INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print), 2222-3045 (Online)
http://www.innspub.net
Vol. 6, No. 1, p. 438-445, 2015

RESEARCH PAPER OPEN ACCESS

Impact of multi-walled carbon nanotubes on seed germination and seedling growth of *Cichorium intybus* L.

Zarin Taj Pilevar, Homa Mahmoodzadeh*, Ali Eshaghi

Department of Biology, Faculty of Science, Mashad Branch, Islamic Azad University, Mashad, Iran

Key words: carbon nanotubes, *Cichorium intybus,* germination indices, Seedling biomass.

Article published on January 19, 2015

Abstract

A medicinal plant was evaluated for the effect of multi-walled carbon nanotubes (CNTs) using germination and seedling growth of *Cichorium intybus* L. The experimental treatments included four concentrations of multi-walled carbon nanotubes (10, 50, 100 and 200 ppm) and control without carbon nanotubes. Results indicated that among the *Cichorium intybus* germination indices, germination percentage, mean germination time and weighted germination index was not affected by treatments, however phytotoxicity was observed at 10 ppm CNTs , since a significant reduction in RGP,GR and GI was observed. In addition, plumule length, radicle length, seedling fresh and dry weight and vigor index were not affected by carbon nanotubes concentrations, significantly. Seedling fresh weight at 100 ppm concentration of carbon nanotubes was higher than the untreated control. It is concluded that treatment with multi-walled carbon nanotubes reatments have more inhibitory effects on germination indices of *Cichorium intybus* in comparison to seedling growth phase.

***Corresponding Author:** Homa Mahmoodzadeh ✉ homa_mahmoodzadeh@yahoo.com

Introduction

Observing and understanding the interactions between vascular plants and engineered nanomaterials (ENMs) has become a current concern. Interest in this field arises from the potential nanotechnological applications of ENMs and their consequent environmental impact. It has been experimentally observed that specifi c types of nanoparticles in low doses are able to activate physiological processes in plants. For example, TiO2 nanoparticles at an optimal concentration were able to improve the growth of spinach plants through activation of photosynthesis (Zheng *et al.*, 2005; Klein *et al.*, 2008). The positive effects of carbon nanotubes on plant growth and development has been described by number of research groups. Thus, increase of root growth in response to carbon nanotubes was documented for onion, cucumber (Canas *et al.*, 2008) and ryegrass(Lin, 2007). It was recently demonstrated that multi-walled carbon nanotubes (MWCNTs) can activate growth of tomato plants(Khodakovskaya *et al.*, 2009) and affect the expression of genes that are essential for cell division and plant development(Khodakovskaya *et al.*, 2009; Khodakovskaya *et al.*, 2011). Liu *et al.* (2009) demonstrated that single walled nanotubes (SWCNTs) can penetrate the walls and membranes of tobacco cells. The ability of nanoparticles to penetrate plant cells has generated interest in the possibility of using nanoparticles as smart treatment-delivery systems in plants(Gonzalez *et al.*, 2008). Torney *et al.*(2007) have reported that goldcapped mesoporous silica nanoparticles (MSNs) are able to penetrate cell walls and deliver DNA into plant cells by using a bombardment method. Nanocapsules can be used to deliver herbicide to plants. Carbon nanotubes (CNTs) have become one of the most studied and exploited ENMs due to the outstanding electronic, mechanical and structural properties that their arrangement of graphite layers confers upon them (Smart *et al.*, 2006).

CNTs are promising nanotools and plant nanotechnology can benefit widely from their manipulation and internalisation in plants. However, to date, studies regarding the mechanism of phytotoxicity and bioaccumulation of CNTs in plants are limited and report contradictory results on species germination and growth (Cañas *et al.*, 2008; Lin *et al.*, 2009; Khodakovskaya *et al.*, 2011). Besides the intrinsic differences regarding the plant species, great variability arises from the CNT material, as CNT samples may differ in CNT length, diameter and, in particular, the content and bioavailability of the metallic impurities present (Guo *et al.*, 2007). The detection and imaging of CNTs in biological tissues is challenging as their carbonaceous composition impedes detection by elemental analysis, while imaging using electron microscopy is hindered by low contrast and small diameter The present work was aimed at studying the effect of MWNTs, on *Cichorium intybus* L. germination indices and seedling growth.

Materials and methods

Cichorium intybus L. seeds were taken from the Pakan Bazr Company, Isfahan Province, Iran. multi-walled carbon nanotubes was supplied by Nutrient Company.The size and topography of multi-walled carbon nanotubes (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 multi-walled carbon nanotubes was shown in Fig. 3. XRD measurement showed that the used multi-walled carbon nanotubes were made by carbon.

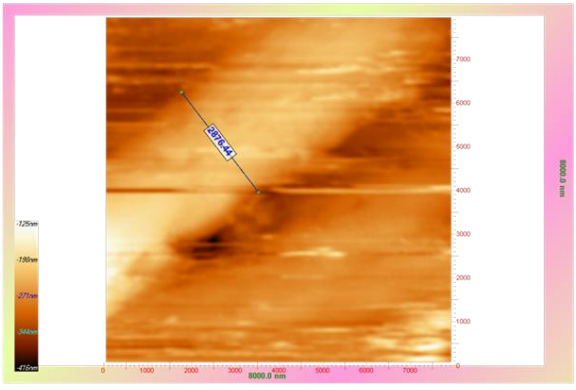

Fig. 1. Image of carbon nanotubes by STM.

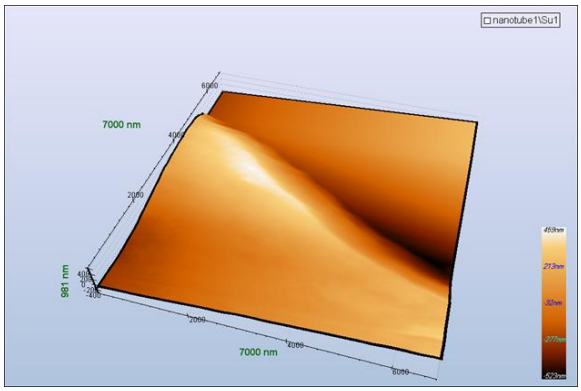

Fig. 2. Topographic image of carbon nanotubes by STM.

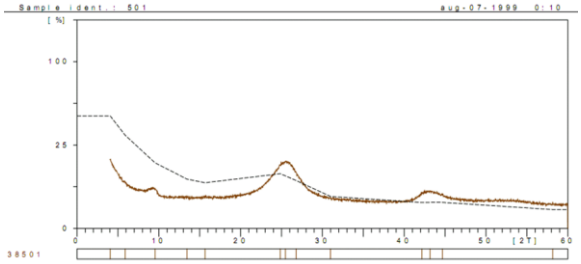

Fig. 3. XRD pattern of carbon nanotubes.

Experimental Design

In order to study the effect of different concentrations of carbon nanotubes on *Cichorium intybus* germination, a randomized completely design with four replications was employed. The experimental treatments included four concentrations (10, 50, 100 and 200 ppm) of carbon nanotubes and untreated control (without carbon nanotubes). The experiment was conducted in laboratory conditions with natural light and an average temperature of 25±1°C at the Faculty of Science, Mashad 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 10 ml of each concentration treatment was added to each Petri dish. For the control, only distilled water was added to Petri dishes. Germination tests were performed according to the rule issued by the International Seed Testing Association. All concentrations of carbon nanotubes 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 1 mm length. In this study, we used following germination parameters: Germination percentage (GP, %), 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(Figueroa and Armesto, 2001; Bu *et al.*, 2007; Wu and Du, 2007).

GP = 100 × GN / SN

GN is the total number of germinated seed, SN is the total number of seeds tested

RGP = GP treatment / GP control × 100

GR= $\sum_n^1 Si/Gi \times 100$

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

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

GI is a synthetic measure designed to reflect the synthetical germination ability including germination rate and germination numbers. Where i is the number of days since the day of sowing and Gi is the number of seeds germinated on day I.

A weighted germination index (WGI) as described by Bu *et al.*(2007) was calculated with maximum weight given to the seeds germinating early and less to those germinating late.

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

where n1, n2, ..., n60 are the number of seeds that germinated on first, second, and subsequent days until the 60th 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 were measured using a ruler. In order for dry biomass to be weighed, the 7-day seedlings were first weighed; then, having been placed in oven at 80°C for 48 h, they were weighed for a second time.

TTC viability tests for root tips

2, 3, 5-triphenylte trazolium chloride (TTC) was used as a histopathologic stain for testing the viability of root tips. The test was as follows: 5 mL of 0.5% solution of TTC was added to test tubes containing root tips, the temperature was kept at 35 ± 1°C. After 5 h in the dark, the TTC solution was removed with a syringe and root tips were thoroughly rinsed with distilled water and then examined. The redcolored root tips were considered to be viable and others were non-viable or dead (Shaymurt *et al*., 2012).

Data Analysis

Significant differences for all statistical tests were evaluated at the level of $P \leq 0.05$ with ANOVA. All data analyses were conducted using SPSS for Windows, Version 13.0.

Results

After seven days, the germination percentage of Cichorium intybus seeds were calculated for each concentration of CNTs. The exposure of the Cichorium intybus seeds to the CNTs showed no significant difference between germination percentage of CNT treatments and control . For the seeds grown on control media without any CNTs, the germination percentage was 84.72%,. The lowest and highest germination percentage (71.25% and 90%) were found in 10 and 50 ppm concentration CNTs, respectively (Table 1). Also the highest germination rate (24.8%) was shown in 50 ppm CNTs that had no significant difference with other treatments, except 10 ppm CNTs . The lowest mean germination time (4 day) was found in 200 ppm concentration CNTs, and the highest(4.16 day) was shown in 10 and 100 treatments that had significant difference with 200 ppm CNTs. In the media containing 50 and 200 ppm CNTs, the relative germination percentage (106.9 and 100 respectively) were higher than Other treatments and had significant difference with 10 ppm treatment(Table 1). The highest germination index (42.28) was found in 100 ppm CNTs treatment and the lowest(40.17) was shown in 10 ppm treatment that had significant difference with the control and other treatments (Table 1). Different concentrations of CNTs did not significantly affect the weighted germination index of *Cichorium intybus* seeds. The effect of studied treatments on plumule and radicle length was not significant. Plumule length at all of treatments of CNTs was higher than control although all CNTs treatments decresead radicle length in comparison with the control (Table 1). The lowest seedling fresh biomass was found in the control and 200 ppm CNTs concentration, that had significant difference with 100 ppm. Experimental treatments not affected seedling dry biomass significantly. The lowest seedling dry biomass (0.009 g) was found in 50, 100 and 200 ppm concentration CNTs, and the highest was shown in the control and 10 ppm treatment(0.01 g)(Table 2). Vigor index was not affected significantly by CNTs concentrations(Tables 2).

Table 1. Effect of different concentrations of carbon nanotubes on seed germination of *Cichorium intybus*.

Carbon nano tube Concentration(ppm)	Germination(%)	RGP	Germination Rate(%Day-1)	MGT(Day)	GI	WGI
10	71.25 b	73.82 b	21.80 b	4.16 a	40.17 b	0.542 b
50	90 a	106.94 a	24.80 a	4.04 ab	42.21 a	0.562 a
100	84.25 ab	94.33 ab	23.80 ab	4.16 a	42.28 a	0.557 ab
200	87.5 a	100 a	24.42 a	4 b	41.70 a	0.557 ab
Control	84.72 ab	------	23.80 a	4.06 ab	42.03 a	0.550 ab

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

Table 2. Effect of Carbon nanotubes concentrations on seedling growth of *Cichorium intybus*.

Carbon nano tube Concentration(ppm)	Plumule Length(cm)	Radicle Length(cm)	Seedling Fresh Biomass(g)	Seedling Dry Biomass(g)	Vigor Index
10	4.05 a	5.09 a	0.29 ab	0.01 a	7.12 a
50	3.74 a	4.33 a	0.30 ab	0.009 a	6.83 a
100	4.03 a	4.66 a	0.32 a	0.009 a	7.52 a
200	3.78 a	4.76 a	0.28 b	0.009 a	8.54 a
Control	3.4 a	5.11 a	0.28 b	0.01 a	8.64 a

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

The TTC tests showed that the effects of carbon nanotubes on root tips not varied with concentrations applied except for 10 ppm treatment(Fig. 4). For 24-h treatment all root tips were colored red, but root tips of 10 ppm treatment were less red than others.

Fig. 4. TTC tests for different concentrations of carbon nanotubes(Right to left: control, 10, 50, 100 and 200 ppm treatments).

Discussion

The major aim of this study was to provide information on MWCNT effect on seed germination and seedling growth of a medicinal plant, *Cichorium intybus*. Previously, limited reports indicated both positive and negative effects of different nanoparticles on plant physiology(Klaine *et al.*, 2004). It was demonstrated that nano-TiO2 treatment in proper concentration accelerated the germination of the aged spinach seeds and increased its vigor (Zheng *et al.*, 2005). Nanoparticles (Pd, Au at low concentrations; SiCu at higher concentrations, and combination of Au and Cu) also had a positive influence on lettuce seed germination, measured in terms of shoot to root ratio and growth of the seedling(Adhikari *et al.*, 2009). Some other studies also support the positive effects of suspensions of nanomaterials on seed germination and root growth of nine different crop species,such as tomato (Mariya *et al.*, 2009), radish (*Raphanus sativus*), rape (*Brassica napus*), rye grass (*Lolium perenne*), lettuce (*Lactuca sativa*), corn (*Zea mays*), cucumber (*Cucumis sativus*) (Lin, 2007), zucchini (Stampoulis *et al.*, 2009), onion, and cucumber (Cañas *et al.*, 2008). There were also negative reports of nanomaterials for seed germination and root growth, such as the inhibition effect on ryegrass and corn(Lin, 2007). Nanomaterials have also been reported to have no influence on the germination and root growth of tomato, cabbage, and carrots (Khodakovskaya *et al.*, 2009) . Effect of MWCNTs on plants has already been described by others(Villagarcia *et al.*, 2012; Liu *et al.*, 2009; Canas *et al.*, 2008; Mariya *et al.*, 2009). Preliminary studies have provided evidence that MWNTs and SWNTs are pathogenic to animals (Poland *et al.*, 2008), yet they have different effects on plants. MWNTs were shown to considerably increase the growth rate of tomato seedlings (Khodakovskaya *et al.*, 2009), have no effect on the growth parameters of wheat (Wild *et al.*, 2009), and inhibit the growth of rice seedlings (Lin *et al.*, 2009). SWNTs have been shown to suppress the growth of tomato roots, but stimulate the root growth of onion and cucumber (Canas *et al.*, 2008). In contrast, MWNTs have a toxic effect on Arabidopsis cultured cells (Lin *et al.*, 2009). The reason for the contradictory conclusions of different reports may be due to the type of plant species, the nature of

nanomaterials, and the concentrations of nanomaterials (Zhao *et al.*, 2008; Poland *et al.*, 2008; Khodakovskaya *et al.*, 2009).

Our results demonstrated, for the first time, that carbon nanotubes had no significantly effect on *Cichorium intybus* seed germination and seedling growth at most used concentrations that were consistent with previous reports(Villagarcia *et al.*, 2012). Distribution of MWCNTs among plant tissues is not homogeneous, they are more accumulated in newly developed leaves and in peripheral areas of leaves, i.e. in areas of active growth. It suggests that CNTs would be transported to plant leaves as if they were nutrients certainly via the sap flow as suggested in other articles(Lin *et al.*, 2009; Zhang *et al.*,2011). If CNTs were perceived by plants as toxicants then their distribution would be characteristic of a detoxification process. For instance they would accumulate in plant trichomes like metals such as Cd or Zn (Isaure *et al.*, 2006; Sarret *et al.*, 2009). At the cell scale CNTs are observed in vacuoles and not in other cell compartments. This distribution suggests that they are sequestered in compartments that keep them away from active metabolic sites. This is a classical mechanism of plant tolerance to exogenous compounds (Memon and Schroder, 2009).

Conclusions

In this article we provide information of MWCNT effects on a medicinal plant, *Cichorium intybus*. On the basis of the results, it is clear that seed germination was not affected by the CNTs and this indicates a compatible nature for the germination of this medicinal plant seeds and non-hazardous nature of the CNTs on them. Also the *Cichorium intybus* was observed as the tolerant plant.

References

Gachon CM, Langlois-Meurinne M, Saindrenan P. 2005. Plant secondary metabolism glycosyltransferases: the emerging functional analysis. Trends in Plant Science **10**, 542-549.

Adhikari RM, Shah BK, Palayangoda SS. 2009. Solvent dependent optical switching in carbazole-based fluorescent nanoparticles. Langmuir **7**, 2402-2406.

Bu HY, Chen XL, Wang YF. 2007. Germination time, other plant traits and phylogeny in an alpine meadow on the eastern Qinghai-Tibet plateau. Community Ecology **8**, 221 – 227.

Canas JE, Long M, Nations S, Vadan R, Dai L, Luo M, Ambikapathai R, Lee EH, Olszyk D. 2008. Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species. Environmental Toxicology and Chemistry **27**,1922-1931

Figueroa JA, Armesto JJ. 2001. Community-wide germination strategies in a temprate rainforest of southern chile: ecological and evolutionary correlates. Austrulian Journal of Botany **49**, 411 – 425.

Guo L, Morris DG, Liu X, Vaslet C, Hurt RH, Kane AB. 2007. Iron bioavailability and redox activity in diverse carbon nanotube samples. Chemistry of Materials **19**, 3472-3478

González-Melendi, P, Fernández-Pacheco R, Coronado MJ, Corredor E, Testillano PS, Risueño MC, Marquina C, Ibarra MR, Rubiales D, Pérez-de-Luque A. 2008. Nanoparticles as smart treatment-delivery systems in plants: assessment of different techniques of microscopy for their visualization in plant tissues. Annals of Botany **101**, 187-195

Isaure MP, Fayard B, Saffet G, Pairis S, Bourguignon J. 2006. Localization and chemical forms of cadmium in plant samples by combining analytical electron microscopy and X-ray spectromicroscopy. Spectrochimica Acta Part B: Atomic Spectroscopy **61**, 1242–1252.

Klaine SJ, Alvarez PJJ, Batley GE. 2008. Nanomaterials in the environment: behavior fate, bioavailability, and effects. Environmental Toxicology and Chemistry **27**,1825–1851.

Khodakovskaya M, Dervishi E, Mahmood M, Xu Y, Li Z, Watanabe F, Biris AS. 2009. Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. ACS Nano **3**,3221–3227 .

Khodakovskaya MV, de Silva K, Nedosekin DA, Dervishi E, Biris AS, Shashkov EV, Galanzha EI , Zharov VP. 2011. Complex genetic, photothermal, and photoacoustic analysis of nanoparticle-plant interactions. Proceedings of the National Academy of Sciences of the United States of America **108**, 1028–1033.

Lin B. 2007. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environmental Pollution **150**,243–250

Lin S, Reppert J, Hu Q, Hudson J S, Reid ML, Ratnikova TA, Rao AM, Luo H, Ke PC. 2009. Uptake, translocation, and transmission of carbon nanomaterials in rice plants. Small **5**, 1128-1132.

Liu Q, Chen B , Wang Q, Shi X, Xiao Z, Lin J, Fang X. 2009. Carbon nanotubes as molecular transporters for walled plant cells. Nano Letters **9**, 1007–1010

Mariya K, Enkeleda D, Meena M. 2009. Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. ACS Nano **3**,3221-3227.

Memon AR, Schroder P. 2009. Implications of metal accumulation mechanisms to Phytoremediation. Environmental Science and Pollution Research **16**, 162–175.

Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WA, Seaton A, Stone V, Brown S, MacNee W, Donaldson K. 2008. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Natural. Nanotechnology **3**,423–428.

Pairis S, Sarret G, Willems G, Isaure MP, Marcus MA, Fakra SC, Frerot H, Geoffroy N, Manceau A, Saumitou-Laprade P. 2009. Zinc distribution and speciation in Arabidopsis halleri ×Arabidopsis lyrata progenies presenting various zinc accumulation capacities. New Phytologist **184**, 581–595.

Shaymurt T, Gu J, Xu C, Yang Z, Zhao Q, Liu Y. 2012. Phytotoxic and genotoxic effects of ZnO nanoparticles on garlic (*Allium sativum* L.): A morphological study. Nanotoxicology, **.6**(3), 241-248.

Stampoulis D, Sinha SK, White JC. 2009. Assay-dependent phytotoxicity of nanoparticles to plants. Environ Sci Technol. **1**,9473–9479

Torney F, Trewyn B, Lin VSY, Wang K. 2007. Mesoporous silica nanoparticles deliver DNA and chemicals into plants. Natural Nanotechnology **2**(5),295-300

Villagarcia H , Dervishi E , de Silva K, Biris AS, Khodakovskaya M. 2012. surface chemistry of carbon nanotubes impacts the growth and expression of water channel protein in tomato plants. Small **8**, 2328-2334 .

Wild E, Jones KC. 2009. Novel method for the direct visualization of in vivo nanomaterials and chemical interactions in plants. Environmental Science & Technology **43**,5290–5294

Wu GL , Du GZ. 2007. Germination is related to seed mass in grasses (poaceae) of the eastern Qinghai – Tibetan plateau, china. Nordic Journal of Botany **25** , 361 – 365

Zhang Z, He X, Zhang H, Ma Y , Zhang P , Ding Y, Zhao Y. 2011. Uptake and distribution of ceria nanoparticles in cucumber plants. Metallomics **3**, 816–822

Zhao Y, Xing G , Chai Z. 2008. Nanotoxicology: Are carbon nanotubes safe? Natural Nanotechnology **4**,191–192

Zheng L, Hong F , Lu S. 2005. Effect of nano-TiO2 on strength of naturally aged seeds and growth of spinach. Biological Trace Element Researsc **104**,83–91.