SHORT COMMUNICATION

EFFECT OF NICKEL ON PHYSIOLOGICAL CHARACTERISTICS OF CASTOR PLANT (*RICCINUS COMMUNIS* L.)

TAPAN ADHIKARI* AND AJAY

Indian Institute of Soil Science, Nabiabagh, Berasia Road, Bhopal-462038, Madhya Pradesh

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A green house study was conducted to analyse the effect of Ni treatment on physiological characteristics of castor plant (*Ricinus communis* L.). Different levels of Ni (0, 10, 40, 80, 120, 160, 180, 200, 250 mg Ni kg^{-1} soil) were applied in the soil. The findings of the study showed that photosynthesis and chlorophyll content decreased with increasing concentration of Ni and to combat the Ni-induced stress, respiration rate of castor plant increased with increasing level of Ni applied. The activities of various metabolic enzymes like NRA (nitrate reductase), POD (peroxidase) and SD (succinate dehydrogenase) decreased with increase in Ni concentration applied. Leaf protein content decreased at the level of 80 mg kg^{-1}, Ni applied while at higher dose of Ni (>80 mg kg^{-1}) phenol and O-dihydric phenols activity showed increasing trend. The study concluded that at lower level of Ni in soil may improve plant growth, but higher level will severely affect the photosynthesis rate as well as enzymatic activities.

Key words: **Castor, enzyme activities, growth, nickel, photosynthesis**

The contamination of agricultural lands caused by heavy metals in and around industrial areas is a serious problem. Nickel pollution is mainly derived from a growing number of diverse anthropogenic sources like industrial effluents and wastes, urban runoff, sewage treatment plants and mining operations. In India, the areas located near glass, cycle and battery industries are prone to Ni pollution. Nickel is not essential nutrient for higher plants (Adriano 1986), but has been reported to stimulate growth at lower concentrations and showed toxic effects at high concentrations (Singh and Verloo 1996). Nickel is usually absorbed in the ionic form Ni^{+2} from soil or culture media and has been reported as a stable species over a wide range of pH and redox conditions (Cotton and Wilkinson 1980). The information regarding the direct application of the inorganic forms of these metals to soil and their uptake by plants is lacking. Castor is a tropical plant that grows under various habitats and has multiple economic uses. It is a fast growing plant and adapt easily to various conditions, dominate quickly a community and produce a monotypic stand. This study was planned to investigate the effect of Ni on growth and physiological characteristics of castor.

A pot culture experiment was conducted in 2007 on a Typic Haplustert soil, which is located at 23^{o}20¢ N latitude and 77^{o}27¢ E longitude at 495 m above mean sea level, having subtropical monsoon like climate (mean annual precipitation 1208 mm) with a hot summer (maximum day temperature 35-45^{o}C) and a mild winter (minimum night temperature 8-17^{o}C). The physicochemical properties of the soil like pH, electrical conductivity (EC), organic carbon (OC), cation exchange capacity (CEC) were estimated following the standard

*Corresponding author, tapan_12000@yahoo.co.uk

methods (Page 1982) and found as following: pH 7.89, electrical conductivity (EC) 0.40 dSm^{-1}, OC 0.56%, CEC 45 cmol (p^+) kg^{-1}, clay 55.50%, and DTPA-Ni 1.0 mg kg^{-1} soil. Castor plants were grown in pots (24 cm x 25 cm) in net house and various concentration of Nickel (Ni) was added as nickel chloride salt solution (0, 10, 40, 80, 120, 160, 180, 200, 250 mg kg^{-1} soil) to 5 kg air-dried soil filled in earthen pots, lined with polythene bags. The plants were watered to maintain field capacity and were left in the greenhouse to allow the added Ni to equilibrate before sowing for one month. A basal dose of 60 mg nitrogen (N) kg^{-1} soil (as urea), 50 mg phosphorus (P_2O_5) kg^{-1} soil (as SSP) and 40 mg potassium (K) kg^{-1} soil (as KH_2PO_4) was added to each pot. All the treatments were replicated thrice. Plants were harvested at 45 days after sowing. The shoots and roots from each pot were pooled, washed thoroughly with distilled and deionized water and weighed after drying at 70°C. Dried plant samples were digested with a mixture of HNO_3-$HClO_4$ acid (3:1). At the end of the experiments, the soil from each pot was sampled and extracted with the DTPA extractant (Lindsay and Norvell 1978). Nickel concentration in the digested plant samples and the soil extracts was determined by Perkin Elmer Double Beam Atomic Absorption Spectrophotometer.

Peroxidase activity was determined according to McCune and Galston (1959). The succinic dehydrogeanse (SD) enzyme activity was determined following the method described by Kun and Abood (1949). The nitrate reductase enzyme activity was determined following the method described by Hageman and Huldesby (1971). Phenol was determined according to Yubedee and Arinze (1994). The supernatant was used to estimate phenol. Ortho-dihydric phenol was determined according to Mahadevan and Sridhar (1986). Net photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO_2 concentration (Ci) were measured in fully matured expanded leaves using a portable photosynthesis system (CID-510, CID inc. USA) between 11-12h under natural sunlight at ambient temperature range of 28-32°C. Intrinsic carboxylation efficiency was derived as the ratio of net photosynthetic rate to intercellular CO_2 concentration (Pn/Ci). Respiration was measured in fully matured leaves using a portable photosynthesis system (CID-510, CID Inc. USA) at night under natural conditions with ambient temperature range of 28-32°C. Pigment contents in fresh leaves were analyzed by adopting the non-macerating using Dimethyl sulfoxide (DMSO) following described by Hiscox and Israelstam (1979). The chlorophyll concentration was calculated using the formula of Arnon (1949). Soluble extract from roots of control and Ni treated castor plant was determined for total protein following Lowry *et al.* (1951). There were three replicates for each observation and data presented as mean of three replicates ± SD.

The findings of the study indicate that castor plants accumulated Ni from Ni contaminated soils. Singh *et al.* (2010) have reported similar results. Dry matter production decreased at its higher level of Ni applied in the soil (Fig. 1). Application of Ni at lower doses (< 80 mg Ni kg^{-1} soil) increased growth of castor plant. Ma *et al.* (2009) reported similar results in *Brassica*. The higher concentrations of Ni have been reported to retard cell division, elongation, differentiation and affect plant growth and development (Baccouch *et al.* 1998). Under Ni deficiency, foliar fertilization of soybean with urea lowers the activity of urease enzyme in the foliage but accumulates toxic levels of urea in the tip region, resulting in leaf tip necrosis (Krogmeier *et al.* 1991). In tomato too, Ni deficiency resulted in chlorosis of the youngest leaves and necrosis of meristem (Checkai *et al.* 1986). Ni application did not show any metal toxicity symptoms in castor plant. Compared with the control (without Ni), the treatments above 200 mg Ni kg^{-1} soil resulted in significant decrease in the yield of tops. Dry-matter yield of castor roots significantly reduced with application of Ni at 200 mg Ni kg^{-1} soil (Fig. 1) and the

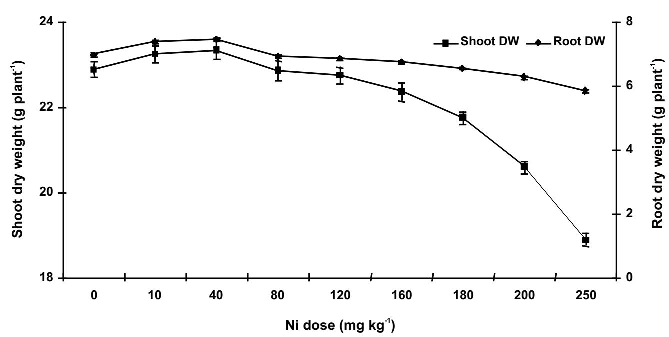

Fig. 1. Effect of nickel on shoot and root dry weight of castor plants

level of reduction was upto 10%. Leaf area and height of castor plant increased with the application of Ni up to 40 mg kg^{-1} soil (Fig. 2) and with further increase in the level of Ni a decreasing trend was observed. The result revealed that plant height reduced at higher levels of Ni (200 mg kg^{-1} soil), which indicated that Ni might have interfered in the growth process of castor plant. Similar trend of effect of Ni application was observed

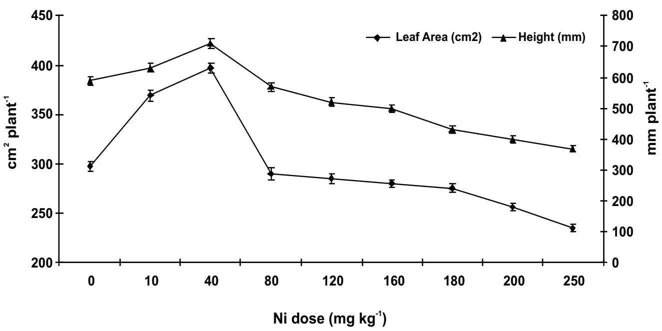

Fig. 2. Effect of nickel on leaf area and height of castor plants

on the leaf area of castor plant. The effect of Ni on above plant growth parameters was also observed on various other physiological characters like photosynthesis, transpiration, stomatal conductance and intercellular CO_2 concentration, which have been related to productivity of the plants (Balasubramanian and Gurumurthi 2001). The rate of photosynthesis increased at lower levels of Ni application, but with increasing levels of Ni, a decreasing trend was observed (Fig. 3). However, Ni acts as a heavy metal for most of the plant species at relatively higher concentrations. Excess of Ni decreases

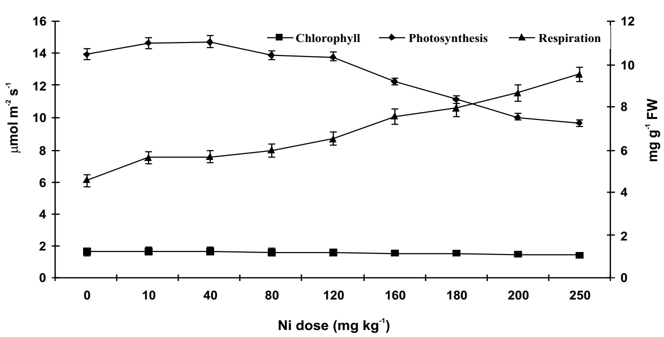

Fig. 3. Effect of nickel on chlorophyll content, photosynthesis and respiration in castor plants

dry matter production of plants (Setia *et al.* 1988). The yield reductions, usually exposed to excess Ni particularly those of root system, occur directly or indirectly inhibiting either photosynthetic production or its translocation out of leaves. Photosynthesis is the key to dry matter production and increasing the photosynthetic efficiency is the most important way of increasing productivity (Gupta 1994). The net photosynthesis rate of castor plant varied between 9.65 µmol m^{-2} s^{-1} to 14.72 µmol m^{-2} s^{-1}. Higher levels of Ni reduced the net photosynthesis rate of castor plant. The role of stomates in determining water use efficiency is also understood (Li 2000). The percentage reduction in total leaf conductance was maximum in 250 mg Ni kg^{-1} soil (Fig. 4). Minimum stomatal conductance (0.40 mol m^2 s^{-1}) was recorded at the highest level of Ni application and lower at 40 mg Ni kg^{-1} soil (0.78 mol m^2 s^{-1}). The leaf conductance decreased on account of higher concentration of Ni.

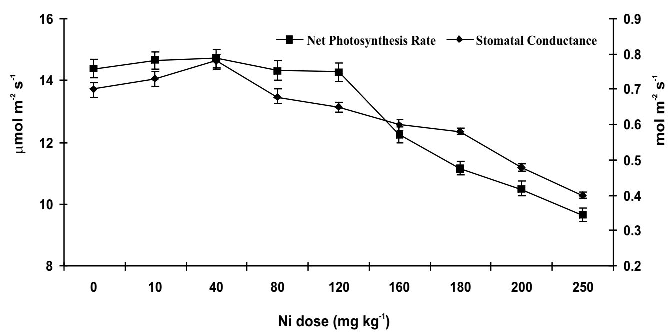

Fig. 4. Effect of nickel on net photosynthesis, and stomatal conductance in castor plants

Reduced leaf conductance is a common feature in stressed plants which results in reduced transpiration as well as photosynthectic rate, and dry matter accumulation. Higher level of Ni application exhibited maximum reduction in chlorophyll content. At 200 mg Ni kg^{-1} soil chlorophyll content of castor plant was 1.29 times less than control. Heavy metal like Ni stimulates the formation of free radicals and reactive oxygen species and caused oxidative stress. The result of the present investigation depicted that peroxidase activity increased at higher levels of Ni (Fig. 5). Peroxidases are involved not only in scavenging of H_2O_2 produced in chloroplast but also in growth and developmental processes (Schutzendubel *et al.* 2002). Peroxidase activities

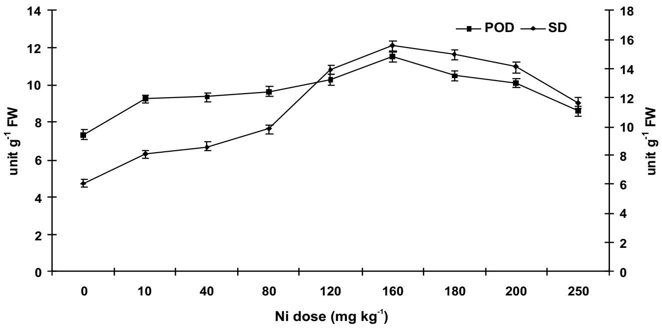

Fig. 5. Effect of nickel on POD and SD enzymes activity in castor plants

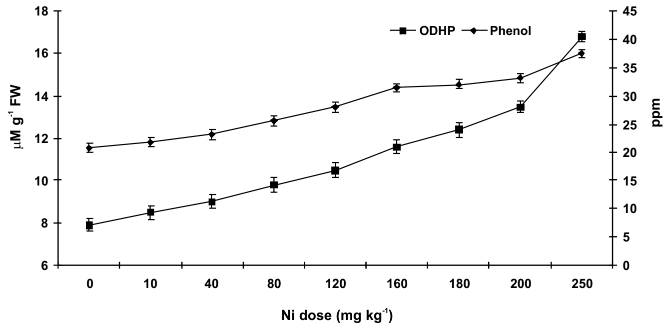

Fig. 7. Effect of nickel on phenol (µM g^{-1} FW), O-DHP (10-2) (ppm) content in castor plants

increased in lower Ni treatments but decreased at its higher levels. Similar changes in POD activity have been reported in various plants (Demiral and Turkan 2005). Ni can induce oxidative stress indirectly by producing disturbances in chloroplasts. Ni stress caused a significant reduction in nitrate reductase activity (NR) (Fig. 6). NR activity decreased with increasing concentration of Ni. Inhibition of NR activity under the influences of heavy metals may be direct effect of heavy metals on protein synthesis because they have a strong

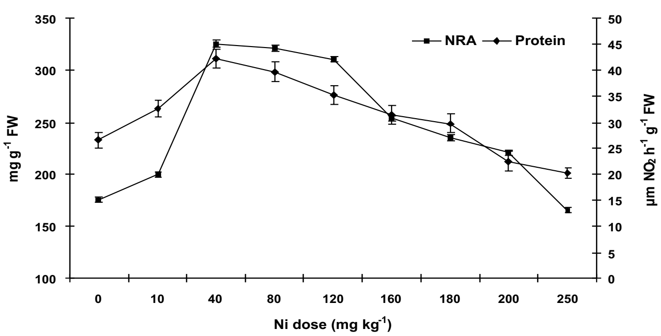

Fig. 6. Effect of nickel on protein and NRA enzyme activity in castor plants

affinity for functional sulphydryl group of enzyme (Singh 1998). The findings of this study indicated that with increasing Ni levels, phenol and O-DHP (Ortho-dihydric phenols) content showed an increasing trend (Fig. 7). In plants, phenolic compounds can act as antioxidants by radical scavenging, in which they break the free radical chain reaction through hydrogen atom donation. The resulting phenoxy radical can be reduced to its parent compound by enzymatic or non enzymatic reactions. The levels of phenols was found higher in this study. So it can be assumed that, the higher phenolic contents of young leaves of both the species may play an important role in the superoxide scavenging activities through free radicals quenching, electron transfer, radical addition or radical recombination. Growth reduction may be, generally linked to a loss of cellular turgor resulting in either a decreasing of mitotic activity and/or an inhibition of cell elongation (Gabbrielli *et al.* 1990).

The study concludes that accumulation of Ni in the root and shoot enable the plant to tolerate metal ions up to 80 mg kg^{-1}, beyond this level plant growth is affected adversely. It also suggests that lower dose of Ni causes less reduction in photyosynthesis rate, chlorophyll content and leaf diffusion resistance. Finally the study concludes that cultivation of castor plant can be advocated to mitigate Ni pollution in those areas.

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