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INTERACTIVE EFFECTS OF NICKEL AND ZINC AMENDMENT IN SOIL ON GROWTH, TISSUE CONCENTRATION AND SOME BIOCHEMICAL RESPONSES OF TOMATO (*Lycopersicum esculentum* L.)

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Abstract: A clay pot experiment was conducted to study the effects of low and high concentrations of Ni (0.5 ppm) and Zinc (5 and 10 ppm) in amendment of soil singly or in combination. Following parameters were selected to observe effects on growth (shoot length, fresh and dry weight), tissue concentration and some biochemical responses (pigments, protein, sugar and proline contents and catalase enzyme activity) of selected Tomato plants (*Lycopersicon esculentum* Mill.-Solanaceae). Visible effects produced on plants were observed regularly. Tomato plants accumulated high content of heavy metal where as tissue concentrations of root and shoot were 80.6 and 50.5 µg Ni/g dry weight respectively. The contaminated soil exhibited phototoxic effects on plants.

Keywords: Biochemical parameters; *Lycopersican esculantum* L.; Nickel; Phytoextraction; Zinc.

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INTRODUCTION

Ni is a constituent of urease, and small quantities of Ni (0.01 to 5 g/g dry weight) are essential for some plant species. On the other hand, Ni is not as important for plant metabolism as Zn. High Ni concentrations may become toxic to plant (Takishima *et al*., 1988). Checkai *et al*. (1986) reported that Ni-deficient tomato plants (*Lycopersicum esculentum* L.) developed chlorosis in the newest leaves and, ultimately, necrosis of the meristem. The earliest report of a growth responses to in addition under controlled experimental conditions (Brown *et al*., 1987b) indicate that Ni deficiency has a wide range of effects on plant growth and metabolism (Marschner, 2000; Chen *et al*., 2009; Yusuf *et al*., 2011). In the literature, indicates that Ni may also have essential functions in grain maturation and plant senescence (Brown *et al*., 1987b). The bioavailability of Zn in soil solution increases at low pH, while organic ligands and hardness cations such as Ca^{2+} decrease Zn availability (Pedler *et al*., 2004). Zn is an essential micronutrient involved in a wide variety of physiological processes (Cakmak, 2000; Reeves and Baker, 2000; Doncheva *et al*., 2001; Stoyanova and Doncheva, 2002; Di Baccio *et al*., 2005; Broadley *et al.,* 2007), yet at concentrations above 0.2 mg/g dry matter the potential phytotoxicity at leaf tissue develops (Ali *et al*., 2000; Bonnet *et al*., 2000). With phytotoxicity rising up reduced yields and stunted growth overcame (Marschner, 1995; Broadley *et al*., 2007). Zn uptake is an active or passive process (Brennan, 2005). Bowen (1969) also demonstrated that Zn absorption in the leaves of sugarcane is strongly depressed due to the inhibition of oxidative phosphorylation, whereas Bowen *et al*. (1974) implied that low temperatures inhibit the absorption of Zn in the roots of Pinus radiate. Zinc play an important role in completion the life cycle of plants and also a key role in nitrogen metabolism, photosynthesis and auxin synthesis (Cakmak, 2000; Vaillant *et al*., 2005)

and involved in diverse metabolic activities, influences the activity of hydrogenase and carbonic anhydrase, synthesis of cytocrome and the stabilization of ribosomal function (Tisdale *et al*., 1984). The integrity of cellular membranes also requires Zn to preserve the structural orientation of macromolecules and keep ion transport system (Dang *et al*., 2010). High levels of chelating agents (e.g. EDTA), particularly when used together with levels of FE (Brown *et al*., 1987b). Successful phytoremediation requires plants with high metal uptake capacity and high biomass production. Since most known hyperaccumulators have a low annual biomass production, considerable research is currently investigating methods that enhance the availability of heavy metals in soils and increase phytoextraction efficiency of potential accumulators (Baker *et al*., 1988). Chelating agents, such as LMWOAs and synthetic chelators, are the most common amendments utilized in chemically assisted phytoextraction of metals from soils (Nascimento *et al*., 2006; Wasay *et al*., 1998; Quartacci *et al*., 2005). Chelates make metal cations being gradually released to a medium solution or absorbed by plants in complexed forms (Wreesmann, 1996). Some differences in yields of this crop were found between the application of Zn chelated and mineral forms (Kozik *et al*., 2009).

EXPERIMENTAL

Seeds of tomato (*Lycopersicum esculentum* L.) were raised in soil filled in polythene tub amended with Ni (0.5 ppm) and two doses of Zn (5 and 10 ppm) to study the interactive effects of Ni and Zn, singly and in combination, on growth, tissue concentration of Ni and Zn and some biochemical constituents. Hoagland nutrient solution (Hoagland and Arnon, 1950), which served as control, or with nutrient solutions containing Ni (0.5 ppm) and two doses of Zn (5 and 10 ppm) which served as treatment solutions were applied in the experiment. Biochemical constituents (pigments content, catalase and peroxidase activity) were determined at day 70 of the sowing, when visible symptoms appeared prominently. Pigment content was estimated by the method by Lichtenthaler and Welburn (1983). Chlorophyll concentrations were expressed as mg g^{-1} fresh weight. Protein content was estimated by the method by Lowry *et al*. (1951). Proline content was estimated colorimetrically by the method of Bates *et al.* (1973). Sugar content was determined by the method of Dubois *et al.* (1956). Catalase activity was measured according to the modified method of Bisht (1976). Plant growth (dry matter yield) was observed at maturity. Data presented in the table are mean values of replications, all data were tested statistically for SE (n=3) and LSD values (Panse and Sukhtme, 1961).

RESULTS AND DISCUSSION

Tomato grown in native soil showed very low Ni and Zn in their tissues. Tissue concentration of Zn increased with increase in Zn dose in soil (Treatment IV > III). Maximum tissue concentration of Ni (80.6 µg/g dry weight in root and 50.5 µg/g dry weights in shoot) were observed at Treatment VI (Table 3). Zn promoted uptake and translocation of Ni in tomato, increased dose of Zn amendment in soil (10 ppm) was found to promote Ni content in tomato. Plants grown at native soil (I) and soil amended with Ni (0.5 ppm) and Zn (10 ppm) (VI) exhibited some visible symptoms, such as stunted plant growth, inter-venial chlorosis on young leaves, and reduced size of leaf lamina and yellowing of older leaves. The severity of these symptoms was increased with increase in growth days. Symptoms developed on tomato at the treatment VI were stunted plant growth, chlorosis in young leaves, some young leaves showed venial chlorosis and some young leaves showed tip burning, observed at day 60 of the treatment. At later stage of the growth, these symptoms also appeared in plants grown at treatment II, but, severity was less. However, no significant visible effects were observed at Zn 5 ppm concentration. Treatment at II, III, IV and V promoted plant growth, while treatment at VI resulted inhibitory effect on tomato growth with respect to shoot length and plant biomass (fresh and dry weight). Maximum effects were observed at treatment V (soil amended with 0.5

ppm Ni and Zn - in combination). This was slightly more than the treatment III (soil amended with 5 ppm Zn only). Tissue concentration of Zn (39.4% in root and 30.6% in shoot) and Ni (46.1% in root and 40.4% in shoot) was observed in experiment V. Increase in growth was found in the order V>III>IV>II>VI. The soil which was deficient in Zn (Table 1) amended with 5 ppm Zn (as $ZnSO_4$) showed more promontory growth as compared to soil amended with 10 ppm Zn and Ni. Nickel (0.5 ppm) amended soil also promoted shoot length and biomass of tomato, but effectiveness was less than the Zn amended soil over control. Nickel 0.5 ppm amendment in soil with 10 ppm Zn suppressed shoot length and biomass of tomato, tissue concentrations were 80.6 Ni µg/g, 50.5 Ni µg/g and 23 Zn µg/g, 40 Zn µg/g dry weight in roots and shoots respectively (Table 3).

Table 1: Interactive effects of Ni and Zn amendment in soil, singly and in combination, on growth of tomato (*Lycopersicum esculentum* L.) at day 60

Parameters	Ni and Zn amendments in soil					
	I	II	III	IV	V	VI
Shoot length (cm)	23.0 ±1.9 (0.0)	25.0 ±1.7 (+8.6)	25.9 ±1.2 (+12.6)	28.8 ±8.6* (+25.2)	29.0 ±2.1 (+26.0)	20.2 ±3.7 (+12.1)
Fresh weight/plant (g)	6.7 ±0.6 (0.0)	7.4 ±1.5 (+10.4)	9.6 ±4.2 (+43.3)	8.5 ±5.9* (+26.8)	9.8 ±2.5** (+46.3)	6.0 ±0.5 (-10.4)
Dry weight/ plant (g)	1.3 ±0.01 (0.0)	1.5 ±0.3 (+12.3)	2.0 ±0.6 (+54.6)	1.9 ±1.7 (+45.3)	2.1 ±1.3* (+61.5)	1.1 ±0.04 (+15.3)

Soil amended with: I- zero Ni and Zn (Control); II- Ni (0.5ppm); III- Zn (5ppm); IV- Zn (10ppm); V- Ni (0.5ppm) + Zn (5ppm); VI- Ni (0.5ppm) +Zn (10ppm); DAS- days after sowing. ± - S.E. value (n=3); *- value significant at P<0.05 and **- value significant at P<0.01 levels.

Table 2: Interactive effects of Nickel and Zinc amendment in soil, singly and in combination, on some biochemical constituents in tomato (*Lycopersicum esculentum* L.) observed at day 60

Parameters	Ni and Zn amendments in soil						LSD P=0.05
	I	II	III	IV	V	VI	
Chlorophyll a (mg/g fresh weight)	1.1 (0.0)	1.2 (+9.0)	1.4 (+27.7)	1.6 (+45.4)	1.7 (+54.5)	0.9 (-18.1)	0.3
Chlorophyll b (mg/g fresh weight)	0.2 (0.0)	0.2 (0.0)	0.3 (+50.0)	0.4 (+100.0)	0.4 (+100.0)	0.3 (+50.0)	0.1
Total chlorophyll (mg/g fresh weight)	1.3 (0.0)	1.4 (+7.6)	1.7 (+30.7)	2.0 (+53.8)	2.1 (+76.9)	1.3 (0.0)	0.3
Carotenoids (mg/g fresh weight)	0.8 (0.0)	0.8 (0.0)	1.0 (+25.0)	1.0 (+25.0)	1.1 (+37.5)	0.7 (-12.5)	0.2
Catalase (ml H_2O_2 hydrolysed/ mg fresh weight)	172.0 (0.0)	316.0 (+141.8)	228.0 (+32.5)	284.0 (+65.1)	248.0 (+44.1)	280.0 (+62.7)	54.3
Protein (mg/g fresh weight)	41.9 (0.0)	42.5 (+1.43)	51.4 (+22.6)	60.9 (+45.3)	65.7 (+56.8)	23.7 (-43.4)	13.3
Sugar (µg/g fresh weight)	10.9 (0.0)	11.4 (+20)	15.3 (+57.8)	19.5 (+105.2)	20.0 (+110.5)	10.0 (+5.2)	4.9
Proline (µg/g fresh weight)	1.2 (0.0)	1.8 (+20.0)	0.3 (-80.0)	0.3 (-80.0)	0.2 (-86.6)	1.9 (+13.3)	0.8

Treatment I- soil without Ni and Zn amendment; II-Ni (0.5ppm); III-Zn (5ppm); IV Zn (10ppm); V-Ni (0.5ppm) + Zn (5ppm) and VI- Ni (0.5ppm) + Zn (10ppm); parenthesis indicate percentage increase (+) or decrease (-) over control.

Table 3: Interactive effects of nickel and zinc amendment in soil, singly and in combination, on tissue concentration of tomato, observed at day 60 of the sowing

Plant parts	Tissue concentration (µg/g dry weight)						
		I	II	III	IV	V	VI
Shoot	Ni	ND	44.8	ND	11.5	40.4	50.5
	Zn	6.5	10.5	36.5	40.3	30.6	40.1
Root	Ni	ND	24.4	ND	23.9	46.1	80.6

	Zn	9.8	17.0	62.5	49.0	39.4	43.0

Treatment I- soil with Ni and Zn amendment; II-Ni (0.5ppm); III-Zn (5ppm); IV-Zn (10ppm); V-Ni (0.5ppm) + Zn (5ppm) and VI- Ni (0.5ppm) + Zn (10ppm). ND- not detectable. ± - S.E. value (n=3); *- value significant at P<0.05 and **- value significant at P<0.01 levels. Parenthesis indicate percentage increase (+) or decrease (-) over control.

Zinc amendments (5 and 10 ppm, treatments III and IV) increased pigment content in tomato leaves. There was no significant effect of Ni (0.5 ppm, treatment II) on pigment contents (chlorophyll a, b, carotenoids and total chlorophyll). Maximum promotory effect on pigment contents was observed at soil amended with Ni and Zn at 0.5 ppm and 5 ppm, treatment V while treatment VI Ni (0.5 ppm) and Zn (10 ppm) decreased pigment contents. Protein content was increased in tomato grown at soil amended with Ni and Zn either singly or in combination only at (0.5 and 5 ppm). Protein content decreased at soil treatment VI amended with Ni and Zn at 0.5 and 10 ppm however at this level concentration of Ni and Zn in shoot was 50.5 and 40 µg/g dry weights respectively. Maximum increase in protein content by 56.8% was observed in V treatment but concentration of Ni and Zn in shoot was 40.4 and 30.6 µg/g dry weight respectively. Sugar content followed similar trend as shown in protein and pigment contents. Maximum increase in sugar content in tomato leaves was observed in treatment V (Ni 0.5 ppm; Zn 5 ppm) whereas decreased sugar content by 105% was found in treatment VI (Ni 0.5 ppm; Zn 10 ppm) amended soil. Proline content was found to be increased in the treatments II and VI but no significant increase recorded in treatments III – V. However elevated level of proline content was found in tomato leaves grown at native soil, deficient in Zn. Catalase activity was found increased in plants grown at soil amended with Ni and Zn. Comparatively, elevated level of catalase activity was observed at soil amended with Ni (0.5 ppm), Zn (10 ppm) and Ni (0.5 ppm) plus Zn (10 ppm). Maximum increase in catalase activity was found at Ni (0.5 ppm) amendment in soil. Plants grown at soil amended with Ni (0.5 ppm), singly, showed tissue concentration 24.4 µg Ni/g dry weight in root and 44.8 µg Ni g^{-1} dry weight in shoot. Uptake and translocation of Zn were increased with increase in Zn concentration in soil. The finding was in accord with (Pandey and Gautam, 2009). Zinc amendment (5 and 10 ppm) with Ni (0.5 ppm) promoted Ni content in tomato. Barman *et al*. (2001) reported antagonistic and synergetic effects in between the metals. Plants grown at soil amendment with Zn (10 ppm) and Ni (0.5 ppm) exhibited visible symptoms of toxicity such as reduced size of leaf lamina, yellowing of older and middle leaves and chlorosis in young of older leaves. These symptoms resembled with earlier report of Singh and Pandey (2011) developed with response to excess Ni in growth medium. Maximum shoot length and biomass of plants were observed at soil amended with Zn (5 ppm) with Ni (0.5 ppm), could be due to the beneficial role of Ni and Zn in plants (Gerendas *et al*., 1999; Alloway, 2004). Plants grown at soil amended with Ni (0.5 ppm) and Zn (10 ppm) was showed poor growth and high Ni content in tissue. The reduction in growth could be due to the high uptake of Ni in presences of Zn through antagonistic effects (Barman *et al*., 2001; Sharma, 2006).

Growth and biochemical parameters (pigments, protein, sugar, and proline contents and activity of catalase) increased in tomato were observed at soil amendment with Ni (0.5 ppm) and (5 and 10 ppm). But, higher dose amendment of Zn (10 ppm) declined above mentioned parameters in tomato. These results could be attributed due to uptake and translocation of high Ni content in plant (Gajewaska *et al*., 2006). The accumulation of Ni in higher concentration in tomato could be due to the uptake of Ni facilitated by Zn in soil (Kabata-Pendias and Pendias, 1992). The uptake and translocation of one metal plants resulted due to the interactive effects of other metal have been reported earlier by several workers (Agarwala *et al*., 1977; Pandey and Sharma, 2002). Production of reactive oxygen species (ROS) due to Ni-stresses stimulates production of H_2O_2, which causes lipid peroxidation to damage the cell membrane.

Catalase activity converts H_2O_2 to H_2O and O_2 which is harmless to plant cell. Under stress conditions, including excess Ni exposure, an imbalance between generation and removal of ROS can arise in plant tissues (Grataq *et al*., 2005). This may subsequently lead to oxidative injuries of important macromolecules like lipids, proteins and nucleic acids (Kehrer, 2002). Increase in biomolecules and enzyme activity could be attributed due to role of Zn against ROS (Cakmake and Marschner, 1993), increased carbonic anhydrase and nitrate reductase for hypertolerance (Ernst *et al*., 1992), Zn exclusion and compartmentalization by mycorrhiza which altered root-to-shoot translocation (Hall, 2002; Ernst *et al*., 1992) and interactive effects of soil properties (Barman *et al*., 2001).

CONCLUSION

Zinc (5ppm) uptake and translocation of Ni in tomato, wild at evaluated it showed toxic effects appeared on leaves and stem of plant. Ni (0.5ppm) amendment in soil with 10 ppm Zn suppressed shoot length, biomass and dry weight of tomato. Zn declined antioxidative defense with respect to (protein, sugar, proline) content, catalase activity and produce visible symptoms of toxicity.

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