76 min^{-1} mg^{-1} protein) and root (0.22, 0.56 and 0.45 min^{-1} mg^{-1} protein) were recorded in 300 mg/l concentration of Nickel treated plants at 30, 60 and 90 DAS respectively.

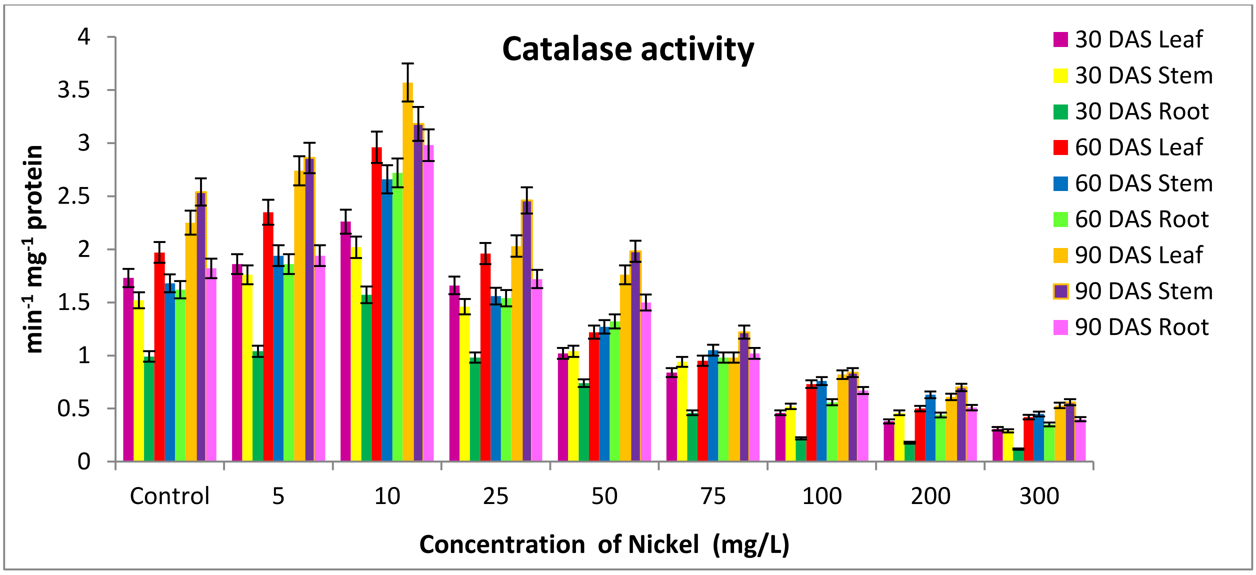

Fig.1. Effect of different concentrations of nickel on catalase content of *Arachis hypogea* L.

Superoxide dismutase

The effect of different concentrations of nickel on reducing superoxide dismutase contents in leaf, stem and root of groundnut at 30, 60 and 90 DAS is given in fig.2. The highest superoxide dismutase content of leaf (3.98, 4.97, 6.18, and 7.26 min^{-1} mg^{-1} protein), stem (3.5, 4.87, 5.81 and 7.01 min^{-1} mg^{-1} protein) and root (4.29, 5.73, 6.29 and 7.39 min^{-1} mg^{-1} protein) were recorded in 10 mg/l concentration of Nickel treated plants at 30, 60 and 90 DAS respectively. The lowest superoxide dismutase content of leaf (1.9, 2.22, 3.27 and 3.94 min^{-1} mg^{-1} protein), stem (1.04, 2.17, 3.53 and 4.86 min^{-1} mg^{-1} protein) and root (1.75, 3.28, 3.89 and 4.22 min^{-1} mg^{1} protein) were recorded in 300 mg/l concentration of Nickel treated plants at 30, 60 and 90 DAS respectively.

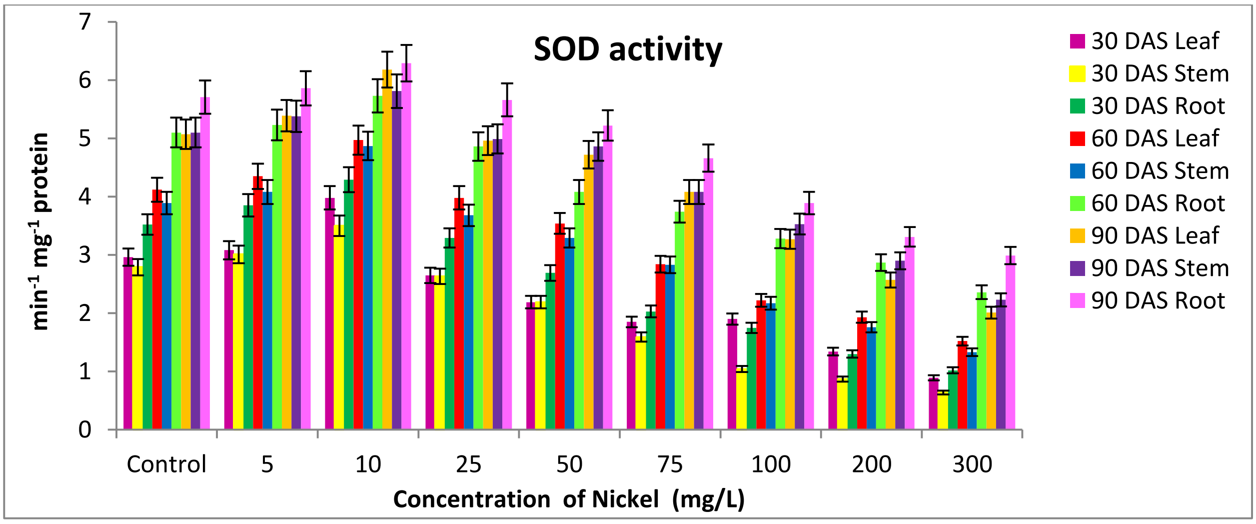

Fig.2. Effect of different concentrations of nickel on Super Oxide Dismutase content of *Arachis hypogea* L.

Peroxidase

The peroxidase activity in the leaf, stem and root of grondnut irrigated with different concentrations of nickel was shown in fig.3. The highest peroxidase acitivity of leaf (1.92, 2.15, 2.76 and 3.48 min^{-1} mg^{-1} protein), stem (1.21, 1.74, 2.39 and 2.96 min^{-1} mg^{-1} protein) and root (0.74, 1.1, 1.57 and 2.29 min^{-1} mg^{-1} protein) were recorded in 10 mg/l concentration of nickel treated plants at 30, 60 and 90 DAS respectively. Similarly, the lowest peroxidase activity of leaf (0.18, 0.34, 0.98 and 1.56 min^{-1} mg^{-1} protein), stem (0.1, 0.12, 0.32 and 0.76 min^{-1} mg^{-1} protein) and root (0.08, 0.10, 0.32 and 0.56 min^{-1} mg^{-1} protein) were recorded in 300 mg/l concentration of nickel treated plants at 30, 60 and 90 DAS respectively.

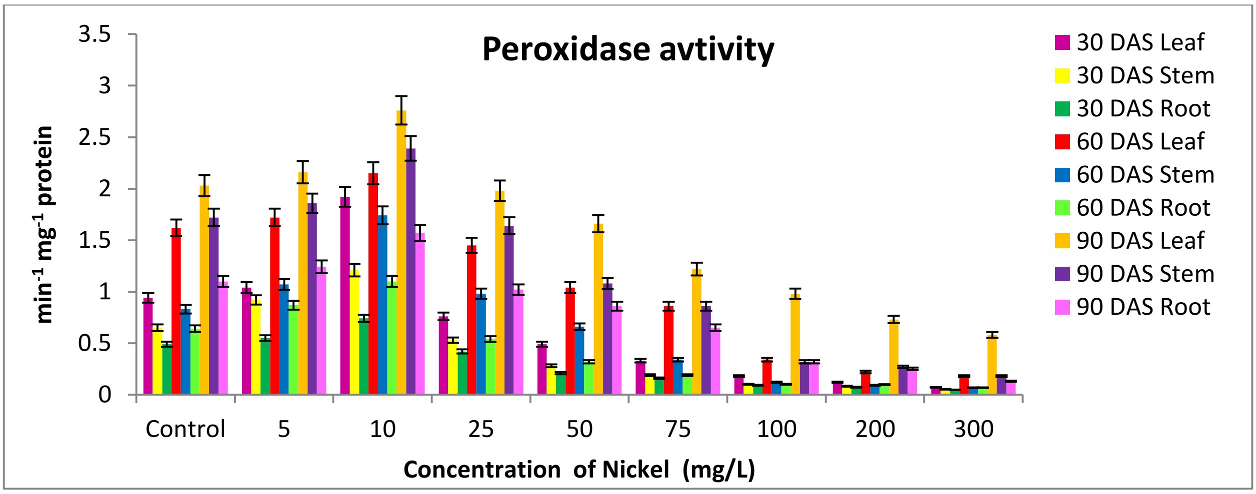

Fig.3. Effect of different concentrations of nickel on Peroxidase avtivity of *Arachis hypogea* L.

Polyphenol oxidase

The impact of different concentrations of nickel on polyphenol oxidase content in leaf, stem and root of grondnut at 30, 60 and 90DAS is given in fig.4. The highest polyphenol oxidase content of leaf (2.57, 3.27, 4.49 and 5.37 min^{-1} mg^{-1} protein), stem (2.05, 2.68, 3.32 and 4.49 min^{-1} mg^{-1} protein) and root (1.86, 2.17, 2.59 and 3.68 min^{-1} mg^{-1} protein) were recorded in 10 mg/l nickel treated plants at 30, 60 and 90 DAS respectively. The lowest polyphenol oxidase content of leaf (0.72, 0.98, 1.72 and 2.24 min^{-1} mg^{-1} protein), stem (0.24, 0.59, 0.76 and 1.62 min^{-1} mg^{-1} protein) and root (0.12, 0.37, 0.41 and 0.84 min^{-1} mg^{-1} protein) were recorded in 300 mg/l concentration of nickel treated plants at 30, 60 and 90 DAS respectively.

Ascorbic acid

The effect of different concentrations of nickel on ascorbic acid contents in leaf, stem and root of grondnut at 30, 60 and 90 DAS is given in fig.5. The highest ascorbic acid content of leaf (1.10, 1.92, 2.26 and 3.18 mg.g-1 fr.wt), stem (1.57, 2.05, 2.86 and 3.26 mg.g-1 fr.wt) and root (0.74, 1.56, 1.96 and 2.67 mg.g-1 fr.wt) were recorded in 10 mg/l concentration of nickel treated plants at 30, 60 and 90DAS respectively. The lowest ascorbic acid content of leaf (0.10, 0.18, 0.46 and 0.84 mg.g-1 fr.wt), stem (0.32, 0.43, 0.75 and 1.04 mg.g-1 fr.wt) and root (0.08, 0.19, 0.35 and 0.75 mg.g-1 fr.wt) were recorded in 300 mg/l concentration of Nickel treated plants at 30, 60 and 90 DAS respectively.

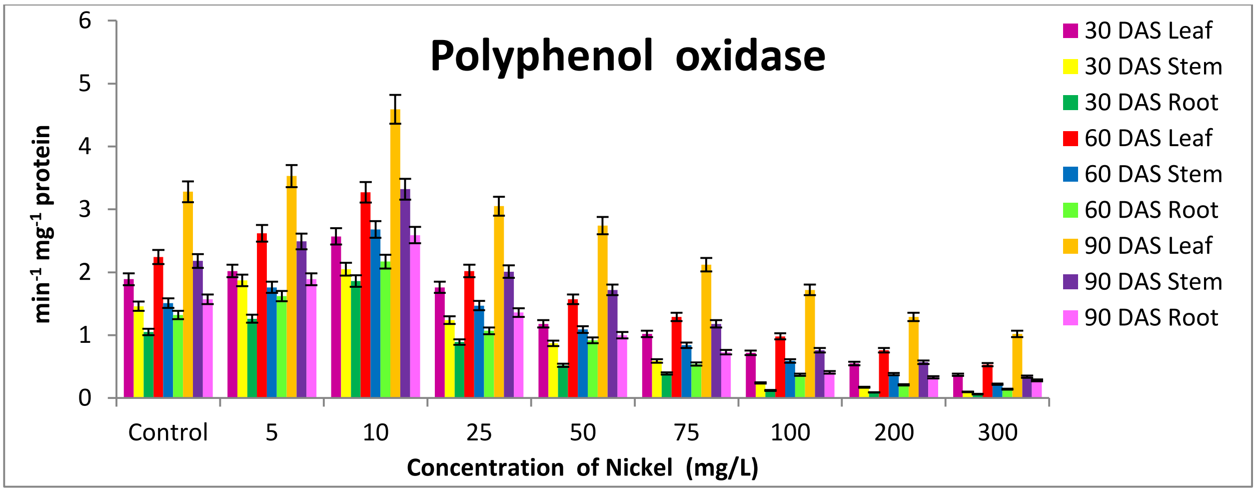

Fig.4. Effect of different concentrations of nickel on Polyphenol oxidase activity of *Arachis hypogea* L.

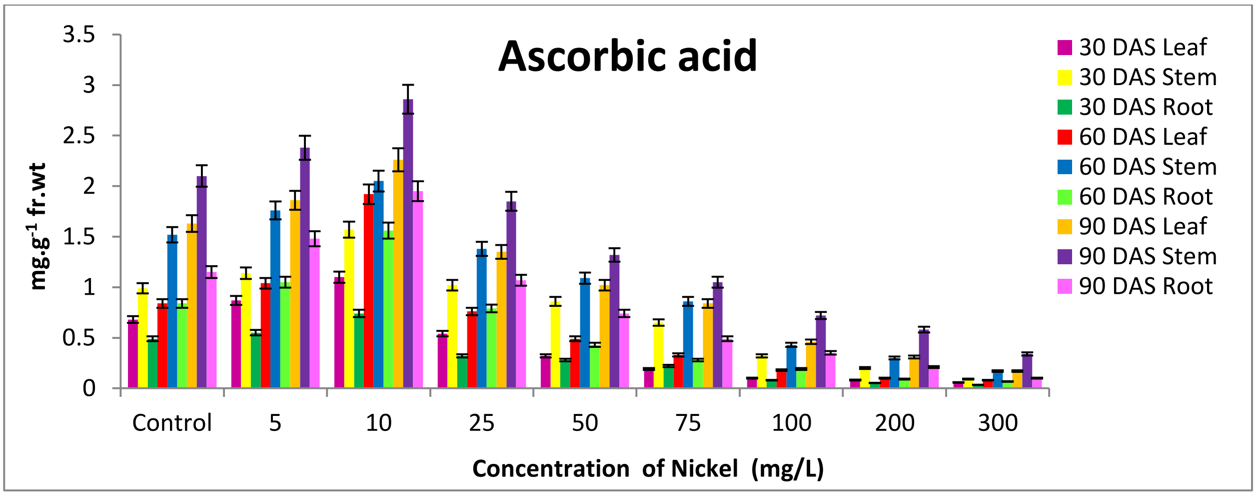

Fig.4. Effect of different concentrations of nickel on Ascorbic acid activity of *Arachis hypogea* L.

DISCUSSION

Catalase and peroxidase

Catalase is one of the most important components of plant protective mechanisms that exists in mitochondria and peroxisomes (Gupta *et al.*, 2009) and has important role in scavenge free radicals specially H_2O_2 generated during photorespiration (Bowler *et al.*, 1992) and stress condition (Foyer and Noctor, 2005). In the present work, increase in catalase activity can be considered as a circumstantial evidence for role of catalase in detoxification of H_2O_2 that induced under heavy metals stress. The catalase and peroxidase activity in the leaf, stem and root of groundnut irrigated with different concentration of nickel. The highest catalase and peroxidase acitivity of leaf, stem and root were recorded in 10 mg/l concentration of nickel treated plants at 30, 60 and 90 DAS respectively. Similarly, the lowest catalase and peroxidase activity of leaf were recorded in 300 mg/l concentration of Nickel treated plants at 30, 60 and 90 DAS respectively. Similar, results were also reported by Dong *et al.*, (2006). H_2O_2 produced during oxidative stress in plant in scavenged by peroxidase and catalase enzymes. The increased activity of CAT in the presence of Pb and Zn indicates the increased amounts of hydrogen peroxide and additionally effective scavenging of H_2O_2.

Peroxidase play a significant role in defense against oxidative stress and are suggested to be indicators of metal toxicity (Radotic *et al*., 2000). The peroxidase up regulation as a response of the plant to pollutants can be already be used for the phytomonitoring contaminated soil. These enzymes have been shown to be quite sensitive the atmospheric pollution with a response that can be stronger than other classical biomarkers (Wu and Von Tiedemann, 2002). On the other hand, the results suggest that there were similar responses in SOD, Apex and GR activities with increasing cadmium concentration by both cultivars. However, POD activity significantly increased at highly toxic cadmium levels (Ekmekci *et al*., 2008).

Super Oxide Dismutase

Super Oxide Dismutase (SOD) is one of the antioxidant enzymes found in various compartments of the plant leaf cell, found in chloroplast (Mittova *et al*., 2000). It is a prime enzyme which dismutates the superoxide anions to H_2O_2 (Kumar *et al*., 2011). It is considered to be the first line of defence against Reactive Oxygen Species (ROS) because it catalyses the dismutation of superoxide radicles to generate O2 and H_2O_2 and POD catalyse the conversion of H_2O_2 to H_2O and O_2 (Zhang *et al*., 2014).

In the present study, the highest Superoxide dismutase contents of leaf, stem and root were recorded in 10 mg/l nickel treated plants and the lowest Superoxide Dismutase content of leaf, stem and root were recorded in 300 mg/l concentration of Nickel treated plants.

5.6.3. Polyphenol oxidase

The highest polyphenol oxidase content of leaf, stem and root was recorded in 10 mg/l nickel treated plants and the lowest polyphenol oxidase content of leaf, stem and root were recorded in con300 mg/l concentration of Nickel treated trol plants. Increased polyphenol oxidase activity is reported under various metal stresses such as rubidium, nickel and cesium (Chinmayee *et al*., 2014) and Vlasyuk and Galinskaya (1970). High polyphenol oxidase activity under stress indicates its ability to oxidize and to degrade the toxic substances such as phenolic components which are generally reported to be accumulating during metal stress.

5.6.4. Ascorbic acid

Ascorbic acid, a natural antioxidant scavenger free radicals generated by pollutants (Halliwell and Gutteridge, 1993). It is considered as a secondary antioxidant that stabilizes the process of cell division and growth by scavenging free radicles which damage cell membrane and induce lipid peroxidation. These activities of ascorbic acid are necessary to maintain plant health and proper growth (Maramag *et al*., 1997).

In this present study, the highest ascorbic acid content of leaf, stem and root were recorded in 10 mg/l concentration of nickel treated plants and the lowest ascorbic acid content of leaf, stem and root were recorded in 300 mg/l concentration of Nickel treated plants.

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