Seed Germination and Growth Response of Chicory (*Cichorium intybus* L.) to Copper Oxide Nanoparticles

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ABSTRACT

Higher plants strongly interact with their atmospheric and terrestrial environments and are expected to be affected as a result of their exposure to nanoparticles. In this study, the effects of different concentrations of nanosized CuO on seed germination, seedling growth and photosynthetic pigments of chicory were investigated in a completely randomized design (CRD) with four replications. The experimental treatments included four concentrations of nanosized CuO (10, 50, 100, and 500 ppm) and control without any CuO. Results indicated that among the chicory germination indices, only germination percentage and weighted germination index were affected positively by nano CuO treatments. All of nano CuO treatments increased chlorophyll and carotenoids contents but this increase was not significantly different. It is concluded that treatment with nano CuO treatments have no effect on germination indices and photosynthetic pigments of chicory in comparison with the control.

Keywords: Chicory, CuO nanoparticles, germination percentage, seedling dry weight, seedling length.

INTRODUCTION

In recent times, nanotechnology is regarded as a revolutionary science with predicted evolution within the next decades that may have equivalence with the ones observed for other industries, such as that recorded for the computer industry during the second half of the last century or earlier with the automobile industry. This emerging nanoparticles (NPs) industry is expected to contribute to diverse products and services and to serve multiple consumers' purposes. However, and despite the success of nanotechnology, the release of NPs to the environment remains unknown, mostly due to the lack of scientific knowledge concerning the potential health and environmental risks associated with nanomaterials (NMS). The effects of NPs have been described in a wide variety of organisms, such as microorganisms (Pelletier et al., 2010; Dimkpa et al., 2011), protozoa (Mortimer et al., 2010), invertebrates (Zhao and Wang, 2011; Valant et al., 2012) and vertebrates (Federici et al., 2007). However, interactions of NPs with plants and other organisms that share similarities with plant cells, such as algae, have been poorly studied, and as a result the general consequences of NPs exposure for plant cells remains unclear (Zhang et al., 2012). The lack of data results in a defective understanding of how NMs are transferred and accumulate in the various food chain levels (Kahru and Dubourguler, 2010). Copper is an active transition metal, involved in many redox processes in plant and animal cells. In plants, copper is a component of regulatory proteins, participates in electron transport in the photosynthetic and respiratory

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chains, and is a cofactor of phenol oxidases, ascorbate oxidase, and superoxide dismutase (Yruela, 2005).

Plants need only trace amounts of copper, hence at high concentrations it becomes toxic. Free copper ions can unspecifically bind to thiol groups of enzyme proteins, which results in the loss of their secondary structure and, therefore, activity (Nekrasova and Maleva, 2007). Copper also exerts its toxic action through the Fenton reaction, i.e., generation of hydroxyl radicals catalyzed by the metal (Yruela, 2005). At high concentrations, copper causes damage to thylakoid membranes, thereby disturbing the functioning of photosystem II and the water oxidizing complex of chloroplasts (Pätsikkä et al., 2002; Yruela, 2005). The action of copper nanoparticles on plant cells has not been studied sufficiently, and the available results are ambiguous. For example, it is known that nanopowders are successfully used as microfertilizers and pesticides (Raikova et al., 2006). It has also been shown that copper nanoparticles are biologically accessible to mung bean and wheat germs (Lee et al., 2008). They exert their toxic effect by penetrating directly into the cell, supposedly by causing oxidative damage to cell structures and molecules. The increased production of nanomaterials and their application in various fields, inevitably lead to additional ecological impact on the environment, which increases the relevance of studies on the bioaccumulation of nanoparticles in plants and animals (Bogatikov, 2003). The purpose of this study was to analyze germination features and growth of *Cichorium intybus* L. under copper oxide nanoparticles.

MATERIALS AND METHODS

Chichory seeds were taken from the Pakan Bazr Company, Isfahan Province, Iran. Copper oxide nanoparticles was supplied by Nutrino Company (Tehran, Iran). The size and topography of Copper oxide nanoparticles (Figs. 1 and 2) were determined by scanning tunneling microscope (STM) in the Central Laboratory of Ferdowsi University of Mashhad, Iran. X-ray diffraction (XRD) pattern of copper oxide nanoparticles was shown in Fig. 3. The XRD measurement showed that the used Copper oxide nanoparticles were made by tenorite.

Experimental Design

A completely randomized design(CRD) with four replications was carried out to study the effect of different concentrations of copper oxide nanoparticles on seed germination of chicory,. The experimental treatments included four concentrations (10, 50, 100, and 500 ppm) of copper oxide nanoparticles and untreated control (without copper oxide nanoparticles). The experiment was conducted under laboratory conditions with natural light and an average temperature of 25±1°C at the Faculty of Science, Mashhad Branch, Islamic Azad University, Mashhad, Iran, in 2014. One hundred seeds of similar size were randomly selected and placed on moistened paper as four groups of seeds in Petri dishes, and then 5 ml of each concentration treatment was added to each Petri dish. For the control, only distilled water was added to Petri dishes. Germination test was performed according to the rule issued by the International Seed Testing Association. All concentrations of copper oxide nanoparticles and the control were run at the same time and consequently under equal light and temperature conditions. The number of germinated seeds was noted daily for 7 days. Seeds were considered as germinated when their radicle showed at least one mm length. In this study, we used following germination parameters: Germination percentage (GP, %), germination rate (GR), relative germination percentage (RGP), mean germination time (MGT), germination index (GI) and weighted germination index (WGI). Final percentage germination (GP) for each treatment was calculated after seven days. The germination index (GI) is

based on number of seeds that germinated and the germination rate (Wu and Du, 2007).

$GP = 100 \times GN / SN$

GN is the total number of germinated seeds. SN is the total number of seeds tested

$GR = \sum G_i / \sum n_i G_i$

Gi is the number of seeds germinated on day I. ni is day I

RGP = GP treatment / GP control × 100

$GI = (\Sigma(N - i) \times G_i) \times 100) / (N \times GN)$

Where i is the number of days since the day of sowing and Gi is the number of seeds germinated on day I.

$WGI = [N \times n_1 + (N-1) \times n_2 + (N-Z) \times n_3 + ...] / N \times N'$

where n1, n2, …, n7 are the number of seeds that germinated on first, second, and subsequent days until the 7th day, respectively; N is total days of experiment; N′ is the total number of seeds placed in incubation.

Vigor index = germination% × seedling length (root + shoot)

After an incubation period of 7 days, plumule and radical length of seedlings was measured. For dry biomass measurement, the 7-day seedlings were first weighed; then placed in oven at 80°C for 48 h, and weighed after drying.

Pot Experiments. The experiment was conducted at the greenhouse of the Faculty of Science , Islamic Azad University, Mashhad, Iran. The design of the experiment was a randomized complete block with four replications. Chicory seeds were sown in pots (30 cm × 40 cm) filled with equal quantity of soil, watered to field capacity and placed in a greenhouse under controlled conditions: day/night photoperiod: 16/8 h; temperature (day/night), 24/20 ± 1°C. Leaves were sprayed with five concentrations of nano CuO (0, 10, 50, 100, and 500 ppm). The experiment was carried out two times. After 45 days from sowing, plants were uprooted gently along with the whole soil mass. Leaf photosynthetic pigments content was measured by Arnon method (1949).

Data Analysis

Significant differences for all statistical tests were evaluated at the level of $P \leq 0.05$ in ANOVA. All data analyses were conducted using MINITAB for Windows, Version 15.0

RESULTS

When chicory seeds were exposed to nano CuO, no significant difference was found between the germination percentage of nano CuO treatments and control. For seeds grown in the control media (without any nano CuO), the germination percentage was 97%. The lowest (92%) was reported for seeds treated with 10 ppm of nano CuO, while the highest germination percentage (100%) were reported for the seeds treated with 100 and 500 ppm of nano CuO (Table 1). The highest germination rate (38.39% Day-1) was shown in 100 and 500 ppm nano CuO which were not significantly different from other treatments. The lowest mean germination time (5 day) was found in the 100 and 500 ppm concentration nano CuO, and the highest (5 day) was shown in the 10 ppm nano CuO treatment which was not significantly different from other treatments. In the media containing 100 and 500 ppm nano CuO, the relative germination percentage (103.59) were higher than other treatments, but this difference was not significantly different (Table 1). The germination index of chicory seeds was not affected by different concentrations of nano CuO. The highest weighted germination index (3.97) was found in the 500 ppm nano- CuO treatment and the lowest (3.56) was shown in the 10 ppm treatments which were significantly different from other treatments of nano - CuO (Table 1).

Table 1 Effect of different concentrations of copper oxide nanoparticles on seed germination of Chicory

Nano-CuO Concentration(ppm)	GP(%)	RGP(Day)	GR(%Day^{-1})	MGT(Day)	GI	WGI
10	91.62 b	94.97a	34.14a	5.015 a	114.72a	3.56b
50	98.32ab	101.79a	37.24 a	5.009a	114.56a	3.87a
100	100 a	103.59a	38.39a	5.004a	114.42a	3.96a
500	100 a	103.59a	38.39 a	5.004a	114.42a	3.97a
Control	96.65ab	------	36.51a	5.009a	114.64a	3.80ab

Means in each column followed by similar letters are not significantly different at the 5% probability level using Tukey's multiple range test

The effect of studied treatments on plumule and radicle length was not significant. The plumule length for all treatments of nano CuO was higher than the control, except for treatment of 100 ppm. Treatments of 50 and 500 ppm of nano CuO decreased the radicle length in comparison with the control (Table 2).

Table 2. Effect of copper oxide nanoparticles concentrations on seedling growth of Chicory

Nano-CuO Concentration(ppm)	Plumule Length(cm)	Radicle Length(cm)	Seedling Fresh Biomass(g)	Seedling Dry Biomass(g)	Vigor Index
10	3.04 a	5.19 a	0.30a	0.003a	7.54a
50	3.21a	4.58a	0.11a	0.003a	7.67a
100	2.71 a	5.69a	0.11a	0.004a	10.01a
500	3.03a	4.87a	0.11a	0.004a	7.97a
Control	3.01a	4.88 a	0.09a	0.003a	7.63a

Means in each column followed by similar letters are not significantly different at the 5% probability level using Tukey's multiple range test

The lowest seedling fresh biomass was found in the control, which was not significantly different from other treatments. Treatments did not significantly affect seedling dry biomass. The lowest seedling dry biomass (0.003 g) was found in the control, 10 and 50 ppm concentration nano CuO, and the highest was shown in 100 and 500 ppm treatments (0.004 g) (Table 2). Vigor index was not affected significantly by nano CuO concentrations (Table 2).

The chlorophyll a content of chicory plants showed an almost linear increase, in response to the increase in nano CuO concentration, which ranged from 0 ppm (the control) to 500 ppm but no significant difference was observed (Table 3).

Table 3 Effect of different concentrations of copper oxide nano particles on photosynthetic pigments of *Chicory* leaves

copper oxide nano particles Concentration(ppm)	Chl.a(mgg^{-1}FW)	Chl.b(mgg-1F)	Chl.a+b(mgg-1FW)	Total chl. (mgg-1FW)	Carotenoide(mgg-1FW)
10	0.5589a	0.4858a	1.0802a	1.2026a	0.4011a
50	0.5478a	0.4161a	0.9639a	1.3403a	0.2039a
100	0.5924a	0.4282a	1.0119a	1.1260a	0.3596a
500	0.7207a	0.5404a	1.2612a	1.4037a	0.4736a
Control	0.4862a	0.3717a	0.8443a	0.9395a	0.3251a

Means in each column followed by similar letters are not significantly different at the 5% probability level using Tukey's multiple range test

Chlorophyll a content was greater than that of chl.b in all studied plants. In comparison to the control, chlorophyll b increased by 31% after treatment with 500 ppm nano CuO; however, it did not significant differ from other treatments. Higher concentrations of nano CuO (500 ppm) increased the chlorophyll a+b and total chl. content compared to the control (Table 3). The highest carotenoid content was measured in the treatment of 500 ppm which was not significantly different from the control and other treatments (Table 3).

DISCUSSION

The biological accessibility of copper nanoparticles to plants has previously been shown in other species, such as mung bean and wheat (Lee et al., 2008) and it was noted that the suspension of nanoparticles remained stable in the course of the experiment. Their mechanism of entry into the plant cell has not been studied sufficiently. It is possible that nanoparticles penetrate the cell wall via the plasmodesmata and then behave as in the animal cell (Garnett, 2007). The impact of nanoparticles depends on the size and/or the shape of the particles, the applied concentrations, the specific conditions of experiments, and more importantly the plant species studied (Lin and Xing, 2007). In a separate study, the EC50 (effective concentration that exhibits half of its maximal effects) dose of nano CuO for seed germination was found to be 13 mg L-1 for lettuce, 398 mg L- 1 for radish, and 228 mg L- 1 for cucumber (Wu et al., 2012). In the present investigation, three different concentrations of nano CuO suspensions (low, 10 ppm; medium, 50 and 100 ppm and high 500 ppm) were selected.

The present study revealed that the copper nanoparticles had no effect on the seed germination traits of the chicory plant; however, the germination percentage and weighted germination index were increased under treatments of 100 and 500 ppm nano CuO. Seed germination is the beginning of a physiological process that requires the imbibition of water. However, in this case, the germination of chicory seeds occurred normally probably due to the seed coat, which can act as protector for the embryo and can totally guard the whole seed. This result is related to the report of Adhikari et al. (2012) who found that germination of soybean and chickpea was not affected by 2,000 ppm concentration of CuO nanoparticles.

Stampoulis et al. (2009) studied the effect of bulk or nano-sized Ag, Cu, Si, multi-walled carbon nanotube (MWCNTs), or ZnO on the seed germination of zucchini and observed that these compounds had no effect on germination even at 1,000 mg L^{-1}. Other experiments carried out with Cu nanoparticles showed that the results obtained are inconsistent, as nano-Cu had an inhibitory effect on plant growth (Lee et al., 2008).

Abdul Hafeez et al. (2015) examined the effect of nano-Cu on wheat germination and yield. They revealed that nano-Cu certainly have potential to enhance the growth and yield of wheat. The application of 30 ppm nano-Cu to soil, may significantly increase the yield of wheat crop, to match the food demand of a growing population. Relatively fewer studies have reported on the application of Cu-NPs. Contrary to our results, Shah and Belozerova (2009) observed that Cu-NPs have a favourable effect on the germination of lettuce seeds.

Several studies have reported the phytotoxic effects of nano Cu on growth, which is contrary to our results. Adverse effects of nano-Cu on root (Adhikari et al., 2012; Stampoulis et al., 2009), seedling growth (Shah and Belozerova ,2009) and shoot growth (Mustante and White, 2010) on different plants including wheat have been reported. Kim et al. (2012) found that the *Cucumis sativus* seedling biomass was significantly decreased to 75% of the control at 1,000 mg/L of CuO nanoparticles. However, it was noted that the phytotoxic effects were

concentration dependent. The concentration of nano Cu used in all the studies reporting toxic effects were higher than 200 ppm (Shah and Belozerova, 2009; Doshi et al., 2008; Lin and Xing, 2008). Exposure of plants to elevated levels of nano-Cu increases bioavailability. Consequently, massive accumulation of nanoparticles in roots and shoots occurs (Adhikari et al., 2012), resulting to phytotoxicity. DNA damage induced by nano-Cu (Atha et al., 2012) may be responsible for the toxic effects. Nanoparticles induced increased activity of chloroplast, rubisco (Hong et al., 2005), antioxidant enzyme system and nitrate reductase (Lu et al., 2002) might be the possible underlying mechanism responsible for enhanced growth and yield. Prior to this study, there was no report on the effects of nano-Cu on the germination of chicory.

The results of this experiment showed that the foliar application of nano CuO on chicory plants produced an increase in photosynthetic pigments. These results are consistent with the reports of other researchers (Zheng et al., 2005). An increase in net photosynthetic rate, Rubisco carboxylase activity and chlorophyll has also been reported for plants treated with other nanoparticles (Gao et al., 2006; Xuming et al., 2008). In conclusion, the results indicated that the germination percentage and weighted germination index were significantly increased by nano CuO treatments. Nano CuO treatments had no effect on germination indices and photosynthetic pigments of chicory, in comparison with the control plants.

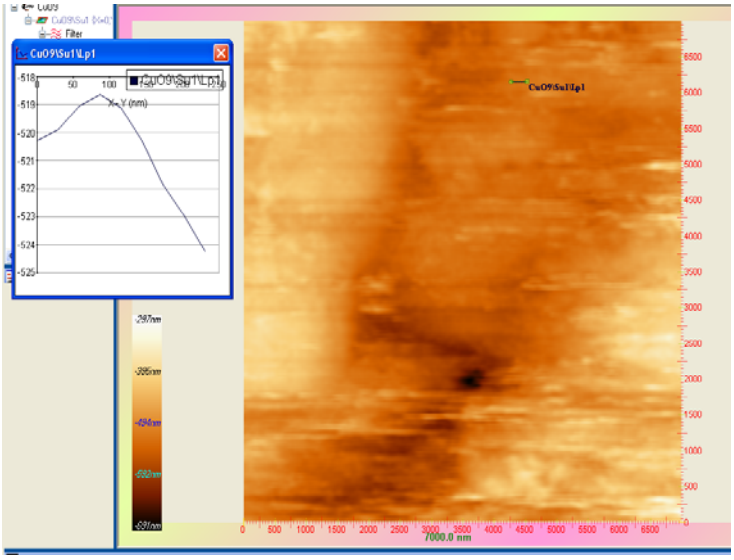

Fig. 1. Image of Copper oxide nanoparticles by STM

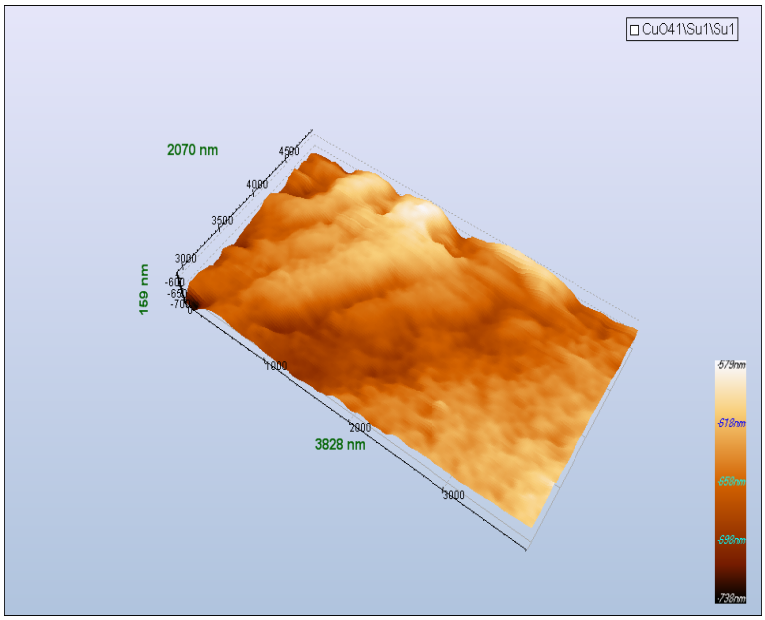

Fig. 2. Topographic image of Copper oxide nanoparticles by STM

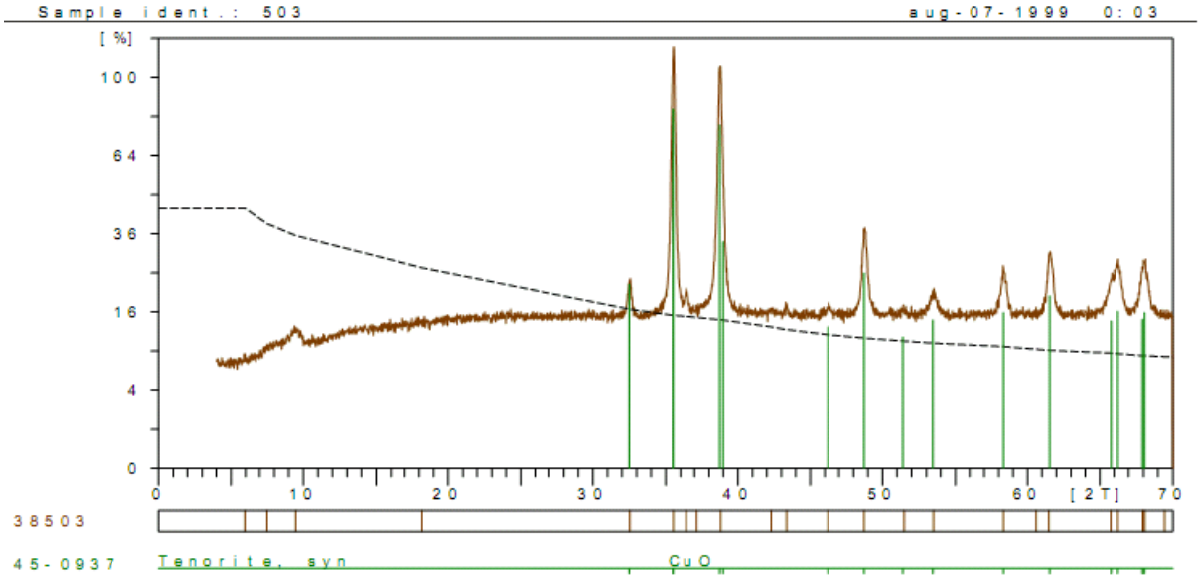

Fig. 3. XRD pattern of Copper oxide nanoparticles

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إنبات البذور واستجابة النمو في نبات الهندباء (*Cichorium intybus* L.) إلى جزيئات أكسيد النحاس النانونية

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ملخص

النباتات العليا تتفاعل بقوة مع بيئتها الجوية والأرضية، ومن المتوقع أن تتأثر نتيجة لتعرضها للجزئيات النانوية. في هذه الدراسة، تمت دراسة تأثير تركيزات مختلفة من جزئيات اكسيد النحاس النانونية على إنبات البذور ونمو البادرات من البذور وأصباغ البناء الضوئي من الهندباء وقد تم دراستها باستخدام التصميم العشوائي الكامل (CRD) مع أربعة مكررات. وقد شملت المعاملات التجريبية أربعة تركيزات من جزئيات اكسيد النحاس النانونية (10، 50، 100، و 500 جزء في المليون) والمعاملة المثالية بدون اكسيد النحاس. وأشارت النتائج إلى أن من بين المؤشرات لإنبات الهندباء البرية، فانه فقط نسبة الإنبات ومؤشر الإنبات الموزون قد تأثرت بشكل إيجابي مع معاملات جزئيات اكسيد النحاس النانوية .جميع معاملات جزئيات اكسيد النحاس النانوية أدت الى زيادة محتويات الكلوروفيل والكاروتينات لكن هذه الزيادة لا تختلف كثيرا بشكل معنوي. وخلصت الدراسة إلى أن المعاملات بجزئيات اكسيد النحاس النانوية ليس لها أي تأثير على مؤشرات الإنبات وأصباغ البناء الضوئي في الهندباء بالمقارنة مع المعاملة المثالية الخالية من جزيئات اكيسد النحاس النانونية.

الكلمات الدالة: الهندباء، جزئيات اكسيد النحاس النانوية ، نسبة الإنبات، وزن الشتلات الجاف، وطول الشتلات.

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