

Effects of Nickel Toxicity on Seedling Growth, Photosynthetic Pigments, Carotenoids and Phenols Contents of Cowpea *Vigna unguiculata* (L.)

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Abstract—Nickel pollution is worldwide problem due to industrial and anthropogenic activities. Seedlings of cowpea (*Vigna unguiculata*) were treated to different ($\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$) concentrations as 0.0 mM, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM salt into distilled water. The seedlings of *V. unguiculata* became less tolerable to nickel at higher concentration 2.4 mM as compared to control treatment. Nickel in trace amount is required for plant growth. In present study, the high level of nickel concentration 1.8 and 2.4 mM significantly ($p < 0.05$) affected seedling growth performance and physiological, biochemical parameters of *V. unguiculata*. The nickel treatments at 1.2 mM concentrations significantly ($p < 0.05$) affected the number of leaves, shoot, seedling length, fresh weight and dry weight of seedling and root/shoot ratio of *V. unguiculata*. The treatment of Ni^{+2} at 1.20 mM also significantly affected shoot root and seedling fresh and dry weight of cowpea. The leaf area, leaf weight ratio and root fresh and dry weight of *V. unguiculata* showed less affect at 0.6 to 2.4 mM treatment of nickel. Nickel treatment at 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM concentrations showed considerable effects on relative water content, chlorophyll 'a', chlorophyll 'b' total chlorophyll, carotenoids and phenols of *Vigna unguiculata*.

Keywords—Cowpea, nickel, photosynthesis pigments, root, seedling growth.

growth and exposure at higher level considered toxic for human health risk and plant growth [13, 17 and 26]. Literature is available on the effects of nickel toxicity in plants. Inhibition in rate of seed germination percentage, photosynthesis, seedling growth, metabolism, growth and development of plants [4, 5, 14, 15, 19, 20, 21, 25 and 33]. In an investigation, the decline in chlorophyll content of the leaves of maize and *Vigna mungo* were recorded [12 and 28]. The nickel stress at 40 mg L^{-1} showed decrease in the photosynthetic pigments of *Vigna radiata* [1]. Nickel content in food may vary considerably from place to place due to the difference in nickel content of the soil while, certain foods are routinely high in nickel content [9]. Studies have shown toxic effects of nickel on seed germination and seedling growth of plants. The concentration of nickel normally considered in soil 5-500 $\mu\text{g}/\text{gram}$, plant tissue 0.5-5 $\mu\text{g}/\text{gram}$, and animal tissue 0.1-5 $\mu\text{g}/\text{g}$ and fresh water 5-100 $\mu\text{g}/\text{liter}$ [2]. Nickel approximately 0.008% available in Earth's crust, and in soil contains 40 ppm on average basis [8]. The concentration of nickel in soil vary due to soil types, use of synthetic fertilizers, pesticides, nickel smelters, industrial effluents and urban wastes [7]. Nickel is an essential element for healthy plant life, and trace amounts naturally found in most vegetables, fruits, nuts and in slightly greater amounts in chocolate and wine [22].

Nickel is tough silvery hard metal with atomic number 28 and found in living organism and mainly in plants. It is generally believe that nickel at higher concentrations in environment produce toxic effects on plant growth. The researchers are working on the impact of heavy metals on plant growth since last few years. This

I INTRODUCTION

Ni^{+2} is a solid silver white hard transition element [23] and used in manufacturing of stainless steel, coin, jewelry, nickel plating, nickel refining and dental care products. The micronutrients are responsible for plant

paper gives information about the effect of nickel toxicity on seedling growth and some physiological and biochemical parameters of an important legume bean crop cowpea (*Vigna unguiculata* L.) cultivating in agricultural areas of Pakistan.

II MATERIALS AND METHODS

The study was conducted in the green house of the Bio saline Research Laboratory, located at the Department of Botany, University of Karachi, Pakistan, during July to August. The sand was collected from Sands pit sand dunes, Karachi. The sand was passed through 2.00 mm sieve to remove gravels and other material. The sand was washed 5 to 6 times with running tap water and later with distilled water in order to make it free from all nutrients and

minerals. The washed sand was filled up to 2/3 in plastic pots measuring 7.3 cm in diameter and 9.6 cm in height. At the bottom of pot, holes were made for the purpose of absorption of nutrients and water. The filter paper was also placed at the bottom of pots before adding sand. Four pots were place in each plastic tray, which contains irrigation medium.

A modified Hoagland solution was prepared according to Epstein [11]. The composition of Hoagland solutions given in Table 1. Different $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ solutions (0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) were prepared in Hoagland solutions. The 0.0 mM nickel solutions considered as control.



Table 1. Composition of modified Hoagland nutrient solution.

Compounds		Concentration of stock solution (mM)	Volume of stock solution per liter of final solution (ml)
Macronutrients	KNO_3	1000	6
	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	1000	4
	KH_2PO_4	1000	2
	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1000	1
Micronutrients	KCL	25	2
	H_3BO_3	12.5	
	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	1.0	
	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	1.0	
	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.25	
	MoO_3	0.25	
Fe Na EDTA		64	1

The average pH and E.C. (Electrical conductivity) of irrigation medium was determined by pH meter (AD 1000 pH/ mv and temperature meter) and conductivity reading meter, respectively.

Table 2. The average EC ($\text{dS} \cdot \text{m}^{-1}$) and pH of the irrigation medium		
$\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ solution (mM)	EC ($\text{dS} \cdot \text{m}^{-1}$)	pH
0	2.375	8.16
0.6	2.415	8.185
1.2	2.50	8.36
1.8	2.56	8.445
2.4	2.615	8.515

The healthy seeds of cowpea (*Vigna unguiculata* L.) were bought from the local market and were surface sterilized with 0.2% solution of sodium hypochlorite (NaOCl) for one minute to avoid any fungal contamination. Then the seeds were imbibed for 30 minutes in distilled water. The seeds were germinated in sterilized Petri plates, moistened with distilled water. Four seedlings of the same size were selected and transplanted into pots at nearly equal distance. The seedlings were initially irrigated with ¼ strength Hoagland solutions for one week. The solution was

replaced after two days interval. Then the seedlings were irrigated with ½ strength Hoagland solution two times at the interval of two days. The irrigation medium was changed to full strength Hoagland solution and the seedlings were established and were subjected to the respective desired Ni^{+2} treatment. The treatment was given twice a week. During the experiment, the range of minimum and maximum temperature and relative humidity were in between 28 to 33 °C and 65 to 74%, respectively The experiment was completely randomize block design

consisted of eight replicates. Four replicate were used for growth analysis while four were used for relative water content and biochemical analysis.

After five weeks, the seedlings were harvested and different growth parameters were determined including number of leaves per plant, leaf area, length of root and shoot, fresh and dry weight of leaves, root and shoot. For dry weights, the root and shoots were dried at 80° C for 48 hours in oven. The root, shoot ratio, leaf weight ratio, specific leaf area and leaf area ratio were determined by the following formula, respectively.

Root/ shoot ratio = root dry weight / shoot dry weight

Leaf weight ratio = leaf dry weight / total plant dry weight

Specific leaf area ($\text{cm}^2 \text{g}^{-1}$) = Leaf area / leaf dry weight

Leaf area ratio = Leaf area / Total plant dry weight

Relative water content (%)

For the determination of relative water contents, fully expanded leaf was excised from one plant of each pot. The dust particle were removed. The leaf sample was immediately weighted to take the fresh weight (FW) and then immersed in distilled water at 4° C for 10 hours. The saturated leaf sample was removed from water and excess water was removed by tissue paper. The leaf sample was weighted to obtain turgid weight (TW) and the dried in an oven at 70° C for 48 h to record dry weight (DW). The R.W.C. of leaf was determined by the following formula

$$\text{R.W.C (\%)} = [\text{FW}-\text{DW}] / \text{TW}-\text{DW}] \times 100$$

Physiological and biochemical analysis:

Phenols were determined by using folin reagent method while, soluble sugars were obtained by Anthrone reagent method.

Soluble phenols were determined by the methods of Singleton and Rossi [29]. The dried leaf powder was homogenized in 80% methanol and centrifuged. In 1 ml of diluted extracts, 5 ml of Folin-Ciocalteu reagent (1.9 ratio in distilled water) and 4 ml of 7.5 % Na_2CO_3 were added. The absorbance was recorded at 765 nm after incubation of 30 minutes at 25° C. The soluble phenols concentration in leaf tissues was determined against Gallic acid and calculated from best-fit standard curve. The concentration of total phenols mentioned in $\mu\text{g.g}^{-1}$ dry weight of leaves.

Biochemical analysis

Photosynthetic pigments

The leaf samples were excised from the plants and immediately frozen in liquid nitrogen and stored at -20° C until used for photosynthetic pigments. The leaf samples (0.1 g) were grounded in liquid nitrogen and then homogenized in 5 ml 80% cold acetone, centrifuged at 3000 rpm for 5 minutes. The supernatant was separated and the residue was again dissolved in 3 ml of 80% cold acetone and centrifuged. The process were repeated until all the photosynthetic pigments were extracted. All supernatant fractions were pooled and final volume was adjusted. The absorbance of the extract was recorded at 649 and 665 nm for chlorophylls determination while 480 and 510 nm for carotenoids determination, respectively. The absorbance was recorded on spectrophotometer the chlorophyll and carotenoid contents were determined according to the equation described by Strain, *et al.*, [30] and Duxbury and Yentsch [10], respectively.

$$\text{Chlorophyll a } (\mu\text{g/ml}) = 11.63 (A_{665}) - 2.39 (A_{649})$$

$$\text{Chlorophyll b } (\mu\text{g/ml}) = 20.11 (A_{649}) - 5.18 (A_{665})$$

$$\text{Total Chlorophyll } (\mu\text{g/ml}) = 6.45 (A_{665}) + 17.72 (A_{649})$$

$$\text{Carotenoids } (\mu\text{g/ml}) = 7.50 (A_{480}) - 2.63 (A_{510})$$

Statistical analysis

The means as well as standard errors were calculated. Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) using personal computer software packages SPSS version 14.0 were statistically analyzed the data. Level of significance for these tests was at $P < 0.05$.

III RESULTS AND DISCUSSIONS

In this paper, the treatment of various concentrations of nickel 0.6, 1.2, 1.8 and 2.4 mM affected number of leaves, root, shoot, seedling length, fresh weight of root, shoot and leaves, dry weights of root, shoot and leaves, leaf area and specific leaf area of *V. unguiculata* as compared to control (0.0 mM). Nickel affects significantly ($p < 0.05$) the seedling length and shoot length of cowpea at 0.6 mM as compared to control. Nickel after two weeks of treatment started leaves tip chlorosis and then in third week necrotic lesions appeared on leaves followed by the wilting of cowpea seedlings. In another study, barley plants grown in 100 μM Ni showed typical visual symptoms of Ni toxicity such as chlorosis, necrosis of leaves and browning of the root system [24]. Nickel in adequate quantities has a vital role in a large variety of physiological processes, from seed germination to productivity [31]. Root, shoot and seedling growth of green gram showed tolerance at low concentration of nickel. Metals toxicity is a problem for all living organisms [18]. The report confirmed that seedlings

treated to Nickel metal at higher concentration induced toxicity and showed reduction in seedling growth of green gram and agrees with the findings of Yang and Zhao [32] who reported that Ni^{2+} treatment affected all growth indices of oilseed rape (*Brassica napus* L.) gradually at higher concentration.

Nickel treatment at 1.2 concentration affected root growth of *V. unguiculata*. While, no significance difference was found in root length of *V. unguiculata* at highest concentration of nickel treatment at 2.4 mM. The treatment of nickel at 1.2 mM significantly affected on number of leaves of *V. unguiculata* as compared to control.

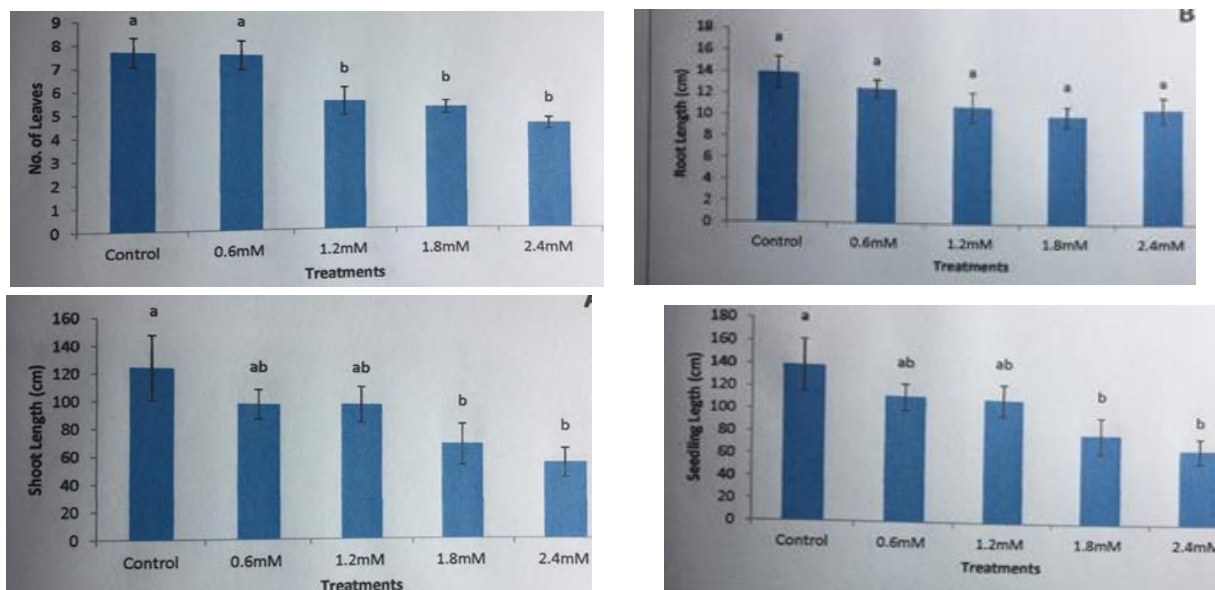


Fig. 1. Effects of different concentrations of nickel (control, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on number of leaves, root, shoot and seedling length (cm) of *Vigna unguiculata*. Statistical difference determined by ANOVA and Values followed by the same letter are not significantly different ($p < 0.05$) according to Duncan's multiple range test.

Nickel treatment at all concentration produced toxic effects on fresh weight of root for *V. unguiculata* (Fig. 2). An increase in concentration of Nickel 1.2 mM caused a gradual decline significantly in fresh weight of root for *V. unguiculata*. Nickel treatment at 1.20 mM also significantly affected shoot, leaves and total seedling fresh weight of *V. unguiculata*.

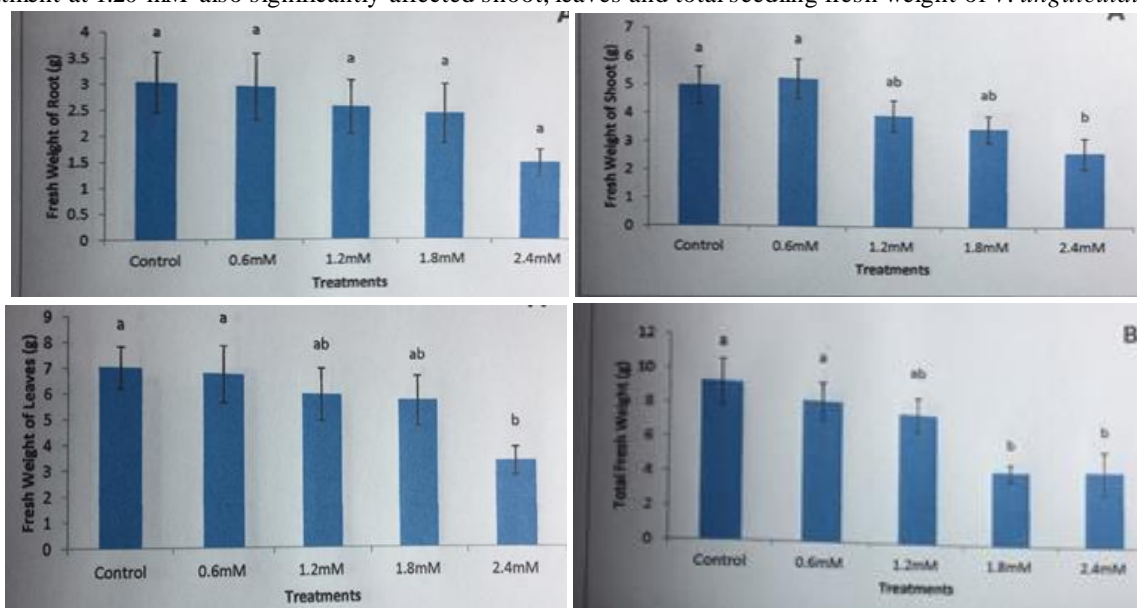


Fig. 2. Effects of different concentrations of nickel (control, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on fresh weight of root, shoot, leaves and total fresh weight (g) of *Vigna unguiculata*. Statistical difference determined by ANOVA and Values followed by the same letter are not significantly different ($p < 0.05$) according to Duncan's multiple range test.

In *V. unguiculata* a constant decline in dry weight of root, shoot, leaves and seedling dry weight was recorded (Fig. 3). The Ni^{+2} treatment at 1.2 mM caused a significant ($p < 0.05$) decrease in seedling dry weight of *V. unguiculata*. An increase in concentration of Nickel to 2.4 mM caused further reduction ($p < 0.05$) in shoot, leaves and seedling dry weight of *V. unguiculata* as compared to control.

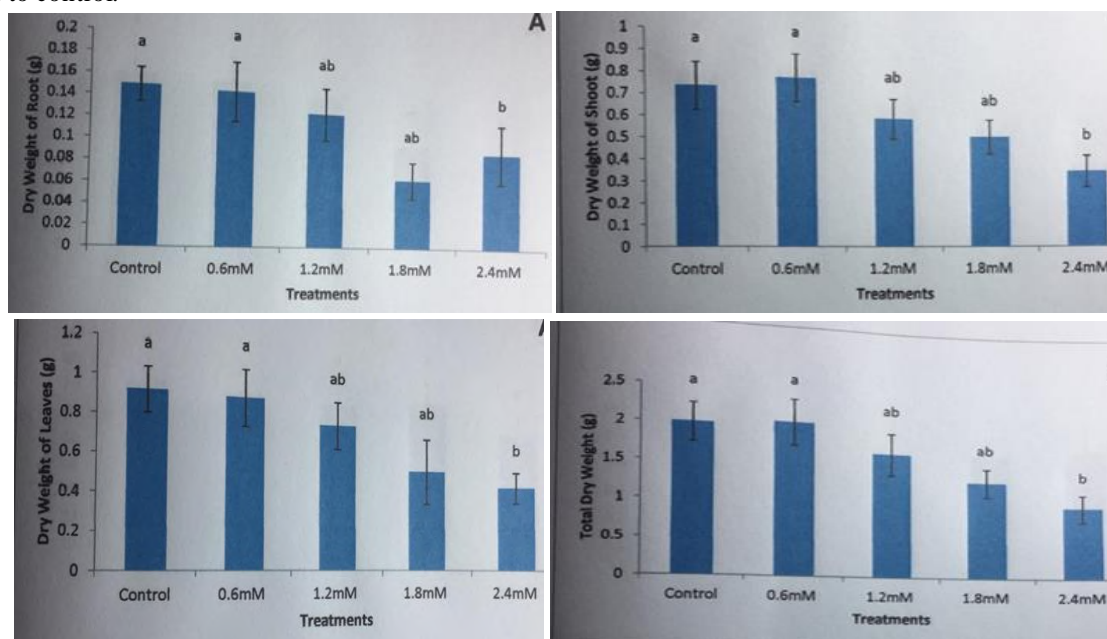


Fig. 3. Effects of different concentrations of nickel (control, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on dry weight of roots, shoot, leaves and total dry weight (g) of *Vigna unguiculata*. Statistical difference determined by ANOVA and Values followed by the same letter are not significantly different ($p < 0.05$) according to Duncan's multiple range test.

In *V. unguiculata* no significance difference ($p < 0.05$) was found in leaf area and root / shoot ratio of *V. unguiculata* with the treatment of nickel at all concentration as compared to control (Fig. 4). An increase in concentration of Nickel 0.6 to 2.4 mM caused a decline in leaf area and root / shoot ratio of *V. unguiculata*. Increase in nickel treatment also decreased the leaf weight ratio, specific leaf area and leaf area ratio of *V. unguiculata*.

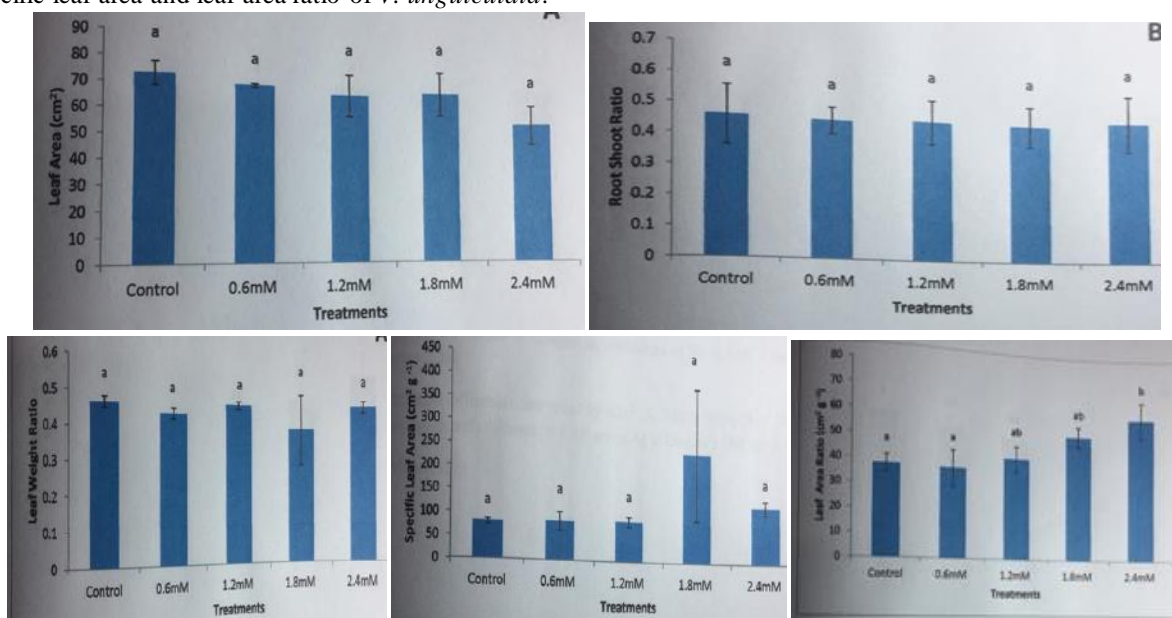


Fig. 4. Effects of different concentrations of nickel (control, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on leaf area, root / shoot ratio, leaf weight ratio, specific leaf area, leaf area ratio of *V. unguiculata*. Statistical difference determined by ANOVA and Values followed by the same letter are not significantly different ($p < 0.05$) according to Duncan's multiple range test.

The effects of different concentrations of nickel 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM on relative water content (%), chlorophyll 'a', chlorophyll 'b' total chlorophyll, carotenoids and phenols $\mu\text{g/ml}$ of *Vigna unguiculata* were recorded. The effects of nickel on relative water content of *V. unguiculata* was less affected (Fig. 6). In, *V. unguiculata* the decreasing trend was found in relative water content with the increase in concentration of nickel. The variations in photosynthetic pigment content of *V. unguiculata* was found. The chlorophyll 'a' and total chlorophyll content was significantly ($p < 0.05$) decreased, but no significant different was found in chlorophyll 'b' content. In a study, Singh and Pandey (2011) [] reported the toxic effect of nickel stresses on uptake, pigments and antioxidative responses of water lettuce, *Pistia stratiotes* L. Carotenoid also showed significant decreasing trend from

control to higher values. i.e. 1.8 mM Ni and 2.4 mM Ni concentration. The reduction was cleared at 2.4 mM Ni concentration. An increase in phenols contents of *V. unguiculata* at higher concentration i.e. 2.40 mM Ni concentration as compared to control was recorded. Such results are in agreement with the findings of Gopal *et al.*, (2002), Pandey, and Sharma (2002) []. These results suggests that nickel might have oxidative damaged to membranous system of chloroplast (Boaccouch *et al.*, [1998). High levels of this micronutrient can alter various metabolic activities of the plant such as the ratio of mineral nutrients, enzyme inhibition, functioning of the stomata, photosynthetic transport of electrons, and degradation of chlorophyll molecules, consequently reducing the photosynthetic rate, growth and chlorophyll content and biological yield of plants [] (Bybordi and Gheibi, 2009).

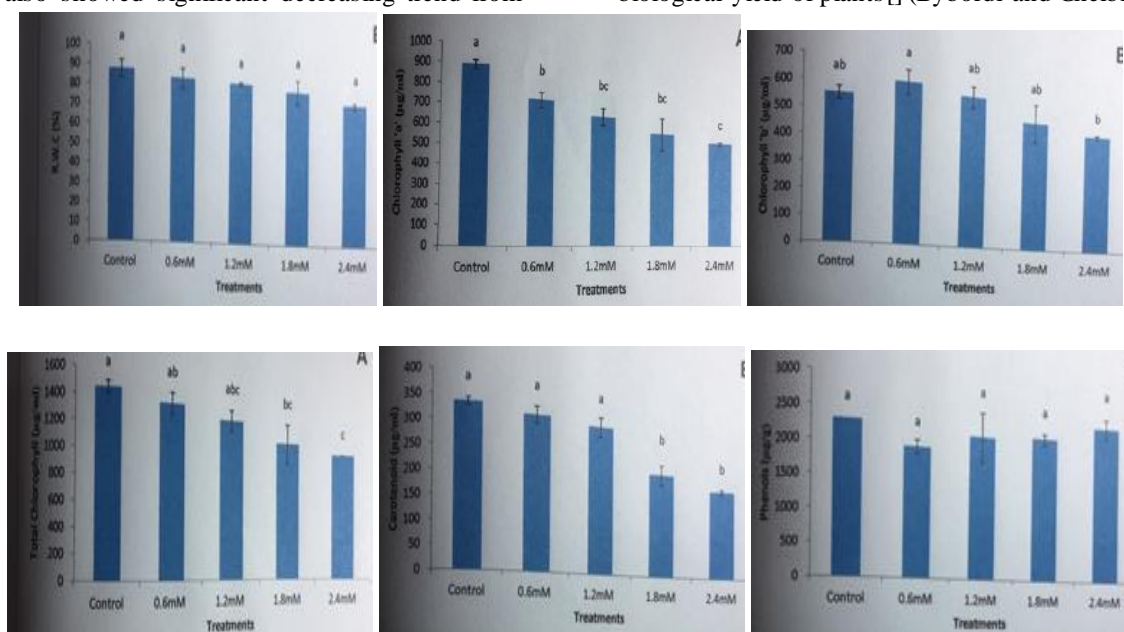


Fig. 6. Effects of different concentrations of nickel (control, 0.6 mM, 1.2 mM, 1.8 mM and 2.4 mM) on relative water content (%), chlorophyll 'a', chlorophyll 'b' total chlorophyll, carotenoids and phenols $\mu\text{g/ml}$ of *Vigna unguiculata*. Statistical difference determined by ANOVA and Values followed by the same letter are not significantly different ($p < 0.05$) according to Duncan's multiple range test.

IV

CONCLUSION

In summary, from the present findings suggest that the nickel treatment at different levels (0.6, 1.2, 1.8 and 2.4 mM) affected seedling growth performance, physical and biochemical parameters of cowpea (*Vigna unguiculata* (L.). Root growth, specific leaf area and leaf area ratio of cowpea seedlings showed tolerance to nickel toxicity at 0.6 mM concentration as compared to control. Seedling fresh and dry weight affected at 1.2 mM Ni concentration. Nickel

concentration at 1.2 mM significantly affected number of leaves of cowpea. Shoot growth of cowpea highly affected than root growth. Relative water content of cowpea in response to nickel levels at 0.6 mM were less affected as compared to control. Ni^{+2} treatment at 0.6 mM significantly affected chlorophyll 'b' and carotenoids ($\mu\text{g/ml}$) content of cowpea. Ni^{+2} treatment significantly increased phenols content of cowpea with the increase in nickel treatment. Overall results suggests that cowpea has a potential of

cultivation in nickel polluted soils having less than 2.4 mM level of nickel and could serve as marker of nickel toxicity.

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