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Acute Toxicity of Nickel on *Ipomoea aquatica* Forsk.

KEYWORDS: *Ipomoea aquatica*, Nickel, Growth, Necrosis.

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ABSTRACT *Ipomoea aquatica plants were exposed to different concentrations of Ni (0.22, 2.23, 4.02, 7.14, 12.51 and 22.33 mg l-1) for 15 days. Significantly less increase in shoot height and number of new nodes and leaves produced, were observed at 12.51 and 22.33 mg l-1 of Ni from day 6 of the exposure when compared to those in the control. Appearance of necrosis leading to stem disintegration was also observed at the higher concentrations of 12.51 and22.33 mg l-1, resulting in the reduction of shoot height and number of new nodes in these exposure groups. Necrosis was observed to eventually lead to the death of the plants.*

Introduction

Nickel (Ni) occurs naturally in igneous rocks as a free metal or along with iron (Chen etal., 2009). Some of the common anthropogenic sources of Ni are kitchen appliances, surgical instruments, steel alloys and automobile batteries (Tariq et al., 2006). Ni is essential for plants at low concentrations (Chen et al., 2009), but at higher concentrations it is toxic and causes chlorosis, necrosis, growth inhibition as well as decreased water and nutrient uptake (Madhava Rao and Sresty, 2000; Gajewska et al., 2006). It causes allergic reactions and dermatitis in humans, and also acts as a carcinogenic agent (Wilhelm et al., 2007, Bocca et al., 2007; Mishra et al., 2010).

Ipomoea aquatica Forsk. (water spinach) is a perennial herb cultivated and distributed in Southeast Asia, India and Southern China (Umar et al., 2007;Göthberg et al., 2002).Young stem and leaves having nutritional benefits are used as a leafy vegetable and also as fodder. This plant possesses neutraceutical as well as therapeutic properties (Prasad et al., 2008; Sivaraman and Muralidaran, 2010). It flourishes in nutrient-enriched water, wetland or cultivated areas that often serve as recipients of heavy metals including Ni due to fertilizer and pesticide application in the crop field and tea garden. Consumption of nickel-contaminated plants can be harmful to animals and humans alike. The present experiment was undertaken to delineate the acutely toxic concentrations of Ni in *I. aquatica* and to assess the effects on growth and development of the plant in a 15 day experiment.

Materials and Methods

Culture of the plants and experimental set up were done by adopting the method of Göthberg et al.(2004) using 50 % Hoagland nutrient solution. Plants with 20-25cm long shoots were carefully wiped with clean tissue paper and their length, number of nodes and number of leaves were measured. They were then placed in graded Ni concentrations of 0.22, 2.23, 4.02, 7.14, 12.51 and 22.33 mg l^{-1} for a 15 day period. All test solutions were prepared in 50% Hoagland nutrient media. Control plants were put in 50 % Hoagland media without added Ni. Six replicates were taken for each treatment. Control and exposed plants were maintained in test chambers provided with a photon flux density of 100-120 µmol m^{-2} s^{-1} with a temperature range of 23.4±2.8°C around the culture vessels following a photoperiod of 12 h/day.

Statistical analysis

Statistical analysis comprised one-way analysis of variance (ANOVA) with Tukey test at $P \leq 0.05$ for multiple comparisons using SPSS 20 for Windows.

Results and Discussion

In this 15 day experiment, both shoot height (SH) and number of new nodes (NN) of *I. aquatica* continued to increase in control plants till the end of the experiment (Table 1). SH in plants exposed to 0.22 – 4.02 mg l^{-1} Ni also increased till the end of the experiment at rates lower than but not significantly different from that in the control. SH increase in plants exposed to 7.14 mg l^{-1} Ni was significantly lower than that in the control on 15 day. In plants exposed to 12.51 and 22.33 mg l^{-1}, SH was significantly lower than that in the control from 6 day onwards (Table 1). NN was significantly lower in plants exposed to 12.51 and 22.33 mg l^{-1} than that in the control from 3 day onwards. NN was also significantly different from that in the control in plants exposed to 7.14 mg l^{-1} on 9 and 15 day. Similar results were obtained when SH was significantly reduced in *Salicornia brachiata* at Ni concentrations of 50-400µM (Sharma et al., 2011). Plants exposed to 2.23 mg l^{-1} also had significantly less NN than that in the control during 3-9 day, but they subsequently recovered from 12 day onwards. At the end of 15 day, SH and NN in plants exposed to 12.51 and 22.33mg l^{-1} Ni were less than that at the beginning of the experiment because of collapse of stem tissue due to necrosis (Table 1). The appearance of necrosis in stem tissue at12.51 and 22.33 mg l^{-1} might have occurred because of stunted root development due to Ni toxicity which in turn made the metal easily accessible to the stem tissues. Studies on Ni also showed that it was mainly accumulated in the roots of most plants as it could cross the endodermal barrier and accumulate in the pericycle (Seregin and Kozhevnikova, 2006) thus hampering lateral root growth. Khellaf et al.(2010) found that *Lemna gibba* exposed to 2 mg l^{-1} Ni showed chlorosis and frond disconnection.The other toxicological endpoints used in the present study were number of new leaves (NL) and number of dead plants (DP). NL kept on appearing in the control as well as in plants exposed to Ni up to 7.14 mg l^{-1} throughout the experiment with no plant dying during this period. New leaves also appeared in plants exposed to 12.51 and 22.33 mg l^{-1} Ni till the end of the experiment, although their numbers were significantly less than that in the control from 6 day onwards. Statistically insignificant DP was also observed in 12.51 mg l^{-1} group from 9 day, while in 22.33 mg l^{-1} group, significant DP occurred on 15 day (Table 2).

Conclusions

Nickel is an essential element for most plants at extremely low concentrations, but turns toxic at higher concentrations the threshold of which may vary among different plant species. The present study indicates that *I. aquatica* was moderately tolerant to Ni toxicity, showing toxic effects at concentrations exceeding 10 mg l^{-1} during a 15 day acute exposure study. Thus Ni toxicity could be a limiting factor for the growth and propagation of this food and fodder species in areas that are affected by even a moderate load of Ni.

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Table 1: Change in shoot height (SH) and number of new nodes (NN) in control and Ni exposed *I. aquatica* during a 15 day experiment.

Ni concentration (mg l^{-1})		Change in SH (cm) and NN (Mean±S.E).				
		3d	6d	9d	12d	15d
Control	SH	3.5±0.8	12.7±2	19.1±1.5	28.2±2.4	31.2±2.2
	NN	2.2±0.7	5±1	6.3±1	7.3±1	8.2±1
0.22	SH	3.8±0.7	11.8±2.8	18.3±4.7	21.2±5.8	22.6±5.7
	NN	1±0.4	2.8±0.8	3.8±1	4.3±1.2	4.3±1.2
2.23	SH	2.1±0.6	8.5±1.3	13.6±1.9	21.2±5.8	22.6±5.7
	NN	0.3±0.2*	1.7±0.4*	2.7±0.7*	3.7±0.8	4.2±1
4.02	SH	3.7±1.1	9.1±2.8	11.9±3.1	16.4±3.2	18.8±2.5
	NN	1.2±0.3	3.2±0.8	4±0.5	4.7±0.4	5.5±0.4
7.14	SH	4.5±0.8	8.2±0.7	11.9±1.6	14.4±2.6	15.3±2.5*
	NN	1.2±0.3	2.8±0.2	3.2±0.3*	3.5±0.4	3.5±0.4*
12.51	SH	0.5±0.3	1.1±0.4*	2.1±0.8*	3±1.4*	-0.1±0.9*
	NN	0.2±0.2*	0.3±0.2*	0.5±0.3*	0.7±0.5*	-1.3±1.2*
22.33	SH	1.3±0.5	2±0.8*	3.4±1.2*	2.1±1.6*	-1.3±1.1*
	NN	0.2±0.2*	0.5±0.3*	1±0.4*	-0.8±1.4*	-2.7±1*

*** Significant difference from corresponding value in control at P<0.05, n=6. d-day**

Table 2: Number of new leaves (NL) and dead plants (DP) in control and Ni exposed *I.aquatica* during a 15 day experiment.

Ni concentration (mg l^{-1})		Appearance of NL and DP (Mean±S.E.)				
		3d	6d	9d	12d	15d
Control	NL	1.2±0.3	3±0.4	4.2±0.3	4.8±0.5	5.7±0.6
	DP	0±0	0±0	0±0	0±0	0±0
0.22	NL	1.5±0.2	3±0.4	3.5±0.6	4±0.7	4.5±0.8
	DP	0±0	0±0	0±0	0±0	0±0
2.23	NL	0.7±0.2	1.8±0.4	2.5±0.6	3.7±0.4	4.3±0.5
	DP	0±0	0±0	0±0	0±0	0±0
4.02	NL	0.8±0.4	2.2±0.6	2.8±0.5	3.5±0.3	4.7±0.5
	DP	0±0	0±0	0±0	0±0	0±0
7.14	NL	1.2±0.3	3±0.3	3.3±0.3	3.7±0.3	4.2±0.4
	DP	0±0	0±0	0±0	0±0	0±0
12.51	NL	0.3±0.2	0.8±0.3*	1.2±0.4*	1.2±0.4*	1.3±0.5*
	DP	0±0	0±0	0.2±0.2	0.2±0.2	0.2±0.2
22.33	NL	0.8±0.4	1±0.4*	1.3±0.3*	1.5±0.4*	1.5±0.4*
	DP	0±0	0±0	0±0	0±0	0.5±0.2*

*** Significant difference from corresponding value in control at P<0.05, n=6. d-day**

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