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ORIGINAL RESEARCH

Growth Inhibition and Oxidative Stress Responses in Root and Shoot of *Vigna radiata* (L.) Wilczek under Nickel Toxicity

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ABSTRACT

An experiment was conducted to investigate the effects of nickel toxicity (100 µM Ni) on shoot and root growth, as well as the oxidative system in green gram (*Vigna radiata* L. Wilczek syn. Var. K-851) over a 16-day period. During this period the relationship between oxidative stress and Ni accumulation in shoot and root was studied. Depression in shoot and root growth was observed after 3d of Ni supply. Enhanced H_2O_2 concentration in roots only 24 hours after Ni supply indicated early induction of oxidative damage. Membrane lipid peroxidation was also observed early and was well related to Ni concentration. The ascorbate concentration in shoot and root showed an increase throughout the period of Ni supply, but dehydroascorbate concentration remained unaffected. The activity of the antioxidative enzymes SOD, APX and GR increased initially but decreased after 6 d of exposure to excess Ni supply. The activity of peroxidase increased up to 4 d but remained unaffected thereafter, and that of catalase decreased with the duration of excess Ni supply. Our findings suggest that oxidative damage, indicated by elevated H_2O_2 and malondialdehyde (MDA) levels, preceded the inhibition of shoot and root growth and was closely linked to the extent of Ni accumulation in the roots. Prolonged Ni exposure led to increased Ni accumulation, ultimately resulting in the breakdown of the antioxidative defense system in green gram.

KEY WORDS: Antioxidative defence mechanisms, Ascorbate concentration, Lipid peroxidation, Nickel phytotoxicity, Oxidative stress, *Vigna radiate*

INTRODUCTION

Nickel holds a unique position among the heavy metals. Unlike cadmium (Cd), lead (Pb), mercury (Hg), and several other metals that are not components of plant enzymes, nickel is an essential constituent of the enzyme urease. Small amounts of nickel (0.01 to 5µg/g dry weight) are necessary for the growth of some plant species. However, at high concentrations, nickel becomes toxic to plants. Nickel has been proposed, though not unequivocally established, as an essential element for higher plants. However, there is convincing evidence that excessive nickel supply leads to phytotoxic effects in plant roots (Boominathan & Doran, 2002; Pathak *et al.,* 2025). The problem of Ni toxicity acquires a serious concern because of agricultural use of sewage sludge that is usually rich in Ni (Coman *et al.,* 2013) and the industrial use of Ni in production of Ni-Cd batteries leading to discharge of Ni rich effluents. Sharma *et al.* (2013) reported that in plants, nickel is taken up by the roots through both active transport and passive diffusion mechanisms. Nickel is a micronutrient which is essential for plant growth and development and it is a major component of metabolically important enzyme urease which is found in higher plants for metabolism of nitrogen (Yadav & Sharma, 2016). When soil is exposed to high concentration of Ni^{2+}, then nickel concentration in tissue of plant is usually higher in roots than in stem and leaves. Due to excess Ni in soil, decrease the uptake of Fe which leads to Fe deficiency in plants and it also reduced the biosynthesis of some Fe related metalloenzymes (Sharma *et al.,* 2018).

At high concentration nickel is a strong phytotoxic element and some common toxicity symptoms of Ni in

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plants are initiation of wilting, chlorosis leads to necrosis, decrease percentage of seed germination (Yadav *et al.,* 2009), inhibits plant growth, reduce the rate of photosynthesis, and interrupt the transport of biomolecules and essential element (Bhalerao *et al.,* 2015). Adverse effects of Ni toxicity have been described by inhibiting the activities of enzyme, increased in production of ROS and show reduction in physiological processes because of competing with other elements for uptake (Kumar *et al.,* 2012). Due to excess Ni, Fe is most affected element which show similarities with Ni regarding to their chemical structure, nature and higher uptake via roots. In younger leaves, heavy metal in excess amount can induce chlorosis (Dubey & Pandey, 2011), and which is most visual symptoms of iron deficiency (Ghasemi *et al.,* 2009). It also decreases efficiency of photosynthesis by inhibiting the activities of enzymes found in Calvin cycle and biosynthesis of chlorophyll. Chen *et al.* (2009) reported that ROS in excess amount leads to excess production of unsaturated lipid peroxidation fatty acids and which may damage cell membrane. It also affects cell metabolism by disturbing enzymes activities, imbalance normal redox of cells and leads to oxidative damage. Accumulation of these heavy metals show significant effects on plants viability respiratory rates and carbohydrate concentration (Kutman *et al.,* 2019).

High nickel (Ni) concentrations have toxic effects on plants. Although Ni is a natural component of soils, human activities such as metal processing, land application of sludge, and the use of certain fertilizers can lead to its accumulation at potentially toxic levels (Kopittke *et al.,* 2010). The analysis of published evidence on Ni toxicity towards plants shows that, in addition to general toxicity displayed by all heavy metals, Ni manifests the specific characteristics due to its characteristic physical and chemical properties. The toxicity of heavy metals may depend on their binding to various ligands; among such ligands in the biological systems, carboxylate ion, imidazole, sulfhydryl group, and aliphatic amine are the most important. To illustrate, heavy metal binding to various functional groups of proteins, primarily SH-groups, would modify protein conformation and result in the loss of activity in many enzymes comprising SH groups in their activity centers. Besides, metal binding to SH-groups of the physiologically active compounds of low molecular weight would also interfere with cell metabolism. Thus, binding to SH-groups alone would already give rise to diverse metabolic disturbance.

Nickel is plays important role in many metabolic processes, including acitogenesis, hydrogen metabolism, ureolysis and methane biogenesis (Lopez & Magnitskiy, 2011). In last few decades, gradual increase in industrialization and urbanization processes increase the contamination of pollutants like, chemical fertilizers, pesticides, heavy metal and petroleum products in the environment like soil, water and air which degrade the quality of the environment and also affect both plants and animals (Singh, 2020). Heavy metals present naturally in the soil but their concentration increase rapidly due to anthropogenic and geological activities that show harmful effects to both plants and animals. Nickel in minute amount play a crucial role in a wide range of physiological processes, from seed germination to their productivity (Pandey *et al.,* 2006).

Nickel in higher concentration can change many metabolic processes of plants like, the ratio of water and mineral uptake, nutrients dynamics, inhibit the enzymatic rate and alter enzyme structure, open and closing processes of the stomata, degrading chlorophyll molecules and disrupt the electron transport in photosynthesis processes, reduce its photosynthesis rate, decrease the chlorophyll amounts and decrease the productivity of plants (Baccouch *et al.,* 2001; Yusuf *et al.,* 2011).

There are many compounds of nickel like Ni $(CH_3CO_2)_2$, $NiCO_3$, $Ni(OH)_2$ and NiO that are commonly used in various industrial process. Nickel is present in water due to biological processes, its solubilisation from soils, and sedimentation from the atmosphere. Freshwater generally contains approximately 300ng dm^{-3} of nickel. In agricultural field it contains approximately 3–1,000 mg kg^{-1} of Ni in soil, but concentration can be increased up to 24,000 mg kg^{-1} Ni in soil near industries or metal refineries and 53,000 mg kg^{-1} Ni in dried sludge. Nickel compounds are relatively soluble in soil at pH 6.5; however, at pH 6.7, they are mostly found in the form of insoluble hydroxides (Bhalerao *et al.,* 2015). Several studies have shown the toxic effects of nickel (Ni) on mitosis by disrupting spindle formation and causing chromosomal aberrations during root meristem cell division in both serpentine and non-serpentine seedlings (Pavlova, 2017).

Nickel in soil exhibits varying levels of ecotoxicity depending on its salt forms, influenced by their associated anions. The toxicity of these nickel compounds is ranked as follows: $NiSO4 < Ni(CH_3COO)_2 <$ Ni(II)-citrate $< NiCl_2 <$ Ni(II)-EDTA (Nie *et al.,* 2015). Some reports have found that nickel (Ni) causes toxicity in many plant species by affecting the activity of enzymes such as protease, amylase, and ribonuclease. This results in reduced seed germination and inhibited seedling growth in several crops (Gajewska *et al.,* 2006). Some studies have also reported that nickel disrupts the digestion and mobilization of food reserves, such as proteins and carbohydrates, in germinating seeds (Gomes-Junior *et al.,* 2006). It also reduces root length, plant height, wet and dry biomass,

chlorophyll content, and alters the activity of the enzyme carbonic anhydrase (Siddiqui *et al.,* 2011). Nickel toxicity also affects the photosynthetic pigments, reduces yields and leads to the accumulation of other ions like $Ca^{2+,}$ Na^{+} and K^{+} in mung bean (Ahmad *et al.,* 2007). Heavy metal toxicity and abiotic stress affects many plant processes such as seed germination, seedling growth, photosynthesis, osmotic homeostasis and carbohydrate metabolism *etc*. (Sethy & Ghosh, 2013). Thus, nickel toxicity negatively affects the growth and yielding of mung beans at various concentration of Ni in soil.

In all wheat cultivars studied, the percentage of seed germination gradually decreased with increasing concentrations of $NiCl_2$. However, at a low concentration of 0.05 mg/L, $NiCl_2$ did not exhibit any noticeable toxic effects on germination. These findings indicate that high concentrations of $NiCl_2$ have a negative effect on seed germination in wheat cultivars and drastically suppress root growth (Shweti *et al.,* 2018). A study on 14-day-old seedlings of *Triticum aestivum* cv. vergina further revealed that increasing $NiCl_2$ concentrations caused most toxicity symptoms to appear in the roots, along with a noticeable reduction in shoot growth (Kumar *et al.,* 2018).

In coriander and milk thistle seedlings, varying concentrations of nickel nitrate [$Ni(NO_3)_2$] had a negative impact on their growth traits. High concentrations of $Ni(NO_3)_2$ significantly reduced radicle growth during the experiment, eventually leading to complete cessation of radicle elongation and seedling death in the final days. Elevated levels of nickel also adversely affected seed germination and the seed vigour index. Moreover, the inhibitory effects of Ni on growth traits were more pronounced in milk thistle seedlings compared to coriander (Poozesh *et al.,* 2014).

MATERIALS AND METHODS

To study the effect of Ni toxicity on the component of the antioxidative defence system, seeds of green gram were grown under controlled glasshouse conditions. The seeds were shown in buckets of 5L capacity polyethylene pots for growth in sand culture. Before soaking the seeds, healthy and uniform sized seeds of green gram were surface sterilized with 0.01% mercuric chloride ($HgCl_2$) for one minute and washed thoroughly in distilled water. Soaked seeds were kept in BOD for 24 hours. Plants of green gram (*Vigna radiata* L) were grown from seeds in purified sand

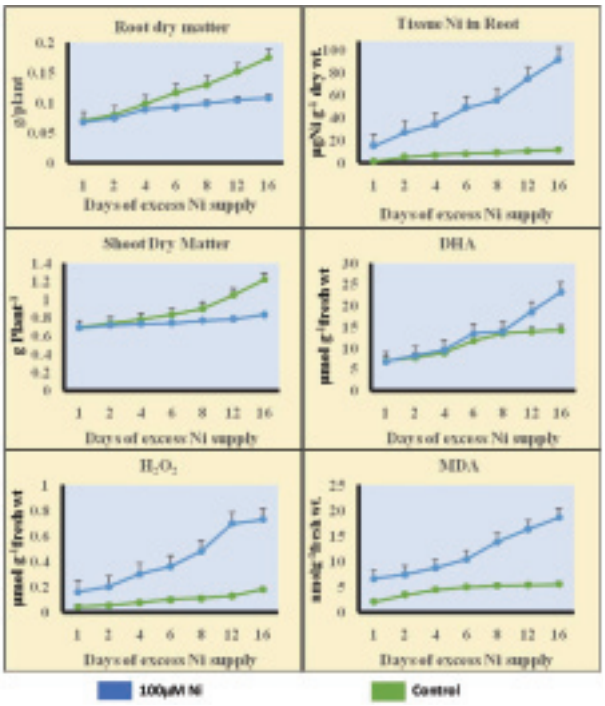

Fig. 1: Effect of $NiCl_2$ on dry matter yield, tissue nickel concentration, DHA content, hydrogen peroxidation and MDA content in green gram plants at different days after Ni treatment.

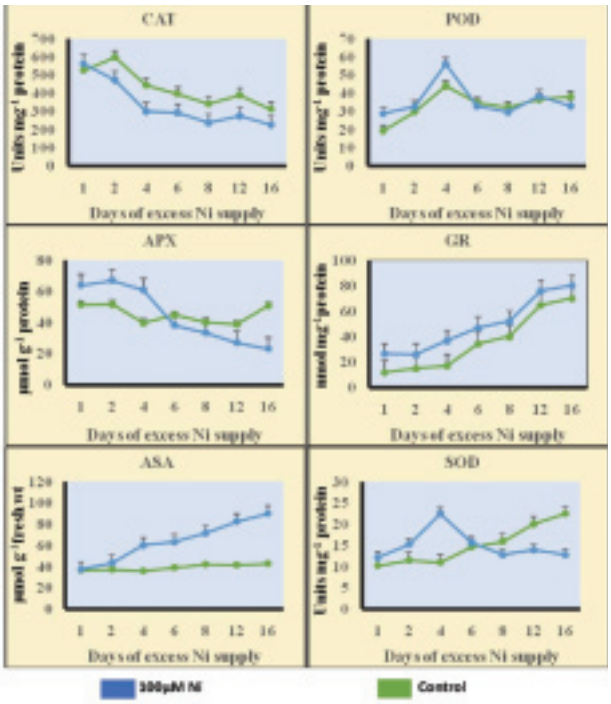

Fig. 2: Effect of $NiCl_2$ on the antioxidant enzymes–Catalase, Peroxidase, Ascorbate Peroxidase, Glutathion reductase, Ascorbate and superoxide dismutase content in leaves of green gram plants at different days after Ni treatment.

under glasshouse conditions and after germination plants were supplied complete nutrient solution. The pH was maintained at 6.7 ± 0.2. After healthy growth for 30 days, Ni was superimposed at 100 µM Ni, over the complete nutrient solution containing- 4mM $Ca(NO_3)_2$; 4mM KNO_3; 2mM $MgSO_4$; 1.33mM NaH_2PO_4; 0.1mM NaCl; 0.1mM Fe-EDTA; 10µM $MnSO_4$; 1µM $ZnSO_4$, 1µM $CuSO_4$; 0.2µM Na_2MoO_4; 0.1µM $CoSO_4$ and 0.1µM $NiCl_2$. After initiation of excess Ni supply at 2, 4 and 8 days plants were assayed for: Tissue Ni: AAS in wet digests (HNO_3:$HClO_4$ 10:1 v/v) of oven dried leaf material. Ascorbate peroxidase: (Nakano and Asada, 1981) oxidation of Ascorbate per min at 290 nm. Superoxide dismutase: (Beauchamp & Fridovich, 1971) Photochemical reduction of NBT at 560 nm. Glutathione reductase: (Madamanchi *et al.,* 1992) NADPH oxidation at 340 nm. Lipid peroxidation: (Heath & Packer, 1968) MDA content was assayed in TCA extracts by reaction with 0.5% thiobarbituric acid. Ascorbate: (Law *et al.,* 1983). Hydrogen Peroxide as described by Pandey *et al.* (2009).

Data given are mean of the three replicates. Visual records were maintained daily. The plants were analysed for chlorophyll, carotenoid, lipid peroxidation, hydrogen peroxide and activities of catalase, peroxidase, ascorbate peroxidase, superoxide dismutase and glutathione reductase. Light ranged between 1050-1180 ìmol $m^{-2}s^{-1}$ at 12:00 noon, relative humidity ranged between 85-92 % at 9:30 am and maximum and minimum temperature ranged between 38-43°C and 22-28°C, respectively during the period of experiment.

RESULTS AND DISCUSSION

The growth rate of plants decreased as the amount of Ni increased, inhibiting seedling growth and normal development due to the inhibition of cell division, cell elongation, or both. This results in the limited uptake and translocation of nutrients and water, ultimately leading to mineral deficiencies in plants (Batool, 2018). Exposure of plant roots to an excessive supply of Ni (100 mM) leads to increased Ni accumulation and inhibition of root growth. Accumulation of 34 µg Ni g^{-1} dry wt. of roots caused inhibition of root growth (d4) and this was visibly manifested as toxicity effects of Ni like chlorosis of leaves (Fig. 3C). Root exposure for beyond 8 d (that increased Ni conc. to 56 mg g^{-1} dry wt.) almost ceased growth of laterals and caused their browning (Fig. 3B). A marked increase in the level of H_2O_2 and a parallel increase in MDA level within 24 h of exposure of excess (100 mM) Ni, suggested oxidative damage as a primary lesion of Ni toxicity. The oxidative damage was related to magnitude of the Ni accumulation in the roots (Fig. 1). The marked increase in the activity of the antioxidative enzymes SOD, APX and GR (Fig. 2) as well as an increase in the concentration of

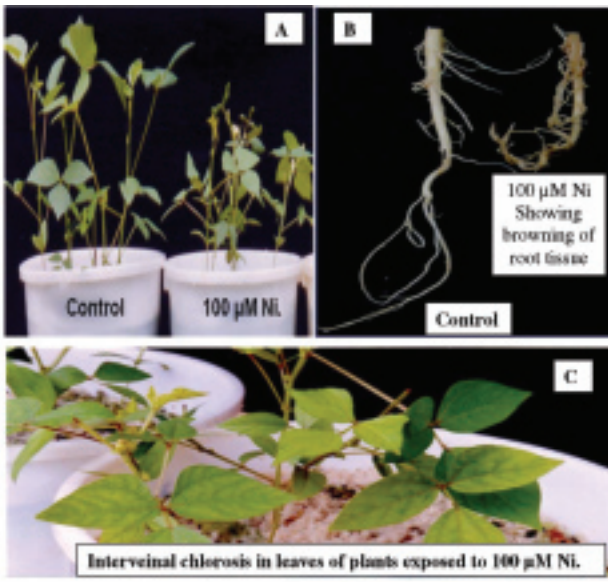

Fig. 3. Effect of Ni toxicity on green gram plants: (A) shoot and (B) root compared to control plants, and (C) interveinal chlorosis observed in leaves of plants exposed to 100 µM Ni.

antioxidants, ascorbate and dehydroascorbate up to d4 indicates induction of the antioxidative system at initial stage following supply of 100 µM Ni.

With continued supply of Ni, the oxidative damage in plants was enhanced, leading to the breakdown of their antioxidative defence systems after day 6. This was evident through the appearance of visible symptoms, increased in lipid peroxidation, elevated H_2O_2 concentration and a relative decline in the activity of antioxidative enzymes. The steady accumulation of H_2O_2 under excess Ni exposure suggests that prolonged Ni stress induced an overproduction of reactive oxygen species, which the antioxidant system fails to scavenge effectively. It is likely that prolong exposure to Ni accelerated the generation of reactive oxygen species by impairing the function of iron/ heme containing complexes in the mitochondrial electron transport chain, as well as by inhibiting antioxidant enzymes that rely on iron or heme cofactors

CONCLUSIONS

This study showed that nickel mediates toxicity in plants by competing with other metal ions and forming chelate complexes with metal ligands. The consequences of nickel toxicity include deficiencies of essential metal ions, delayed seed germination, disruption of cell structure, wilting, induction of reactive oxygen species (ROS), metabolic disturbances, and ultimately, growth inhibition and reduced yields. Nickel exerts its phytotoxic effects

through multiple mechanisms. The interference with iron (Fe) absorption and catalytic utilization emerged as a key mechanism underlying the toxicity of excess nickel. This was demonstrated by the reduced activity of both non-heme and heme enzymes, along with the limited effectiveness of increasing Fe supply in the nutrient medium. Another mechanism by which nickel induced toxicity was by reducing tissue hydration, with the observed increase in proline production likely resulting from both metal ion interference and dehydration stress. To mitigate the environmental impact of nickel contamination, its removal from soil and water is essential. Further research on nickel-hyperaccumulating plants is needed to enhance phytoremediation strategies for the clean-up of contaminated sites

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