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Original Research Article

Effect of nickel toxicity on growth, photosynthetic pigments and dry matter yield of *Cicer arietinum* L. varieties

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Abstract

Effects of nickel toxicity on photosynthetic pigments and dry matter yield of *Cicer arietinum* L. (Chickpea) varieties were observed. Nickel as $NiCl_2$ was applied to the soil in solution form @ 0mgL^{-1}(Control), 25mg L^{-1}, 50mg L^{-1}, 100mg L^{-1} and 150mg L^{-1}. All the treatments were replicated six times. Experiment was laid down in Completely Randomized Design (CRD) with two factor-factorial arrangement. A significant decrease in growth, chlorophyll a, b, total chlorophyll and carotenoid contents was observed with increasing concentrations of nickel application. A similar decreasing trend was also noted for dry matter yield of the plants which may be attributed to decreased pigment contents and decreased photosynthetic activity. Chickpea was found to be an indicator of nickel toxicity showing its inhibitory effects on various growth and biochemical parameters.

Keywords: Nickel, Chlorophylls, Carotenoids, Dry Matter, Chickpea

Introduction

Bioaccumulation of heavy metals in the environment has become a danger for plants and other living organisms (Emamverdian et al., 2015). Nickel, a heavy metal is an essential plant nutrient (Harasim and Filipek 2015; Chen et al., 2009) and is needed in very low quantities for normal growth of plants (Chen et al., 2009). In legume plants, it is required by hydrogenase enzyme during nitrogen fixation (Gerendȧs et al., 1999; Seregin and Kozhevnikova, 2006), and is the component of urease enzyme which brings about hydrolysis of urea (Seregin and Kozhevnikova, 2006). Deficiency of nickel results in inhibited activity of urease which leads to accumulation of toxic levels of urea in shoots (Yusuf et al., 2011) causing necrosis of leaf tips or chlorosis of older leaves (Seregin and Kozhevnikova, 2006; Yusuf et al., 2011).

At higher levels, nickel is highly toxic and causes alterations in antioxidant enzymes (Dubey and Panday 2011; Bhalerao et al., 2015) and may form reactive oxygen species which can cause oxidative stress (Bhalerao et al., 2015).

Its toxicity adversely affects the growth of plants causing inhibition of shoot growth (Gajewska et al., 2006), and reduction of fresh biomass of root and shoot (Lu et al., 2010). Dry matter yield is also reduced due to the accumulation of higher concentrations of the nickel (Rathor et al., 2014).

Nickel at elevated levels causes reduction in photosynthetic activity (Hussain et al., 2013). It causes destruction of photosynthetic apparatus as it damages the chloroplast grana structure and reduces the size of grana (Chen et al., 2009). Chlorophyll a, chlorophyll b, and total chlorophyll contents decrease due to nickel toxicity (Singh, 2011; Lu et al., 2010) which results in chlorosis of leaves (Singh, 2011; Lu et al., 2014). Carotenoid contents also decrease due to nickel toxicity (Hassanpour and Rezayatmand, 2015; Lu et al., 2010; Singh et al., 2012).

Cicer arietinum L. commonly called chickpea is a legume crop of family Fabaceae. It is mostly used as seed food (Ibricki et al., 2003), as a rich source of proteins and other nutrients (Ibricki et al., 2003; Al-Snafi, 2016). Dried seeds contain 20% proteins, 61% carbohydrates and seed coat serves as the source of crude fiber. Seeds also contain certain minerals such as Ca, P and K alongwith vitamins A, B and C (Al-Snafi, 2016).

It has been reported that 10ppm concentration of nickel is damage threshold for chickpea and elevated levels resulted in reduced seed germination, growth, yield and pigment contents (Khan and Khan, 2010).

The experiment aims at providing some basic mechanisms regarding toxic effects of varying concentrations of nickel on plants.

Material and Methods

The present study was conducted in Botanical Garden of University of Agriculture, Faisalabad, Pakistan. Sixty earthen pots (30x30cm) were used allocating six pots for each treatment. 8kg homogeneously mixed and sun dried soil was used for each pot. Eight seeds of each variety of Cicer arietinum L. (Punjab 2000 and Bittal 98) were sown in each pot separately. 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 30 days after the germination of plants. There were five treatments viz., T_0, T_1, T_2, T_3 and 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. The experiment was laid down in Completely Randomized Design (CRD) with two factor-factorial arrangement.

Growth parameters were studied at three successive harvests at an interval of two weeks each starting from treatment application. At the time of sampling, three plants from each treatment were taken to determine mean values.

Growth analysis

Values of growth parameters were subjected to growth analysis using Radford's formulae (1967).

1. Relative growth rate (RGR) (g/g/day)

RGR= $(\log W_2 - \log W_1) / t$

Where

W_1 = Dry weight of preceding harvest

W_2 = Dry weight of following harvest

t = Time interval between two harvests

Using above formula, following were also calculated:

2. Relative increase in plant height
3. Relative increase in number of branches
4. Relative increase in number of leaves

These parameters were not subjected to statistical analysis.

The chlorophyll (chl. a, chl. b and total) and carotenoid contents were determined when plants were 87 days old. 0.5 g fresh leaves were chopped in small pieces and extracted with 10 ml of 80% acetone. The chlorophyll contents were determined by the method of Arnon, (1949) and carotenoid contents following Davies, (1979) by a spectrophotometer.

At maturity of the crop, dry biomass of the plants was determined. For this purpose, the sample plants were kept in an oven at 70^{o}C for 4 days and then mean values were calculated.

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

Results

Data for various growth parameters are presented in Fig.1, Fig.2, Fig.3 and Fig.4 which showed a gradual decrease with increasing levels of nickel application.

ANOVA for different parameters are presented in Table.1 while Table.2 and Table.3 showed comparison of means for variety Punjab 2000 (V_1) and variety Bittal 98 (V_2) respectively. For chlorophyll 'a', T_1 showed maximum contents of chl.'a' (0.615) and (0.628) for (V_1) and (V_2) respectively whereas T_4 showed the minimum (0.570) for (V_1) and (0.591) for (V_2). Chlorophyll 'b' contents revealed the gradual decrease of 2.49%, 4.53%, 6.90% and 9.17% in T_1, T_2, T_3 and T_4 respectively for variety Punjab 2000. Similar trend was noted for variety Bittal 98 showing a decrease of 1.84%, 3.68%, 6.09% and 7.24% for T_1, T_2, T_3 and T_4 respectively.

For total chlorophyll, there was a little decrease at T_1 and T_2 but then more pronounced at T_3 and T_4. The maximum decrease of 17.79% and 7.29% was observed for (V_1) and (V_2) respectively at 150 mg L^{-1} of nickel treatment. For carotenoids, (V_1) showed maximum decrease of 16.83% at T_4 while minimum of 6.57% was noted for T_1. T_2 and T_3 showed intermediate values. (V_2) also had maximum decrease of 12.80% at T_4 and minimum decrease of 3.20% at T_1.

Maximum dry matter yield was recorded for control which gradually decreased in all other treatments of nickel for both varieties.

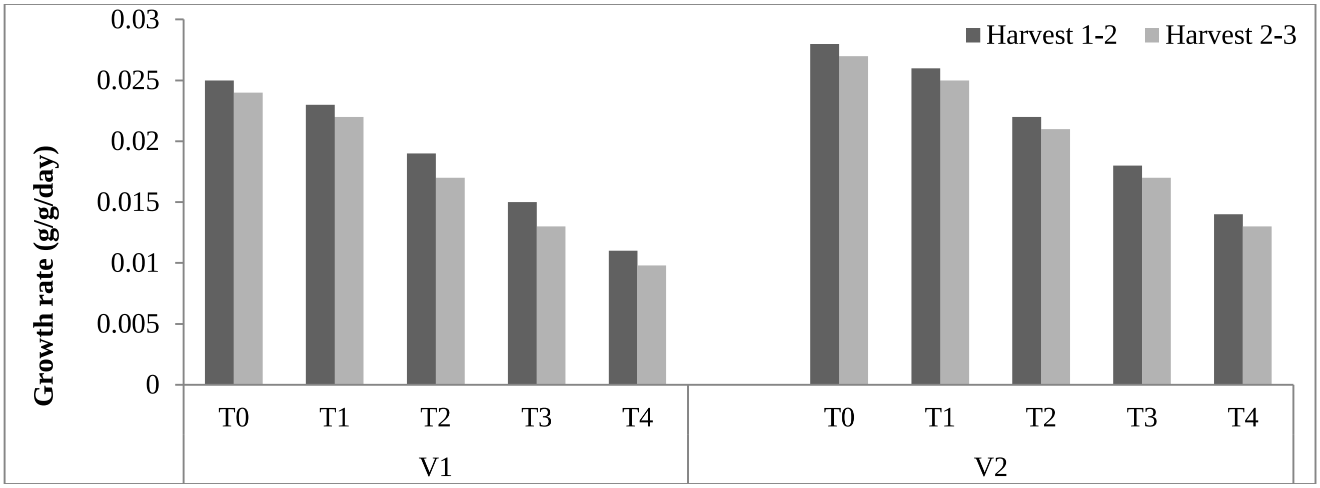

Fig.1. Relative growth rate as influenced by nickel of two chickpea varieties

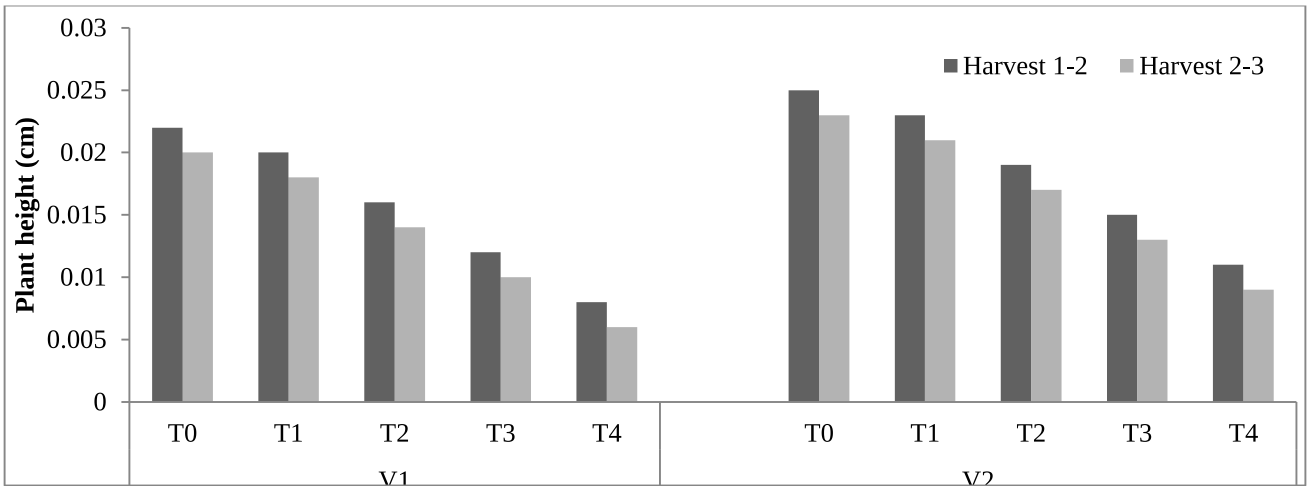

Fig.2. Relative increase in plant height (cm) as influenced by nickel of two chickpea varieties

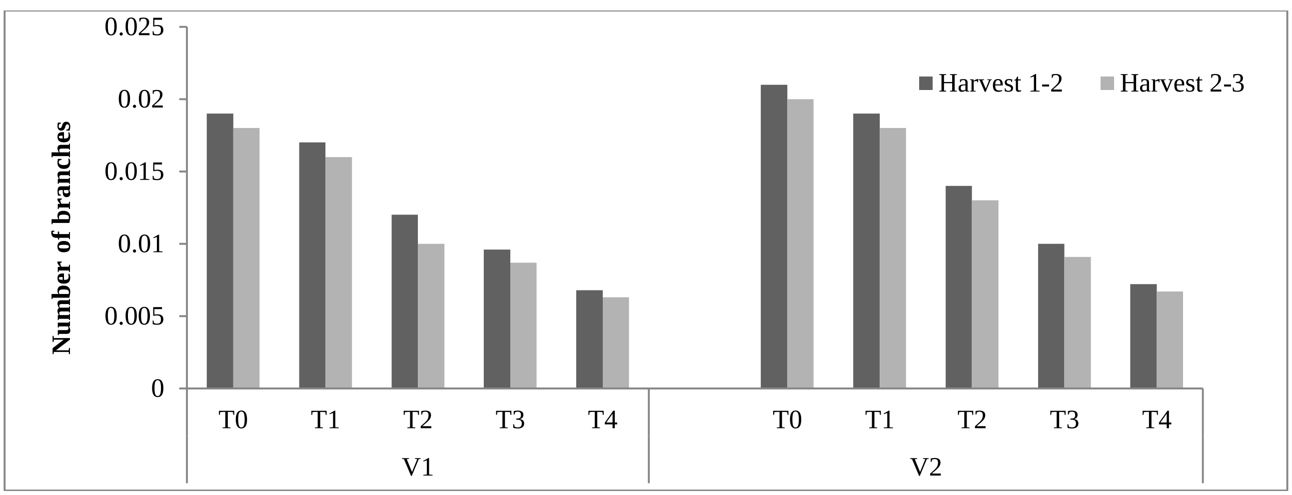

Fig.3. Relative increase in number of branches as influenced by nickel of two chickpea varieties

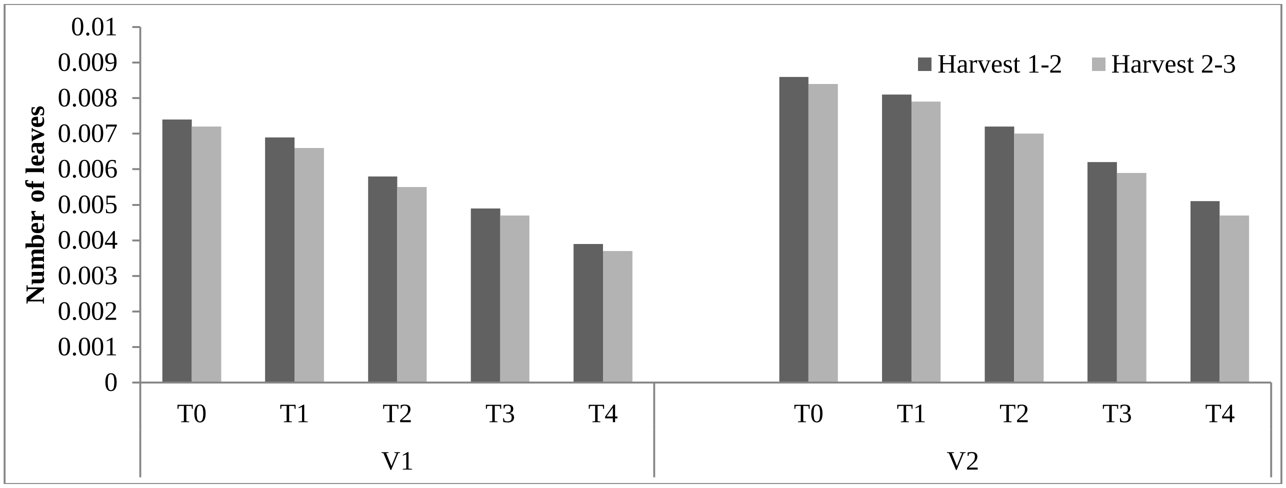

Fig.4. Relative increase in number of leaves as influenced by nickel of two chickpea varieties

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

Source of variation	df	Chlorophyll 'a' (mg g^{-1})	Chlorophyll 'b' (mg g^{-1})	Total chlorophyll (mg g^{-1})	Carotenoids (mg g^{-1})	Dry matter yield (g)
Varieties (V)	1	0.002**	0.000**	0.004ns	0.007**	0.172**
Treatments (T)	4	0.002**	0.005**	0.036**	0.005**	0.847**
Interaction (V x T)	4	0.000**	0.000**	0.009ns	0.000*	0.004*
Error	20	0.000	0.000	0.008	0.000	0.001

**= Highly Significant   *= Significant   ns= Non Significant

Table. 2. Effect of different concentrations of nickel on photosynthetic pigments and dry matter yield of chickpea variety Punjab 2000.

Treatments	Chlorophyll 'a' (mg g^{-1})	Chlorophyll 'b' (mg g^{-1})	Total chlorophyll (mg g^{-1})	Carotenoids (mg g^{-1})	Dry matter yield (g)
T_0	0.620a	0.883a	1.669a	0.487a	3.31a
T_1	0.615b	0.861b	1.480ab	0.455b	3.07b
T_2	0.608c	0.843c	1.452b	0.435c	2.87c
T_3	0.598d	0.822d	1.411b	0.421d	2.64d
T_4	0.570e	0.802e	1.372b	0.405e	2.41e

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

Table. 3. Effect of different concentrations of nickel on photosynthetic pigments and dry matter yield of chickpea variety Bittal 98.

Treatments	Chlorophyll 'a' (mg g^{-1})	Chlorophyll 'b' (mg g^{-1})	Total chlorophyll (mg g^{-1})	Carotenoids (mg g^{-1})	Dry matter yield (g)
T_0	0.638a	0.869a	1.507a	0.500a	3.51a
T_1	0.628b	0.853b	1.481ab	0.484b	3.24b
T_2	0.616c	0.837c	1.453b	0.472c	3.07c
T_3	0.608d	0.816d	1.424b	0.459d	2.74d
T_4	0.591e	0.816d	1.397b	0.436e	2.51e

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

Discussion

The present study was an attempt to evaluate the toxic effects of nickel on photosynthetic pigments and dry matter yield of Cicer arietinum L. Experiment clearly showed that principal photosynthetic pigments, chlorophyll ‘a’ and chlorophyll ‘b’ as well as total chlorophyll contents gradually decreased with increasing levels of nickel toxicity (Rastgoo et al., 2014; Shafeeq et al., 2012). However, chlorophyll ‘b’ was found to be more sensitive to nickel than chlorophyll ‘a’ (Aldoobie and Baltgai, 2013), showing more decrease over control than that of chlorophyll ‘a’. This reduction in chlorophyll contents might be due to inhibition of enzymes for chlorophyll biosynthesis (Kaveriammal and Subramani, 2013), such as protochlorophyll-dereductase and δ-aminolevulinic acid dehydratase (Younis et al., 2015), and also due to decrease in Mg uptake (Chen et al., 2009). It has been reported that excess nickel resulted in decreased nitrogen contents in chickpea plants (Athar and Ahmad, 2002) which might be a contributing factor towards reduced cholorophyll contents. Carotenoids, which are the accessory photosynthetic pigments also reduced due to higher concentrations of nickel (Sai Kachout et al., 2015; Flemotomou et al., 2011).). Photosynthetic activity has been reported to be inhibited due to decrease in stomatal conductance and destruction of mesophyll tissue (Hussain et al., 2013), decrease in cholorophyll contents (Chen et al., 2009) and reduction in number of leaves (Hussain et al., 2013; Chen et al., 2009). This reduction in photosynthesis adversely affected the growth and fresh biomass (Younis et al., 2015; Ishtiaq and Mahmood, 2011) as well as dry matter yield of plants (El-Enany et al., 2000).

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