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Research article

Toxic level of Ni and its combination with micronutrient (B and Cu) on antioxidative enzymes and synthesis of sugar and protein in brinjal (*Solanum melongena* L.) metabolism

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Abstract: Brinjal (*Solanum melongena*) var NBH-774 were grown in pots at ambient temperature 20– 30°C. The 200 µM, 500 µM, 1000 µM of nickel, boron (150 µM, 300 µM) and copper test solutions (50 µM, 100 µM) were prepared in pure distilled water after quantification of nickel, boron and copper as per percent availability in $NiSO_4$, HBO_3, $CuSO_4$. Pure distilled water was used as control. The 250 ml of test solutions were supplied daily in each pot, which was enhanced to 500 ml having same concentration of nutrients at the time of flowering stage. Increased activity of catalase (antioxidative enzymes) was recorded on 35th day of Ni treatment from 200–1000 µM, whereas on 70th day of Ni treatment the catalase activity was increased up to 500 µM of Ni, While the recovery treatments using B and Cu reduced the activity on 70th day of treatment as compared to lone Ni. The activity of peroxidase (an antioxidative enzymes) on 35th and 70th days of Ni supply in leaves of brinjal showed increased activity except 1000 µM of Ni on 70th day of treatment. Eventually reduced activity of peroxidase was observed in recovery treatment using B and Cu as compared to respective lone Ni supply (500 µM and 1000 µM). Excess Ni supply (200 µM to 1000 µM) significantly decreased the protein in brinjal leaves at 35th and 70th day of treatment. Whereas, 500 and 1000 µM of Ni in combination with each concentration of B (150 µM, 300 µM) and Cu (50 µM, 100 µM) improved protein content in leaves. Total sugar was decreased with an increase in Ni supply from 200 µM to 1000 µM. The reduced sugar in 500 µM and 1000 µM of Ni was improved in recovery treatment on both the time points of analysis.
Keywords: Nickel - Catalase - Peroxidase - Protein - Sugar.

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INTRODUCTION

Heavy metal pollution is a worldwide problem with serious environmental consequences. Amongst heavy metals, nickel (Ni) is an essential micronutrient for plant growth. Trace elements are necessary for normal metabolic functions in plants, but higher concentrations of these metals are toxic and may severely interfere with many physiological and biochemical processes of plants (Seregin & Kozhevnikova 2006, Chen *et al.* 2009). Heavy metal affects plants in two ways. First, it alters reaction rates and influences the kinetic properties of enzymes leading to changes in plant metabolism. Second, excessive heavy metals lead to oxidant stress. During the period of metal treatment, plants develop different resistance mechanisms to avoid or tolerate metal stress, including the changes of lipid composition, the profiles of isozymes and enzyme activity, sugar or amino acid contents, and the level of soluble proteins and gene expressions. These adaptations entail qualitative and/or quantitative metabolic changes that often provide a competitive advantage, and affect plant survival (Schützendübel & Polle 2002). The threshold concentration of nickel that leads to toxicity strongly depends on the type of plant under investigation, because plants differ drastically in their ability to deal with nickel toxicity (Manusadzianas *et al.* 2002).

The major environmental factors that affect heavy metal uptake by plants are the soil acidity, its cation exchange capacity, the content of organic substance and lime, moisture potential, granulometric composition

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and concentration of macro- and micro- nutrients. The effect of these factors on the uptake of many heavy metals are mostly nonspecific (Merkusheva *et al.* 2001, Kukier *et al.* 2004). The presence of Cu, Zn significantly declined the uptake of nickel in *Thlaspi montanum* (Boyd *et al.* 1998). For instance the concentration of one element may affect the level of accumulation of other metals or may modify the toxic effects of other elements (Beckett & Davis 1978).They also stated that the nickel-zinc interaction and nickel-copper interactions were ineffective in reducing the nickel toxicity, that is varying concentrations of zinc or copper neither reduced nor increased the effect of nickel. Moreover copper and boron are micro- nutrients and their deficiency causes wilting, melanism, white twisted tips, reduction in panicle formation, infertile pollen grains (Shkolnik 1981). While boron deficiency causes chlorosis and browning of young leaves, death of growing point and inhibition of multiplication of cells.(Bergmann & Cumakov 1977). Therefore, the investigations were carried out to explore the nickel toxicity and to quantify the toxic responses of excess nickel in terms of antioxidative enzymes, protein and sugar content. Whereas attempts were also made to find out the effect of interaction of nickel with boron and copper in brinjal (*Solanum melongena*).

MATERIAL AND METHODS

Brinjal (*Solanum melongena* L.) var NBH-774 were grown in pots at ambient temperature 20 –30°C. The 20 seed were sown in prepared pots and five were maintained later. The 200 µM, 500 µM, 1000 µM of nickel, (150 µM, 300 µM) boron and (50 µM, 100 µM) copper solutions were prepared in pure distilled water after quantification of nickel, boron and copper as per percent availability in $NiSO_4$, HBO_3, $CuSO_4$. Pure distilled water was used as control for the study in triplicate. The 250 ml of treatments were supplied daily in each pot, which was enhanced to 500 ml at the time of flowering stage. The activity of enzymes (Catalase, peroxidase), total protein and total sugar was analyzed on 35th and 70th day of treatment supply.

Catalase and peroxidase activities were determined by the methods of Bisht (1989) and Luck (1963). Total protein was estimated by the method of Lowry *et al.* (1951) and total sugar by the method of Dubais *et al.* (1956). The data observed in the experiment, were statistically analyzed for the calculation of standard error (S.E). Student‘t’ test was administered for testing the hypothesis with the help of computer software sigma stat 2.0 programme.

RESULTS AND DISCUSSION

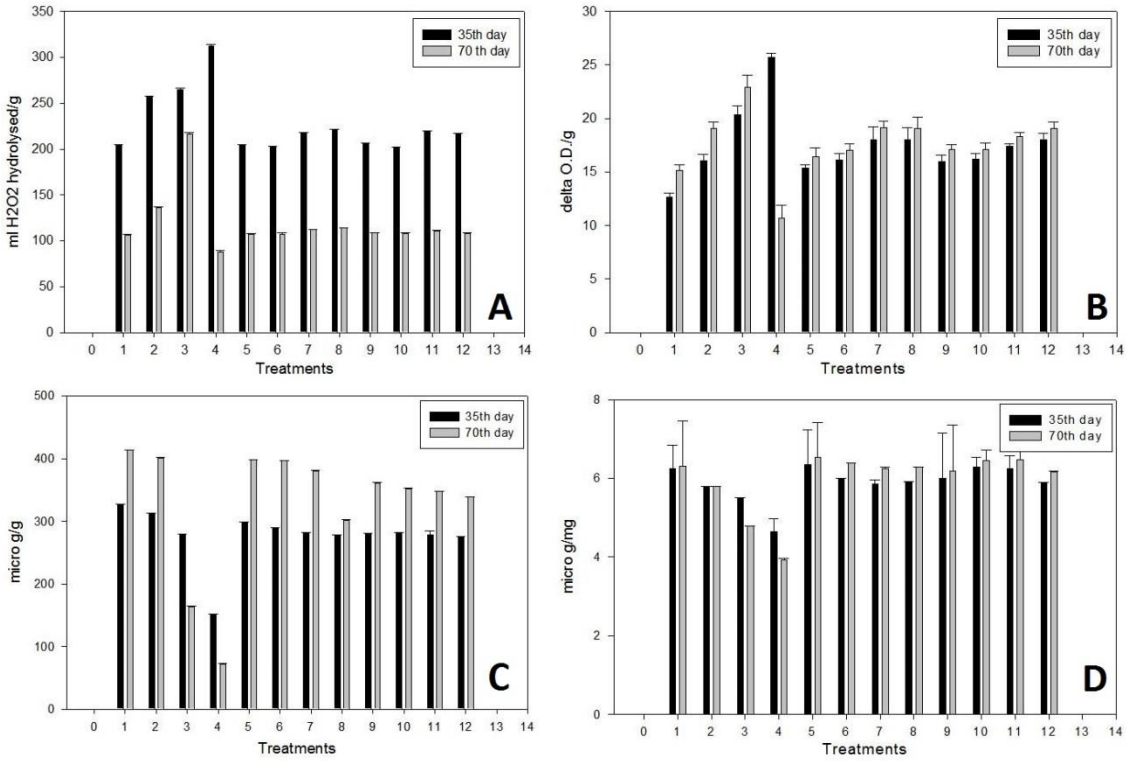

Figure 1. Effect of Ni on brinjal: A, Catalase activity; B, Peroxidase activity; C, Total protein; D, Sugar content. (1. Control. 2. 200 µM Ni. 3. 500 µM Ni. 4. 1000 µM Ni. 5. Ni + B 500 +150 µM. 6. 500 + 300 µM. 7. 1000 +150 µM. 8.1000+ 300 µM. 9. Ni + Cu 500 + 50 µM. 10. 500 +100 µM. 11.1000+ 50 µM. 12. 1000+ 100 µM)

Analysis of catalase activity on 35 and 70 days of treatment is show in table 1 and Fig 1A. Increase activity of catalase was recorded on 35 day of Ni treatment from 200 µM to 1000 µM, whereas on 70 days of Ni treatment the catalase activity was increased up to 500 µM of Ni. While the recovery treatment reduce the activity except the combination of 1000 µM Ni with B (150 µM, 300 µM) and Cu (50 µM, 100 µM) on 70 days of treatment as compare to lone concentration. However the activity of catalase was marked more on 35 days as compare to 70 days of metal treatment.

Table 1. Effect of increasing grade of Ni and its recovery treatment with B and Cu on catalase, peroxidase, total protein and total sugar in Brinjal (*Solanum melongena* L.) at 35 and 70 days of treatment.

Treatments	35th days				70th days			
	Catalase (ml H_2O_2 hydrolysed.g^{-1})	Peroxidase (ΔO.D.g^{-1})	Total Protein (µg.g^{-1})	Total Sugar (µg.mg^{-1})	Catalase (ml H_2O_2 hydrolysed.g^{-1})	Peroxidase (ΔO.D.g^{-1})	Total Protein (µg.g^{-1})	Total Sugar (µg.mg^{-1})
Control	204.427±0.415	12.667±0.333	326.320±0.574	6.250±0.583	106.517±0.457	15.160±0.503	413.230±0.580	6.300±1.149
200 µM Ni	256.887±0.419*	16.073±0.582*	312.090±0.615*	5.780±0.005	136.323±0.577*	19.077±0.582*	401.353±0.328*	5.790±0.005
500 µM Ni	264.493±1.772*	20.330±0.883*	278.013±1.155*	5.510±0.005	216.790±0.962*	22.893±1.163*	164.213±1.158*	4.780±0.005
1000 µM Ni	312.447±1.445*	25.743±0.371*	151.087±0.861*	4.647±0.328	87.793±1.925*	10.677±1.202*	72.217±1.175*	3.927±0.031
Ni + B								
500+150 µM	204.003±0.574	15.387±0.318*	298.010±0.583*	6.353±0.886	107.013±0.580	16.417±0.877	398.013±0.574*	6.543±0.878
500+300 µM	202.603±0.897	16.130±0.586*	289.877±0.333*	6.000±0.005	107.417±1.136	17.047±0.560	396.687±0.876*	6.380±0.005
1000+150µM	217.050±0.562*	18.047±1.160*	281.007±1.530*	5.863±0.088	112.010±0.580*	19.160±0.627*	380.740±1.153*	6.253±0.026
1000+300µM	220.713±0.649*	17.983±1.155*	278.127±0.630*	5.910±0.005	113.777±0.667*	19.070±1.097*	302.007±0.580*	6.280±0.005
Ni + Cu								
500+50µM	206.513±0.298*	16.003±0.577*	280.373±0.873*	6.000±1.152	108.473±0.319*	17.117±0.475*	361.003±1.526*	6.190±1.158
500+100µM	202.010±0.577*	16.240±0.499*	282.207±0.586*	6.277±0.267	108.183±0.639	17.123±0.623*	352.010±0.580*	6.447±0.267
1000+50µM	219.777±0.338*	17.433±0.207*	278.073±6.336*	6.247±0.332	110.343±0.882*	18.333±0.328*	347.683±0.881*	6.470±0.257
1000+100µM	216.437±0.719*	18.030±0.578*	274.887±0.667*	5.890±0.005	108.010±0.572	19.080±0.579*	338.903±0.577*	6.170±0.005

Note: The average of three replicate ± S.E and (*) statistically significant at p <0.05 level.

The activity of peroxidase both on 35 and 70 days of Ni supply in leaves of brinjal show increased activity except 1000 µM of Ni on 70 days of treatment. Eventually reduce activity was observed in recovery treatment as compare to respective lone concentration of 500 µM and 1000 µM Ni supply (Table 1 and & Fig. 1B). Excess Ni supply (200 µM to 1000 µM) significantly decreased the protein in brinjal leaves at 35 and 70 days of treatment. Whereas 500 and 1000 µM of Ni in combination with each concentration of B (150 µM, 300 µM) and Cu (50 µM, 100 µM) improve protein in brinjal leaves (Table 1 & Fig. 1C). Total sugar was decreased with an increase in Ni supply from 200 µM to 1000 µM (Table 1 & Fig. 1D). The reduce sugar in 500 µM and 1000 µM of Ni were improve in recovery treatment on both days of analysis.

Increase activity of catalase and peroxidase was recorded on 35th and 70th day of Ni treatment from lower to higher concentration. Catalase, which catalyses conversion of hydrogen peroxide into water and oxygen, is the major H_2O_2-scavenging enzyme in all aerobic organisms (Willekens *et al.* 1995). Yan *et al.* (2008) reported that Ni treatment resulted in a significant increase in catalase activities of plant, while the results of MadhavaRao & Sresty (2000) showed that the activity decreased significantly in pigeon pea seedlings grown at higher Ni levels. These results suggested that catalase activities in plant tissues are correlated with the tested Ni concentrations. The present results suggested that the catalase activities are remarkably increased in plant tissue under excessive Ni stress, and these results are in agreement with the previous results. Accumulating evidence indicates that catalase plays an important role in the protection against oxidative damage by breaking down hydrogen peroxide (Mittler 2002). Induction of peroxidase activity after Ni treatment of plants was reported previously (Gomes-Junior *et al.* 2006). Enhancement of peroxidase activity under metal stress was explained by its role in building up physical barrier against toxic metals entering the cell as well as in scavenging H_2O_2 (Passardi *et al.* 2005). Therefore, this peroxidase serves as a parameter of metabolism activity against nickel toxicity.

Total proteins and sugar were significantly decreased by increasing Ni concentration in brinjal at both times of observation. The decrease in protein content may be cause by enhanced protein degradation as a result of increase protease activity under stress conditions (Palma *et al.* 2002). Sridhar *et al.* (2005) conducted experiment on wheat genotypes and found that total sugar decrease in all developing grains. Reduction in total sugar content induced by heavy metal treatments may be due to its inhibitory effect on photosynthetic activities, photosynthetic pigment concentrations, as well as on the activity of ribulose diphosphate carboxylase leading to decrease in all sugar fractions (Stibrova *et al.* 1986).

In the recovery experiment the toxic effect of Ni were overcome by using boron and copper. The interaction of boron with other metals is not very much studied but it is antagonistic or synergistic with Mo and Fe and possible antagonism with Cr. Lou *et al.* (1991) found that Ni concentration and uptake was markedly reduced by copper application. The presence of Cu, Zn significantly declined the uptake of nickel in *Thlaspi montanum* (Boyd *et al.* 1998). Furthermore Cu involved in numerous physiological functions as a component of several enzymes, mainly those which participate in electron flow, catalyze redox reactions in mitochondria and chloroplasts (Hansch & Mendel 2009).

CONCLUSIONS

The result of the research showed that nickel treatment caused oxidative damage in brinjal as well as synthesis of protein and sugar. Although use of boron and copper can help the plants to get rid of this inhibition up to certain limit.

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