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EFFECT OF NICKEL ON SEED GERMINABILITY OF SOME ELITE SUNFLOWER (*HELIANTHUS ANNUUS* L.) CULTIVARS

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Abstract

Seeds of 5 sunflower cultivars viz., Nstt-160, Mehran-II, Hyssun-33, M-3260 and SF-187 were exposed to a range of nickel levels (0, 10, 20, 30, 40, 50 and 60 mg L^{-1}) prepared in Hoagland's nutrient solution. The low concentration (10 and 20 mg L^{-1}) of nickel significantly promoted seed germination and improved early seedling growth while higher levels (40, 20 and 60 mg L^{-1}) caused a significant inhibition in germination and resulted in a considerable delay to achieve 50% germination. Although, plumule and radicle length, and fresh and dry weights of germinating seeds were also improved at lower concentrations, they were significantly reduced in all cultivars at higher levels of nickel. Magnesium (Mg) contents in plumule and radicle also increased at lower levels of nickel but they were significantly reduced at higher Ni levels. However, plumule and radicle K contents consistently reduced with increasing concentration of Ni in the growth medium. The maximum nickel tolerance level in all cultivars appeared to be 40 mg L^{-1} at which all parameters were reduced more than 50%. Overall, cv. Hysun-33 was more tolerant whereas, Nust-160 the sensitive. All other cultivars were intermediate in nickel tolerance at the germination stage.

Introduction

Nickel, being one of the important metal pollutants is of considerable concern, because its concentration is rapidly increasing in soils of different parts of the world (Echevarria *et al*., 1998; Faryal *et al*., 2007; Atiq-ur-Rehman & Iqbal, 2008). Nickel is emitted in the environment from a variety of natural and anthropogenic processes and consequently its concentration becomes considerably high in the environment. Natural process includes weathering of minerals and rocks, whereas different compounds of nickel (such as nickel acetate, nickel carbonate, nickel hydroxide and nickel oxide) are used in a variety of industrial process (Cempel & Nikel, 2006). These compounds ultimately accumulate in the soil and environment, and can be easily taken up by plants. Thus, they can enter in the food chain and cause deleterious effects on animals and human lives (Nieboer & Nriagu, 1992; Cempel & Nikel. 2006).

The lower concentration of nickel has been reported to play a variety of roles in plant growth and metabolism. However, it shows deleterious effects at high concentration (Eskew *et al*., 1983; Kochian, 1991; Welch, 1995; Hasinur *et al*., 2005). Although, the toxic effects of excess nickel are evident through crop development, the germination stage is regarded as the most sensitive particularly to nickel toxicity (Viano *et al*., 2005). For example, the increasing concentration of Ni^{2+} has been shown to inhibit seed germination and seedling growth of different plant species (Espen *et al*., 1997; Viano *et al*., 2005, Farooqi *et al*., 2009). The Ni-induced growth inhibition has been ascribed to down-regulation of protein synthesis and activities of some key enzymes responsible for mobilization of food reserves taking place during seed germination (Foy *et al*, 1978; Bishnoi *et al*., 1993). In addition, Ni is known to be an active competitor of 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; 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, tolerance potential of different species varies greatly within different species or even among different genotypes of a same species. Thus, the objectives of this study were to evaluate the intra-cultivar differences for nickel tolerance in sunflower at the initial growth stages such as germination stage which is more vulnerable to any stress than latter growth stages of most plant species. In addition, the effect of Ni stress on uptake of some important nutrients such as K and Mg was also assessed in this study.

Materials and Methods

The achenes (seeds) of five sunflower cultivars, Nstt-160, Mehran-II, Hyssun-33, M-3260 and SF-187 were obtained from the Plant Genetic Resource Institute (PGRI) of the National Agricultural Research Centre (NARC), Islamabad. Seeds were surface sterilized with 0.1% $HgCl_2$ for two minutes and sown in Petri-plates lined with double filter paper. Different levels of nickel (0, 10, 20, 30, 40, 50 and 60 mg L^{-1}) were prepared in Hoagland's nutrient solution (Hoagland & Arnon, 1950) and 10 ml of each solution were applied to each Petri-plate. The solutions were changed every-day to ensure constant level of nickel. The Petri-plates were placed under continuous white fluorescent light (*PAR* 300 μmol m^{-2} s^{-1}) in a growth room at 25ºC ± 2ºC. The experiment was laid down in a completely randomized fashion with three replicates.

Seed germination was counted daily for two weeks. A seed was considered germinated when both plumule and radical emerged from the seeds. These values were used to calculate germination percentage. Germination index was calculated by using the formula given by Association of Official Seed Analysis (1983) whereas, time to achieve 50% germination was calculated by using the formula given by Coolbear *et al*., (1984). The plumule and radicles were detached and their lengths and fresh weights measured. For the determination of their dry weights, they were wrapped in paper bags and dried in an oven at 70^{o}C to a constant dry weight. The concentration of K and Mg was determined by acid digestion method. Dried and well-ground plumule and radicle material (0.5 g each) was digested with wet digestion method (H_2SO_4 and H_2O_2) following the method of Wolf (1982). The concentration of K^+ was determined with a flame photometer (Jenway, PEP-7), whereas that of Mg and Ni was determined with an atomic absorption spectrophotometer (AAnalyst 300, Perkin-Elmer, Germany). The data so obtained were subjected to a two-way analysis of variance (ANOVA) using a COSTAT computer package (CoHort Software, 2003, Monterey, California). Least significance difference (LSD) values (5%) were also calculated to compare significance of interaction means.

Results

In this study, nickel stress significantly affected the percent germination, germination index and days to 50% germination of all sunflower cultivars. However, the CxT interaction term was significant only for germination index, whereas, it was non-significant for germination percentage and days to 50% germination (Table 1). All these parameters were significantly promoted in 10 and 20 mg L^{-1} Ni. Thereafter, a decreasing trend was observed and all these parameters were increasingly reduced at 50 and 60 mg L^{-1} of nickel. Of the cultivars, Hysun-33 showed better germination rate, index as well as it took less days to achieve 50% germination. However, Nustt-160 was poor in all germination parameters appraised in this study. All other cultivars showed an intermediate response to exogenous nickel application (Fig. 1).

Nickel stress had a significant effect on plumule and radicle lengths and fresh and dry weights of seedlings of all sunflower cultivars used in this study. However, CxT interaction term was non-significant for all growth parameters of seedlings except radicle length (Table 1). Although all these parameters were little improved in low levels of nickel (10 and 20 mg L^{-1}), they decreased markedly under high nickel levels in all cultivars. Hysun-33 performed better under nickel stress as compared to all other cultivars as it exhibited less decrease in all seedling growth parameters. Nustt-160 was the least tolerant as it showed a marked seedling growth reduction under nickel stress except for plumule length, which was less in M-3260. All cultivars showed severe inhibition of seedling growth above 40 mg L^{-1} Ni of the growth medium (Figs. 2, 3, 4).

K content in plumule and radicles of the Ni-stressed seedlings was significantly reduced. The CxT interaction term was non-significant for both plumule as well as radicles (Table 1). It was observed that Ni stress led to a progressive decrease in plumule and radicle K content of seedlings of all sunflower cultivars examined in this study. It was observed that the sunflower cultivar Mehran-II accumulated the maximum and M-3260 the least K contents in plumule. In contrast, in radicles, Mehran-II accumulated higher levels of K contents at the least Ni concentrations (control and at 10 mg L^{-1}) and thereafter, the decrease was higher in this cultivar as compared to Hysun-33. SF-187 accumulated the lowest amount of K in radicles of all cultivars (Fig. 5).

Nickel stress significantly reduced Mg contents in plumule and radicles of the Ni-stressed seedlings. The CxT interaction term was also highly significant for Mg contents in plumule and radicles of Ni-stressed sunflower seedling (Table 1). The Mg contents in both plumule and radicle of seedlings of all sunflower cultivars increased up to 10 mg L^{-1} nickel, afterwards, a progressive decrease in Mg contents was observed with increase in concentration of the metal in the growth medium. The sunflower cultivar Hysun-33 accumulated the maximum Mg contents in plumule and radicles, while SF-187 accumulated the minimum Mg of all cultivars. All other cultivars were intermediate in Mg contents of plumule and radicles (Fig. 6).

The Ni contents of the sunflower seedlings increased significantly under Ni-stress (Table 1). The uptake of nickel appeared to be a concentration dependent phenomenon as it increased multi-folds under high nickel application. In plumule, Nustt-160 accumulated the maximum Ni contents, whereas M-3260 accumulated the minimum of all lines. The sunflower cultivar Mehran-II was the second lowest in plumule Ni contents while Hysun-33 the second highest in this attribute. In radicle, SF-187 accumulated the maximum Ni-contents, while Mehran-II the second highest in Ni contents. The lowest Ni content in radicle was observed in Hysun-33, while Nust-160 was the second lowest in radicle Ni contents. The sunflower cultivar M-3260 was intermediate in Ni contents in both plumule and radicles (Fig. 7).

Table 1. Analysis of variance of the data for germination and ionic relations in germinating seeds of five sunflower cultivars subjected to varying levels of Ni.

Sources of variation	d.f.	Germination percent	Germination index	Days to 50% germination (t_{50})	Plumule length	Radicle length
Cultivars (C)	4	3782.38***	30.84***	20.77***	22.13***	7.46***
Treatments (T)	6	1509.84***	15.51***	22.13***	8.32***	10.01***
Interaction	24	39.60na	0.69*	0.64ns	0.12ns	0.12*
Error	**70**	**60.95**	**0.38**	**0.55**	**0.10**	**0.07**
		Plumule fresh weight	**Radicle fresh weight**	**Plumule dry weight**	**Radicle dry weight**	**Plumule K content**
Cultivars (C)	4	0.032***	0.0050***	0.00077***	0.000101***	210.28***
Treatments (T)	6	0.042***	0.0035***	0.00069***	0.000068***	94.32***
Interaction	24	0.001ns	0.0001ns	0.00001ns	0.000001ns	2.30ns
Error	**70**	**0.001**	**0.0001**	**0.00001**	**0.000001**	**2.59**
		Radicle K content	**Plumule Mg content**	**Radicle Mg content**	**Plumule Ni content**	**Radicle Ni content**
Cultivars (C)	4	69.54***	6.88***	4.16***	0.0086***	0.0048***
Treatments (T)	6	210.69***	8.42***	4.58***	0.0676***	0.0238***
Interaction	24	2.95ns	0.32ns	0.12*	0.0008***	0.0002*
Error	**70**	**2.39**	**0.14**	**0.06**	**0.0001**	**0.0001**

*, **, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively. ns = Non-significant

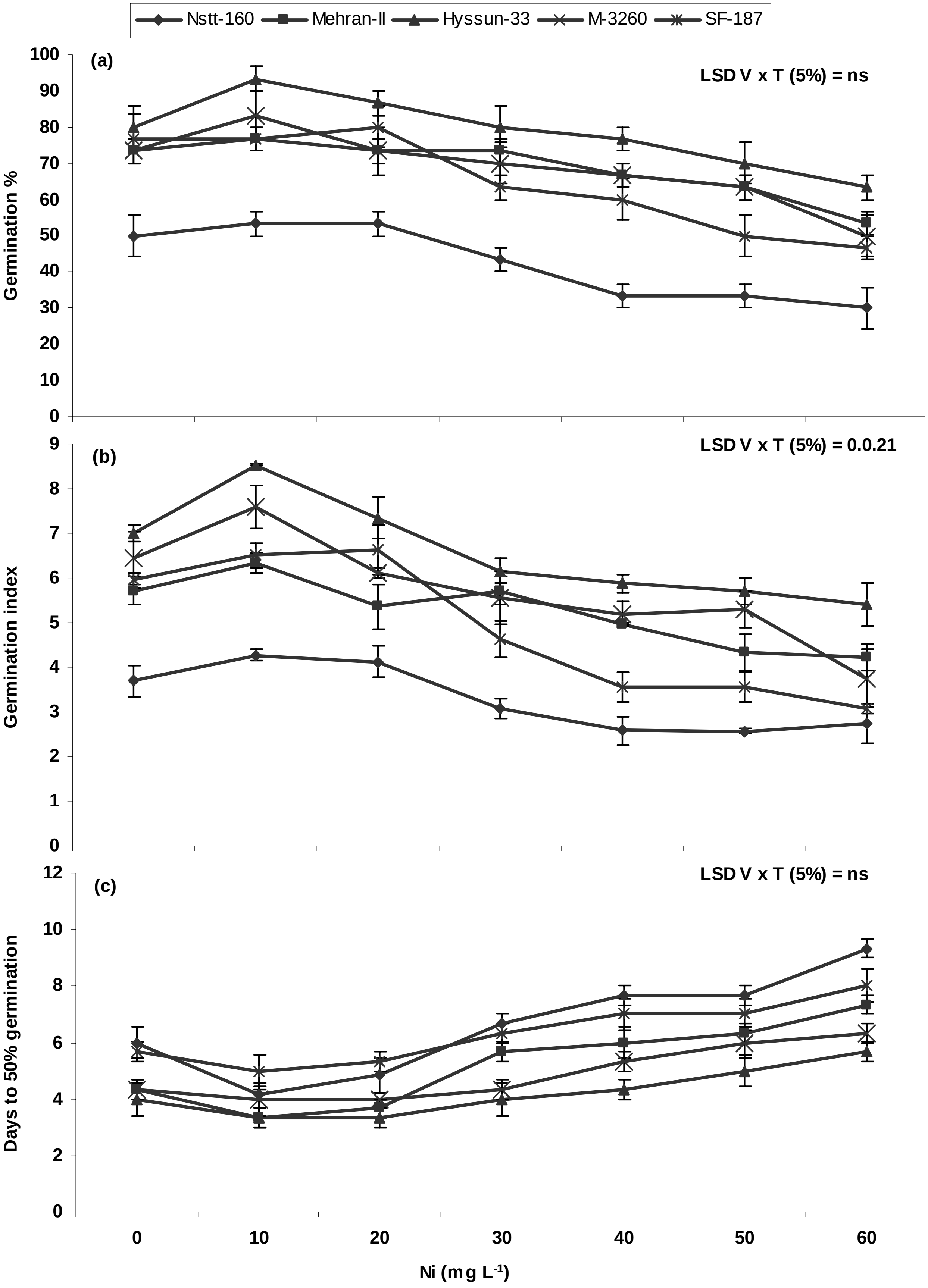

Fig. 1. Effect of different levels of nickel on germination %age (a), germination index (b) and days to achieve 50% germination (c) of different sunflower cultivars.

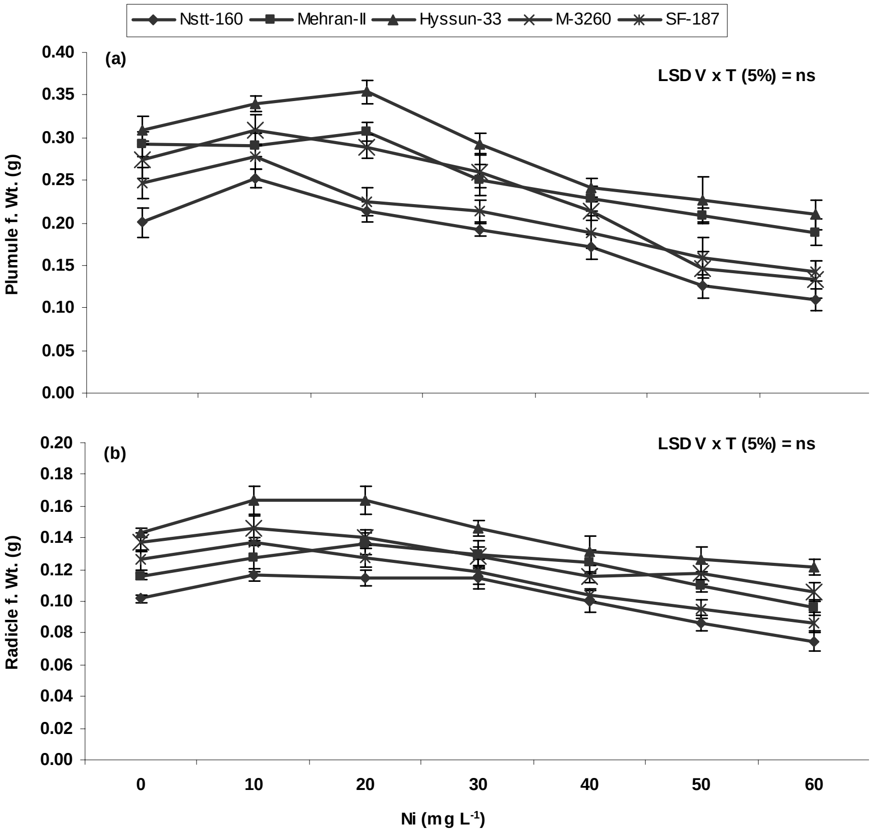

Fig. 2. Effect of different levels of nickel on plumule (a) and radicle (b) fresh weight of different sunflower cultivars.

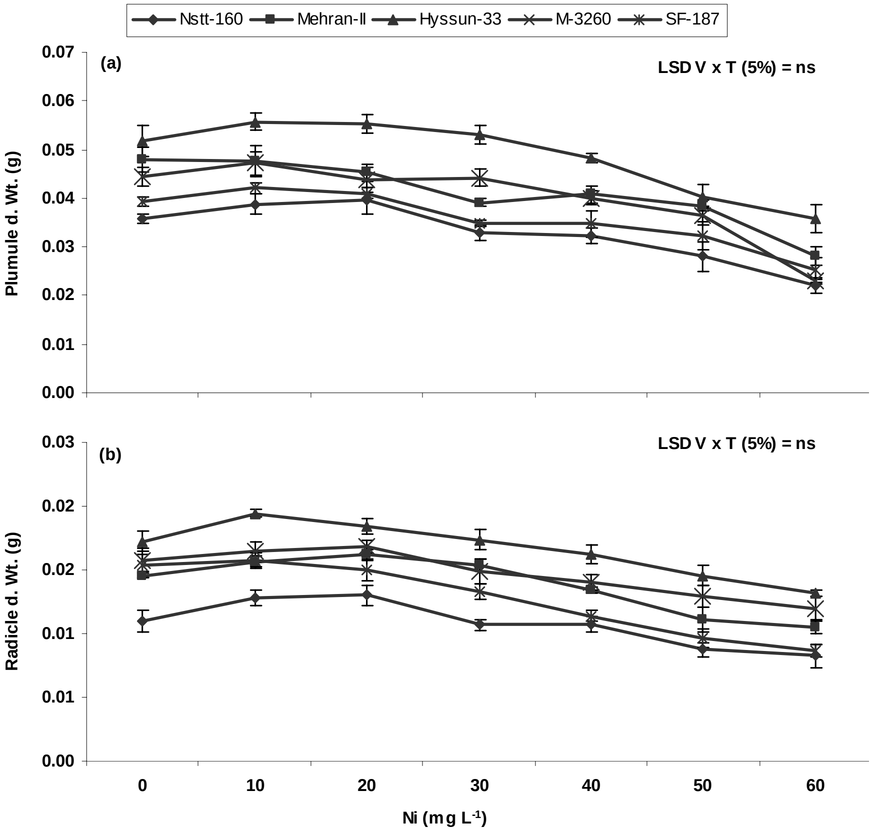

Fig. 3. Effect of different levels of nickel on plumule (a) and radicle (b) dry weight of different sunflower cultivars.

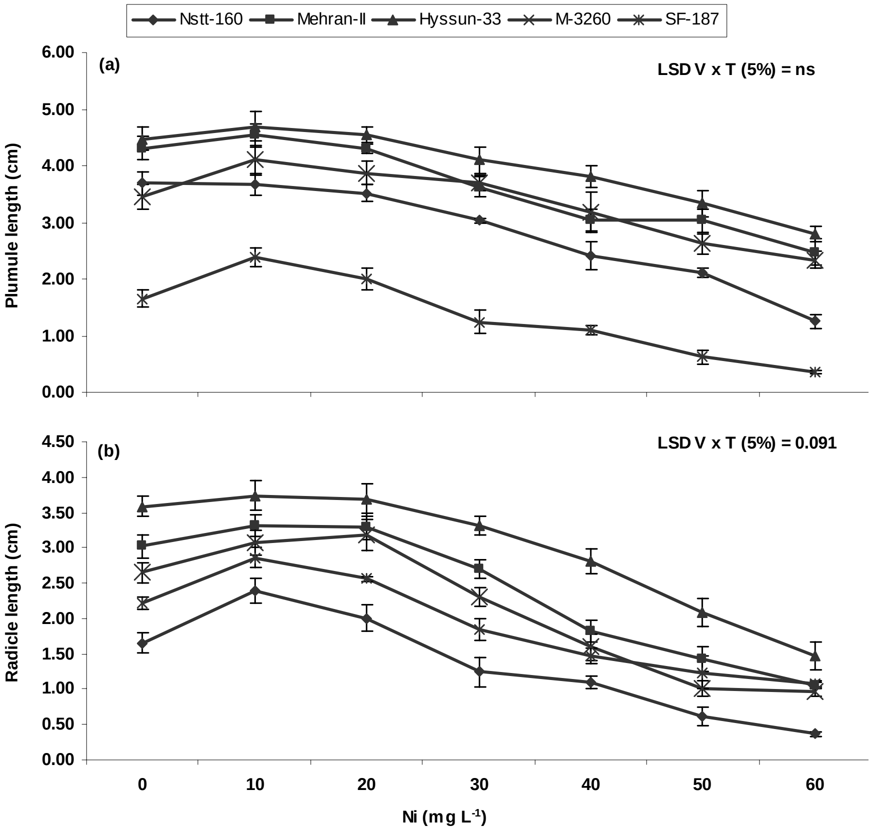

Fig. 4. Effect of different levels of nickel on plumule (a) and radicle (b) length of different sunflower cultivars.

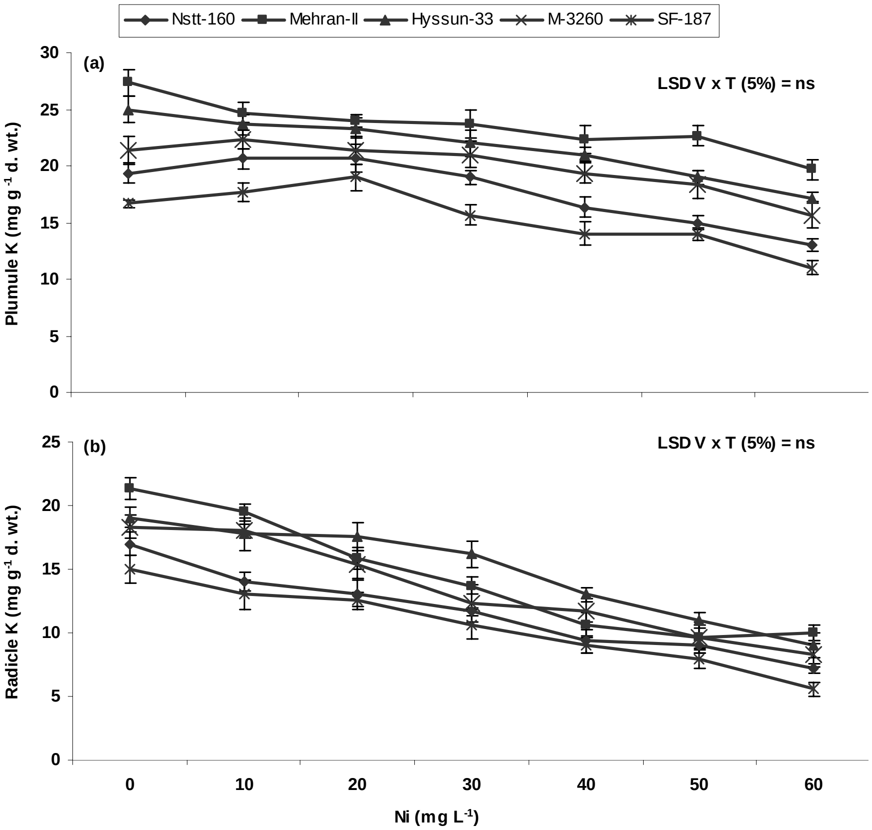

Fig. 5. Effect of different levels of nickel on plumule (a) and radicle (b) K content of different sunflower cultivars.

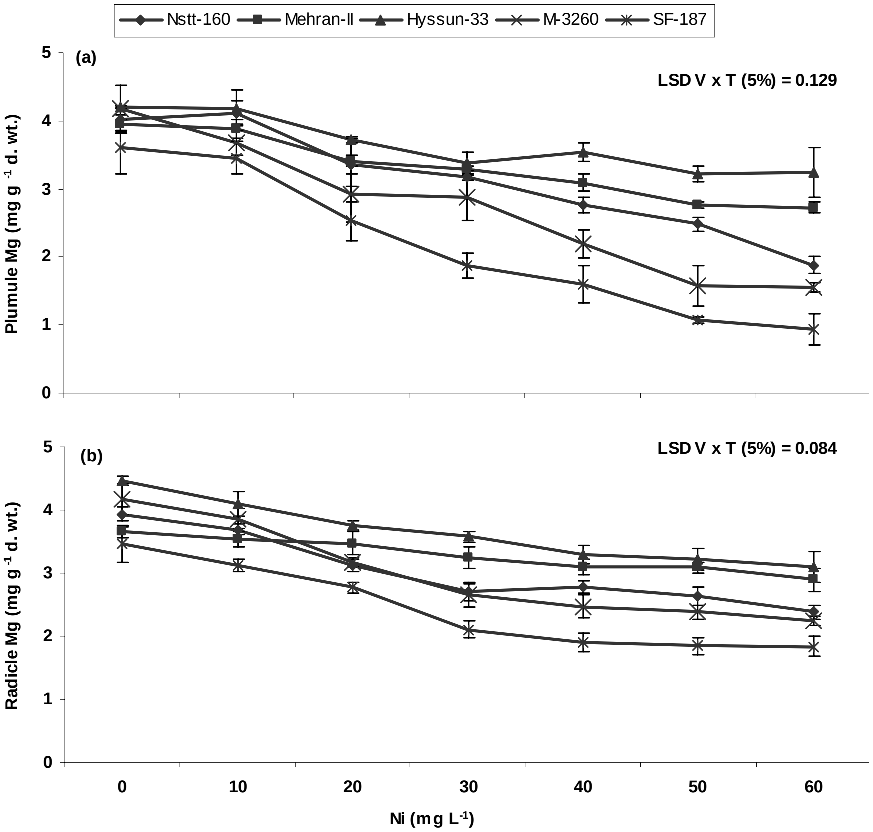

Fig. 6. Effect of different levels of nickel on plumule (a) and radicle (b) Mg content of different sunflower cultivars.

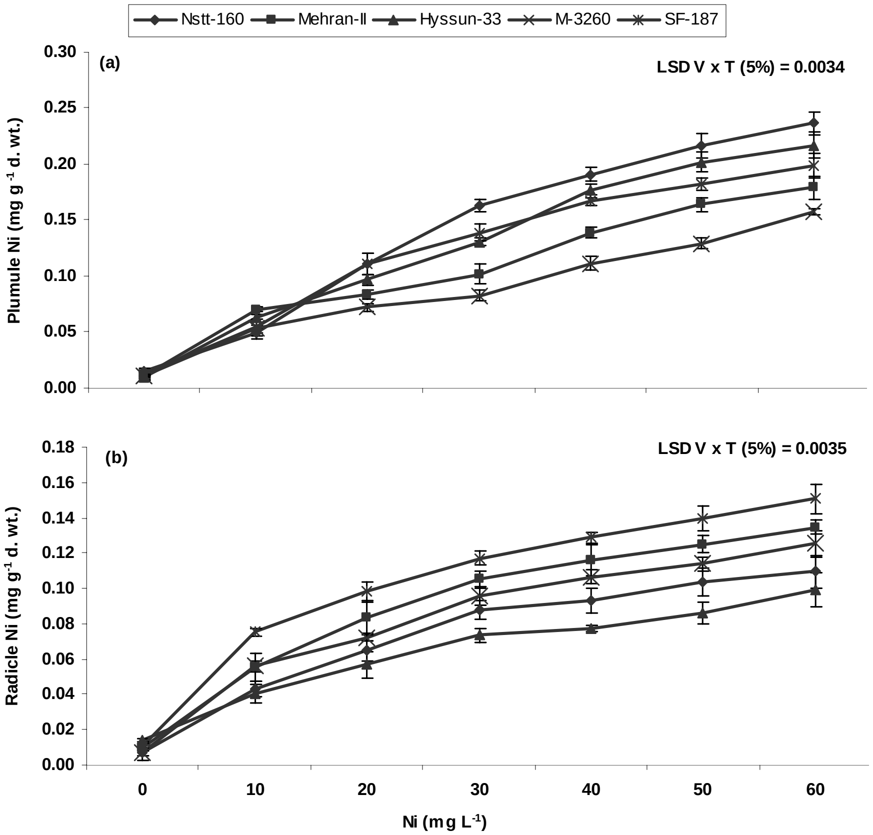

Fig. 7. Effect of different levels of nickel on plumule (a) and radicle (b) Ni content of different sunflower cultivars.

Discussion

The application of nickel at low concentrations (up to 20 mg L^{-1}) significantly promoted germination rate and index, and shortened the time to achieve 50% germination. Moreover, plumule and radicle lengths were also significantly increased at lower Ni levels. However, high levels of Ni (40, 50 and 60 mg L^{-1}) led to a marked inhibition of germination, considerable delay in achieving 50% germination, and decreased the plumule and radicle lengths and fresh and dry weights of all sunflower cultivars. Nickel has been reported to be an important micro-nutrient in a number of studies (Eskew *et al*., 1983; Brown *et al*., 1987; Asher, 1991; Kochian, 1991; Welch, 1995) and it may have beneficial effects on seed germination at very low concentration (Homer *et al*., 1991; Welch, 1995; Rout *et al*., 2000; Peralta *et al*., 2001). It has been shown that nickel is a constituent of a number of metaloenzymes such as *urease* and some *superoxide dismutases* and *hydrogenases* (Ermler *et al*., 1998; Küpper & Kroneck, 2007). Therefore, Ni is proposed to participate in some key metabolic reactions such as ureolysis (N metabolism), hydrogen metabolism, methane biogenesis and acitogenesis (Maier *et al*., 1993; Collard *et al*., 1994; Ragsdale, 1998; Mulrooney & Hausinger, 2003). In addition, lower levels of nickel have been reported to improve the nutritional status such as that of iron and other minerals and thus can lead to improvement in germination capability of seed (Mishra & Kar, 1974; Brown *et al*., 1987a; 1987b; Gerendás *et al*., 1999). Lower levels of Ni may also have a role in phytoalexin synthesis and thus confer resistance to diseases and other environmental stresses (Graham *et al*., 1985; Barker, 2006; Barker & Pilbeam, 2006; Wood & Reilly, 2007).

Despite the fact that Ni application improved seed germination of sunflower seeds at lower concentrations, the higher levels severely inhibited seed germination and uptake of micro- and macro-nutrients such as K and Mg (Nedhi *et al*., 1990; Madhava Rao & Sresty, 2000). Such inhibitory effects of high levels of Ni have been reported to be due to the inhibition in enzyme activity (protease and α-amylase), protein synthesis, carbohydrate metabolism, and mobilization of food reserves (Bishnoi *et al*., 1993; Lin & Kao, 2006; Maheshwari & Dubey, 2007). In addition, high levels of Ni have been reported to have a strong interference with uptake of mineral nutrients, thereby leading to their deficiency in germinating seed (Gabbrielli *et al*., 1990; Rubio *et al*., 1994; Molas, 1997; Ahmad *et al*., 2007). Since most of the micro- and macro-nutrients including K and Mg are involved in a variety of metabolic processes and some of them are even required as co-factor and enzyme activator, their deficiency may result in the suppression of a number of metabolic phenomena involved in regulation of seed germination (Gerendás *et al*., 1999; Barker, 2006; Taiz & Zeiger, 2006).

In conclusion, the lower levels of nickel (10 and 20 mg L^{-1}) significantly promoted seed germination and reduced the time to achieve 50% germination. However, higher levels of nickel had a significant inhibitory effect on seed germination and delayed the time to achieve 50% germination. The improvement in seed germination was found to be associated with improvement in Mg contents at lower levels, whereas reduction in seeds germination at higher levels was directly correlated with reduction in K contents and high concentrations of nickel in plumule and radicle of sunflower seedlings.

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