Acta Sci. Pol. Agricultura 20(3) 2021, 103–112

www.agricultura.acta.utp.edu.pl pISSN 1644-0625 eISSN 2300-8504 DOI: 10.37660/aspagr.2021.20.3.2

ORIGINAL PAPER

Received: 20.08.2021
Received in revised form: 22.11.2021
Accepted: 29.12.2021

IMPACT OF BULK AND NANO-SIZED Al_2O_3 ON *Cynara scolymus* SEED GERMINATION AND SEEDLING GROWTH

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ABSTRACT

Background. Nanoparticles have been applied worldwide, posing substantial effects on the environment and its living organisms. Plants as sessile organisms are always exposed to considerable fluctuations of nanoparticles concentrations. Here, the effects of different concentrations of bulk and nanosized Al_2O_3 on seed germination and seedling growth of *Cynara scolymus* were investigated in a randomized completely design with four replications.
Material and methods. The experimental treatments included four concentrations of bulk Al_2O_3 (10, 50, 100 and 500 ppm), four concentrations of nanosized Al_2O_3 (10, 50, 100 and 500 ppm), and control without any Al_2O_3.
Results. The results indicated that among the *Cynara scolymus* germination indices, only mean germination time and weighted germination index was not affected by treatments. In addition, plumule length, radicle length, seedling fresh and dry weight were affected by bulk and nanosized Al_2O_3concentrations, significantly. Radicle length at 50 ppm concentration of nanosized Al_2O_3 was higher than those of the untreated control and bulk Al_2O_3 at 10, 100 and 500 ppm concentrations. It is concluded that treatment with bulk Al_2O_3 treatments have more inhibitory effects on germination indices of *Cynara scolymus* in comparison to nanosized Al_2O_3.
Conclusion. Overall, we found through our study that bulk and nano sized Al_2O_3 in high concentrations have a negative impact on the seed germination and seedling growth of *Cynara scolymus*. We saw an overall significant reduction in plumule length, seedling dry biomass, and vigor index of *Cynara scolymus* seedlings after being exposed to different concentrations of bulk and nano sized Al_2O_3, but no significant change in the mean germination time, weighted germination index and radicle length of the seedlings. The two common possibilities are either the bulk and nano sized Al_2O_3 will adhere to the roots impeding any uptake of water and nutrients, or that the nanoparticles are up taken and translocated within the plant therefore causing toxicity internally.

Key words: *Cynara scolymus*, Al_2O_3 nanoparticles, bulk Al_2O_3, germination indices, seedling biomass, seedling length

INTRODUCTION

Nanotechnology is a fast-developing industry, posing sub-stantial impacts on economy, society and environment. Particles in such a size (<100 nm) fall in the transitional zone between individual atoms or molecules and the corresponding bulk material, which can modify the physicochemical properties of the material (e.g., performing exceptional feats of conductivity, reactivity, and optical sensitivity). Therefore, such materials can generate adverse biological effects in living cells (Nel *et al*., 2006).

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There is an increasing amount of research on the toxicology of nanomaterials. Some researchers have shown the toxicity of nanoparticles, such as fullerene, carbon nanotubes and metal oxides to human cells, bacteria, and rodents (Hussain *et al*., 2005; Jia *et al*., 2005; Brunner *et al*., 2006; Lam *et al*., 2006; Soto *et al*., 2006). Nano-aluminum is being used in increasing quantities as energetic material (Navrotsky, 2003; Argonide, 2004). Aluminum nanoparticles are the basis for superior fuels for space launch vehicles and other rockets or missiles. A single Space Shuttle launch requires approximately 226 800 kg of Al, all in the form of particles with sizes greater than 10 mm. Replacement by the faster-burning nano-aluminum particles in the Space Shuttle's solid rocket boosters will allow motors to be packed more efficiently which may reduce motor size and thereby increase mission payload. Virtually all food, water, and air contain some aluminum which nature is well adapted to handle. It is reported that Al is poorly absorbed by the body and efficiently eliminated; however, when absorption occurs, Al is distributed mainly in bone, liver, testicles, kidneys, and brain. Aluminum has been linked to Alzheimer's disease. The respiratory system appears to be the primary target following inhalation exposure to Al (Sharma and Mishra, 2005). Toxicity of dissolved aluminum to aquatic organisms has been reported (Ward *et al*., 2006). Aluminum cation (Al^{3+}) is very unfriendly to agriculture as it injures plant root cells and thus interferes with root growth and nutrient uptake in crops (Quing *et al*., 2006). Most aluminum-containing compounds have low solubility in water unless the water is acidic. After investigating the phytotoxicity of nano-scale alumina (nano-Al_2O_3) powders with or without phenanthrene coating, Yang and Watts concluded that uncoated alumina particles inhibited root elongation of corn, cucumber, soybean, cabbage and carrot (Yang and Watts, 2005). This study attracted attentions from scientists and media, and was used to claim that nanoparticles can exert a negative effect on plants (Murashov, 2006). But the authors did not identify dissolution of nano-Al_2O_3 in solution, thus, failed to clarify, if the phytotoxicity was from nano-Al_2O_3 or aluminum ion in the aqueous solution. Therefore, this study aimed to provide new information about phytotoxicology of nanoparticles by investigating the effect of bulk and nano-Al_2O_3 on seed germination and seedling growth of medicinal plant, *Cynara scolymus*.

MATERIAL AND METHODS

Description of Materials

Cynara scolymus (var. Pishtaz) seeds were taken from the Pakan Bazr Company, Isfahan Province, Iran. Nanosized Al_2O_3 powder was supplied by Nutrient Company. The size and topography of Al_2O_3 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 Al_2O_3 nanoparticles was shown in Fig. 3. XRD measurement showed that the used Al_2O_3 nanoparticles were made by Al_2O_3 and Corundum. Bulk Al_2O_3 was supplied by merk Company.

Experimental Design and Data Observation

In order to study the effect of different concentrations of bulk and nanosized Al_2O_3 on *Cynara scolymus* germination, a randomized completely design with four replications was employed. The experimental treatments included four concentrations (10, 50, 100 and 500 ppm) of bulk and four concentrations (10, 50, 100 and 500 ppm) of nanosized Al_2O_3 and untreated control (without any Al_2O_3 types). The experiment was conducted in laboratory conditions with natural light and an average temperature of 25±1ºC 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 10 ml of each 16-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 Al_2O_3 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. These parameters were also calculated from the formulas proposed by (Figueroa and Armesto, 2001; Bu *et al.*, 2006; Wu and Du, 2007):

$$GP = 100 \times GN / SN \quad (1)$$

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

$$RGP = GP\ treatment / GP\ control \times 100 \quad (2)$$

$$GI = (\sum(N-i) \times Gi \times 100)/(N \times GN) \quad (3)$$

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* was calculated with maximum weight given to the seeds germinating early and less to those germinating late (Bu *et al.*, 2007):

$$WGI = [N \times n_1 + (N-1) \times n_2 + (N-Z) \times n_3 + ...] / N \times N' \quad (4)$$

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) (5)

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.

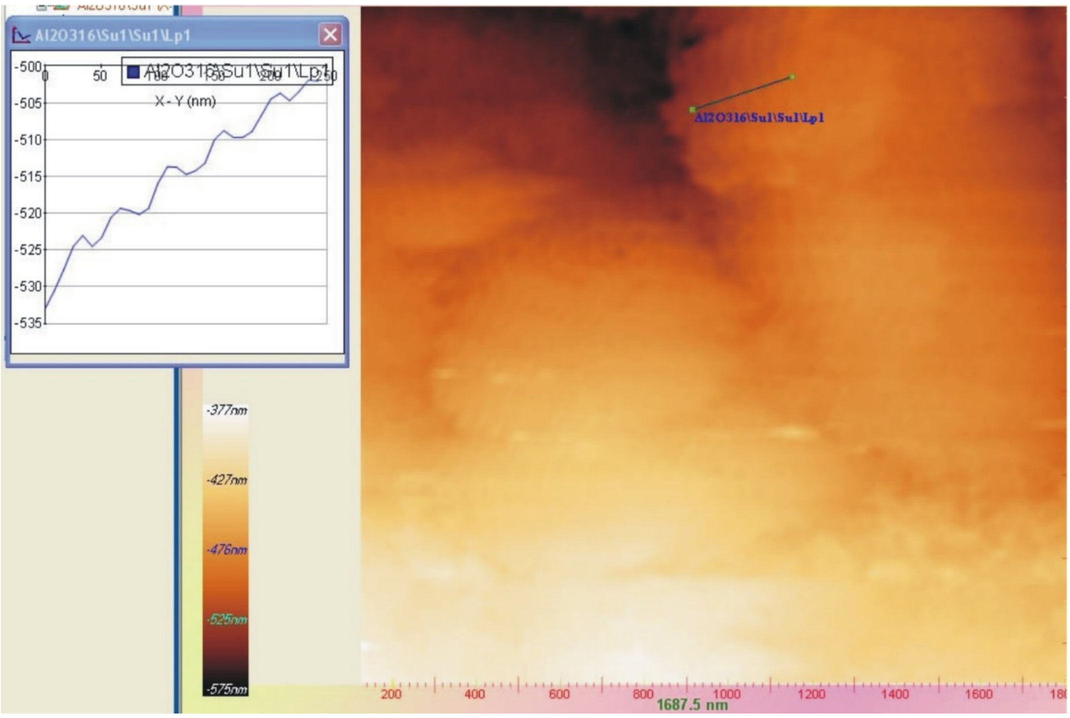

Fig. 1. Image of nanosized Al_2O_3 by STM

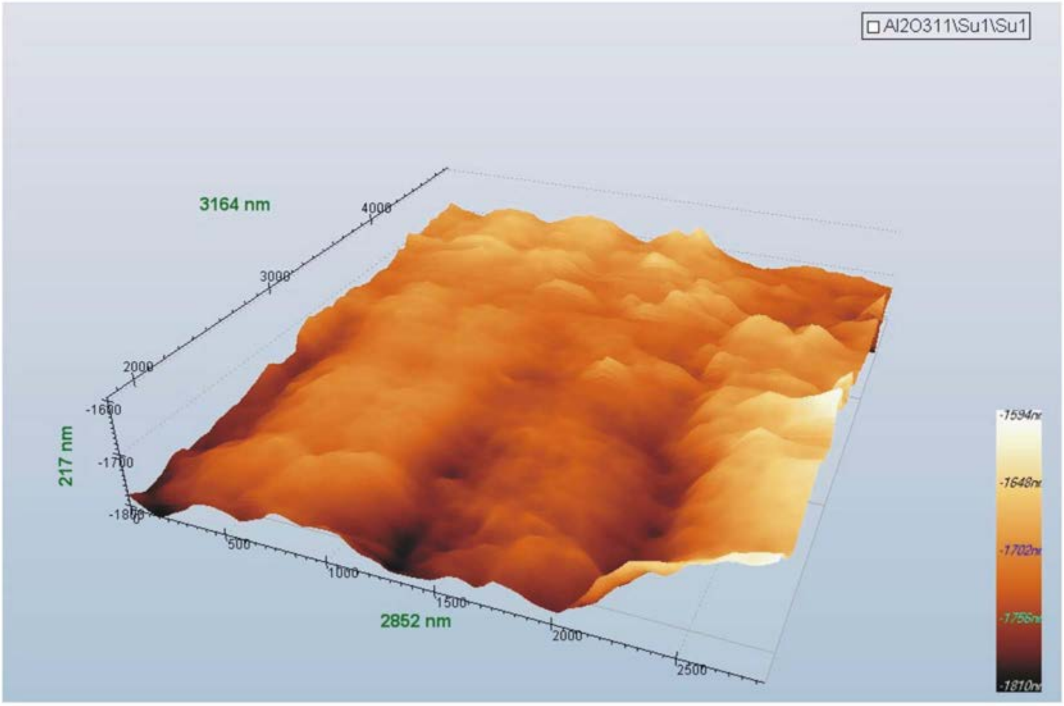

Fig. 2. Topographic image of nanosized Al_2O_3 by STM

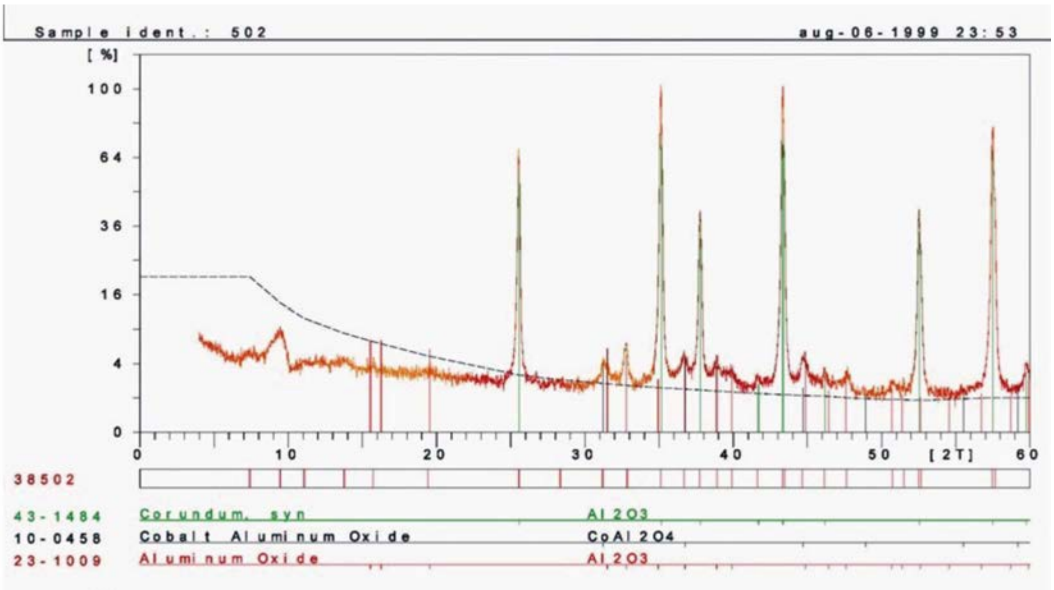

Fig. 2. 3 XRD pattern of nano Al_2O_3 particles

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 red colored root tips were considered to be viable and others were non-viable or dead (Shaymurat *et al*., 2012).

Statistical 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

Once the *Cynara scolymus* seeds were plated, it took approximately three to five days for them to germinate. After seven days, the germination percentage of the *Cynara scolymus* seeds were calculated for each concentration of bulk and nano aluminum oxide. For the seeds grown on control media without any aluminum oxide, the germination percentage was 97.5%, therefore almost all of the *Cynara scolymus* seeds germinated. The lowest germination percentage (80%) was found in 500 ppm concentration nanosized Al_2O_3 (Table 1). Although the highest germination rate (99.4%) was shown in the control treatment, but had no significant difference with other treatments, except 50 and 500 ppm bulk Al_2O3. The lowest mean germination time (5.08 day) was found in 50 ppm concentration nanosized Al_2O_3, and the highest (5.24 day) was shown in the control treatment. Therefore 50 ppm concentration nanosized Al_2O_3 treatment reduced mean germination time by 3% in comparison to untreated control. Although there was some variation between the mean germination times of seeds grown in media with differing concentrations of bulk and nanoparticles aluminum oxide, this variation was not statistically significant, In the media containing 10 and 500 ppm bulk Al_2O_3, the relative germination percentage (105.9 and 106.2, respectively) were higher than other treatments and had significant difference with 50 and 500 ppm nanosized Al_2O_3 treatments (Table 1). The highest germination index (29.2) was found in the control treatment and the lowest (25.2) was shown in 500 ppm bulk Al_2O_3 treatment. Therefore 500 ppm concentration bulk Al_2O_3 treatment reduced germination index by 14% in comparison to untreated control, while other treatments did not significantly reduce germination index in comparison with control (Table 1). Different concentrations of bulk and nano Al_2O_3 did not significantly affect the weighted germination index of *Cynara scolymus* seeds. The effect of studied treatments on root length was not significant, but they had a significant effect on plumule length. plumule length at all of treatments of nano and bulk Al_2O_3 was lower than control. The lowest plumule length was achieved at 500 ppm nanosized Al_2O_3 (Table 1). All nano sized Al_2O_3 treatments decreased seedling fresh biomass significantly. The lowest seedling fresh biomass was found in 50 and 500 ppm nano. Al_2O_3 concentrations seedling fresh biomass significantly. Bulk Al_2O_3 treatments have no significant effect on seedling fresh biomass. Experimental treatments affected seedling dry biomass significantly. The lowest seedling dry biomass (0.011 g) was found in 500 ppm concentration nanosized Al_2O_3, and the highest was shown in the control treatment (0.029 g). Therefore 500 ppm concentration nanosized Al_2O_3 treatment reduced seedling dry biomass by 62% in comparison to untreated control, while 500 ppm concentration bulk Al_2O_3 decreased seedling dry biomass by 34% in comparison with control (Table 2). Vigor index was affected significantly by bulk and nanosized Al_2O_3 concentrations except 10 and 50 ppm bulk and 50 ppm nano treatments (Table 2). The lowest vigor index was shown in 500 ppm nano Al_2O_3 that reduced vigor index by 84% in comparison with the control (Table 2). The TTC tests showed that the effects of bulk and nano Al_2O_3 on root tips not varied with concentrations applied (Fig 4). For 24-h treatment all root tips were colored red.

Table 1. Effect of different concentrations of bulk and nanosized Al_2O_3 on seed germination of *Cynara scolymus*

Concentration ppm	Germination %	RGP	Germination Rate %Day^{-1}	MGT Day	GI	WGI
Bulk Al_2O_3						
10	90 abc	105.9 a	86.2 ab	5.17 a	26.1 ab	0.358 a
50	85 bc	100 ab	82.8 b	5.18 a	27.6 ab	0.361 a
100	90 abc	106.2 a	89.7 ab	5.20 a	25.6 ab	0.428 a
500	82.5 c	97.5 ab	83.9 b	5.22 a	25.2 b	0.392 a
Nano Al_2O_3						
10	95 ab	97.7 ab	96.8 ab	5.20 a	27 ab	0.412 a
50	87.5 abc	89.7 bc	93.8 ab	5.08 a	27.3 ab	0.415 a
100	90 abc	92.5 abc	90.1 ab	5.16 a	26.1 ab	0.404 a
500	80 c	82.2 c	85.22 ab	5.17 a	25.8 ab	0.400 a
Control	97.5 a	----	99.4 a	5.24 a	29.2 a	0.418 a

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 bulk and nanosized Al_2O_3 concentrations on seedling growth of *Cynara scolymus*

Concentration ppm	Plumule Length cm	Radicle Length cm	Seedling Fresh Biomass, g	Seedling Dry Biomass, g	Vigor Index
Bulk Al_2O_3					
10	0.47 cd	3.45 abc	0.155 ab	0.012 cd	457.3 a
50	0.70 ab	4.45 a	0.155 ab	0.015 cd	488.0 a
100	0.54 bcd	3.85 ab	0.156 ab	0.021 b	301.3 bc
500	0.52 bcd	3.02 bc	0.161 ab	0.017 bc	367.3 b
Nano Al_2O_3					
10	0.67 abc	2.52 c	0.148 b	0.012 cd	317.6 bc
50	0.47 cd	3.42 abc	0.142 b	0.015 cd	461.3 a
100	0.47 cd	2.43 c	0.147 b	0.012 cd	234.6 c
500	0.41 d	2.41 c	0.143 b	0.011 d	80.6 d
Control	0.82 a	3.13 bc	0.173 a	0.029 a	516.6 a

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

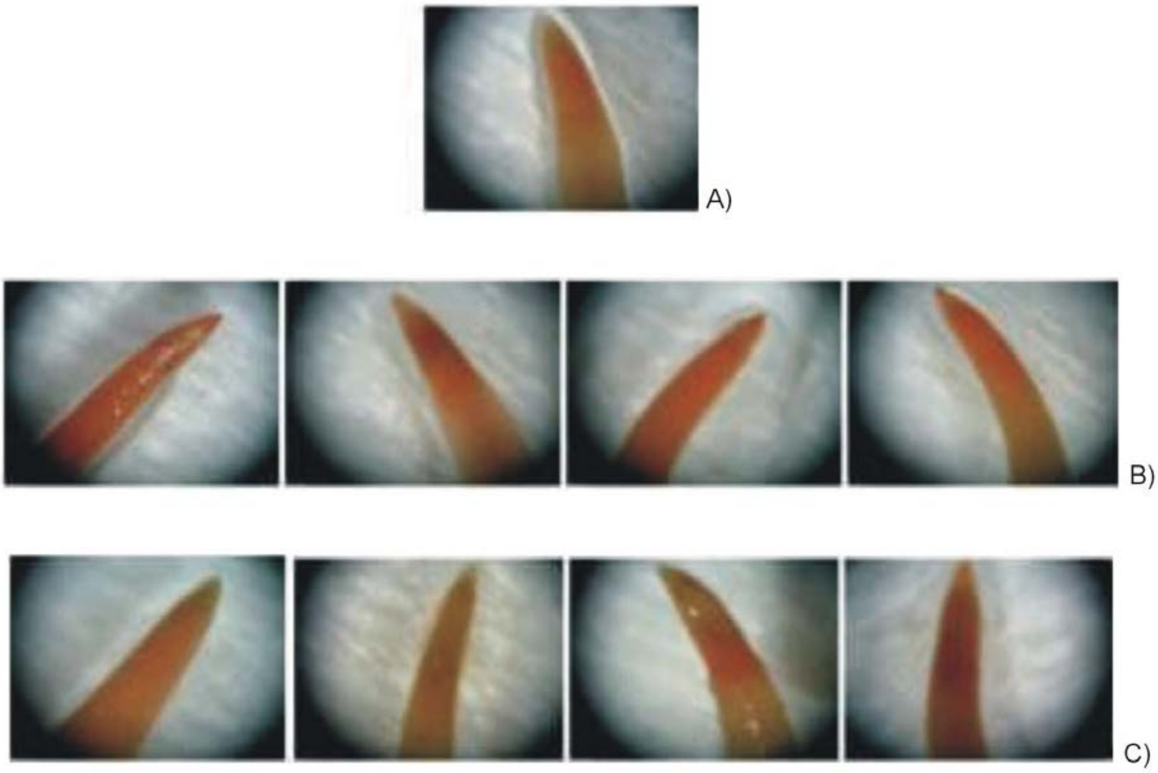

Fig. 4. TTC tests for different concentrations of bulk and nano-sized Al_2O_3, A (control), B (Bulk Al_2O_3(10, 50, 100 and 500 ppm)), C(Nano-sized Al_2O_3(10, 50, 100 and 500 ppm))

DISCUSSION

Nanotoxicology, an emerging discipline, is receiving increasing attraction. Nanotoxicity has been the research focus of many publications, including several reviews (Oberdorster *et al*., 2005; Nel *et al*., 2006; Wiesner *et al*., 2006). Some nanoparticles, such as fullerene, SWCNT and TiO_2, have been widely used as test materials to reveal their nanotoxicity mechanisms. However, available information on the is topic too scarce to search anytopic is too scarce to reach any consensus on nanotoxicity and its mechanism, particularly for the nanoparticle used in our study. Phytotoxicity in higher plants should be investigated in order to develop a comprehensive toxicity profile for nanoparticles. Seed germination and root elongation is a rapid and widely used acute phytotoxicity test with several advantages: sensitivity, simplicity, low cost and suitability for unstable chemicals or samples (Munzuroglu and Geckil, 2002). Germination is normally known as a physiological process beginning with water imbibition by seeds and culminating in the emergence of the rootlet (Kordon, 1992). However, there are different definitions of seed germination according to its root length emergence of root, >1mm or >5mm (Kordon, 1992; Ren *et al*., 1996; Munzuroglu and Geckil, 2002, Murata *et al*., 2003). In this study, seeds showing emergence of radicle coming out of the seed coat were recorded as being germinated. Seed coat plays a very important role in protecting the embryo from harmful external factors. Seed coats can have selective permeability (Wierzbicka and Obidzinska, 1998). Pollutants, though having obviously inhibitory effect on root growth, may not affect germination if they can not pass through seed coats. This may explain that seed germination in this study was not greatly altered by nanoparticles. These findings agree with recent reports stating that seed germination for different plant species was not affected by Al_2O_3 (Yang and Watts, 2005; Lin and Xing, 2007; Lee *et al*., 2010). It has also been reported that the process of seed germination in wheat is not affected by the presence of aluminum ion under experimental settings (Jamal *et al*., 2006).

Our results also were consistent with a study which investigated the effects of five different types of nanomaterials (multi-walled carbon nanotubules, aluminum oxide, zinc oxide, aluminum, and zinc) on the seed germination rate and root length of five agriculturally important crops (radish, rape, ryegrass, lettuce, corn, and cucumber. They found that out of all five crops, that only the seed germination rate of ryegrass and corn were affected by nano-Zn and nano-ZnO, respectively. In short, Lin and Xing found that in response to 200 $g \cdot dm^{-3}$ Al_2O_3 nanoparticles that none of the germination rates from all 5 plants they tested were significantly affected (Lin and Xing, 2007). These results mirror our results because 2000 $g.dm^{-3}$ converts to the concentration of 0.2 g of Al_2O_3 nanoparticles per 100 mL of media. This concentration fits approximately in the middle of the range of Al_2O_3 nanoparticle concentrations that we used (0.1 g, 0.5 g, and 1 g of Al_2O_3 nanoparticles per 100 mL of media). Lin and Xing state that the seed coat of the tobacco seeds was most likely not permeable to the aluminum oxide nanoparticles, therefore the germination rate was not affected greatly (Lin and Xing, 2007). Thus, it would not be until after the seedlings started to emerge from the seed coat, that they would be affected by aluminum oxide nanoparticles. As shown in Table 2, radicle growth was promoted at 10, 50 and 100 ppm of bulk Al_2O_3. The positive effect of Al_2O_3 on root elongation in *Arabidopsis thaliana* has been reported by Lee *et al*. (2010). Improvement in wheat root growth has also been reported at low doses of Al (Aniol, 1984). On the other hand, the radicle length in presence of 10, 100 and 500 ppm nano Al_2O_3 was reduced slightly (not significantly) as compared to control radicle length. This observation can be attributed to high Al content in the roots. The decrease in root elongation in the presence of nAl_2O_3 for various plant species has been reported (Yang and Watts, 2005; Lin and Xing, 2007). In contrast, plumule length was affected by bulk and nano Al_2O_3 (Table 2) which may be a consequence of high transportability of this material to the shoot. Therefore, it may be concluded that the more uptake of these particles, the more adverse effects on the growth of seedlings.

The TTC tests showed that after 24-h treatment, all root tips were colored red that confirmed bulk and nano Al_2O_3 could not cause the root tip cells to death. It was also found that the nano Al_2O_3 roots were less red than bulk Al_2O_3 treatments (Fig 4). These results showed that nano Al_2O_3 treatments had more toxicity effects than bulk Al_2O_3 treatments.

CONCLUSIONS

Overall, we found through our study that bulk and nano sized Al_2O_3 in high concentrations have a negative impact on the seed germination and seedling growth of *Cynara scolymus*. We saw an overall significant reduction in plumule length, seedling dry biomass, and vigor index of *Cynara scolymus* seedlings after being exposed to different concentrations of bulk and nano sized Al_2O_3, but no significant change in the mean germination time, weighted germination index and radicle length of the seedlings. The two common possibilities are either the bulk and nano sized Al_2O_3 will adhere to the roots impeding any uptake of water and nutrients, or that the nanoparticles are up taken and translocated within the plant therefore causing toxicity internally. Aluminum oxide exists commonly in the soil as aluminum is one of the most abundant elements found in the earth's crust. It is not known how much of the aluminum oxide found in the soil exists as nanoparticles, therefore more research needs to be conducted to determine if the increasing use of aluminum oxide nanoparticles in industry is drastically affecting the concentrations of aluminum oxide that is already present in the soil. More research also needs to be conducted in order to elucidate the mechanisms of aluminum oxide nanoparticle toxicity and to determine the exact mode of phytotoxicity in plants.

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WPŁYW MASY I NANOCZĄSTEK Al_2O_3 NA KIEŁKOWANIE I WZROST SIEWEK *Cynara scolymus*

Streszczenie

Nanocząstki są stosowane na całym świecie, wywierając znaczny wpływ na środowisko i żywe organizmy. Rośliny jako organizmy ukorzenione są zawsze narażone na znaczne wahania stężeń nanocząstek. Zbadano wpływ różnych stężeń Al_2O_3 w masie i nanocząsteczkach na kiełkowanie nasion i wzrost siewek *Cynara scolymus* w całkowicie losowym projekcie z czterema powtórzeniami. Zabiegi doświadczalne obejmowały cztery stężenia Al_2O_3 w masie (10, 50, 100 i 500 ppm), cztery stężenia Al_2O_3 w postaci nanocząstek (10, 50, 100 i 500 ppm) oraz kontrolę (bez Al_2O_3). Wyniki wskazują, że spośród wskaźników kiełkowania *Cynara scolymus* zabiegi nie miały wpływu na średni czas kiełkowania i ważony wskaźnik kiełkowania. Na długość pędu embrionalnego, długość korzonków, świeżą i suchą masę siewek istotnie wpływały stężenia Al_2O_3 w masie i nanoskali. Długość rodnika przy stężeniu 50 ppm Al_2O_3 w postaci nanocząstek była wyższa w porównaniu z kontrolą i masowym Al_2O_3 przy stężeniach 10, 100 i 500 ppm. Stwierdzono, że traktowanie dużą ilością Al_2O_3 ma bardziej hamujący wpływ na wskaźniki kiełkowania *Cynara scolymus* niż stosowanie Al_2O_3 w postaci nanocząstek. W badaniach określono, że Al_2O_3 w dużych ilościach i w wysokich stężeniach nanocząsteczek ma negatywny wpływ na kiełkowanie nasion i wzrost siewek *Cynara scolymus*. Zaobserwowano znaczne zmniejszenie długości pędu embrionalnego, suchej masy sadzonek i wskaźnika wigoru siewek *Cynara scolymus* po ekspozycji na różne stężenia Al_2O_3 w rozmiarze masowym i nano, ale bez znaczących zmian w średnim czasie kiełkowania, ważonym wskaźniku kiełkowania i długości korzonków siewek. Wynika to albo z przywierania Al_2O_3 do korzeni w rozmiarze masowym i nanometrowym, utrudniając wchłanianie wody i składników odżywczych, albo nanocząsteczki są pobierane i przemieszczane w obrębie rośliny, powodując w ten sposób toksyczność wewnętrzną.

Słowa kluczowe: biomasa siewek, *Cynara scolymus,* długość siewek, masa Al_2O_3, nanocząstki, wskaźniki kiełkowania