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Research Article

THE EFFECT OF DIFFERENT CONCENTRATIONS OF NICKEL ON GERMINATION AND GROWTH OF CORIANDER (*CORIANDRUM SATIVUM*) AND MILK THISTLE (*SILYBUM MARIANUM*) SEEDLINGS

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ABSTRACT
Heavy metal pollution is a major environmental stress that can affect plant growth. The toxic effects of nickel on germination and growth coriander (*Coriandrum Sativum*) and Milk thistle (*Silybum Marianum*) seedling were investigated for 8 days. The results of analysis of variance and mean comparison showed that with treatment of 3000 mg/l Ni to control, percentage and rate of germination were reduced significantly by 80% after 8 days. Growth rate was measured on the last day with the measurement of radicle and plumule length. Growth of the seedling parts, especially radicle, was greatly reduced for concentrations above 1000 mg/l $NiNO_3$. Also, the effect of Ni treatments on the seedling length and seed vigor of coriander and Milk thistle was statistically significant at the 5% level. Different concentrations of nickel nitrate caused a more significant decrease in growth parameters of the Milk thistle specie than the coriander plant specie.

Keywords*: *Heavy Metal, Nickel Nitrate, Plumule, Radicle, Seed Vigor

INTRODUCTION
One of the major challenges in the environment is gradually increase in the concentration of heavy metals in soils that can lead to reduce growth and production of reactive oxygen species. Heavy metals are one group of elements with a density greater than 5 grams per cubic centimeter, and they are one the most important environmental contaminants that are increasing (Benavides *et al*., 2005; Sanita and Gabbrielli, 1999). This type of pollution is mainly caused by industrial activities such as metal mining, metal refining, metallurgy, fuel, use of fertilizer and pesticide, and recycling of waste (Kabata-Pendias and Pendias, 2001). When heavy metals absorb by plants and accumulate in tissues, then they cause toxicity in two ways: 1) indirectly through competition with other essential nutrients and degradation enzymes and pigments. 2) directly damage the cell structure. The presence of heavy metals causes oxidative stress and increased production of reactive oxygen species (ROS), which in turn can cause various toxic effects on plants, such as reduced growth, reduced chlorophyll content, inhibit the activity of enzyme, damage to biomolecules as lipids, proteins and nucleic acids (Chaoui and Ferjani, 2005; Mishra *et al*., 2006). Heavy metal ions when present in large amounts in the environment absorb by plant roots and transfer to the shoots that result in impaired metabolism and reduced growth (Prasad, 2004).
In addition, large amounts of heavy metals in soil can result in decreased performance, the quality of products and increase its concentration in agricultural production which is dangerous to human health or animal consumption (Das *et al*., 1998). 53 elements of 90 elements found in nature are known as heavy metals that among them nickel can be named (Weast, 1984). Nickel is one of the natural elements and can has various forms in the environment. Soil and sediment are the primary source of nickel (Smialowicz *et al*., 1984; Smialowicz *et al*., 1988).
Studies have shown that nickel is toxic to the respiratory tract and immune system of animals (Smialowicz *et al*., 1984; Smialowicz *et al*., 1988) and has a negative impact on women's fertility and fetal development (Chashschin *et al*., 1994). Studies have shown that the required amount of nickel for human is less than 100 micrograms per day (Goyer, 1991). Nickel plays an important role in plants. At low concentrations, there are no toxic effects on plants. But in high concentrations is toxic to plants (Baycu *et al*., 2006). The amount of nickel in the soil is between 5 to 500 gr. per kg. Nickel may be

absorbed by clay minerals and compete with the cations as calcium, magnesium, iron and zinc. Thus, high levels of nickel in the root environment, may lead to Fe and Zn deficiency (Brooks, 1998).

Germination and growth of seedlings is milestone of one complete plant and also the most sensitive to changes in plant life surrounding. So study this process in plants that have been exposed to contaminants such as heavy metals is considered as solutions suitable for understanding the toxic effects on plants. Coriander (*Coriandrum sativum)* is one annual herbaceous plant of the Apiaceae. The origin of this plant is in the southwestern region of Asia (Ramadan and Morsel, 2002; Singh *et al*., 2003). Leaves and fruits of Coriander contain essential oils that are used in the food industry, pharmaceutical, etc (Ramadan and Morsel, 2002; Zargari, 1997). Milk thistle (*Silybum marianum*) has also been known to be used as food and pharmaceutical. It is a biennial plant of the Asteraceae (Zargari, 1996). Given the importance of these two species of different aspects of agronomic – medical, the purpose of this study is to observe the distribution of Ni and their toxic effects on germination and growth of seedling until to be examined the ability of this species as a purified plant.

MATERIALS AND METHODS

Healthy seeds of the coriander (*Coriandrum Sativum*) and Milk thistle (*Silybum Marianum*) sodium were to be sterilized in hypochlorite solution 20% for 10 min, then they were rinsed three times with distilled water and were disinfected in a Benomyl solution of one part in a thousand for 20 minutes. All appliances including Petri dishes and filter paper were autoclaved. This study was done in one randomized complete block design with four replications in Damghan University of Iran. For the preparation of nickel treatments, nickel nitrate was used and their concentrations were 0, 500, 1000, 3000 milligrams per liter. After preparing Petri dishes, 50 seeds were placed between two filter paper in each Petri and different Ni treatments were applied.

Distilled water was used for control treatment. Petri dishes were incubated at 25 ° C temperature. The daily numbers of germinated seeds in each plot were counted to estimate the rate of germination. The seeds were counted in each plot until the change in the number of germinated seeds was not observed for three consecutive days.

After the time of planting seeds (8 days) and the optimum growth of the seedlings, germination rate, germination percentage, radicle length, plumule length, seedling length and vigor index were measured. Germination percentage (Camberato and Mccarty, 1999), germination rate (Maguirw, 1962) and seedling vigor index (Agraval, 2005) were calculated according to the following relationships:

(1) $GP = (G / N) * 100$

GP = Germination percentage

G = the number of germinated seeds until X day

N = Total number of seeds

(2) $$GR = \sum_{i=1}^{n} (S_i / D_i) * 100$$

GR = Germination rate

S_i = The number of germinated seeds in each counting

D_i = Number of days to n counting

n = Number of numeration

(3) Seedling vigor index = the final of germination percentage * seedling length

Data were subjected to analysis of variance. Data was analyzed using Graphpad 6 software. Significance differences between means was done using Duncan test at the 5% level.

RESULTS AND DISCUSSION

The results of the analysis of variance showed that the levels of nickel nitrate had a significant effect on radicle and plumule length, seedling length, seed vigor, germination rate and germination percentage of both species. With increasing concentration of nickel nitrate from 0 to 3000 mg per liter, seed germination percentage of Coriander and Milk thistle was declined (Figure 1 & 2).

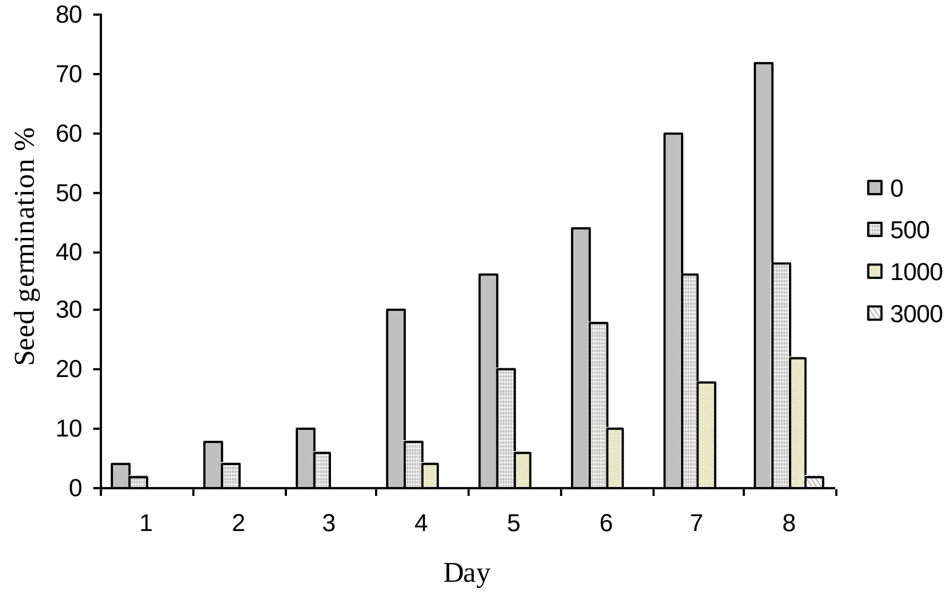

Figure 1: Seed germination percentage of coriander in various concentration of nickel nitrate (mg / l)

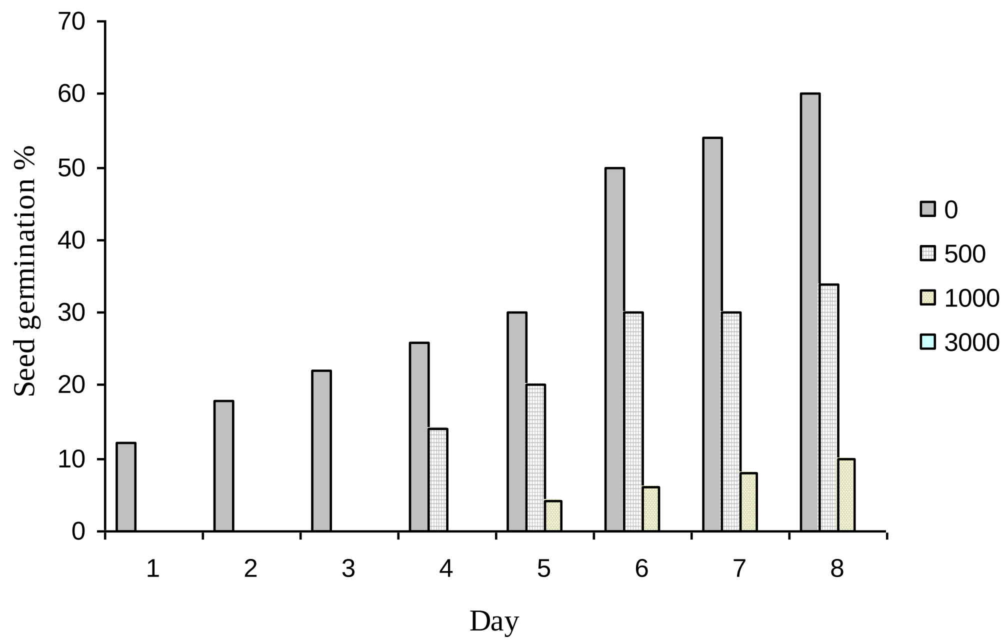

Figure 2: Seed germination percentage of Milk thistle in various concentration of nickel nitrate (mg / l)

The reduction in germination percentage of milk thistle was more significant compared with coriander (Figure 3). One of the crucial steps in the plant growth is the process of seed germination that crop production and higher productivity is determined by this step. Resistance to heavy metals on stages of seed germination and seedling growth is the key to establishing plants under restrictive conditions (Almansouri *et al*., 2001).

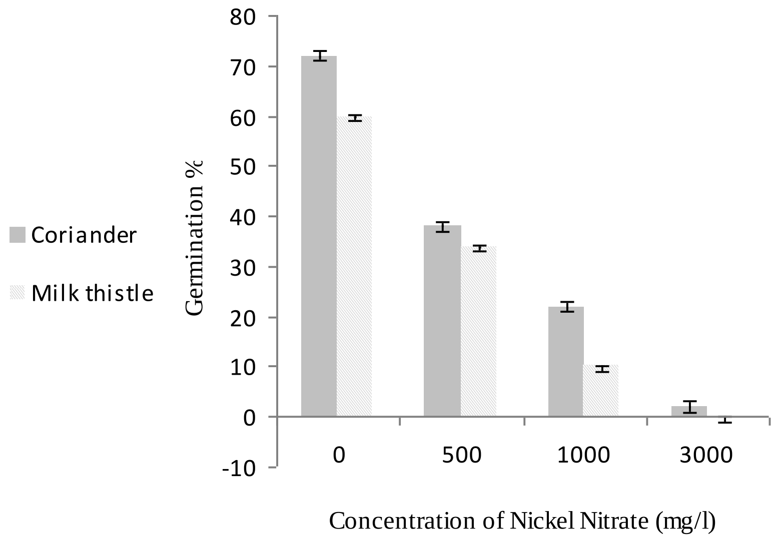

Figure 3: Interactive effects of different concentrations of nickel nitrate on seed germination (%) of coriander and Milk thistle

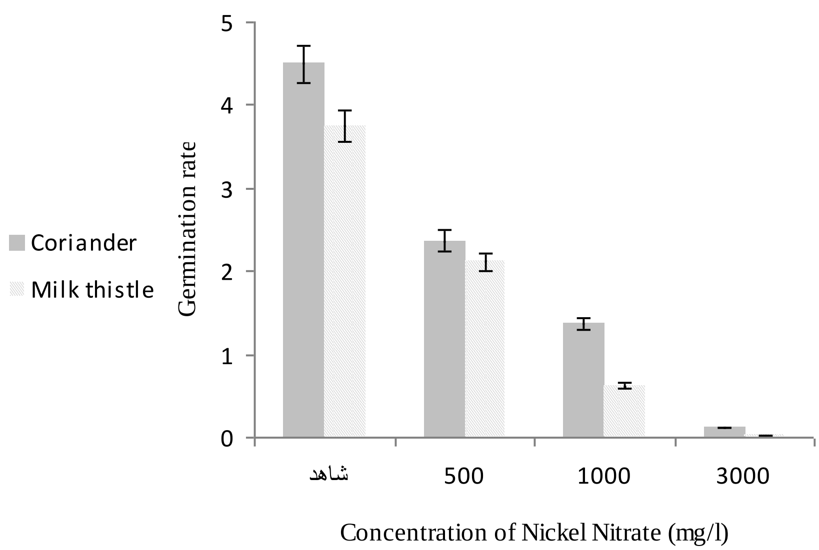

Figure 4: Interactive effects of different concentrations of nickel nitrate on germination rate of coriander and Milk thistle

Based on the results of the analysis of variance with increasing concentration of nickel nitrate from 0 to 3000 m /l, the germination rate of the Coriander and Milk thistle was declined (Figure 4). Mishra and Chouhuri (1997) stated that the inhibitory effects of heavy metals on seed germination were applied on different ways. Rahman *et al.*, (2010) have been reported that germination reduced in the presence of nickel and cobalt (Rahman and Mahmud, 2010). According Shafiq *et al.*, (2008) the use of nickel can reduce seed germination due to the decomposition of food stored in the seed (Shafiq *et al.*, 2008).

The growth of radicle and plumule of the plants slowed down for nickel concentrations greater than 500 mg / l. Generally the radicles are more affected than the plumules; so that the maximum and minimum value was the control and the treatment of 3000 (mg/l) Ni, respectively (Figure 5). Milk thistle radicle growth was stopped in 3000 (mg/l) treatment thoroughly. The radicle growth of coriander and Milk thistle was more inhibited than that of the plumule parts, which can be related to a higher content of nickel in the root, as other authors have shown (Cataldo *et al.*, 1978; Liibben and Sauerbeck, 1991). It is generally recognized that the inhibition of root growth is an early effect of heavy metals phytotoxicity (Barceló and Poschenrieder, 1990).

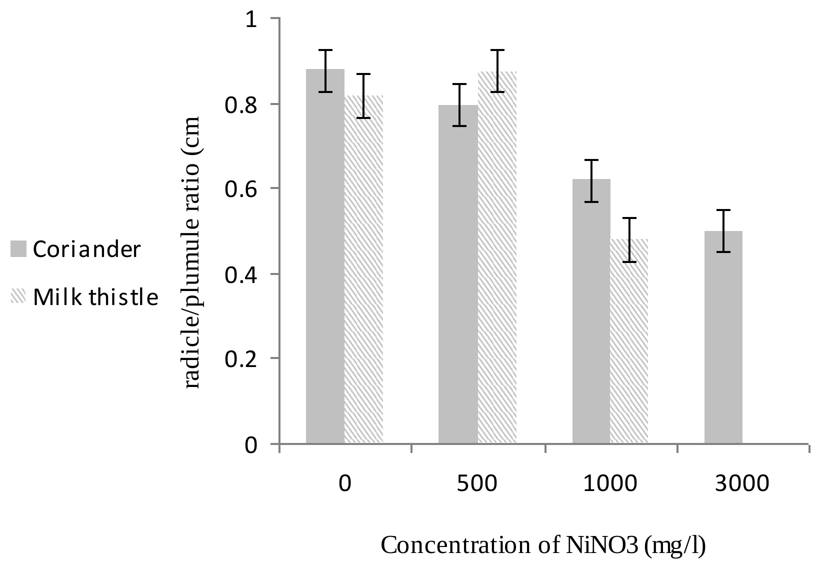

Figure 5: Interactive effects of different concentrations of Ni on length of radicle/plumule ratio of coriander and Milk thistle

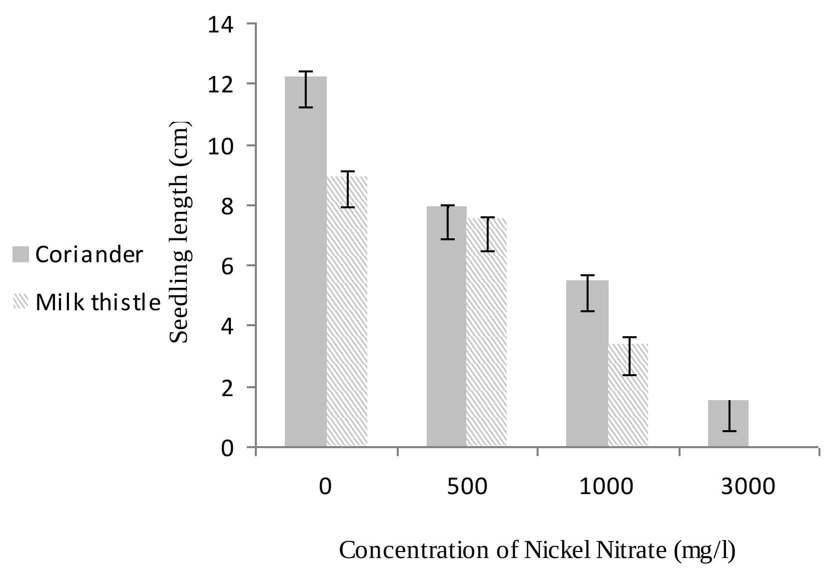

Figure 6: Interactive effects of different concentrations of Ni on seedling lenght of coriander and Milk thistle

Inhibitory the effects on root growth of species *Brassica pekinensis* and *Lulium perenne* have been reported in response to heavy metals (Wong and Bradshaw, 1982; Xiong, 1998). Note that the inhibitory effect of nickel concentration in the roots was more than shoots. That's why researchers are considered the root length as a criterion for determining the tolerance to heavy metals (Xiong, 1998). It has been shown that inhibition of the underground part of the plant is more than shoots (Bashmakov *et al*., 2005). The inhibitory effects of metals on root growth and shoot growth have been reported in many plants, although plant response varies according to the species (Peralta *et al*., 2000). It has been shown that reduction of radicle and plumule growth in the present of heavy metals could result in reduced of meristematic cells in the region of the cell membrane and certain enzymes in the cotyledon and the endosperm. Or is the involvement of heavy elements in the process of cell division (Jiang *et al*., 2001; Kabir *et al*., 2008; Liu *et al*., 2003).

In Figure 6 the comparison of the average seedlings length has been studied. Different treatments of heavy metal nickel in both species were tested, indicating that seedlings length is reduced with increasing Ni concentration; as the lowest length is related to the treatment of 3000 (mg / l) Ni element. It has been shown that nickel treatment; especially in high concentrations have had negative effects on seedling growth (Khatib *et al*., 2008).

Nickel was also affected seed vigor index (Figure 7). Compare vigor index in both species showed that with increasing Ni concentration, vigor index significantly decreased in both species. It has been shown that in concentration of 3000 mg/l NiNO3, seed survival index was close to zero for both species (Figure 7). Thus, it can be shown that the process of seed deterioration in the presence of high concentrations of nickel is accelerating. Rasouli *et al*., (1392) also showed that increasing Ni concentration was decreased seed vigor index for fox berry plant (Rasouli *et al*., 2003).

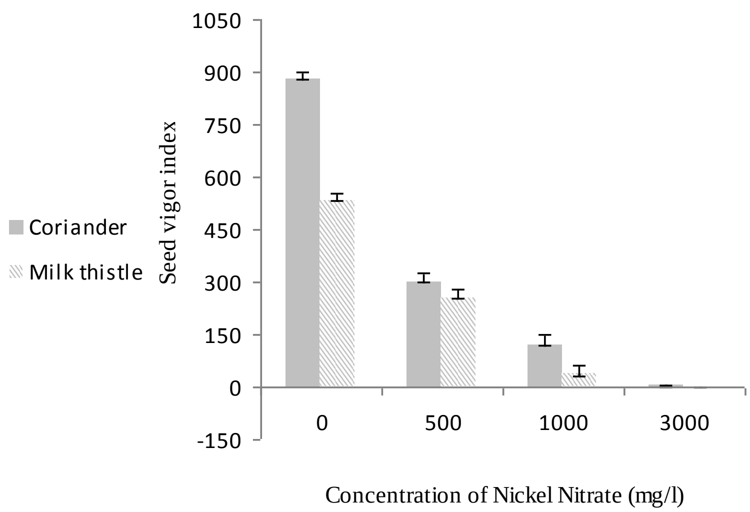

Figure 7: Interactive effects of different concentrations of Ni on seed vigor index of coriander and Milk thistle

Mechanisms of plant resistance to heavy metals are to separate toxic metals from their main metabolic pathway and their accumulation in the plant. Heavy metals accumulation in the roots reduces water absorption and nutrients, and metabolic activity; as a result reduce root growth (Cheng and Huang, 2006; Pandey and Sharma, 2002). These results are consistent with the findings of many researchers (Bashmakov *et al*., 2005; Gulfraz *et al*., 2003; Jeliazkova *et al*., 2003; Mahmood *et al*., 2005; Peralta *et al*., 2000).

Conclusion

The overall results of this study indicate that different levels of nickel nitrate have negative effects on growth traits in coriander and Milk thistle seedlings; So that high concentrations of nickel stopped radicle growth in the final days of the experiment and causes radicle death. Also, seed vigor index and seed germination were strongly influenced by the nickel contamination. The results indicate that the inhibitory effect of high concentrations of nickel on growth traits in Milk thistle seedling was more dramatic than coriander seedling. Given the important species of medicinal plants which are used widely in the pharmaceutical and healthcare industries, this research can be understood in terms of mechanisms of action of nickel concentrations and finding solutions to prevent the penetration of these elements to plants that are important for medicinal value. The results also indicate that probably, these species particularly coriander may be used in areas with low to moderate pollution of nickel metal that can also properly considered as phytoremediation. However, the review and further testing is required.

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