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THE INFLUENCE OF NICKEL SULPHATE ON SOME PHYSIOLOGICAL ASPECTS OF TWO CULTIVARS OF *RAPHANUS SATIVUS* L.

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Abstract - In this study two cultivars of radish *Raphanus sativus* cv. *longipinnatus* (white radish) and *Raphanus sativus* cv. *Cherry Belle* (red radish) were treated with different concentrations of nickel sulphate (0.0-50-100-150-200 ppm). The fresh and dry weight of shoots and roots, photosynthetic pigments, some antioxidant enzymes, total carbohydrates, total proteins and the SDS-PAGE protein profile of both cultivars were determined after 32 days. The results showed that increasing nickel sulphate concentrations decreased the fresh and dry weights of the shoots and roots, photosynthetic pigments, total carbohydrates and total protein in both cultivars. Higher concentrations of nickel sulphate increased the activity of catalase, peroxidase and polypenol oxidase. Electrophoresis banding profiles of proteins revealed qualitative and quantitative changes, and also the appearance or disappearance of some bands of the two cultivars.

Keywords: Radish, protein profile, antioxidant enzymes

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INTRODUCTION

Nickel is an essential element for higher plant nutrition (Brown et al., 1987) as it is a component of the urease enzyme (Dixon et al., 2004) required for nitrogen metabolism in higher plants (Eskew et al., 1984; Brown et al., 1987,1990). Nickel deficiency depressed the urease enzyme activity (Eskew et al., 1984) and other enzymes responsible for nitrate reduction (Brown et al., 1990). As a result, a clear disruption in protein synthesis accompanied by a pronounced reduction in the level of total nitrogen has been noticed with nickel deficiency (Brown et al., 1990).

Natural process includes the weathering of minerals and rocks, whereas different compounds of nickel (such as nickel acetate, nickel carbonate, nickel hydroxide and nickel sulphate) are used in a variety of industrial processes. These compounds ultimately accumulate in the soil and environment and can be easily taken up by plants. Thus, they can enter into the food chain and cause deleterious effects on animals and humans (Nieboer and Nriagu, 1992). A lower concentration of nickel has been reported to play a variety of roles in plant growth and metabolism. However, it shows harmful effects at high concentration (Eskew et al., 1984; Kochian, 1991; Welch, 1995 and Hasinur et al., 2005). For example, the increasing concentration of Ni^{2+} has been shown to inhibit the seed germination and seedling growth of different plant species (Espen et al., 1997 and Farooqi et al., 2009). The Ni-induced growth inhibition has been ascribed to down-regulation of protein synthesis and the activities of some of the key enzymes responsible for the mobilization of food reserves which takes place during seed germination (Foy et al., 1978 and Bishnoi et al., 1993). In addition, Ni is known to be an active competitor for a number of essential micro- and macro-elements and it may reduce the uptake of elements in germinating seeds thereby resulting in poor germination and seedling establishment (Cataldo et al., 1978; Korner et al., 1987 and Kochian, 1991).

Although, some plants can tolerate relatively high levels of nickel in the environment, most of the

cultivated plant species are sensitive to metal stress in the environment. However, the tolerance potential of different species varies greatly within different species or even among different genotypes of the same species. Thus, the objectives of this study were to evaluate the intra-cultivar differences for nickel tolerance in the radish at the initial growth stages such as germination, which is more vulnerable to any stress than latter growth stages of most plant species.

In this study nickel sulphate was used to determinate the heavy metal stress on two cultivars of radish.

Nickel sulfate (NiSO4) exists as a hexahydrate, initially in the alpha-form, which changes into the ß-form at 53.3°C. Nickel sulfate is soluble in water, ethanol, and methanol.

The radish is a herbaceous plant (*Raphanus sativus*) belonging to the family Cruciferae (mustard family), with an edible, pungent root sliced in salads or used as a relish. There are many varieties, with white, red, or black roots of different shape and size, some of which are quite large. Radishes grow easily and quickly throughout temperate regions; they are a staple food in Egypt, Japan and China. Radishes are classified in the division Magnoliophyta, class Magnoliopsida, order Capparales, family Cruciferae.

Radish *Raphanus sativus* cv. *longipinnatus* (white radish) and *Raphanus sativus* cv. *Cherry Belle* (red radish) were treated with different concentrations of nickel sulphate and then planted. After 32 days the effect of nickel sulphate on the photosynthetic pigments, activity of some antioxidant enzymes, total carbohydrates, total proteins and electrophoretic SDS-PAGE of the two *Raphanus sativus* L. cultivars were investigated.

MATERIALS AND METHODS

In this study two cultivars of radish red and white were soaked overnight in different concentrations of nickel sulphate (0.0-50-100-150-200 ppm) and then the seeds were planted, and after 32 days the following was determined:

Growth criteria

After 32 days, the shoots and roots of the two cultivars were detached and their fresh weights measured. For the determination of their dry weights, they were wrapped in paper bags and dried in an oven at 70°C to a constant dry weight.

Estimation of photosynthetic pigments

The spectrophotometric method recommended by Metzner et al., (1965) was used to estimate chlorophyll a, chlorophyll b and carotenoids

Determination of total carbohydrates

Total carbohydrates were determined using the colorimetric methods described by Dubois *et al.,* (1956) with some modification after Orabi (1994).

Determination of total protein

Total protein content of plant tissue was estimated according to the method of Bradford (1976).

Polyacrylamide Gel Electrophoresis (SDS-PAGE):

Protein extracts from the leaves of two cultivars of red and white radish grown under Niso4 stress were subjected to SDS-PAGE according to the method of Laemmli, (1970).

Determination of (catalase, peroxidase and polyphenol oxidase)

Catalase, peroxidase and polyphenol oxidase extracted from the leaves was determined using the method of Kar and Mishra (1976).

Statistical analysis:

The obtained data was subjected to a statistical analysis using the SAS Statistical Analysis System (2006). The statistical analysis model used was two-way analysis of variance with interaction at L.S.D. 5%.

RESULTS

The effect of nickel on growth parameters

Table 1 shows that nickel sulphate significantly decreased the fresh weights of shoots of both radish cultivars, and in particular, high concentrations of nickel sulphate. Moreover, the fresh weight of the root in the red radish was significantly decreased in comparison with untreated samples: on the other hand, the fresh weight of the root of the white radish was not significantly different, whatever the concentration.

Table 2 shows that the shoot dry weight of white radish significantly decreased with moderate and high concentrations, but that of the red radish was significantly decreased at moderate concentration (100 ppm). Moreover, the dry weight of the root of the white radish showed no significant different at any concentration, but in the red radish it significantly decreased at only higher concentration, compared with the control.

The effect of Nickel on photosynthetic pigments

Table 3 shows the effect of nickel sulphate on photosynthetic pigments. Different concentrations of nickel sulphate significantly decreased the contents of chlorophyll (a), chlorophyll (b), carotenoids and total pigments of both cultivars of radish.

Table 1. Effect of nickel sulphate on the shoot and root fresh weights (gms) of two cultivars of radish *Raphanus sativus* cv. *longipinnatus* (white radish–R1) and *Raphanus sativus* cv. *Cherry Belle* (red radish-R2) .

cv / Con.ppm	F.W. of Shoot		F.W. of Root	
	R1	R2	R1	R2
0	3.68 DC	6.70 A	0.30 DF	0.63 A
50	3.15 DFE	5.38 B	0.22 FE	0.53 BA
100	2.73 DFE	4.43 C	0.20 FE	0.36 DC
150	2.36 GF	3.87 DC	0.19 FE	0.30 DF
200	1.99 G	3.59 DCE	0.16 F	0.30 DE
	L.S.D 5% 0.88		L.S.D 5% 0.11	

Means with the same letter are not significantly different.

Table 2. Effect of nickel sulphate on the shoot and root dry weights (gms) of two cultivars of radish *Raphanus sativus* cv. *longipinnatus* (white radish –R1) and *Raphanus sativus* cv. *Cherry Belle* (red radish-R2).

cv / Con. ppm	Dry W. of Shoot		Dry W. of Root	
	R1	R2	R1	R2
0	1.39 A	0.65 CB	0.016 DCE	0.07 A
50	0.31 DE	0.60 C	0.009 DE	0.06 BA
100	0.22 DE	0.50 DE	0.008 DE	0.04 DC
150	0.20 E	0.46 CD	0.007 E	0.03 BC
200	0.20 E	0.41 CDE	0.005 E	0.02 DCE
	L.S.D 5% 0.26		L.S.D 5% 0.03	

Means with the same letter are not significantly different.

The effect of nickel on the total carbohydrate content

Table 4 reveals that nickel sulphate treatment significantly decreased the total carbohydrate content of the white radish with moderate and higher concentrations (50.100.150.200 ppm) compared to the untreated samples, but the red radish - R2 - showed significant decrease only with a higher concentration.

Effect of Nickel on total protein

Table 5 shows that different treatments of nickel sulphate significantly decreased the total proteins of both cultivars of radish.

SDS-PAGE

Table 6 and Fig. 1 show that different treatments of nickel sulphate on the protein banding of white radish did not affect the total number of protein bands except at 50 ppm. Nickel sulphate decreased the number of bands in comparison with untreated samples, but a new band at 35.29 kDa appeared. At

Table 3. Effect of nickel sulphate on the photosynthetic pigments (mg/g fresh weight) of leaves of two cultivars of radish *Raphanus sativus* cv. *longipinnatus* (white radish –R1) and *Raphanus sativus* cv. *Cherry Belle* (red radish-R2) .

cv / Con.ppm	Chlorophyll (a)		Chlorophyll (b)		Carotenoid		Total pigment	
	R2	R1	R2	R1	R2	R1	R2	R1
0	6.68 A	4.42 B	2.87 A	2.40 B	0.96 B	1.40 A	10.51 A	8.20 B
50	3.83 C	3.62 C	1.87 C	1.90 C	0.90 C	0.65 D	6.60 C	6.17 DC
100	3.33 DC	2.95 DE	1.76 DC	1.82 DC	0.63 D	0.35 F	5.72 D	5.12 E
150	2.50 FE	2.30 F	1.74 DC	1.75 DC	0.49 E	0.27 G	4.33 F	3.52 G
200	1.73 G	1.53 G	1.63 D	1.20 E	0.16 H	0.22 G	3.52 G	2.96 G
	L.S.D 5% 0.57		L.S.D 5% 0.23		L.S.D 5% 0.05		L.S.D 5% 0.60	

Means with the same letter are not significantly different.

100 ppm 2 new bands at 183.98 and 69.70 kDa appeared, while at 150 and 200ppm new bands at 198.05 , 118.85 and 37.12 kDa, respectively, appeared. However, at 150 and 200 ppm the band at 100.14 kDa disappeared but new bands at 63.62 kDa at both high concentrations of nickel sulphate appeared. In addition, protein bands at100, 150 and 200 ppm of nickel sulphate produced new bands at 12.31 and 11.60 kDa, respectively.

Table 7 Fig. 2 reveal the appearance of certain protein bands which are considered as common for treated and untreated red radish plants at 222.23, 128.11 and 16.40 kDa, while treated plants were characterized by the disappearance of 7 protein bands at 135.11, 111.61, 63.62, 55.11, 37.12, 18.76 and 12.31 kDa.

In addition, it was found that the new band at 78.61 kDa appeared in the red radish plants treated at Ni concentrations of 150 and 200 ppm .

Also, the plants treated with 150 ppm nickel sulphate are characterized by the presence of new

Table 4. Effect of nickel sulphate on the total carbohydrate(mg/g) dry weight of two cultivars of radish *Raphanus sativus* cv. *longipinnatus* (white radish–R1) and *Raphanus sativus* cv. *Cherry Belle* (red radish-R2).

cv / Con.ppm	R1	R2
0	120.77 C	144.26 A
50	89.07 D	137.66 BA
100	70.96 E	124.23 BC
150	51.07 F	110.50 C
200	48.27 F	62.70 FE
	L.S.D 5% 16.64	

Means with the same letter are not significantly different.

Table 5. Effect of nickel sulphate on the total protein (mg/gm) of two cultivars of radish *Raphanus sativus* cv. *longipinnatus* (white radish–R1) *and Raphanus sativus* cv. *Cherry Belle* (red radish-R2).

cv / Con.ppm	R1	R2
0	0.91 A	0.80 BAC
50	0.81 BA	0.67 BDEC
100	0.75 BDAC	0.57 FE
150	0.71 BDEC	0.63 FDEC
200	0.59 FDE	0.47 F
	L.S.D 5% 0.18	

Means with the same letter are not significantly different.

bands at 65.65 and 11.13 kDa, and those treated with 200 ppm at 143.43 and 17.62 kDa Nickel treatment of 50 and100 ppm created new bands at 48.73, 20.46 and 13.62 kDa, respectively, and at 50 ppm a new band at 25.33 kDa appeared.

Table 6. SDS-PAGE of protein patterns of *Raphanus sativus* cv. *longipinnatus* (white radish –R1)treated with different concentrations (50ppm-100ppm-150ppm-200ppm) of nickel sulphate.

Band No.	MW (kDa)	control	50ppm	100ppm	150ppm	200ppm
1	353.77	+	-	-	-	-
2	222.23	+	+	+	+	+
3	189.05	-	-	-	+	-
4	183.98	-	-	+	-	+
5	128.11	+	+	+	-	-
6	118.85	-	-	-	-	+
7	100.14	+	+	+	-	-
8	90.45	+	+	+	+	+
9	73.94	+	+	+	+	+
10	69.70	-	-	+	-	-
11	65.65	+	-	-	-	-
12	63.62	-	-	-	+	+
13	55.14	-	-	+	+	-
14	48.73	+	+	-	-	-
15	39.08	-	-	+	+	-
16	37.12	-	-	-	-	+
17	35.29	-	+	-	-	-
18	29.31	+	-	-	-	-
19	24.10	-	-	+	+	+
20	22.19	+	-	-	-	-
21	16.40	-	+	+	+	+
22	13.62	+	+	-	-	-
23	12.31	-	-	+	+	+
24	11.60	-	-	+	+	+
Total		11	9	13	11	11

Table 7. SDS-PAGE of protein patterns of *Raphanus sativus* cv. *Cherry Belle* (red radish-R2) treated with different concentrations (50ppm-100ppm-150ppm-200ppm) of nickel sulphate.

Band No.	MW (kDa)	control	50ppm	100ppm	150ppm	200ppm
1	222.23	+	+	+	+	+
2	183.98	-	+	+	+	+
3	171.22	+	-	-	+	+
4	143.43	-	-	-	-	+
5	135.11	+	-	-	-	-
6	128.11	+	+	+	+	+
7	111.61	+	-	-	-	-
8	93.26	-	-	+	+	+
9	90.45	+	+	-	-	-
10	78.61	-	-	-	+	+
11	73.94	+	+	+	-	-
12	65.65	-	-	-	+	-
13	63.62	+	-	-	-	-
14	55.14	+	-	-	-	-
15	48.73	-	+	+	-	-
16	37.12	+	-	-	-	-
17	35.29	-	-	+	-	-
18	25.33	-	+	-	-	-
19	24.10	+	-	-	+	+
20	20.46	-	+	+	-	-
21	18.76	+	-	-	-	-
22	17.62	-	-	-	-	+
23	16.40	+	+	+	+	+
24	13.62	-	+	+	-	-
25	12.31	+	-	-	-	-
26	11.60	+	-	-	-	+
27	11.13	-	-	-	+	-
Total		15	10	10	10	11

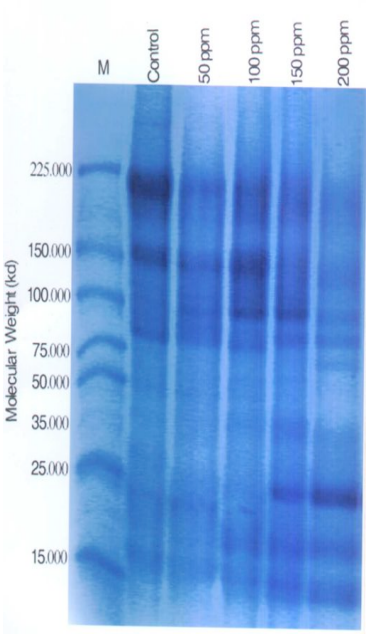

Fig. 1. Electrophoresis banding profiles of proteins of leaves *Raphanus sativus* cv. *longipinnatus* (white radish–R1) treated with different concentrations (50ppm-100ppm-150ppm-200ppm) of nickel sulphate.

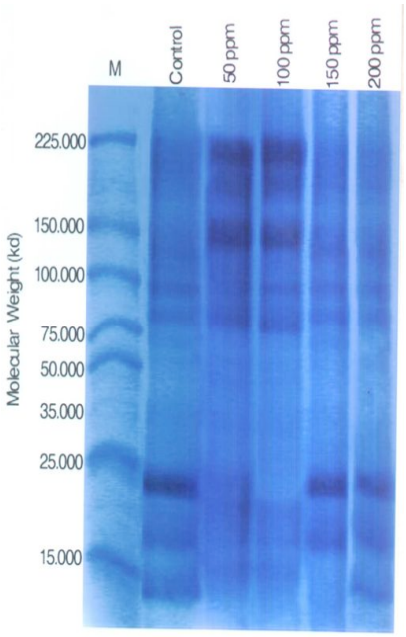

Fig. 2. Electrophoresis banding profiles of proteins of leaves *Raphanus sativus* cv. *Cherry Belle* (red radish-R2)treated with different concentrations (50ppm-100ppm-150ppm-200ppm) of nickel sulphate.

The effect of nickel on some antioxidant enzymes (catalase, peroxidase and polyphenol oxidase)

Tables 8, 9 and 10 show that different concentrations of nickel sulphate increased the activity of catalase (CAT; EC 1.11.1.6), peroxidase (POX; EC 1.11.1.7) and polyphenol oxidase (PPO; EC 1.10.3.1) in both cultivars of radish, particularly white radish where the increase was more significant in response to to nickel sulphate than in red radish.

Table 8. Effect of nickel sulphate on peroxidase (gm/fw.equv./hour) of two cultivars of radish *Raphanus sativus* cv. *longipinnatus* (white radish–R1) and *Raphanus sativus* cv. *Cherry Belle* (red radish-R2).

Con.ppm \ cv	R1	R2
0	32.31 E	6.30 I
50	37.56 D	12.45 H
100	47.52 C	18 G
150	51.39 B	26.40 F
200	57.30 A	50.10 B
L.S.D 5% 2.50		

Means with the same letter are not significantly different.

Table 9. Effect of nickel sulphate on Catalase(gm/fw.equv./hour) of two cultivars of radish *Raphanus sativus* cv. *longipinnatus* (white radish–R1) and *Raphanus sativus* cv. *Cherry Belle* (red radish-R2).

Con.ppm \ cv.	R1	R2
0	2085 G	1387.3 H
50	3232.50 E	2655 F
100	4275 D	3585 E
150	6112.50 B	5430 C
200	7275 A	5887 B
L.S.D 5% 441.24		

Means with the same letter are not significantly different.

Table 10. Effect of nickel sulphate on Polyphenol oxidase(gm/fw.equv./hour) of two cultivars of radish *Raphanus sativus* cv. *longipinnatus* (white radish–R1) and *Raphanus sativus* cv. *Cherry Belle* (red radish-R2).

Con.ppm \ cv	R1	R2
0	1.84 G	1.52 H
50	2.10 FE	1.92 FG
100	3.52 D	2.19 E
150	4.81 B	3.42 D
200	5.62 A	3.80 C
L.S.D 5% 0.24		

Means with the same letter are not significantly different.

DISCUSSION

The presented results show that nickel sulphate caused a reduction of the fresh and dry weights of roots and shoots of white and red radish plants, particularly with high concentrations of nickel sulphate. Similar results were reported by previous authors, who showed that the influence of relatively higher amounts of Ni in wheat cv. *Vergina* resulted in depressed shoot growth (Athar and Ahmad, 2002). This suggests that heavy metals inhibit root and shoot growth directly by inhibiting cell division or cell elongation, or a combination of both (Mocquot et al., 1996; El-Sheekh et al., 2003 and Vijayarengan and Dhanavel, 2005).

Chlorophyll a, chlorophyll b and carotenoids showed a significant decrease when the concentrations of nickel sulphate were increased in both cultivars of the radish. This indicates that biosynthesis was inhibited by metals in higher plants (Prasad and Prasad, 1987).

The effect of heavy metals on photosynthetic pigments may be due to the heavy metals entering the frond chloroplast with a resulting over-accumulation locally causing oxidative stress and subsequent damage through the peroxidation of the chloroplast membranes (Clemens et al., 2002). Also, heavy metals can directly destroy the structure and function of chloroplast by binding to the -SH groups of enzymes and affect chlorophyll biosynthesis (Baker, 1981).

Heavy metals inhibit the uptake and transportation of other metal elements such as Mn, Zn and Fe by antagonistic effects and cause the fronds to lose the capacity of pigment synthesis (Cobbett, 2000). Heavy metals may also activate the pigment enzyme and accelerate the decomposition of pigment.

The soaking of seeds in different concentrations of nickel sulphate had a significant effect on the total carbohydrate content of both cultivars of radish. The observed decline with respect to the high level of Ni may be due to its role on the enzymatic reactions related to the cycles of carbohydrate catabolism (Rabie et al., 1992). The decrease in the total carbohydrate content corresponded with the photosynthetic inhibition or stimulation of the respiration rate (Tzvetkova and Kolarov, 1996 and John et al., 2008).

Total proteins were significantly decreased by increasing nickel sulphate concentrations in both cultivars. Biotic stress may inhibit the synthesis of some proteins and promote others (Ericson and Alfinito 1984). Our results coincide with Costa and Spitz (1997) who also reported a decrease in soluble protein content under heavy metal stress. The decrease in protein content may be caused by an enhanced protein degradation as a result of increased protease activity under stress conditions (Palma et al., 2002). Also, these heavy metals may have induced lipid peroxidation and fragmentation of proteins due to the toxic effects of reactive oxygen species leading to a reduction in protein content (Davies et al., 1987). Such inhibitory effects of high levels of Ni have been reported to be the result of inhibition of protein synthesis and changes in carbohydrate metabolism (Bishnoi et al., 1993; Lin and Kao, 2006 and Maheshwari and Dubey, 2007).

In the present investigation the occurrence of new protein bands in both cultivars of radish could be stress proteins produced to overcome the toxic effect of heavy metals. On the other hand, the

disappearance of some protein bands in both cultivars of radish could be due to lowered protein synthesis and/or the depletion of reserve proteins to overcome the stress caused by nickel.

The effect of nickel sulphate on the activity of some antioxidant enzymes (catalase (CAT; EC 1.11.1.6) ,peroxidase (POX; EC 1.11.1.7) and polypenol oxidase (PPO; EC 1.10.3.1) showed significant increase at higher concentrations of nickel in both cultivars of radish. Peroxidase (POX) is a heme protein, and a member of the oxidoreductase class. It catalyzes the oxidation of a wide variety of organic and inorganic substrates in the presence of hydrogen peroxide (Köksal and Gülçin,2008).

Peroxidase and catalase are two major systems for the enzymatic removal of H_2O_2 and the peroxidative damage of cell walls is controlled by the potency of the antioxidative peroxidase enzyme system (Sreenvasulu et al., 1999 and Velikova et al., 2000). Previous studies have found a positive relationship between increased POX and CAT enzyme activity and the amounts of heavy metals such as Cu, Pb and Zn in plant tissue (Girotti, 1985 and Mocquot et al., 1996). These enzymes remove superoxide radicals, which are harmful to cell membranes. Gad et al., (2007) showed that the increase in the activity of catalase and peroxidase with high levels of nickel is known to enhance plant respiration and this may cause further consumption of plant net photosynthesis and enhanced plant catabolism. Polyphenol oxidase is responsible for enzymatic browning of raw fruits and vegetables (Mathew and Parpia 1971). It was shown that increasing the metal concentration causes an increase in peroxidase and polypenol oxidase activities (Cheruth Abdul Jaleel et al., 2008)

CONCLUSION

It could be concluded that red radish was more tolerant to the inhibitory effect of Ni than the white radish.

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