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RESEARCH ARTICLE

EFFECT OF NI ON SEEDLING GROWTH, PHYSIOLOGICAL ATTRIBUTES IN BLACK GRAM (*VIGNA MUNGO* L.) IN LEAVES

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ABSTRACT

Nickel (Ni) is an indispensable micronutrient for plants. At higher concentration Ni becomes toxic for various plant species. Black gram (*Vigna mungo* L.) seed was grown on different concentration of Ni to study its toxic effect on seedling growth and biochemical parameters. The objective of this study was to investigate the effect of nickel on photosynthetic pigment, protein and sugar content and catalase activity in black gram leaves. One day old seedlings of black gram were subjected to different concentrations of nickel sulphate (0.01, 0.5, 5 and 50 ppm) every alternate day with nutrient solution. Plants were harvested after 15th days for determined photosynthetic pigment, protein and sugar content and enzymes activities. In growth parameters i.e. radical and plumule length, fresh weight and dry weights were also found to increase with further decrease in concentration of Ni up to 5 ppm in crop. The tolerance in *Vigna mungo* with respect to physiological attributes (chlorophyll a, b and total; protein and sugar content; catalase activity) were increased with decrease in concentration of Ni upto 5 ppm, whereas, these parameters were increase with further decrease in Ni concentration (50 ppm).

INTRODUCTION

Nickel (Ni) is among the abundant heavy metals and it constitutes about 0.08% of the earth crust thus it is ubiquitously distributed in soil and water (Kupper and Kroneck, 2007). Ni toxicity is of serious concerns to agriculture, ecosystem and human health (Jarup, 2003). Rapid industrialization and high anthropogenic pressures in the developing countries have encountered excessive amount of Ni in the environment. The most common symptoms of Ni toxicity in plants are inhibition of growth, photosynthesis, seed germination, sugar transport (Ali *et al*., 2009; Leon *et al*., 2005; Ahmad *et al*., 2009) and induction of chlorosis, necrosis and wilting (Madhava Rao and Sresty, 2000; Pandey and Sharma, 2002; Nakazawa *et al.*, 2004). It is known Ni originates more frequently from the non-ferrous metal industry, mining, production and disposal of batteries (Boularbah *et al*., 2006). In addition, untreated municipal wastewater, sludge disposal, application of pesticides and phosphate fertilizers are also important contributors of Ni pollution (Pandey, 2006). However, like many other essential elements its supra-optimal concentrations are strongly phytotoxic (Gautam and Pandey, 2008).

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Excessive Ni can induce alterations of plant metabolism that leads to the inhibition of germination and growth. It is known to produce stunted growth, chlorosis and necrosis of leaf which are visible symptoms associated with Ni toxicity (Seregin and Kozhevnikova, 2006). High concentration of Ni can inhibit dry matter production and chlorophyll biosynthesis (Ahmed *et al*., 2010). Ni has been classified among essential micronutrients (Brown *et al*., 1987). It is found associated with some metallo-enzymes which are necessary for various plants processes (Giridhara and Siddaramappa, 2002). Previous investigation demonstrated that the effects of Ni on antioxidant enzyme systems have already been studied in some plant species (Gajewska *et al*., 2006; Gajewska and Sklodowska, 2007; Yan *et al*., 2008; Wang *et al*., 2010). *Vigna mungo* L. (black gram) is a bean native to Central Asia. Since mungo beans and sprouts contain high amount of easly digestable proteins, they are good substitute for soya protein in diets (Fery *et al*., 2002). We investigated the influence of high Ni concentration on the activities of enzymes (protein and catalase), Photosynthetic pigment in leaves at 15th day after Ni application.

MATERIALS AND METHODS

Black gram (*Vigna mungo* L. A-65) was used for the Petri dish culture experiment. Seeds were surface sterilized with 0.1 % sodium hypochlorite solution for 10 min and then rinsed with double distilled water. After soaking for 24 h in water, twenty seeds were placed on filter paper in each Petri dish and 10 ml solution was used and the experiment was under observation for two weeks. The experiment was performed in replicate. This served as control or Ni solutions of (0.01, 0.5, 5 and 50 ppm) concentrations which served as treatment solutions. Seeds were germinated in 2 days. The fresh solutions were applied every day for the prevention contaminants and maintenance of proper concentration. Hoagland nutrient solution (Hoagland and Arnon, 1950), which served as control, or with nutrient solutions containing 0.01, 0.5, 5 and 50 ppm Ni which served as treatment solutions were applied in the present experiment. Growth traits were measured in terms of number of healthy plantlet, radical and plumule length (cm) and fresh weight after 15 days of treatment. The analysis of pigment, sugar and protein content and catalase activity were carried out after 15 days when toxicity symptoms were visible on plants. Chlorophyll a, b and total were estimated by the method of Lichtenthaler and Wellburn (1983); protein by Lowry *et al*. (1951); catalase activity by the modified method of Bisht (1976). Pigment was determined in 80% acetone extract absorbance of clear supernatant was measured after centrifugation (10,000 *g*, 20 minutes), at 663, 645 and 652 nm for chlorophyll a, b and total. Results were expressed on fresh weight basis in mg g^{-1}. For protein content, 500 mg of test plants were crushed in 5ml of 10% trichloro acetic acid and centrifuged at 10000 rpm for 10 minutes. After decanting the supernatant, pellets were washed with 5ml of 1N NaOH twice, again centrifuged in 5ml of 1N NaOH and final supernatant was collected. Reagent A (50 ml) and B (1ml) were added to reagent C to make 100ml. 5ml of above (A+B+C) solution was added to final supernatant (0.5ml) and kept for 10-15minutes at 30^0C. Reagent D (0.5ml) was finally added and thoroughly mixed. After 45 minutes, the absorbance was recorded at 750 nm. Bovine serum albumin (sigma) was used as standard. Sugar was determined by the method of Dubois *et al*. (1956). 500 mg leaves were homogenized with 10 ml 80% ethanol, and centrifuged at 2000 rpm for 20 minutes. The supernatant, collected separately, was added to 1.0 ml alcoholic extract, then 1.0 ml 5% phenol solution was added and mixed. 5.0 ml of 96% sulphuric acid was added rapidly, Each tube was gently agitated during the addition of acid and allowed to stand in a water bath at 26-30^{o}C for 20 minutes. The optical density (OD) was measured at 490 nm in a spectrometer after setting for 100% transmission against the blank. Standard curve was prepared by using known concentrations of glucose. The quantity of sugar was expressed as µg g^{-1} fresh weight of tissue. For catalae activity, 100 ml substrate mixture containing 50 mM H_2O_2 and 10 mM phosphate buffer (pH 7.0) was taken in a 50 ml test tube and stabilized at 25^0 C for 5 minutes. One ml of suitably diluted enzyme extract was added to initiate the reaction and was allowed to continue after 5 minutes. The reaction was stopped by adding 10 ml of 2% H_2SO_4 after one minute of incubation at 20^0C. In corresponding blanks, H_2SO_4 was added prior to addition of enzyme extracts. The acidified reaction mixture with or without enzyme extract was titrated against 0.01 N $KMnO_4$ to determine the quantity of H_2O_2 utilized by the enzymes. The catalase activity was expressed as ml H_2O_2 hydrolyzed mg^{-1} fresh weight. Data presented was statistically analyzed (Panse and Sukhatme, 1961) for mean (n=5) values. Significance of treatment effects were tested by least significant difference ($p<0.05$).

RESULTS AND DISCUSSION

Seedling growth of black gram (Table 1) had shown effects when Ni was supplied in different concentrations. It was observed that in comparison of control, there was gradual increase in growth (Radicle & Plumule) with the increase in concentrations of Ni from 0.01 to 5 ppm however 50 ppm concentrations retarded growth of plant. There was gradual reduction in fresh and dry weight of biomass with the increase of concentrations from 0.01 to 5 ppm and even at 50 ppm. In terms of percentage maximum increase in length of radical and plumule was observed at 5 ppm concentration (+16.8%) and (+63.3 %) while minimum of (+0.9%) and (+30.0%) was at 0.01ppm however increase of concentration had retarded growth of length of radical and plumule at 50ppm concentration (-20.1%) & (-10.0%) reduction had been experienced in the present experiment. Growth promotion in plants at low concentration of Ni and retardation in excess concentration of Ni have been reported by (Gerendas, *et al.*, 1999) and (Tripathi *et al*., 1981). Reduction in fresh weight and dry weight by (-17.9% to -48.2% & -3.5% to -28.6%) was

Table 1. Effect of different concentrations of nickel on black gram (*Vigna mungo* L.) seedling growth and fresh and dry matter

		Ni supply (ppm)				
Growth parameters	Days	0	0.01	0.5	5	50
Radical length (cm)	7	10.1±0.5	10.2±2.0	11.1±1.6	11.8±0.8	8.0±1.1
		(0.0)	(+0.9)	(+9.9)	(+16.8)	(-20.1)
Plumule length (cm)	7	3.0 =1.0	3.9±1.2	4.5±1.8	4.9±2.2	2.7±1.1
		(0.0)	(+30.0)	(+50.0)	(+63.3)	(-10.0)
Fresh weight (g)	15	5.6 ±1.9	4.6 ±1.1	3.6 ±0.3	3.0 ±3.0	2.9 ±3.1
		(0.0)	(-66.1)	(-35.7)	(-46.4)	(-48.2)
Dry weight (g)	15	0.28 ±0.7	0.27 ±0.1	0.25 ±0.2	0.23±0.3	0.20=0.6
		(0.0)	(-3.5)	(-10.7)	(-17.9)	(-28.6)

±- S.E. value (n=3); *-value significant at $P<0.05$ level.

Table 2. Effect of nickel on black gram leaves, pigments protein, sugar content and catalase activity at 15th day after treatment

Parameters	Ni supply (ppm)					LSD P=0.05
	0	0.01	0.5	5	50	
Chlorophyll a ($mg\ g^{-1}$ fr.wt.)	1.86 (0.0)	1.61 (-0.13)	1.34* (-27.9)	1.12* (-39.7)	0.90* (-51.6)	0.47
b ($mg\ g^{-1}$ fr.wt.)	1.65 (0.0)	1.42 (-13.9)	1.34 (-18.8)	1.23* (-25.5)	0.87* (-47.3)	0.35
total ($mg\ g^{-1}$ fr.wt.)	1.22 (0.0)	0.98 (-19.6)	0.81* (-33.6)	0.74* (-39.3)	0.55* (-54.9)	0.31
Protein ($\mu g\ g^{-1}$ fr.wt.)	52.28 (0.0)	63.37 (+21.2)	84.76 (+62.1)	87.13 (+66.6)	16.63* (-68.2)	35.74
Sugar ($\mu g\ g^{-1}$ fr.wt.)	0.7 (0.0)	1.2 (+71.4)	2.0 (+185.7)	4.4* (+528.5)	0.4 (-42.8)	1.9
Catalase (ml H_2O_2 hydrolysed mg^{-1} fr.wt.)	213 (0.0)	243 (+14.1)	256 (+20.2)	294* (+38.0)	182 (-14.5)	52.97

*- value significant at P<0.05 and **- value significant at P<0.01 levels.

observed with the gradual increase of concentration of Ni at (0.01, 0.5,5 & 50ppm). Reduction in bio-mass may be associated with the lignifications of cell walls, as reported in heavy metal stressed plants (Diaz *et al*., 2001). Gajewaska *et al*. (2006) have reported metal-induced decline the tissue water content, which could be the reason for bio-mass reduction. Beneficial role of low Ni has been described earlier in different plants (Gerendas *et al*., 1999; Pandey, *et al*., 2009). Different concentrations of nickel significantly decreased the content of chlorophyll a, chlorophyll b and total in leaves. Chlorophyll a, b and total chlorophyll contents were decreased by -51.6%, -47.3% and -54.9% respectively at 15th day after Ni supply as compared to the control value. Protein contents in black gram leaves increased with the increase in Ni supply upto 50ppm as shown in Table 2. Some amino acids and proteins, involved in anti-oxidative defense mechanism in plants, combine with heavy metals to form metallo-protein complex and provide tolerance in plants against heavy metal-stress (Sharma, 2006).Sugar contents increased gradually in 0.01 to 5 ppm Ni supply but decreased at the concentration of 50 ppm Ni supply. Reduction in sugar content could be attributed to decrease in chlorophyll synthesis and photosynthesis rate (Moya *et al*., 1993). Catalase can eliminate H_2O_2 and play a key role in the elimination of oxygen. Protein content and catalase activity increased by 66.6% and 294% compared to control, at 15 day after Ni supply while the sugar content increased by 528.5% (Table 2).

The increase of catalase activity suggests elevation level of H_2O_2, formed by dis-mutation of superoxide radicals. This is indicative of protection against heavy metal-stress by plants (Gajewaska and Sklodowaska, 2007). Increased catalase activity is generally regarded as a response to heavy metal stress due to the generation of Reactive Oxygen Species (ROS) in plant cells (Schützendübel and Polle, 2002). In present experiment typical symptoms of Ni toxicity developed 10-12 days after the beginning of treatment. Chlorosis, a common symptom of Ni toxicity (Pandey and Sharma, 2002) was seen in the leaves of plants treated with 0.01ppm, 0.5 ppm, 5 ppm and 50 ppm Ni concentrations. No visible symptoms of Ni injury were observed in the case of plants treated with 0.01 ppm. Growth promotion and retardation have been reported in plants at low and high concentration of Ni (Gerendas *et al.*, 1999; Tripathi *et al*., 1981). Present experiment data shows that the nickel treatment caused a reduction of biosynthesis of photosynthetic pigment. Prasad and Prasad (1987) reported the biosynthesis inhibition by metals in higher plants. Clemens *et al*. (2002) mentioned the oxidative stress and subsequent damage through the peroxidation of the chloroplast membranes as major causes for photosynthetic pigments biosynthesis. Chlorophyll contents reduction in leaves exposed to Cd stress were also reported in *Vigna mungo* (Singh *et al*., 2008). Reduced chlorophyll content due to nickel toxicity has been documented by (Pandey and Pathak, 2006) in green gram and similar results have been found in the present experiments. Our results showed a stimulation of catalase activity after 15th day of nickel treatment.

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