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IMPACT OF BIOACCUMULATION OF NICKEL ON GROWTH, SEED YIELD AND MINERAL UPTAKE OF CHICKPEA (*CICER ARIETINUM* L.) VARIETIES

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Abstract

A pot experiment was conducted to investigate the bioaccumulation of nickel in chickpea *Cicer arietinum L.* and its impact on growth, seed yield and mineral contents. $NiCl_2$ as nickel treatment was applied in solution form (25 mg L^{-1}, 50 mg L^{-1}, 100 mg L^{-1} and 150 mg L^{-1}) to the soil. A significant decreasing trend in shoot length, number of branches, number of leaves and biomass yield was observed for all treatments as compared to control one. Accumulation of nickel in plant shoots was gradually increased with increasing concentrations of nickel application. Contents of Na^+, K^+ and Ca^{2+} in shoots were significantly reduced for all treatments which might be due to some interaction of nickel with the uptake of mineral nutrients by the roots and their distribution through the shoots.

Key words: Nickel, Growth, Yield, Nutrient contens, Bioaccumulation.

Introduction

Heavy metals are found to be among the most important environmental pollutants (Tangahu *et al*., 2011) which are causing major health problems due to their bioaccumulation through food chain (Gill, 2014). These metals are very dangerous as they cannot be degraded biologically as well chemically (Tangahu *et al*., 2011). Plants show reduction in growth, biomass production and changes in metabolic reactions when grown in metal polluted soils (Nagajyothi *et al*., 2010).

Among heavy metals, nickel holds an important place due to its specific physical and chemical characteristics (Seregin & Kozevnikova, 2006). The uptake of nickel by the plants depends upon its concentration in the soil (Chen *et al*., 2009) which mainly carried out through the root system (Sharma and Dhiman, 2013) and then transported to the aerial parts of the plants (Chen *et al*., 2009).

Nickel is an essential as well as a toxic element for the plants (Yusuf *et al*., 2011). Being a component of many enzymes (Chen *et al*., 2009), it plays a vital role in cellular metabolic processes (Yusuf *et al*., 2011) and is required in very small quantity for normal growth and development of the plants (Sareekanth *et al*., 2013). However, nickel has phytotoxic effects at higher concentrations (Harasim & Filipeck, 2015; Bhalerao *et al.,* 2015) which include inhibition of enzymes, photosynthesis and water relations (Hussain *et al*., 2013).

Bioaccumulation of nickel inhibits seed germination and seedling growth (Poozesh & Tagharobian, 2014; Ahmad *et al.,* 2009), root and shoot length (Kaveriammal & Subramani, 2013) and biomass production (Latif, 2010). It also interacts with plant mineral nutrition (Sharma & Dhiman, 2013; Sareekanth *et al.,* 2013) causing inhibition of mineral uptake which results in reduction of nutrient contents and even their deficiency (Chen *et al.,* 2009).

Chickpea (*Cicer arietinum* L.) is an important seed crop belonging to family Fabaceae and is cultivated in nearly all parts of the world (Al-Snafi, 2016). It plays an important role to improve soil fertility (Aslam *et al.,* 2003) by increasing nitrogen contents through atmospheric nitrogen fixation (Turpin *et al*., 2002) and by increasing organic carbon contents (Aslam *et al.,* 2003) which may be helpful to increase the yield of following crops such as wheat (Aslam *et al.,* 2003; Fatima *et al.,* 2008).

Keeping in view the role of nickel as a toxic element and ecological importance of chickpea, the present study has been conducted to evaluate the phytotoxic impact of nickel on chickpea varieties.

Materials and Methods

Plant material and treatment application: The present experiment was conducted in Botanical Garden of University of Agriculture, Faisalabad, Pakistan. Sixty earthen pots were used allocating six pots for each treatment. Homogeneously mixed and sun dried soil was used for each pot. Seeds of two chickpea varieties named Punjab 2000 and Bittal 98 were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad. Eight seeds of each chickpea variety were sown in each pot. After germination, the plants were thinned to maintain five seedlings in each pot. The plants were irrigated with tap water at alternate days. Nickel as $NiCl_2$ was applied to soil in solution form when plants were 30 days old. There were five treatments viz. T_0, T_1, T_2, T_3, T_4 @ 0 mg L^{-1} (Control), 25 mg L^{-1}, 50 mg L^{-1}, 100 mg L^{-1} and 150 mg L^{-1} respectively. Each treatment was replicated six times. The experiment was laid down in Completely Randomized Design (CRD) with two factor-factorial arrangement.

At maturity of the crop, plant height, number of branches, number of pods per plant, seed yield per plant and 100-seed weight were determined.

Ion analysis: Three plants from each treatment were randomly selected and used for ion analysis. For this purpose, dried plant material (stem) was chopped into small pieces and 0.1 g of that material was digested with sulfuric acid and hydrogen peroxide (Wolf, 1982).

Determination of Ca^{2+}, K^+ and Na^+ contents (mg g^{-1} dry weight): Mineral ions (Ca^{2+}, K^+ and Na^+) were tested by using flame photometer (Jenway, PEP-7). A

graded series of standards (10 ppm, 20 ppm, 30 ppm) of $Ca^{2+,}$ K^+ and Na^+ were run and standard curves were drawn. The values of Ca^{2+}, K^+ and Na^+ from flame photometer were compared to standard curves and final amounts were calculated.

Determination of Ni^{2+} contents (mg g^{-1} dry weight): Concentration of Ni^{2+} was determined with an atomic absorption spectrophotometer (Varian AA 10/20). A graded series of standards (10 ppm, 20 ppm, 30 ppm) were run for the construction of standard curves. Final values of Ni^{2+} were calculated by comparing with standard curves.

Statistical analysis

The data were analyzed statistically by applying ANOVA. To find out significant difference among treatment means, Duncan's Multiple Range Test (Steel & Torrie, 1986) was also applied.

Results

Data regarding ANOVA for different parameters is presented in Table 1. Highly significant results have been obtained for varieties and treatments while interaction between varieties and treatments showed non-significant results.

Growth and seed yield: Table 2 shows comparison among treatment means for growth parameters and seed yield of variety Punjab 2000 (V_1) and Bittal 98 (V_2) respectively. Plant height gradually decreased with increasing levels of nickel application. However, there was a little decrease at T_1 and T_2 but then was more pronounced at T_3 and T_4 as compared to control one. The maximum decrease of 21.92 % in variety Punjab 2000 and 20.31% in variety Bittal 98 over untreated control was observed at 150 mg L^{-1} of nickel treatment.

All treatments of nickel above 25 mg L^{-1} showed pronounced negative impact on number of branches. The most drastic effect was noted at 150 mg L^{-1} which showed maximum decrease (55.55 %) in V_1 and (63.40%) in V_2 as compared to control one.

As regards number of pods per plant, no significant difference has been found at 50 mg L^{-1} and 100 mg L^{-1} concentrations of nickel. However, 150 mg L^{-1} concentration was found to be most effective having 51.75 % decrease for (V_1) and 53.09% for (V_2) over control.

Seed yield was maximum at T_1 whereas T_4 showed minimum seed yield in both varieties. T_2 and T_3 were found to be intermediate having a decrease of 12.88 % and 20.85 % respectively for (V_1) and 13.48% and 19.66% respectively for (V_2) as compared to T_0.

For100-seed weight, all the treatments varied significantly from one another. Nickel at 25 mg L^{-1} had comparatively less negative impact with a decrease of only 4.32 % followed by T_2, T_3 and T_4 for variety Punjab 2000 as compared to control. Variety Bittal 98 showed a similar trend having 3.42 % decrease at T_1 and 22.56 % at T_4.

Accumulation of Ni^{2+} and mineral contents: Accumulation of Ni^{2+} was found to be increased with increasing levels of nickel application whereas concentration of Ca^{2+}, K^+ and Na^+ decreased gradually with increasing accumulation of nickel in plant shoots. Nickel at concentration of 25 mg L^{-1} and 50 mg L^{-1} had less negative impact on mineral contents whereas 100 mg L^{-1} and 150 mg L^{-1} concentrations of nickel were more effective in reducing mineral contents in both varieties (Table 3).

Table 1. ANOVA for effect of nickel on various measured parameters of two chickpea varieties.

Source of variation	df	Plant height (cm)	Number of branches	Number of pods per plant	Seed yield per plant (g)	100-seed weight (g)	Ni^{2+} (mg g^{-1})	K^+ (mg g^{-1})	Ca^{2+} (mg g^{-1})	Na^+ (mg g^{-1})
Varieties (V)	1	20.833 **	4.800**	1.633ns	0.107**	6.440**	0.001**	0.411ns	1.408**	3.008**
Treatments (T)	4	56.065 **	27.700**	24.917**	0.196**	33.321**	0.034**	32.704**	18.492**	11.737**
Interaction (V x T)	4	0.208ns	0.633ns	0.217ns	0.001ns	0.075ns	0.000**	1.094ns	0.117ns	0.029ns
Error	20	0.113	0.553	0.567	0.001	0.048	0.000	0.525	0.150	0.100

** = Highly significant; ns= Non significant

Table 2. Effect of different concentrations of nickel on various growth and yield parameters of chickpea varieties.

Varieties	Treatments	Plant Height (cm)	Number of branches	Number of pods per plant	Seed yield per plant (g)	100-seed weight (g)
Punjab 2000 (V_1)	T_0	36.76a	9.00a	9.66a	1.63a	26.13a
	T_1	34.66b	7.33b	8.33b	1.54b	25.00b
	T_2	33.46c	6.33c	7.33c	1.42c	23.76c
	T_3	31.20d	5.33d	6.33c	1.29d	22.73d
	T_4	28.70e	4.00e	4.66d	1.20e	20.10e
Bittal 98 (V_2)	T_0	37.90a	10.00a	10.66a	1.78a	27.16a
	T_1	36.56b	8.66b	9.00b	1.64b	26.23b
	T_2	35.16c	7.33c	7.33c	1.54c	24.43c
	T_3	33.30d	6.33d	6.66c	1.43d	23.50d
	T_4	30.20e	3.66e	5.00d	1.29e	21.03e

Values in columns followed by same letters indicate non-significant difference according to DMR Test

Table 3. Bioaccumulation of nickel and mineral contents in shoots of chickpea varieties (mg g^{-1} dry weight).

Varieties	Treatments	Ni^{2+} (mg g^{-1})	K^{+} (mg g^{-1})	Ca^{2+} (mg g^{-1})	Na^{+} (mg g^{-1})
Punjab 2000 (V_1)	T_0	0.41a	19.99a	5.66a	6.32a
	T_1	0.49b	17.65b	4.83b	5.49b
	T_2	0.52c	15.49c	3.66c	4.32c
	T_3	0.55d	15.15d	2.50d	3.65d
	T_4	0.60e	13.99e	1.66e	2.82e
Bittal 98 (V_2)	T_0	0.40a	19.66a	6.33a	6.82a
	T_1	0.46b	17.82b	5.50b	6.15b
	T_2	0.51c	17.15c	4.00c	5.15c
	T_3	0.55d	15.32d	3.00d	4.32d
	T_4	0.60e	13.49e	1.66e	3.33e

Values in columns followed by same letters indicate non-significant difference according to DMR Test

Discussion

Inhibition of plant growth is the most obvious impact of nickel toxicity. In both varieties of chickpea, shoot length and number of branches were decreased with increased contents of nickel in soil medium. This reduction in growth might be due to inhibition of cell division (Yusuf *et al.,* 2011; Sharma & Dhiman, 2013) and cell elongation (Sharma & Dhiman, 2013). The reduction in plant growth has been reported in number of previous reports (L'Huillier *et al.,* 1996; Gautum & Panday, 2008; Siddiqui *et al.,* 2011). This includes inhibition of seed germination (Khan & Khan, 2010; Ishtiaq & Mahmood, 2011) by effecting the activities of amylases and proteases enzymes thereby inhibiting the digestion and mobilization of stored food in germinating seeds (Ahmad & Ashraf, 2011), reducing shoot length (Rubio *et al.,* 1994; Gajewska *et al.,* 2006; Khan & Khan 2010; Hussain *et al.,* 2013; Ali *et al.,* 2015) and effecting branching system (Ahmad & Ashraf, 2011).

Excess amounts of nickel had negative impact on development of pods and seeds (Chen *et al.,* 2009). Nickel exposure of plants led to decrease in number of pods per plant (Malan & Farrant, 1998; Khan & Khan, 2010; Yusuf *et al.,* 2012), seed yield per plant and 100-seed weight (Chen *et al.,* 2009; Yusuf *et al.,* 2012). This reduction in yield might be due to reduced supply of nutrients to the reproductive parts of the plants (Chen *et al.,* 2009). In this experiment, exposure of chickpea to nickel resulted in decreased number of pods per plant, seed yield per plant and 100-seed weight in both varieties.

As plant height, number of branches, number of pods and seed yield are criteria of chickpea vigor (Islam *et al.,* 2008), this experiment clearly indicates the toxic effects of nickel on chickpea varieties.

Bioaccumulation of nickel in plants depends upon its availability in soil medium (Sharma & Dhiman, 2013). Shoot contents of nickel have been found to be increased with increasing levels of nickel application (Khan & Khan 2010; Ishtiaq & Mahmood 2011; Ali *et al.,* 2015) as nickel in divalent form is taken up by the plants very easily (Siedlecka, 1995) and can move readily through vascular tissues and transported from roots to shoots (Emamverdian *et al.,* 2015. The present study clearly showed a linear relationship between nickel application and its accumulation in the shoots.

One of the functional mechanisms of nickel stress is its interference with mineral uptake (Rubio *et al.,* 1994; Chen *et al.,* 2009) and displacement of other essential ions (Sharma & Dhiman, 2013). In present experiment, exposure of chickpea to varying levels of nickel resulted in decreased contents of K^{+}, Ca^{2+} and Na^{+} in the shoots. This disturbance in mineral uptake might be due to nickel induced inhibition of membrane bound enzymes such as ATP-ase (Gill, 2014) and changes in composition of plasma membrane lipids (Yusuf *et al.,* 2011) such as sterol and phospholipids (Seregin & kozhevnikova, 2006). Moreover, nickel competes with other essential metal ions during their absorption and utilization due to some similar characteristics (Chen *et al.,* 2009; Yusuf *et al.,* 2011; Sharma & Dhiman, 2013). Many earlier reports of decreased K^{+} contents (Rubio *et al.,* 1994; Ahmad *et al.,* 2009; Matraszek *et al.,* 2016), Ca^{2+} contents (Rubio *et al.,* 1994; El-Enany *et al.,* 2000; Matraszek *et al.,* 2016) and Na^{+} contents (Palacios *et al.,* 1998) in shoots in response to nickel toxicity confirrned the present results.

In conclusion, nickel has been found to be a toxic heavy metal causing reduction in growth and yield of chickpea. Mineral contents such as K^{+}, Ca^{2+} and Na^{+} also decreased with increasing levels of nickel treatment.

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