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Full Length Research Paper

The Physiological responses of sunflower (*HELIANTHUS ANNUUS* L.) to $NiSO_4$

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The effects of different concentrations of nickel sulfate ($NiSO_4$) on some physiological parameters in sunflower (*HELIANTHUS ANNUUS* L.) were studied. Sunflower plants were treated with $NiSO_4$ at 0, 4, 8, 16, 32 and 40 µM in Hoagland solutions. With the increasing concentrations of $NiSO_4$, all the growth parameters increased. The highest significant increase of NAR and RGR were obtained with 16 µM $NiSO_4$. Control and treated plants had similar chlorophylls and unsoluble sugars contents. Total soluble sugars and protein contents were increased in high concentrations of $NiSO_4$. Addition of all nickel concentrations had a significant promotive effect on Mn, Fe and Ni contents in leaves and roots as compared to that of control. Malondialdehyde (MAD) and respiration rate were significantly increased in high concentrations, however, photosynthetic rate and RWC decreased in all concentrations of $NiSO_4$.

Key words: Sunflower, nickel, malondialdehyde, growth parameters, gas exchange.

INTRODUCTION

Nickel (Ni), the most recently discovered essential element (Brown et al., 1987), is unique among plant nutrients in that its metabolic function was determined well before it was determined that its deficiency could disrupt plant growth. Subsequent to the discovery of its essentiality in the laboratory, Ni deficiency has now been observed in field situations in several perennial species (Wood et al., 2004). The interest of plant scientists in the role of nickel was initiated following the discovery in 1975 (Dixon et al., 1975) that it was a critical constituent of the plant enzyme, urease. Urease contains two Ni ions at the active site (Ciurli, 2001). Ni can also replace Zn or Fe, and other metal ions, in certain other metalloenzymes of lower plants (Mulrooney and Hausinger, 2003). The ultimate determination that nickel was essential for plant growth (Brown et al., 1987) depends heavily on the development of new techniques to purify growth media and to measure extremely low concentrations of nickel in plants. The establishment of nickel as an essential element, however, highlights the limitations of the current definition of essentiality of nutrients as applied to plants (Arnon and Stout, 1939). It has been argued, for example, that even though nickel is clearly a normal and functional constituent of plants, it does not fulfill the definition of essentiality, since urease is not essential for plant growth and nickel deficiency apparently does not prevent the completion of the life cycle of all species, even though that criterion has not been explicitly satisfied for any element (Gerendas et al., 1999). Several authors (Gerendas et al., 1999; Epstein and Bloom, 2004) now suggest that the criteria for essentiality should be modified to include elements that are normal functional components of plants.

Ni deficiency caused urea accumulation in foliage of soybean (*Glycine max* (L.) Merr) and cowpea (*Vigna unguiculata* (L.) Walp) (Eskew et al., 1983, 1984; Walker et al., 1985), affected amino acid metabolism in cowpea (Walker et al., 1985) and reduced urease activity, induced metabolic nitrogen deficiency, and affected

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Abbreviations: LA, Leaf area; **LFM,** leaf fresh matter; **LWCA,** leaf water content area; **LDM,** leaf dry matter; **NAR,** net assimilation rate; **RDM,** root dry matter; **RFM,** root fresh matter; **RGR,** relative growth rate; **RLGR,** relative leaf growth rate; **RWC,** relative water content; **SDM,** stem dry matter; **SFM,** stem fresh matter; **SLA,** specific leaf area.

amino acids, amides (Gln and Asn), and urea cycle intermediates (Arg, Orn, and citrulline) in several nonwoody species (rye (*Secale cereale*), wheat, soybean, rape (*Brassica tournefortii* Gouan), zucchini (*Cucurbita pepo* L.) and sunflower (*Helianthus annuus* L.) (Gerendas and Sattelmacher, 1997). At cellular level, high concentrations of Ni, stimulate the production of reactive oxygen species (ROS) such as $O_2^{\cdot -}$ and H_2O_2 (Gajewska and Sklodowska, 2007) and induce oxidative stress as evidenced by peroxidation of membrane lipids (Baccouch et al., 1998, 2001). For protection against ROS- caused oxidative damage, plant cells posses both enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and nonenzymatic antioxidants such as glutathione and ascorbic acid (Mittler et al., 2004).

The aim of the present work is to investigate further the effect of nickel sulfate on certain physiological parameters in sunflower plants.

MATERIALS AND METHODS

Plant materials and treatments

Sunflower (*H. annuus* L.) seeds were prepared from Agricultural Research Center, Tehran, Iran. Seeds were sterilized in 20% (W/V) sodium hypochlorite (10 min) and washed five times with sterile distilled water. Seeds germinated in pots containing sands in a growth chamber at 24 (±1)°C temperature and at a relative humidity of 70%. Germinated seeds were transferred to pots in growth chamber with 17 h light periods and 200 µmol quanta m^{-2} sec^{-1} light intensity, day/ night temperatures of 25(±1)/18(±1) °C respectively and irrigated with Hoagland´s solution. Seventeen days old plants transplanted into the nutrient solutions containing 0, 4, 8, 16, 32 and 40 µM nickel sulphate, and the nutrient solution was renewed every week. The plants were grown under controlled environment (17 h light periods, 200 µmol quanta m^{-2} sec^{-1} light intensity, day/night temperatures of 25(±1)/18(±1)°C respectively) in a greenhouse. After 26 days of experimental period, for physiological analyses, four plants were harvested from each treatment.

Growth analyses

At the end of the experiment, four plants were harvested from each treatment, to determine the leaf area, leaf and stem fresh weights. Dry weights of leaves, stems and roots were determined by oven drying the tissues at 95°C for 24 h. RGR, NAR, RLGR, SLA and LWCA were carried out using the equations (Watson, 1952; Evans and Hughes, 1962).

Relative water content (RWC)

Leaf fresh weight (F) was measured and then the leaf was placed in a petri dish with distilled water. The petri dishes were maintained at 4°C in the dark for 24 h, leaves were blotted dry and then the turgor fresh weight (T) was determined, and the leaf dried at 95°C for 24 h. Finally, the dry weight (D) was measured .The relative water content was calculated by the equation:

((F-D)/(T-D))×100

Pigments assays

150 mg of fresh leaf tissue of each treatment was extracted in 15 cm^3 80% acetone and absorbance of extracts was recorded at 470, 646.8 and 663.2 nm respectively (Lichthenthaler, 1987).

Gas exchanges assay

Photosynthetic and respiration rates were determined in intact plants, employing an Infrared Gas (CO_2) Analyzer (IRGA) (Khavari-Nejad, 1980, 1986).

Sugars assay

500 mg of dried leaves of each treatment was extracted with 80% ethanol and after using $Ba(OH)_2$ and $ZnSO_4$ for removing pigments from extracts and adding %5 phenol and sulfuric acid, absorbance of extracts was recorded at 540 nm (Hellubust and Craigie, 1978).

Protein assay

Protein concentration was determined according to Bradford using bovin serum albumin as standard (Bradford, 1976).

Estimation of lipid peroxidation

The level of lipid peroxidation products in root samples was expressed as malondialdehyde (MDA) content and was determined as described by Heath and Packer (1968).

Estimation of Mn, Fe and Ni level in leaves and roots

The contents of Fe, Mn and Ni in roots and leaves were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, ultima, JY Horiba, Edision, NJ, U.S.A) after the samples were digested with nitric acid.

Statistical analysis

The research was conducted using completely randomized design with four replications. The significance of treatments was analyzed by analysis of variance (AVOVA) using SAS software (SAS Instiute Inc., Cary, NC).

RESULTS

The effects of different $NiSO_4$ concentrations (0, 4, 8, 16, 32 and 40 µM $NiSO_4$) on some growth parameters were evaluated. Table 1 shows that all Ni concentrations increased fresh and dry weights significantly, however, SLA decreased. NAR, RGR and RLGR were increased with increasing $NiSO_4$ concentrations. Leaf relative water content (RWC) decreased in all treatments, with respect to control plants (Table 3). Chlorophyll contents did not show significant changes. However, in 40 µM $NiSO_4$, Chl.*b* content significantly decreased (Table 2). In this study, soluble sugars and gas exchanges were little affected by Ni, in all concentrations of $NiSO_4$, soluble

Table 1. Effects of $NiSO_4$ on growth parameters, means (± SE) of four replicates, numbers followed by the same letter are not significantly different (P>0.05).

Growth parameters	$NiSO_4$(µM)					
(mean final values)	0	4	8	16	32	40
LFM(g)	3.6232 ± 0.468 c	5.5035 ± 0.461 ab	6.5152 ± 0.417 a	4.8010 ± 0.699 b	6.6660 ± .235a	5.4820 ± 0.762 ab
SFM(g)	11.7642 ± 0.612 c	14.6405 ± 1.713 bc	15.4577 ± 0.758 b	13.4300 ± 1.497 bc	19.4392 ± 0.520 a	16.5787 ± 1.298 ab
RFM(g)	5.5617 ± 0.563 b	7.4345 ± 0.804 ab	8.4852 ± 0.281 a	8.5552 ± 0.926 a	9.0592 ± 1.164 a	8.7670 ± 0.692 a
LDM(g)	0.4370 ± 0.064 c	0.7535 ± 0.0961 ab	0.8232 ± 0.045 ab	0.6917 ± 0.091 b	0.9065 ± 0.062 a	0.9157 ± 0.042 a
SDM(g)	1.003 ± 0.022 c	1.5192 ± 0.117 ab	1.6032 ± 0.187 a	1.3352 ±0.075 abc	1.5657 ± 0.289 ab	1.1030 ± 0.147 bc
RDM(g)	0.5622 ± 0.077 b	0.8165 ± 0.050 b	0.9952 ± 0.169 b	1.8742 ± 0.294 a	1.1395 ± 0.203 b	0.9817 ± 0.117 b
LA(cm^2)	207.431 ± 8.601 b	341.006 ± 35.130 a	330.281 ± 27.888 a	321.213 ± 55.643a	354.151 ± 43.736 a	210.504 ± 27.281 b
NAR(g $m^{-2}day^{-1}$)	4.885 ± 0.287 b	5.478 ± 0.195 ab	6.254 ± 0.329 ab	7.403 ± 0.625 a	6.422 ± 1.073 ab	7.643 ± 1.172 a
RGR(g $kg^{-1}day^{-1}$)	69.535 ± 1.687 b	79.560 ± 1.947 a	82.118 ± 1.376 a	84.992 ± 2.462 a	82.739 ± 3.546 a	79.002 ± 1.281 a
RLGR($cm^2m^{-2}day^{-1}$)	449.665 ± 9.893 b	564.769 ± 24.884 a	558.331 ± 21.013a	544.322 ± 39.367a	572.062 ± 29.893 a	447.315 ± 32.795 b
SLA(m^2kg^{-1})	51.476 ± 9.317 a	45.802 ± 2.851 a	39.914 ± 1.305 a	45.892 ± 2.377 a	38.954 ± 3.451 a	23.172 ± 3.073 b
LWCA($g(H_2O)m^{-2}$)	152.486±15.869ab	140.906 ± 7.493 ab	174.716 ± 13.889 ab	129.411± 6.092 b	170.012 ± 20.770 ab	244.267 ± 75.289 a

Table 2. Effects of $NiSO_4$ on chlorophylls contents and chl.*a*/chl.*b* ratio contents, means (± SE) of four replicates, numbers followed by the same letter are not significantly different (P>0.05).

Treatment($NiSO_4$ µM)	Chl.*A*(mg g^{-1} f.w.)	Chl.*B*(mg g^{-1} f.w.)	Chl.*A*+Chl.*B*(mg g^{-1} f.w.)	Chl.*A*/Chl.*B* ratio
Control	1.123 ± 0.053 a	0.600 ± 0.071 a	1.7239 ± 0.105 a	1.930 ± 0.178 a
4	0.950 ± 0.104 a	0.460 ± 0.043 ab	1.410 ± 0.145 a	2.060 ±0.071 a
8	0.898 ± 0.045 a	0.461 ± 0.047 ab	1.359 ± 0.093 a	1.983 ± 0.108 a
16	1.030 ± 0.059 a	0.518 ± 0.030 ab	1.548 ± 0.088 a	1.989 ± 0.035 a
32	1.024 ± 0.072 a	0.526 ± 0.035 ab	1.551 ± 0.106 a	1.947 ± 0.061 a
40	0.937 ± 0.035 a	0.438 ± 0.023 b	1.375 ± 0.057 a	2.142 ± 0.067 a

Table 3. Effects of $NiSO_4$ on RWC, soluble sugar and unsoluble sugars contents, means (± SE) of four replicates, numbers followed by the same letter are not significantly different (P>0.05).

Treatment ($NiSO_4$µM)	RWC(%)	Soluble sugar (mg g^{-1} d.w.)	Unsoluble sugar (mg g^{-1}d.w.)
Control	132.086± 20.447 a	7.292 ± 0.944 b	10.941± 1.454 ab
4	87.073± 7.644 b	13.073 ± 1.865 a	13.637 ± 1.139 a
8	68.439± 8.354 b	12.300 ± 1.210 a	10.300 ± 0.36 ab
16	77.793± 2.926 b	11.856 ± 0.397 a	11.157 ± 1.068 ab
32	64.839± 2.542 b	9.841 ± 0.772 ab	12.930 ± 1.184 a
40	66.646± 5.478 b	7.438 ± 0.575 b	11.653 ± 0.911 ab

sugars and respiration rate were increased. However, photosynthesis rate decreased (Tables 3 and 4). Total protein content increased in plants treated with 4 to 32 µM $NiSO_4$ (Table 5). Lipid peroxidation was monitored by measuring the concentration of MDA in roots of control and Ni-treated plants. The degree of lipid peroxidation varied very little in low concentrations of $NiSO_4$, however, in 40 µM $NiSO_4$, MDA significantly increased (Table 5). The contents of elements were modified in Ni-treated plants. In all treatments, Fe, Mn and Ni accumulation in roots and leaves were significantly increased (Table 6). The highest contents of Fe and Mn in roots and leaves were observed in 16 µM $NiSO_4$. Ni accumulation in leaves and roots were observed in 32 and 40 µM $NiSO_4$.

In 32 and 40 µM $NiSO_4$, Fe and Mn contents in leaves and roots decreased, as compared to other concentrations of $NiSO_4$.

DISCUSSION

In the majority of natural environments, the concentration

Table 4. Effects of $NiSO_4$ on photosynthetic rate and respiration rate, means (± SE) of four replicates, numbers followed by the same letter are not significantly different (P>0.05).

Treatment ($NiSO_4$ µM)	Photosynthetic rate (µmolCO_2 dm^{-2} s^{-1})	Respiration rate (µmolCO_2 dm^{-2} s^{-1})
Control	6.325 ± 0.422 a	4.717 ± 0.233 c
4	5.973 ± 0.284 ab	3.932 ± 0.0338 d
8	5.156 ± 0.333 b	5.203 ± 0.211 bc
16	5.396 ± 0.380 b	6.033 ± 0.088 a
32	5.970 ± 0.287 ab	6.142 ± 0.153 a
40	5.830 ± 0.235 ab	5.735 ± 0.217 ab

Table 5. Effects of $NiSO_4$ on protein and MDA contents, means (± SE) of four replicates, numbers followed by the same letter are not significantly different (P>0.05).

Treatment ($NiSO_4$µM)	Protein(mg g^{-1}fw)	MDA(µmol g^{-1}fw)
Control	212.24 ± 28.37 cd	4.47 ± 0.388 e
4	233.23 ± 44.35 b	4.72 ± 0.208 cd
8	216.12 ± 21.74 bc	4.64 ± 0.136 de
16	288.78 ± 34.12 a	4.91 ± 0.231c
32	225.8 ± 18.03 bc	5.81 ± 0.128 b
40	196.00 ± 30.34 d	6.57 ± 0.263 a

Table 6. Effects of $NiSO_4$ on Mn, Ni and Fe contents in leaves and roots, means (± SE) of four replicates, numbers followed by the same letter are not significantly different (P>0.05).

Treatment	Fe (mg kg^{-1}d.w.)		Mn (mg kg^{-1}d.w.)		Ni (mg kg^{-1}d.w.)	
(Ni SO_4µM)	Leaves	Roots	Leaves	Roots	Leaves	Roots
Control	2.78 ±0.201 d	2.34 ±0.406 e	2.019 ± 0.009 e	1.191 ± 0.003 f	0.0012 ± 0.0002 e	0.0015 ± 0.0005 d
4	5.71 ±0.255 b	12.38 ±0.567 d	2.119 ± 0.01 d	1.391 ± 0.003 e	0.0097± 0.00025 d	0.0112± 0.0003 cd
8	6.66 ±0.388 a	14.25 ±0.548 d	2.267 ± 0.007 c	1.806 ± 0.005 d	0.016± 0.0001 c	0.027 ±0.0004 bc
16	6.36 ±0.506 a	160.67 ± 14.31 a	2.354 ± 0.003 a	3.743 ± 0.005 a	0.026± 0.0002 b	0.0331± 0.0002 ab
32	4.67 ±0.117 c	113 ± 4.726 b	2.309 ± 0.011 b	3.463 ± 0.007 b	0.026± 0.0001 b	0.044± 0.0037 a
40	4.298 ±0.108 c	89.83 ± 1.302 c	1.831 ± 0.035 f	2.717 ± 0.018 c	0.044± 0.0001 a	0.038± 0.022 ab

of heavy metals in the soil is low and does not normally cause any significant phytotoxic effects (Gratao et al., 2005). Nickel (Ni) has one essential role in plants, which is to form the active metallocenter of the hexameric enzyme urease (EC 3.5.1.5.) (Gerendas et al., 1999). Ni is not toxic at low concentrations, but it becomes toxic at high concentrations (Poulik, 1997; Yan et al., 2008). In the present study, the changes of growth parameters in plants exposed to nickel are shown that low concentrations of Ni usually lead to increasing growth. A similar result was observed in *Lycopersicon esculentum* Mill. (Gad et al., 2007) *Albizia* (Tripathi et al, 1999). Numerous reports demonstrate that Ni supply increases the yield of crop plants (Sengar et al., 2008). The first indications of importance of Ni in increasing the crop yield was given by Roach and Barclay (1946). In culture experiments with pine seedling, Ni has proved to be an essential element for growth (Polacco, 1977a). Presowing treatment of wheat seeds with Ni at a concentration of 100 ppm resulted in maximal growth of shoots and roots. Nickel treatment increased the mass of root as well as of the aerial parts of cotton plants. Growth and development of paprica and tomato plants are stimulated by the application of Ni at a concentration less than 1 µg L^{-1} (Sengar et al., 2008).

Accumulation of MDA as a consequence of lipid peroxidation, the main effect of oxidation damage, has also been observed during the imposition of other abiotic stresses such as those responsible for seed deterioration in sunflower (Bailly et al., 1996) and in chill-stressed roots of *Coffea arabica* L. (Queiroz et al., 1998). These results demonstrate that MDA content increased in 32 and 40 µM $NiSO_4$ and in low concentrations MDA decreased. It can also conclude that there is a direct correlation

between MDA and increasing $NiSO_4$ in the environment. In plants treated with $NiSO_4$ the chlorophyll content was not decreased significantly. As described earlier (Mishra and Kar, 1974) an increase in total chlorophyll content with 100-200 µg g^{-1} Ni has been observed in presowing treatment of maize and oat seeds. Presowing treatment of potato tubers with Ni solutions of low concentrations increased the synthesis of chlorophyll, carotene and xanthophyll. There are a number of reports that Ni ion (Ni^{2+}) prevents the destruction of chlorophyll during senescence of detached wheat and rice leaves in the dark (Roach and Barclay, 1946; Singh et al., 2001). With increasing $NiSO_4$, photosynthetic rate decreased and respiration rate increased. The metal is known to inhibit photosynthesis and over all gas exchange in some plants such as maize and sunflower (Lo and Chen, 1994; Mishra et al., 1973).

Sheoran and Singh (1993) have suggested that the metal inhibit photosystem (PS) II more effectively possibly at the oxidizing site. $NiCl_2$ at lower concentration increased the respiratory rate of maize mitochondria but at high concentrations the respiratory reaction was blocked (Miller et al., 1970). Observed changes in RWC in plants treated with high concentrations of $NiSO_4$, showed development of water stress. Heavy metal induced changes in water relations of plants which have been reviewed recently (Shah and Dubey, 1997; Poschenrieder and Barcelo, 1999). Increase in stomatal resistance or decrease in stomatal conductance has been reported in plants exposed to excess supply of Ni^{2+} (Carlson et al., 1975; Alia and Pardha Saradhi, 1991). Increased accumulation of soluble sugar, which has been ascribed an osmoregulatory role, provides added evidence for development of water stress in $NiSO_4$ treated plants. The significant increase of accumulation of total proteins in all the plants treated with $NiSO_4$ was observed (Table 5). As described earlier (Dixon et al., 1980) Ni is a component of urease enzyme and Ni is an essential element not only of nitrogen metabolism but also for protein synthesis in higher plants (Karlowski, 1990).

The study results showed that Ni and Fe contents of the leaves and roots increased in plants were treated with $NiSO_4$. Ni is very mobile and can be accumulated in vegetative and reproductive parts (Soon et al., 1980). During vegetative growth, most of the Ni is translocated and accumulated in leaves (Sengar, et al., 2008). In the present study, in 40 µM $NiSO_4$, the accumulation of Ni in leaves was higher than that of roots; however, Fe content in roots of plants treated with nickel sulfate was higher than that of leaves. Also, Mn content in low concentrations of $NiSO_4$ increased but in 40 µM $NiSO_4$, the Mn content decreased, which is supported by Khalid and Tinsley (1980). Manganese (Mn), copper (Cu) and Zinc (Zn) concentrations were also higher in the presence of 50 mg Ni L^{-1}. One thousand mg Ni L^{-1} induced symptoms of Ni toxicity and this effect may account for the lower concentration of potassium(K) in the shoots and roots of plants exposed to this concentration, as well as for inducing higher concentration of soluble protein in the shoots (Sengar et al., 2008).

It is concluded that sunflower (*H. annuus* L.) plants are tolerant to 40 µM $NiSO_4$ and their growth is higher in low concentrations of nickel sulfate than that of plants grown in control conditions.

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