이학석사학위논문

Effects of nanoparticles TiO_2 and ZnO on growth and antioxidative responses of tomato and kidney bean

나노 산화티타늄과 나노 산화아연이 토마토와
강낭콩의 생장과 항산화 반응에 미치는 영향

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서울대학교 대학원

생명과학부

전희주

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이 논문을 이학석사 학위논문으로 제출함
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전희주의 이학석사 학위논문을 인준함
2012년 6월

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Abstract

Although representative environmental pollution research institutions, the USEPA (US Environmental Protection Agency) and the OECD (Organization for Economic Cooperation and Development), have been doing research in many fields, research concerning the effects of nanoparticles on plants is still rare. In this study, I studied the effects of nanoparticles nano-TiO_2, nano-ZnO on the tomato (*Lycopersicon esculentum*) and kidney bean (*Phaseolus vulgaris*) plants.

The effects of two types of nanoparticles (nano-TiO_2, nano-ZnO) on seed germination and root growth of two higher plant species, the tomato (*Lycopersicon esculentum*) and kidney bean (*Phaseolus vulgaris*) plants were investigated. The concentration range of the nanoparticles spanned from 0 to 5000 mg/L. In order to account for agglomeration and precipitation, a filter paper in a petri dish with distilled water (DW) was used.

At all concentration levels, both nano-TiO_2 and nano-ZnO did not significantly affect the seed germination of the tomato (*Lycopersicon esculentum*) and kidney bean (*Phaseolus vulgaris*) plants. However, significant inhibition of root length appeared in the treatment of nano-ZnO on the tomato (*Lycopersicon esculentum*) plant except at the highest concentration level of nano-ZnO. The same nanoparticle, nano-ZnO, significantly hampered the root length of the kidney bean (*Phaseolus vulgaris*) plant at high concentration levels (1000, 2500 and 5000 mg/L).

The plant seeds' uptake of the nanoparticles was also analyzed. In regard to nano-TiO_2, after an exposure of 48 hours (mg/kg), it was observed that when the concentration of nano-TiO_2 on the tomato (*Lycopersicon esculentum*) seeds increased, the uptake of nano-TiO_2 by the tomato (*Lycopersicon esculentum*) seeds increased in a linear relationship although it was not significantly different. In addition, the kidney bean (*Phaseolus vulgaris*) seeds, at 100 mg/L, nano-TiO_2 was not detected and at other higher nano-TiO_2 concentrations it was detected. In the uptake analysis of nano-ZnO by the kidney bean (*Phaseolus vulgaris*) seeds, after an exposure of 48 hours (mg/kg), kidney bean (*Phaseolus vulgaris*) seeds also showed a

significantly increased nano-ZnO uptake when the concentration of nano-ZnO on kidney bean (*Phaseolus vulgaris*) seeds increased.

In addition to seeds, nanoparticle uptake by the tomato (*Lycopersicon esculentum*) and kidney bean (*Phaseolus vulgaris*) seedlings after an exposure of 15 days (mg/kg) was also measured. Although the tomato (*Lycopersicon esculentum*) seedlings didn't show significant differences with concentration levels of nano-TiO_2, the kidney bean (*Phaseolus vulgaris*) seedlings showed significantly increased nano-TiO_2 uptake at the highest concentration level (5000 mg/L). In nano-ZnO uptake by the tomato (*Lycopersicon esculentum*) and kidney bean (*Phaseolus vulgaris*) seedlings, after an exposure of 15 days (mg/kg), tomato (*Lycopersicon esculentum*) seedlings showed significantly increased nano-ZnO uptake at the highest level (5000 mg/L) and in the case of the kidney bean (*Phaseolus vulgaris*) seedlings, the uptake of nano-ZnO significantly increased with increased concentration levels.

To determine the size of the nano-TiO_2 and nano-ZnO in solution, after 14 days the hydrodynamic diameter of the nano-TiO_2 and nano-ZnO uptake in a petri dish was measured. A FE-SEM (Field Emission Scanning Electron Microscope) was used to measure the diameter. For solutions which showed a particle diameter over 1000 nm, an Axio Zeiss Imager A1 with a differential interference contrast (DIC) microscope was used. In the case of nano-TiO_2, the particle's hydrodynamic diameter at the highest concentration significantly showed the largest diameter. In the case of nano-ZnO, the hydrodynamic diameter of nano-ZnO significantly increased with increased concentration levels. Also, after 7 days in a petri dish, according to the hydrodynamic diameter of the nano-TiO_2 uptake, the hydrodynamic diameter of nano-TiO_2 at the highest nano-TiO_2 concentration significantly showed the largest diameter.

Mature tomato (*Lycopersicon esculentum*) plants only showed the significant difference by nano-TiO_2 at the highest Superoxide dismutase activity (SOD) in the highest (1000 mg/L) treatment. Also, mature tomato (*Lycopersicon esculentum*) plants and mature kidney bean (*Phaseolus vulgaris*) plants showed no significant differences by concentration levels of nano-ZnO. The chlorophyll contents of mature tomato (*Lycopersicon esculentum*) plants after either nano-TiO_2 or nano-ZnO exposure of 7 days (mg/L) showed no significant differences with different

concentrations of nanoparticles (nano-TiO_2, nano-ZnO). And chlorophyll contents of mature kidney bean (*Phaseolus vulgaris*) plants after a nano-ZnO exposure of 7 days (mg/L) also showed no significant differences with different concentrations of nanoparticles (nano-TiO_2, nano-ZnO).

After an exposure of 5 weeks (mg/L), nano-TiO_2 uptake by the mature tomato (*Lycopersicon esculentum*) plants was measured dividing plant parts by root, stem, leaf and fruit. At the root, leaf, fruit parts, there were no significant differences with different nano-TiO_2 concentration levels. However, at the stem part, when nano-TiO_2 concentration levels increased, the nano-TiO_2 uptake of the mature tomato (*Lycopersicon esculentum*) plants decreased inversely. Also, after an exposure of 5 weeks (mg/L), the nano-ZnO uptake of the mature tomato (*Lycopersicon esculentum*) and kidney bean (*Phaseolus vulgaris*) plants was measured and only the mature tomato (*Lycopersicon esculentum*) plants showed significant differences. When the concentration of nano-ZnO increased, the uptake of nano-ZnO by the mature tomato (*Lycopersicon esculentum*) plants also increased.

To confirm the results of the hydrodynamic diameter of the nano-TiO_2 and nano-ZnO uptake, Pchem (Physical chemistry) data (hydrodynamic diameter and zeta potential) were measured by ELS. In the case of nano-TiO_2 in DW, when the concentration of nano-TiO_2 increased, the hydrodynamic diameter of nano-TiO_2 decreased. Nano-TiO_2 in a Hoagland solution showed an increased hydrodynamic diameter of nano-TiO_2 when the concentration of nano-TiO_2 increased. Then, we also measured the hydrodynamic diameter of nano-ZnO in DW. When the concentration level of nano-ZnO increased, the hydrodynamic diameter of nano-ZnO decreased.

Key words: nano-TiO_2 , nano-ZnO, *Lycopersicon esculentum*, *Phaseolus vulgaris*, antioxidant enzyme activities, Pchem

List of Contents

List of Figures

List of tables

Ⅰ. Introduction

Although representative environmental pollution research institutions, the USEPA (US Environmental Protection Agency) and the OECD (Organization for Economic Cooperation and Development) have been doing research in many fields, research concerning the effects of nanoparticles (NPs) on plants is still rare compared to research concerning the effects of nanoparticles (NPs) on animals and microbes (Peralta-Videa et al. 2011). The stability support program of OECD includes some NP research, investigating manufactured nano-TiO_2, AgNP and nano-SiO_2 in the Republic of South Korea, and there are various papers that explore the interaction of plants with NPs—mostly experimenting seed germination rate and root elongation rate. However overall, these research papers are small in number. Additionally, the species of plants regularly used in these types of experiments lack diversity and are more often seedlings as opposed to the matured plant.

In particular, with respect to the interaction of plants with the nano-TiO_2 and the nano-ZnO particle, research papers are quite insufficient, and they inadequately evaluate the effects of the nanoparticles (nano-TiO_2 , nano-ZnO) on varied plants. For example, in the case of nano-TiO_2 in a certain study, negative effects on plant growth and mitosis were reported (Ruffini Castiglione et al. 2011). However, other research, incongruous to the aforementioned study, revealed that nano-TiO_2 promoted plant growth, increased formation of chlorophyll, and enhanced photosynthetic rate (Zheng et al. 2005). An additional study reported that nano-TiO_2 generated an increase in fresh weight, dry weight and protein (Yang et al. 2007). In the case of nano-ZnO, various studies reported its negative effects on plant growth (Lin and Xing 2007; Lin and Xing 2008; Lee et al. 2010; López-Moreno et al. 2010).

Commercial industries are increasingly using NPs for purposes such as fillers, opacifiers, catalysts, semiconductors, cosmetics, microelectronics, and drug carriers (Nel et al. 2006). Therefore, while nanotechnology can have drastic effects on the economy, society and environment, its potential health and environmental impact on humans, non-human biota and ecosystems is not yet fully assessed (Kumari et al. 2009). In addition, there can be environmental regulations on NP products because of their negative effects. A recent study concerning AgNPs shows that the elution of

Ag^+ creates toxic effects (Bae et al. 2010). Unfortunately, in many work places, workers are exposed to NPs like AgNP and lack information about potential risks, which urgently calls for managing, supervising, and informed regulations in their work places (국립환경과학원 2011).

Nano-TiO_2 is widely used in several fields. Cosmetics often use nano-TiO_2 because of its ability to scatter light in the UVA and UVB range. In addition, nano-TiO_2 has a photocatalytic capacity, which allows it to remove harmful substances. Other general products and industrial goods also utilize nano-TiO_2 at low concentration levels. Likewise, nano-ZnO is used in a range of applications such as sunscreens, other personal care products, electrodes, biosensors, photocatalysis and solar cells (Kumar and Chen 2008). However, as nano-TiO_2 and nano-ZnO become more frequently exposed to the surroundings and begin to accumulate in the environment, the need for research concerning their effect on the ecosystem heightens. Because sunscreen includes 20% of UV scattering nano-TiO_2 and nano-ZnO, their exposure level to humans is larger than any other NP (Huang et al. 2009). To protect sunscreen's white turbidity, the sizes of these NPs' are decreased so they easily penetrate the skin's pores (Huang et al. 2009). As these NPs accumulate, they move through blood vessels and spread throughout the entire body (Huang et al. 2009).

Through their production, consumption and disposal, NPs, at a size of 100 nm or less, are released into the air, water and soil (Lin and Xing 2007). Similarly, plant cell walls, which are semipermeable, have pore sizes smaller than several nanometers (Carpita et al. 1979). This pore size allows selected NPs to pass through the cell wall. At times, the small size of NPs allows them to even pass through the cell membrane; Reports have detected NPs in cytoplasm (Lee et al. 2008). Therefore due to their small size, NPs can potentially be more toxic than larger materials (Lee et al. 2008). Moreover, NPs increase peroxidation of lipid membranes by producing ROS (Reactive oxygen species) (Nel et al. 2006) and these ROS induce the oxidative stress of cells.

Generally, crops plants are routinely used in plant research because of the importance of crop plants for food (Lee et al. 2008). In a certain review paper, the effects of NPs on food crops were analyzed as positive, negative and no effects

(Rico et al. 2011). Therefore, in this research, target species were determined as the tomato (*Lycopersicon esculentum*) and kidney bean (*Phaseolus vulgaris*) plants.

The study site of this research for the interaction of the tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) plants with nano-TiO_2 and nano-ZnO was limited to a plant growth chamber except for the interaction of the tomato (*L. esculentum*) with nano-TiO_2. The condition of a plant growth chamber was advantageous to the experiment because many crop species are sensitive to light. In the plant growth chamber, plants have a controlled environment. Thus, this experimental condition was determined to make the guideline of the effects of NPs (nano-TiO_2 , nano-ZnO) to the tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) plants. Also, through this, we can control the pollution of other areas and adjust the environment of other matters.

There are some factors which affect the behavior of NPs on plants. First, the physicochemical properties of NPs is determined by size, shape, charge, surface area and reactivity, purity and chemical composition. Second, applied concentrations, the specific conditions of the experiment, plant species and the uptake mechanisms of plants also influence the bioavailability of NPs (Xia et al. 2006; Darlington et al. 2009; Ruffini Castiglione et al. 2011).

In this research, to test the effects of nano-TiO_2 and nano-ZnO on the tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) plants, seed germination, root elongation, uptake of NPs, antioxidant enzyme activities and chlorophyll contents were measured. In addition, Pchem and SEM were used to evaluate the agglomeration and precipitation of NPs in different concentrations.

Ⅱ. Methods

2.1. Preparation of Particles and Cultures

The critical components of this experiment were obtained as follows: manufactured nano-TiO_2, (aerosol, ≥99.5 %, anatase: rutile 80: 20, 27 nm particle size) named 'AEROXIDE TiO_2 P 25' was purchased from Evonik Industries, Germany, manufactured nano-ZnO (ZnO/Al_2O_3, <50 nm) was purchased from Sigma Aldrich, USA and the tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) seeds were purchased from a local Syngenta (Syngenta AG, Switzerland) agent. Before any experimental use, the seeds were vernalized for two weeks and then sterilized for 10 minutes in a 10 % sodium hypochlorite solution (USEPA, 1996).

2.1.1. Germination Research

For the germination research, seeds were soaked in nano-TiO_2 solutions of concentrations of 0, 50, 100, 1000, 2500 and 5000 mg/L. The nano-ZnO solutions had concentrations in the same range. These nanoparticle concentrations were determined by referencing previous studies that observed the effects of nano-TiO_2 (Ruffini Castiglione et al. 2011) and nano-ZnO (Lee et al. 2010; López-Moreno et al. 2010) to growth of plants. Many previous studies detected toxicity of both nano-TiO_2 and nano-ZnO at 2000 mg/L and experimented with values up to 4000 mg/L. These results helped establish the upper concentration range in this study, whereas the lower concentrations were determined according to results of several other studies which reveal that nano-ZnO precipitated at 100 mg/L (Lin and Xing 2008; Shaymurat et al. 2012). During the experimentation process, nano-ZnO as well as nano-TiO_2 indeed formed precipitate at a concentration level of 100 mg/L.

In this experiment, the seeds were soaked in solution for 48 hours (Zheng et al., 2005) while gently shaken by an orbital shaker at 150 rpm. Then, all samples were washed thoroughly with DW and transferred into 100 mm petri dishes containing one piece of filter paper (90 mm) and 5 ml of DW (Lin and

Xing, 2007). Each petri dish (10 replicates) contained 5 seeds, and each seed was laid 1 cm or more apart to avoid any inhibition of growth (Yang and Watts 2005). Germination rates were investigated every 3 days (4 times). During this process, the seeds were tested in growth chambers under OECD guidelines (OECD, 2003): temperature: 24 ℃, humidity: 70 % ± 25 %, photoperiod: 18 hours of light, and light intensity: 300 μE/m^2/s. After 12 days, seeds were harvested, washed thoroughly with DW and dried for analysis of the accumulation of NPs in each species.

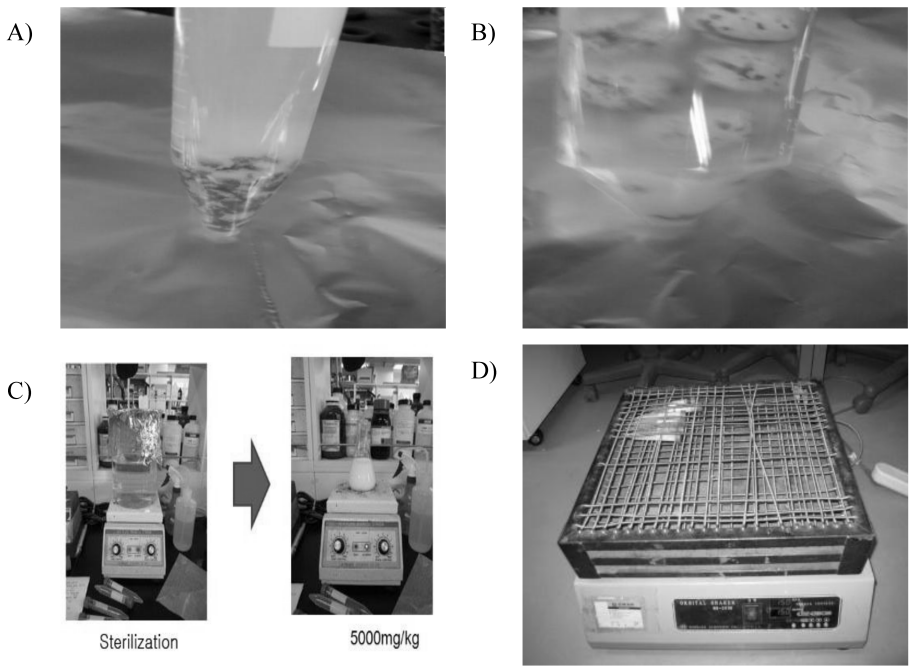


Fig. 1. A) aggregation and precipitation of NPs with tomato (*L. esculentum*), B) aggregation and precipitation of NPs with kidney bean (*P. vulgaris*), C) sterilization of nanoparticles on stirrer, D) shaking by the orbital shaker at 150 rpm

2.1.2. Root Elongation Research

In order to conduct root elongation research, germinated seeds were harvested. Each petri dish contained 5 seedlings and 5 ml of a test medium (nano-TiO_2 or nano-ZnO). Root lengths of the seedlings were measured every 3 days (for 5 trials). Other conditions including concentrations and chamber conditions remained consistent with values from the germination research discussed above. After 15 days, seedlings were harvested, washed thoroughly with DW and dried for accumulation of NP analysis.

2.1.3. Pot Research

The research conducted with potted plants was carried out in two different manners. In the first scenario, research concerning the tomato (*L. esculentum*) and nano-TiO_2 involved tomato (*L. esculentum*) seedlings in a greenhouse grown in pots (4 replicates) filled with 200 g of sunshine mix # 5 (Sun Gro, Canada). After 6 weeks, 200 ml of nano-TiO_2 solution at concentrations of 1000 or 5000 mg/L were given to each pot. After a week, antioxidant enzyme activity and chlorophyll content of the plants were measured. Five weeks after treatment, plants were harvested for accumulation research. During the experiment, the average temperature of the greenhouse was 25.2 ℃ and the average humidity was 66.8 % (measured by the HOBO U10 Temperature Relative Humidity Data Logger, Onset, Southern MA, USA). In the case of tomato (*L. esculentum*) with nano-ZnO and kidney bean (*P. vulgaris*) with either nano-TiO_2 or nano-ZnO, seedlings of tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) were grown in a chamber in 50-well pots filled with 10 g of sunshine mix # 5 (Sun Gro, Canada). After 4 weeks, 10 ml of nano-TiO_2 or nano-ZnO were given to each pot. A week after treating the plants with solution, antioxidant enzyme activity and chlorophyll content of the plants were measured. Five weeks after treatment, plants were harvested for accumulation research. The average temperature of the chamber during the experiment was 24 ℃ and the average humidity was 70 % ± 25 %.

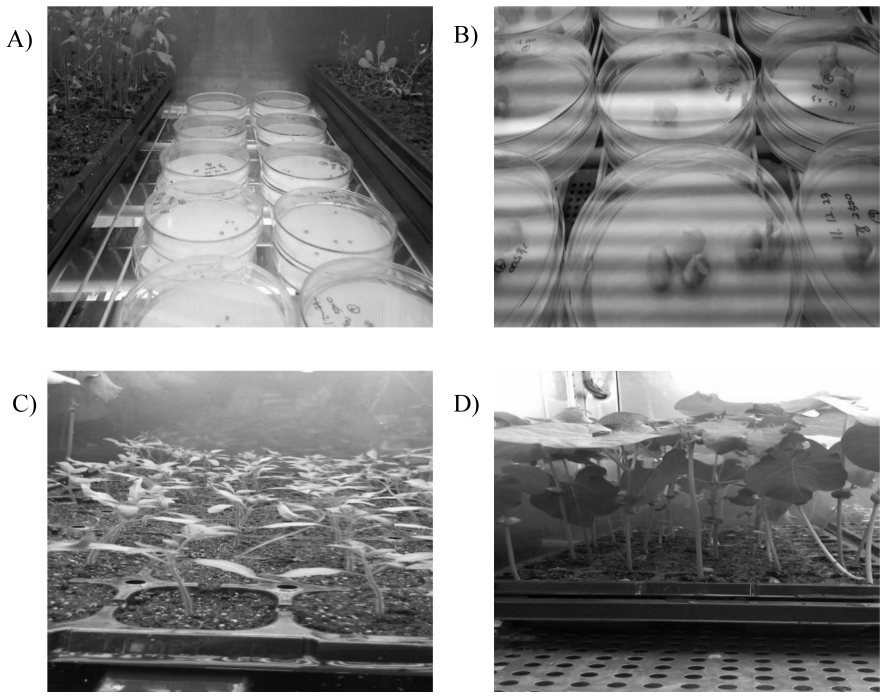


Fig.2. A) root elongation research on newly rooted tomato seeds (*L. esculentum*), B) root elongation research on newly rooted kidney bean seeds (*P. vulgaris*), C) pot research of growing tomato seedlings(*L. esculentum*), D) pot research of growing kidney bean seedlings (*P. vulgaris*)

2.2. Analyzing methods

2.2.1. Measurement of Antioxidant enzyme activity

Antioxidant enzyme activity of the plants was measured by protocols of (Song and Lee, 2010). For the extraction of enzymes, 0.1 g of plant leaf was washed with liquid nitrogen, pulverized, and transferred to a 15 ml Falcon tube with 50 mM of potassium phosphate buffer (pH 7.5). After sitting for 30 minutes, this solution was centrifuged at 4 ℃ at 14000 rpm for 15 minutes. The supernatant of the solution was used to measure the total protein content, TAC (total antioxidant capacity) and SOD (superoxide dismutase activity) of the plants' enzymes. The total protein content was measured by the (Bradford 1976) which uses BSA as a standard.

In order to measure the TAC, Glutathione (Sigma Aldrich) was used as a standard for capacity. Five hundred and eighty-five µl of 20 µM bathocuproine solution purchased from Sigma Aldrich and 15 µl of extract solution were mixed in an 1 ml tube. Two-hundred µl of mixed solution were transferred to a 96-plate well and measured at 490 nm (S1). After, 50 µl of $CuCl_2$ (Sigma Aldrich) were added. After 2 minutes, 1 mM of EDTA (stop solution) was added and measured at 490 nm (S2). TAC was measured as (S2-S1) divided by the total protein content.

SOD was measured using the (Peskin and Winterbourn 2000) method. Also, a SOD assay kit (Dojindo Molecular Technologies, Inc) was used. Twenty µl of the extract solution were moved to blank 2 and a sample well and 20 µl DW were moved to blank 1 and blank 3. 200 µl WST solutions were moved to all wells. Dilution buffer was put into blank 2 and blank 3, while enzyme working solution was moved to a sample well and blank 1. After a 20 minute wait, the absorbance was measured at 450 nm. This method was developed governed by the principle that WST-1 degrades superoxide anion with SOD which is SOD's substrate.

$$\text{SOD activity} = [(A_{blank1}-A_{blank3})-(A_{sample}-A_{blank2})]/(A_{blank1}-A_{blank3}) \times 100$$

2.2.2. Measurement of Chlorophyll contents

Chlorophyll contents of the plants were measured by the DMSO (dimethyl sulphoxide) extraction method (Hiscox and Israelstam 1979; Tait and Hik 2003). The DMSO method has two principal advantages compared to other extraction methods (Hiscox and Israelstam 1979; Richardson et al. 2002). First, it is faster, largely because grinding and centrifuging is not required in this method (Richardson et al. 2002). Second, the chlorophyll extracts are more stable in DMSO and do not break down as quickly as those in acetone do (Richardson et al. 2002). For the extraction of chlorophyll, 0.01 g of plant leaf was placed into e-tubes with 1 ml of DMSO. Then, the e-tube were placed in a water bath and heated for 4 hours at 65 ℃. Finally, 0.3 ml of the extract were moved to a 96-well plate and measured by a UV/visible spectrophotometer (Spectramax Plus 384, Molecular Devices, CA, USA) at 663 and 645 nm. Chlorophyll contents were calculated using the equations proposed by (Arnon, 1949). Arnon's equations are shown below:

Chla (mg/kg) = 12.7 A_{663} – 2.69 A_{645}
Chlb (mg/kg) = 22.9 A_{645} – 4.68 A_{663}
total Chl (mg/kg) = 20.2 A_{645} + 8.02 A_{663}

2.2.3. ICP analysis

In the analysis of Ti and Zn, 0.5 g of dried and milled plants were pretreated with 60 % HNO_3 for 24 hours and heated to 80 ℃ for 2 hours. Then, 10 ml of 70 % perchloric acid was added and the solution was heated to 200 ℃ until it turned clear. The samples were then filtered with Whatman 44 and an ICP emission spectrometer (ICPS-1000IV, Shimadzu, Japan) analyzed their contents.

2.2.4. FE-SEM and Image J analysis

To determine NP size, a Pertri dish with filter paper and 5 ml of test medium, but no seeds or seedlings, was set up. The solution collected for 14 days. A drop of collected nano-solution was then dried on a microscopic slide glass and used for particle size research by the FE-SEM (Field Emission Scanning Electron Microscope), (AURIGA, Carl Zeiss, German). The procedure was conducted under consultation of the Nano-imaging laboratory of The National Instrumentation Center for Environmental Management (NICEM) in Korea. Before measuring samples, FE-SEM required preprocessing. Samples were placed into a jar exhausted with vapor deposition and by platinum vaporing in tungsten. A platinum layer coated the surface of the samples, where platinum's thickness ranged from 50～200 Å. For solutions that showed particle diameters over 1000 nm, the Axio Zeiss Imager A1 with a differential interference contrast (DIC) microscope (Carl Zeiss, Oberkochen, Germany) performed the measurement. The Image J program (National Institutes of Health, USA) analyzed the diameters of the particles by selecting 50 random particles from the obtained image.

2.2.5. Pchem analysis

The ELS (ELS-Z2, Otsuka electronics, Japan) measured Pchem (Physical chemistry) data (hydrodynamic diameter and zeta potential) at 24℃ under dark conditions.

2.3. Statistical analysis

A one-way ANOVA was performed to identify the significant difference between treatments. When a significant difference was detected, a post hoc Tukey's Studentized Range (HSD) Test was assessed using the SAS 9.1 program (SAS Institute Inc, USA). Differences were considered significant when $p < 0.05$.

Ⅲ. Results

3.1. Seed germination rate of nano-TiO_2 and nano-ZnO treated tomato (*L. esculentum*) and kidney bean (*P. vulgaris*)

Fig. 3. shows the effect of A) nano-TiO_2 and B) nano-ZnO on seed germination of the tomato (*L. esculentum*) plant. Values represent mean ± SE of 10 replicates. Symbols having the same letter are not significantly different at the 0.05 level. For germination research, the seeds were soaked in nano-TiO_2 solutions with concentrations of 0, 50, 100, 1000, 2500 and 5000 mg/L and tested for 12 days (every 3 days). And the other seeds were soaked in nano-ZnO solutions with concentrations of 0, 100, 500, 1000, 2500 and 5000 mg/L and tested for 12 days (every 3 days). Both nano-TiO_2 and nano-ZnO treated tomatoes with different concentration gradients had no significant dependences in seed germination.

Fig. 4. shows the effect of A) nano-TiO_2 and B) nano-ZnO on seed germination of the kidney bean (*P. vulgaris*) plant. Values represent mean ± SE of 10 replicates. Symbols having the same letter are not significantly different at the 0.05 level. Both nano-TiO_2 and nano-ZnO treated kidney beans with different concentration gradients had no significant dependences in seed germination.

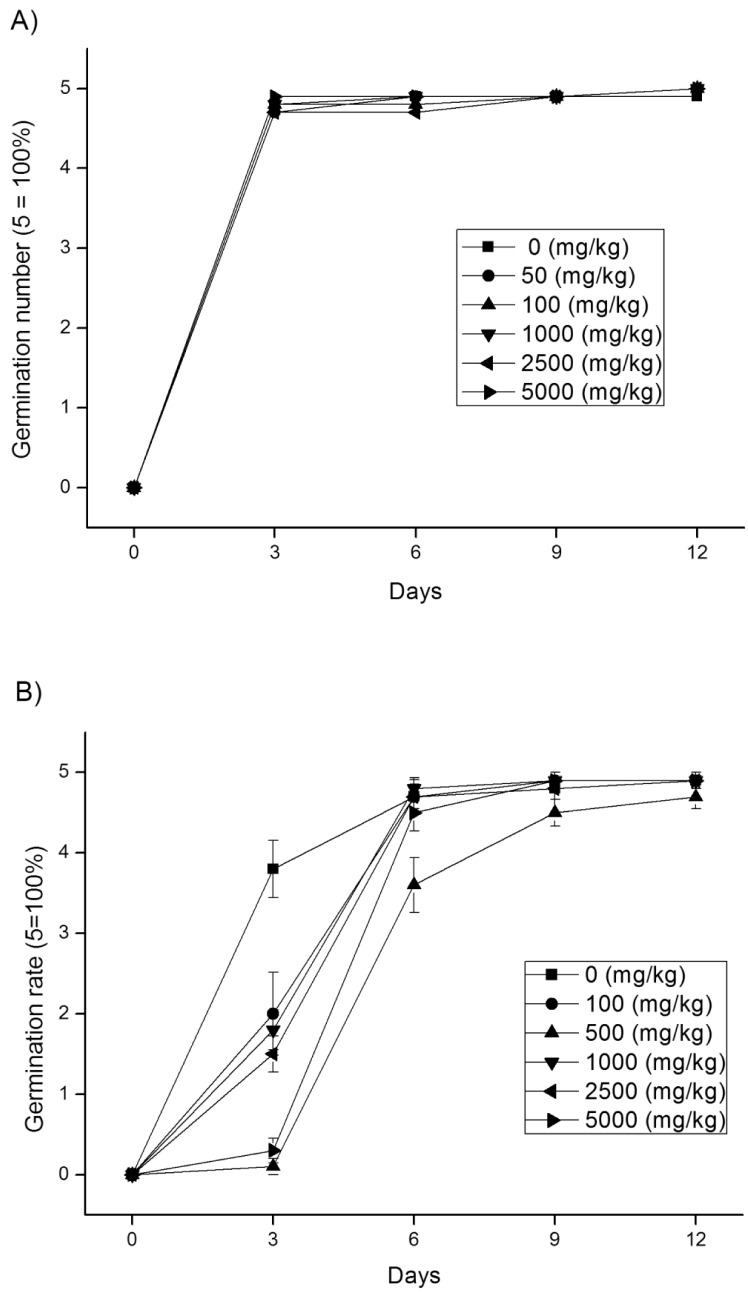


Fig. 3. Effect of A) nano-TiO_2 and B) nano-ZnO on seed germination of tomato (*L. esculentum*). Values represent mean ± SE of 10 replicates. Symbols having the same letter are not significantly different at the 0.05 level.

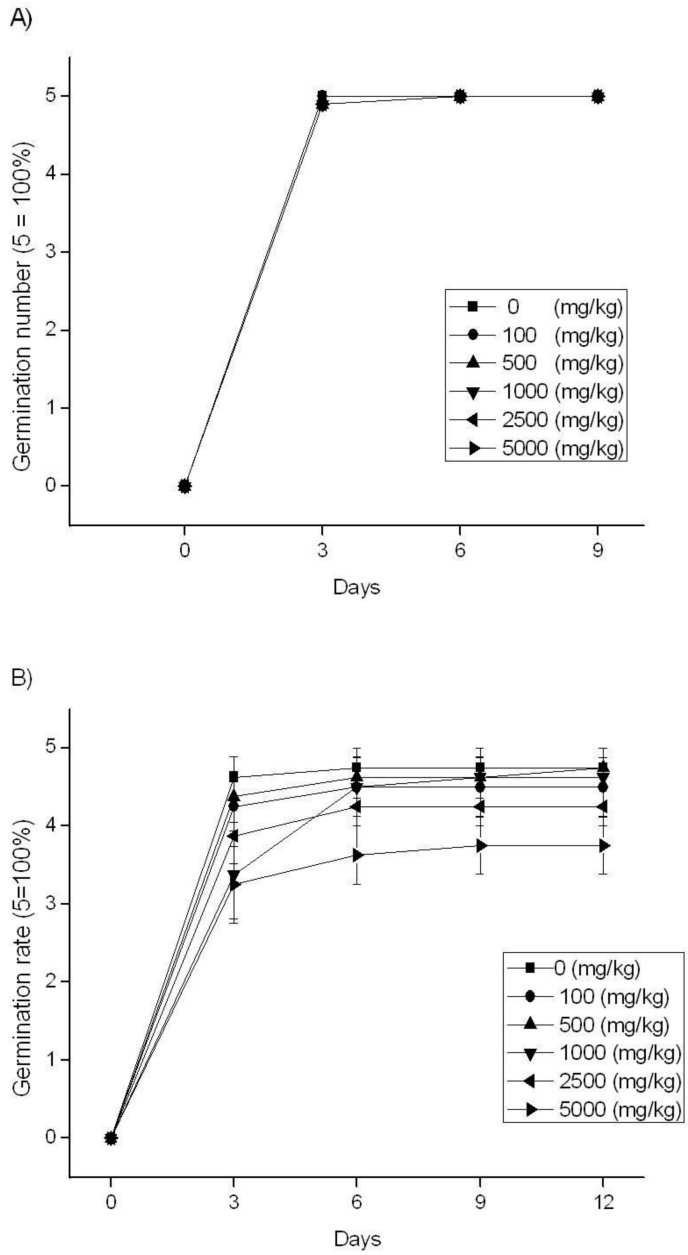


Fig. 4. Effect of A) nano-TiO_2 and B) nano-ZnO on seed germination of kidney bean (*P. vulgaris*). Values represent mean ± SE of 10 replicates. Symbols having the same letter are not significantly different at the 0.05 level.

3.2.1. nano-TiO_2 and nano-ZnO uptake by tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) seeds

Table 1. shows the uptake of nano-TiO_2 by the tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) seeds. The concentrations of nano-TiO_2 were treated by 100, 1000 and 5000 mg/L. The amount of nano-TiO_2 uptake was measured by ICP emission spectrometer (ICPS-1000IV, Shimadzu,Japan). Tomato seeds showed; 100 mg/L nano-TiO_2: 26.4 ± 4.6 mg/kg, 1000 mg/L nano-TiO_2: 97.1 ± 18.2 mg/kg, 5000 mg/L nano-TiO_2: 163.4 ± 53.5 mg/kg. Kidney bean seeds with the surface removed showed; 100 mg/L nano-TiO_2: 1.08 ± 0.21 mg/kg, 1000 mg/L nano-TiO_2: 0.92 ± 0.23 mg/kg, 5000 mg/L nano-TiO_2: 0.75 ± 0.15 mg/kg. And kidney bean seeds with the surface included showed; 1000 mg/L nano-TiO_2: 0.59 ± 0.15 mg/kg, 5000 mg/L nano-TiO_2: 0.74 ± 0.05 mg/kg. Kidney bean seeds treated with 100 mg/L nano-TiO_2 showed no detection of nano-TiO_2. Values represent mean ± SE of 3 replicates and values having the same letter are not significantly different at the 0.05 level. Values of nano-TiO_2 treatments indicate concentration of Ti. These results show that the nano-TiO_2 concentration has no significant effect on uptake of tomato seeds and kidney bean seeds except the surface included kidney bean seeds. In this case, 100 mg/L nano-TiO_2 had the different letter compared with 1000 and 5000 mg/L nano-TiO_2. And according to values, tomato seeds absorbed more nano-TiO_2 than kidney bean seeds while the surface removed kidney bean seeds and the surface included kidney bean seeds didn't show any other differences.

Table 3. nano-TiO_2 uptake of tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) seeds after exposure of 48 hours (mg/kg).

Treatment	nano-TiO_2 Uptake (mg/kg)
tomato 100 mg/L	26.4 ± 4.6
tomato 1000 mg/L	97.1 ± 18.2
tomato 5000 mg/L	163.4 ± 53.5
kidney bean (no surface) 100 mg/L	1.08 ± 0.21
kidney bean (no surface) 1000 mg/L	0.92 ± 0.23
kidney bean (no surface) 5000 mg/L	0.75 ± 0.15
kidney bean 100 mg/L	*NM*[b]
kidney bean 1000 mg/L	0.59 ± 0.15[a]
kidney bean 5000 mg/L	0.74 ± 0.05[a]

NM: Not measured
Values represent mean ± SE of 3 replicates.
Values having the same letter are not significantly different at the 0.05 level.
Values of nano-TiO_2 treatments indicate concentration of Ti.

Table 2. shows the uptake of nano-ZnO by the kidney bean (*P. vulgaris*) seeds. The concentrations of nano-ZnO were treated by 100, 1000 and 5000 mg/L. Kidney bean seeds showed; 100 mg/L nano-ZnO: 1.39 ± 0.08 mg/kg, 1000 mg/L nano-ZnO: 7.30 ± 1.26 mg/kg, 5000 mg/L nano-ZnO: 16,47 ± 5.67 mg/kg. Values represent mean ± SE of 3 replicates and values having the same letter are not significantly different at the 0.05 level. Values of nano-ZnO treatments indicate concentration of Zn. These results show that the amount of nano-ZnO uptake by kidney bean seeds has a significant difference with concentration gradients. When the concentration of treated nano-ZnO increased, the amount of nano-ZnO uptake increased.

Table 4. nano-ZnO uptake of kidney bean (*P. vulgaris*) seeds after exposure of 48 hours (mg/kg).

Treatment	nano-ZnO Uptake (mg/kg)
kidney bean 100 mg/L	55.45 ± 3.34[b]
kidney bean 1000 mg/L	291.90 ± 50.50[ab]
kidney bean 5000 mg/L	658.65 ± 226.61[a]

Values represent mean ± SE of 3 replicates.
Values having the same letter are not significantly different at the 0.05 level.
Values of nano-ZnO treatments indicate concentration of Zn.

3.2.2. Effect of nano-ZnO on the increase rate of kidney bean (*P. vulgaris*)'s seed biomass

Fig. 5. shows the effect of nano-ZnO on the increase rate of kidney bean (*P. vulgaris*)'s seed biomass 48 hours after treatment. Bars having the same letter are not significantly different at the 0.05 level. The concentration levels were setted as 0, 100, 500, 1000, 2500 and 5000 mg/L. The level of significance was different with some concentration levels. Kidney beans treated with 0 and 100 mg/L nano-ZnO showed the same letter. In addition, kidney beans treated with 500 and 1000 mg/L nano-ZnO showed the same letter but 2500 and 5000 mg/L nano-ZnO showed different letters that were different from the letters before explained. When the concentration of treated nano-ZnO increased, increase rate of seed biomass decreased generally.

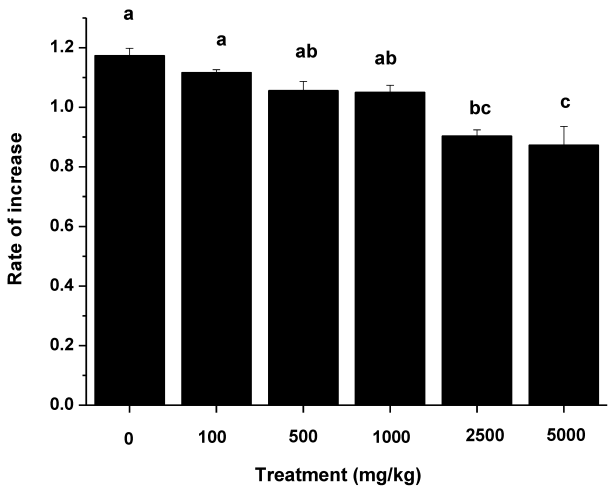


Fig.5. Effect of nano-ZnO on the increase rate of kidney bean (*P. vulgaris*)'s seed biomass 48 hours after treatment. Bars having the same letter are not significantly different at the 0.05 level.

3.3. Root elongation rate of nano-TiO_2 and nano-ZnO treated tomato (*L. esculentum*) and kidney bean (*P. vulgaris*)

Fig. 6. shows the effect of A) nano-TiO_2 and B) nano-ZnO on root elongation of the tomato (*L. esculentum*) plant. Values represent mean ± SE of 50 replicates. Symbols having the same letter are not significantly different at the 0.05 level. For root elongation research, the rooted seeds were soaked in nano-TiO_2 with concentrations of 0, 50, 100, 1000, 2500 and 5000 mg/L and tested for 15 days (every 3 days). And the other rooted seeds were soaked in nano-ZnO with concentrations of 0, 100, 500, 1000, 2500 and 5000 mg/L and tested for 12 days (every 3 days). While nano-TiO_2 solutions on tomatoes had no significant dependence with concentration gradients, nano-ZnO solutions on tomatoes had a significant difference between 0 mg/L and other concentration gradients. In this case of nano-ZnO, except for the tomatoes of 0 mg/L concentration, the other tomatoes of the rest concentrations almost all died in early days of experiments.

Fig. 7. shows the effect of A) nano-TiO_2 and B) nano-ZnO on root elongation of the kidney bean *(P. vulgaris)* plant. Values represent mean ± SE of 50 replicates. Symbols having the same letter are not significantly different at the 0.05 level. While nano-TiO_2 solutions on kidney beans had no significant difference with concentration gradients, nano-ZnO solutions on kidney beans had significant differences with concentration gradients.

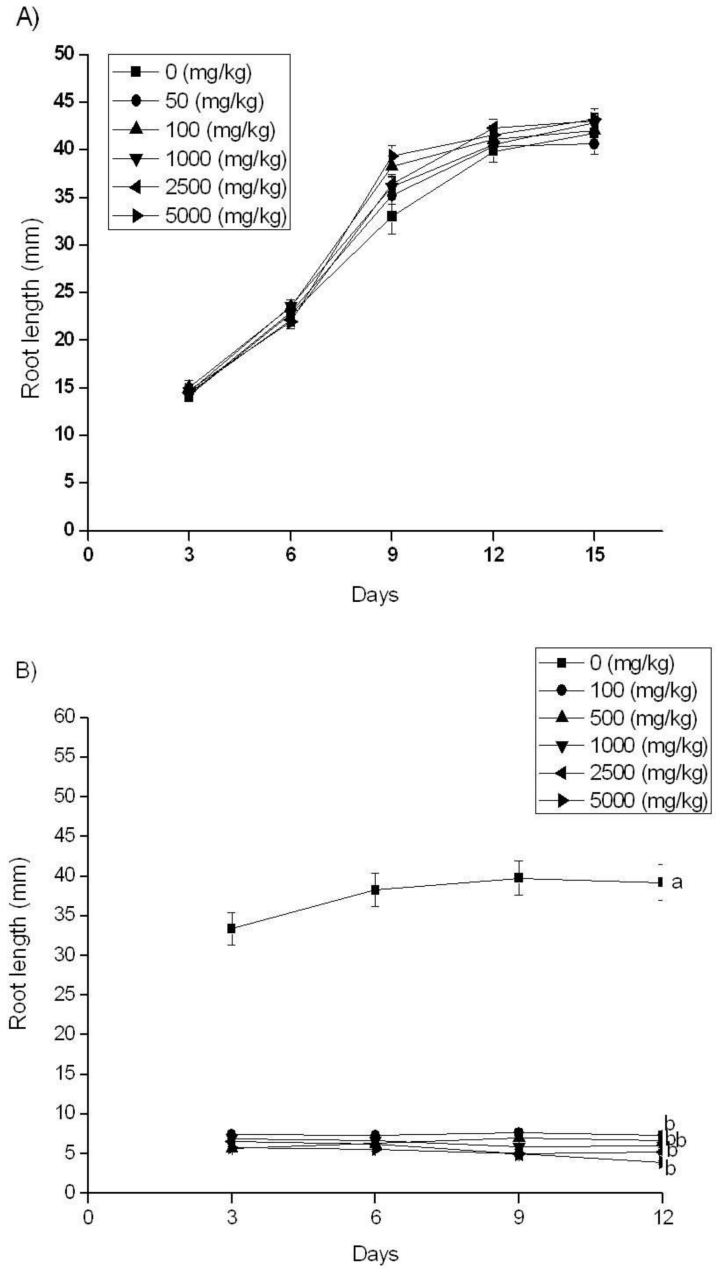


Fig. 6. Effect of A) nano-TiO_2 and B) nano-ZnO on root elongation of tomato (*L. esculentum*). Values represent mean ± SE of 50 replicates. Symbols having the same letter are not significantly different at the 0.05 level.

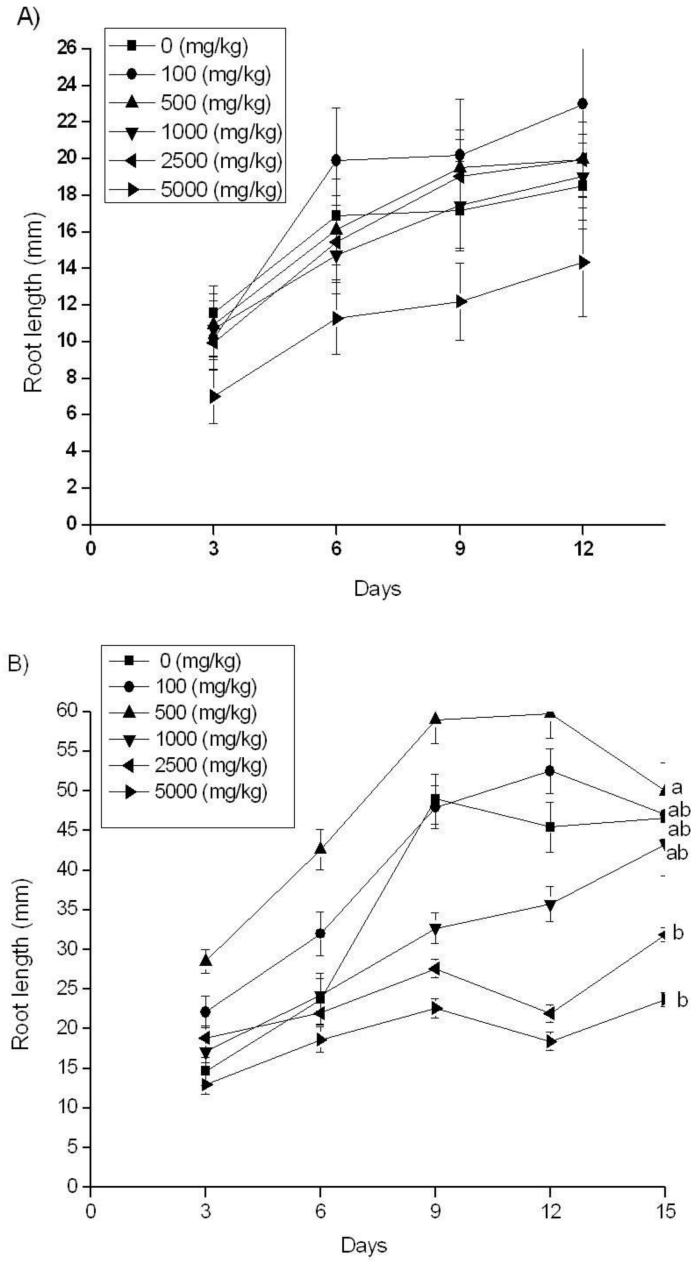


Fig. 7. Effect of A) nano-TiO_2 and B) nano-ZnO on root elongation of kidney bean (*P. vulgaris*). Values represent mean ± SE of 50 replicates. Symbols having the same letter are not significantly different at the 0.05 level.

3.4.1. nano-TiO_2 and nano-ZnO uptake by tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) seedlings

Table 3. shows nano-TiO_2 uptake of tomato (*L. esculentum*) seedlings and kidney bean (*P. vulgaris*) seedlings after exposure of 48 hours (mg/kg). Values of nano-TiO_2 treatments indicate concentration of Ti. In the case of tomato seedlings, there were no significant dependence with concentration gradients of nano-TiO_2. But nano-TiO_2 on kidney bean seedlings had significant differences with concentration gradients of 100, 1000 mg/L nano-TiO_2 solutions and 5000 mg/L nano-TiO_2 solution. Kidney bean seedlings showed; 100 mg/L nano-TiO_2: 1.41 ± 0.48 mg/kg, 1000 mg/L nano-TiO_2: 1.93 ± 0.23 mg/kg, 5000 mg/L nano-TiO_2: 20.43 ± 1.47 mg/kg. At the highest concentration (5000 mg/L), the nano-TiO_2 uptake of kidney bean seedlings was the highest.

Table 4. shows nano-ZnO uptake of tomato (*L. esculentum*) seedlings and kidney bean (*P. vulgaris*) seedlings after exposure of 48 hours (mg/kg). Values of nano-ZnO treatments indicate concentration of Zn. Tomato seedlings showed; 100 mg/L nano-ZnO: 5.61 ± 0.12 mg/kg, 1000 mg/L nano-ZnO: 6.90 ± 0.62 mg/kg, 5000 mg/L nano-ZnO: 10.84 ± 1.29 mg/kg. The uptake of tomato seedlings showed difference between 1000 mg/L nano-ZnO and other treatments of nano-ZnO. Kidney bean seedlings showed; 100 mg/L nano-ZnO: 1.13 ± 0.04 mg/kg, 1000 mg/L nano-ZnO: 8.47 ± 0.09 mg/kg, 5000 mg/L nano-ZnO: 14.62 ± 0.52 mg/kg. This result means that kidney bean seedlings have significant differences among concentrations of 100, 1000 and 5000 mg/L nano-ZnO.

Table 3. nano-TiO_2 uptake of tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) seedlings after exposure of 48 hours (mg/kg).

Treatment	nano-TiO_2 Uptake (mg/kg)
tomato 100 mg/L	5.7
tomato 1000 mg/L	8.4
tomato 5000 mg/L	25.5
kidney bean 100 mg/L	1.41 ± 0.48^{b}
kidney bean 1000 mg/L	1.93 ± 0.23^{b}
kidney bean 5000 mg/L	20.43 ± 1.47^{a}

Values of nano-TiO_2 treatments indicate concentration of Ti.
(In the case of tomato, there are no replicates because the biomass of seedlings were too small.)

Table 4. nano-ZnO uptake of tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) seedlings after exposure of 48 hours (mg/kg).

Treatment	nano-ZnO Uptake (mg/kg)
tomato 100 mg/L	5.61 ± 0.12^{b}
tomato 1000 mg/L	6.90 ± 0.62^{b}
tomato 5000 mg/L	10.84 ± 1.29^{a}
kidney bean 100 mg/L	1.13 ± 0.04^{c}
kidney bean 1000 mg/L	8.47 ± 0.09^{b}
kidney bean 5000 mg/L	14.62 ± 0.52^{a}

Values of nano-ZnO treatments indicate concentration of Zn.

3.4.2. Effect of nano-TiO_2 on the seedling biomass of tomato (*L. esculentum*)

Fig. 8. shows the effect of nano-TiO_2 on the seedling biomass of tomato (*L. esculentum*) 15 days after treatment. Values represent mean ± SE of 50 replicates. Bars having the same letter are not significantly different at the 0.05 level. (The biomass is fresh weight.) The concentration levels were setted as 0, 50, 100, 1000, 2500 and 5000 mg/L. It shows that the seedling biomass of tomatoes is not significantly different with concentration gradients.

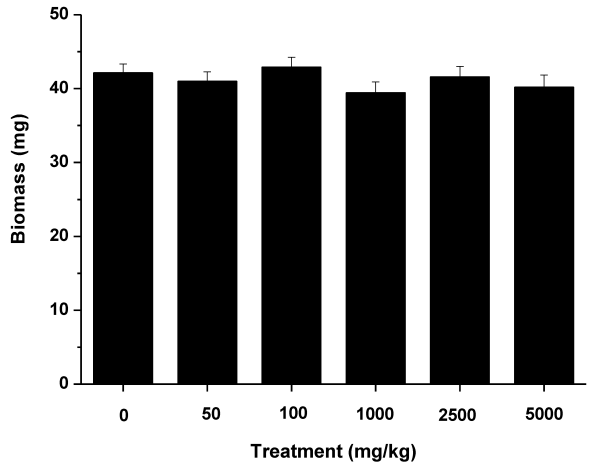


Fig. 8. Effect of nano-TiO_2 on the seedling biomass of tomato (*L. esculentum*) 15 days after treatment. Values represent mean ± SE of 50 replicates. Bars having the same letter are not significantly different at the 0.05 level. (The biomass is fresh weight.)

3.5. Hydrodynamic diameter of nano-TiO_2 and nano-ZnO uptake in a petri dish

Table 5. shows the hydrodynamic diameter of nano-TiO_2 and nano-ZnO uptake after 14 days in a petri dish. It was measured on the glasses as we see from Fig. 9 and 10. shows the SEM images of nano-ZnO according to concentration levels. Values represent mean ± SE of 50 replicates. The hydrodynamic diameter of nano-TiO_2 showed; 100 mg/L nano-TiO_2: 3422.9 ± 478.1 mg/kg, 1000 mg/L nano-TiO_2: 5834.3 ± 517.8 mg/kg, 5000 mg/L nano-TiO_2: 8869.7 ± 1036.3 mg/kg. And the hydrodynamic diameter of nano-ZnO showed: 100 mg/L nano-ZnO: 3057.6 ± 672.2 mg/kg, 1000 mg/L nano-ZnO: 26772.2 ± 3840.7 mg/kg, 5000 mg/L nano-ZnO: 38205.5 ± 3704.9 mg/kg. Nano-TiO_2 showed no significant difference between the hydrodynamic diameter of 100 and 1000 mg/L but showed significant differences between the hydrodynamic diameter of 100, 1000 mg/L and 5000 mg/L. Also, nano-ZnO showed significant differences among the hydrodynamic diameter of 100, 1000 and 5000 mg/L. When the concentration of nano-TiO_2 and nano-ZnO increased, the hydrodynamic diameter of nano-TiO_2 and nano-ZnO increased and nano-ZnO were larger than nano-TiO_2 at 1000 and 5000 mg/L. At 1000 and 5000 mg/L, nano-ZnO was 4 times larger than nano-TiO_2.

Table 6. shows the hydrodynamic diameter of nano-TiO_2 uptake after 7 days in petri dish. The hydrodynamic diameter of nano-TiO_2 showed: 100 mg/L nano-TiO_2: 63.2 ± 3.7 mg/kg, 1000 mg/L nano-TiO_2: 66.2 ± 3.9 mg/kg, 5000 mg/L nano-TiO_2: 11047.7 ± 1151.4 mg/kg. Nano-TiO_2 showed no significant difference between the hydrodynamic diameter of 100 and 1000 mg/L but showed significant differences between the hydrodynamic diameter of 100, 1000 mg/L and 5000 mg/L. When the concentration of nano-TiO_2 increased, the hydrodynamic diameter of nano-TiO_2 also increased.

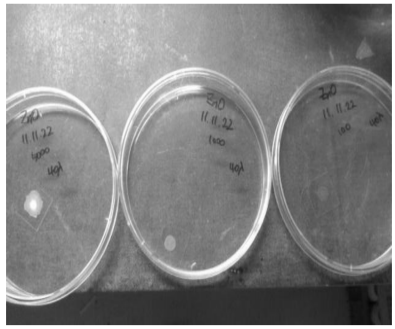
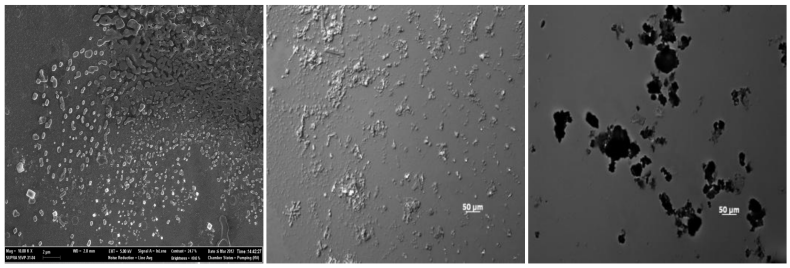


Fig. 9. Preparation of nano-ZnO particles dried on slide glasses.



A) B) C)

Fig. 10. SEM of nano-ZnO particles with concentration gradients.

A) 100 mg/L nano-ZnO, B) 1000 mg/L nano-ZnO, C) 5000 mg/L nano-ZnO

Table 5. Hydrodynamic diameter of nano-TiO_2 and nano-ZnO uptake after 14 days in petri dish.

Treatment	Hydrodynamic diameter (nm)
TiO_2 100 mg/L	3422.9 ± 478.1[b]
TiO_2 1000 mg/L	5834.3 ± 517.8[b]
TiO_2 5000 mg/L	8869.7 ± 1036.3[a]
ZnO 100 mg/L	3057.6 ± 672.2[c]
ZnO 1000 mg/L	26772.2 ± 3840.7[b]
ZnO 5000 mg/L	38205.5 ± 3704.9[a]

Values represent mean ± SE of 50 replicates.

Table 6. Hydrodynamic diameter of nano-TiO_2 uptake after 7 days in petri dish.

Treatment	Hydrodynamic diameter (nm)
TiO_2 100 mg/L	63.2 ± 3.7[b]
TiO_2 1000 mg/L	66.2 ± 3.9[b]
TiO_2 5000 mg/L	11047.7 ± 1151.4[a]

Values represent mean ± SE of 50 replicates.

3.6. Chlorophyll contents of mature tomato *(L. esculentum)* and kidney bean *(P. vulgaris)* after nano-TiO_2 and nano-ZnO exposure of 7 days (mg/L)

Table 7. shows chlorophyll contents of the mature tomato (*L. esculentum*) plant after nano-TiO_2 and nano-ZnO exposure of 7 days (mg/L). Values represent mean ± SE of 4 replicates. Values having the same letter are not significantly different at the 0.05 level. There were no significant differences in chlorophyll a, chlorophyll b and total chlorophyll with concentration gradients of nano-TiO_2. And also, there were no significant differences in chlorophyll a, chlorophyll b and total chlorophyll with concentration gradients of nano-ZnO.

Table 8. shows chlorophyll contents of the mature kidney bean (*P. vulgaris*) plant after nano-ZnO exposure of 7 days (mg/L). Values represent mean ± SE of 4 replicates. Values having the same letter are not significantly different at the 0.05 level. Likewise with the chlorophyll contents of tomatoes of Table 7, there were no significant differences in chlorophyll a, chlorophyll b and total chlorophyll with concentration gradients of nano-ZnO.

Table 7. Chlorophyll contents of mature tomato (*L. esculentum*) after nano-TiO_2 and nano-ZnO exposure of 7 days (mg/L).

Treatment	Chl a	Chl b	Total Chl
TiO_2 0 mg/L	5.82 ± 0.24	2.48 ± 0.07	8.30 ± 0.31
TiO_2 1000 mg/L	4.55 ± 0.38	2.43 ± 0.19	6.97 ± 0.54
TiO_2 5000 mg/L	5.88 ± 0.96	2.74 ± 0.37	8.62 ± 1.33
ZnO 0 mg/L	1.24	1.06	2.29
ZnO 1000 mg/L	4.18	1.99	6.16
ZnO 5000 mg/L	2.91	1.62	4.53

Values represent mean ± SE of 4 replicates.
Values having the same letter are not significantly different at the 0.05 level.
(In the case of nano-ZnO, there are no replicates because the biomass of tomato leaves were too small.)

Table 8. Chlorophyll contents of mature kidney bean (*P. vulgaris*) after nano-ZnO exposure of 7 days (mg/L).

Treatment	Chl a	Chl b	Total Chl
ZnO 0 mg/L	8.05 ± 0.75	2.47 ± 0.19	10.92 ± 0.94
ZnO 1000 mg/L	7.28 ± 0.43	2.75 ± 0.11	10.04 ± 0.54
ZnO 5000 mg/L	8.86 ± 1.26	3.20 ± 0.34	12.07 ± 1.61

Values represent mean ± SE of 4 replicates.
Values having the same letter are not significantly different at the 0.05 level.

3.7. Antioxidant enzyme activitity of tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) by nano-TiO_2 and nano-ZnO

3.7.1. Total antioxidant capacity (TAC)

Fig. 11. shows the Total antioxidant capacity (TAC) of A) nano-TiO_2 treated mature tomato (*L. esculentum*) plant, B) nano-ZnO treated mature tomato (*L. esculentum*) plant and C) nano-ZnO treated mature kidney bean (*P. vulgaris*) plant. Values represent mean ± SE of 4 replicates. Bars having the same letter are not significantly different at the 0.05 level. There was no significant difference with different concentration gradients in both nano-TiO_2 treated tomatoes and nano-ZnO treated tomatoes and kidney beans.

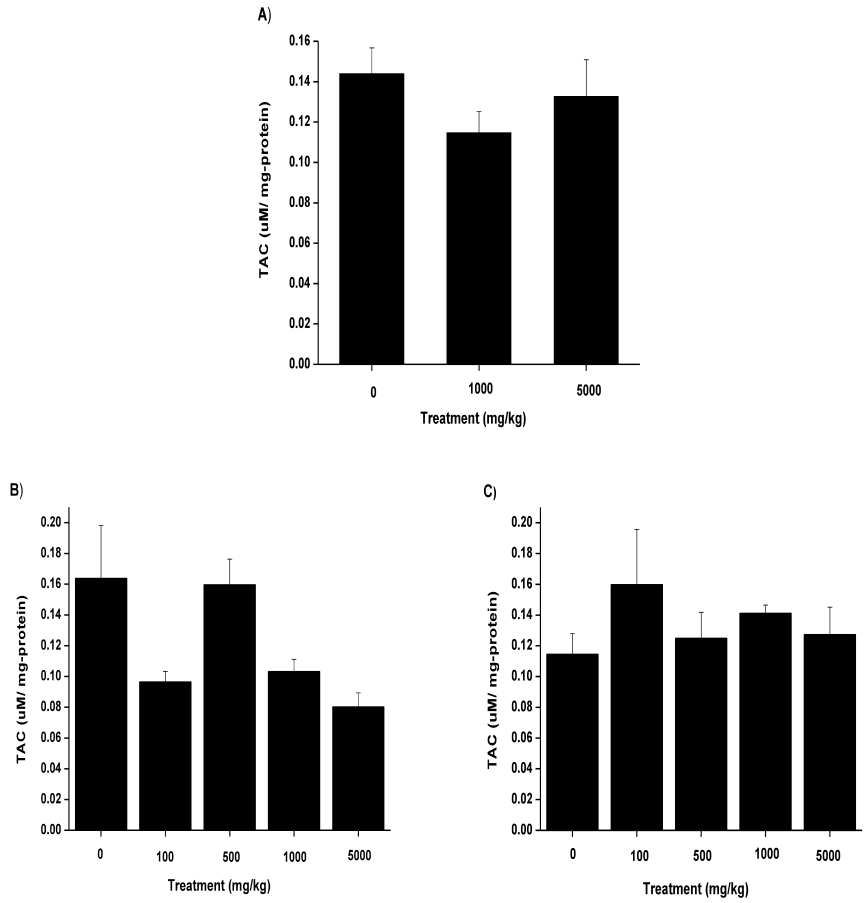


Fig. 11. Total antioxidant capacity (TAC) of A) nano-TiO_2 treated mature tomato (*L. esculentum*), B) nano-ZnO treated mature tomato (*L. esculentum*) and C) nano-ZnO treated mature kidney bean (*P. vulgaris*). Values represent mean ± SE of 4 replicates. Bars having the same letter are not significantly different at the 0.05 level.

3.7.2. Superoxide dismutase activity (SOD)

Fig. 12. shows the Superoxide dismutase activity (SOD) of A) nano-TiO_2 treated mature tomato (*L. esculentum*) plant, B) nano-ZnO treated mature tomato (*L. esculentum*) plant and C) nano-ZnO treated mature kidney bean (*P. vulgaris*) plant. Values represent mean ± SE of 4 replicates. Bars having the same letter are not significantly different at the 0.05 level. Firstly, The SOD of nano-TiO_2 treated tomatoes showed significant differences at 1000 mg/L compared with other concentration gradients. But secondly, the SOD of nano-ZnO treated tomatoes and kidney beans showed no significant difference with concentration gradients.

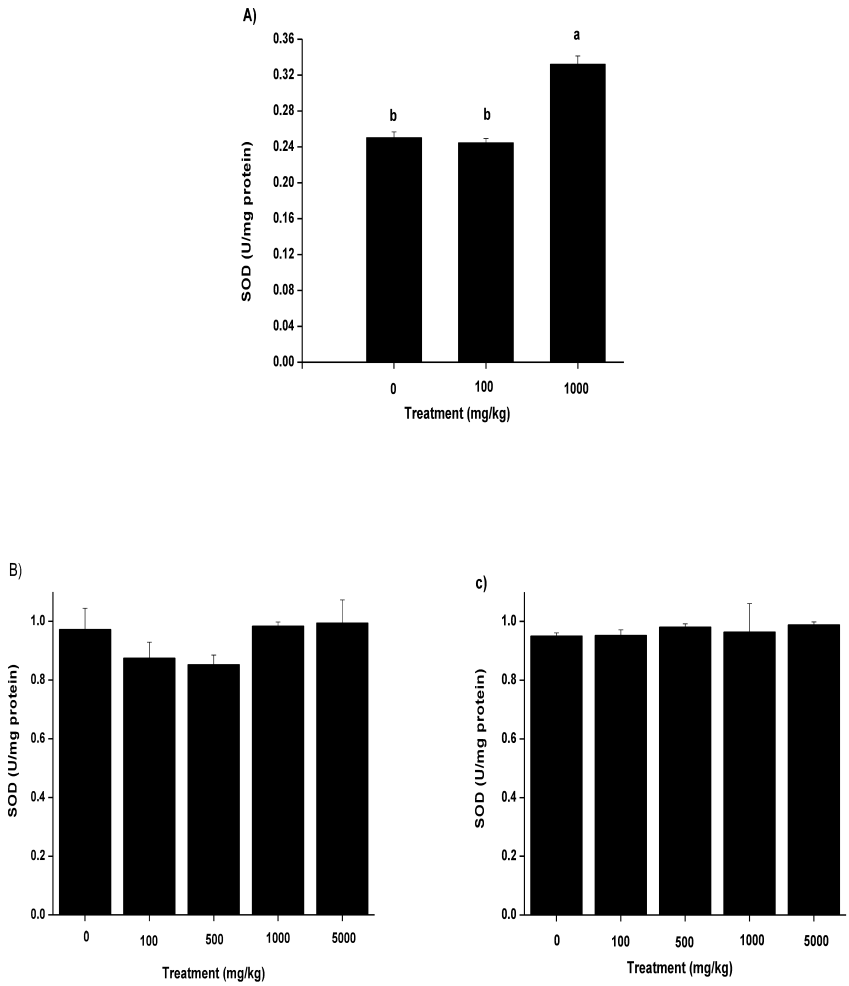


Fig. 12. Superoxide dismutase activity (SOD) of A) nano-TiO_2 treated mature tomato (*L. esculentum*), B) nano-ZnO treated mature tomato (*L. esculentum*) and C) nano-ZnO treated mature kidney bean (*P. vulgaris*). Values represent mean ± SE of 4 replicates. Bars having the same letter are not significantly different at the 0.05 level.

3.8.1. nano-TiO_2 and nano-ZnO uptake by mature tomato (*L. esculentum*) and mature kidney bean (*P. vulgaris*)

Table 9. shows nano-TiO_2 uptake of mature tomato (*L. esculentum*) plant after exposure of 5 weeks (mg/L). Values represent mean ± SE of 3 replicates. Values having the same letter are not significantly different at the 0.05 level. The ICP analysis was made by 4 parts of the mature plants; root, stem, leaf and fruit. The concentrations of nano-TiO_2 were 1000 and 5000 mg/L. They showed no significant differences in root, leaf and fruit but showed the significant increase in stem at 1000 mg/L compared with 5000 mg/L. The nano-TiO_2 uptake of tomato stems showed: 1000 mg/L nano-TiO_2: 10.76 ± 1.29 mg/kg, 5000 mg/L nano-TiO_2: 5.76 ± 0.49 mg/kg.

Table 10. shows nano-ZnO uptake of mature tomato (*L. esculentum*) plant and kidney bean (*P. vulgaris*) plant after exposure of 5 weeks (mg/L). Values represent mean ± SE of 3 replicates. Values having the same letter are not significantly different at the 0.05 level. The nano-ZnO uptake of tomatoes showed: 1000 mg/L nano-ZnO: 7.41 ± 1.44 mg/kg, 5000 mg/L nano-ZnO: 20.98 ± 3.25 mg/kg. These 2 values showed the significant increase in tomatoes at 5000 mg/L compared with 1000 mg/L but there was no significant difference in kidney beans.

Table 9. nano-TiO_2 uptake of mature tomato (*L. esculentum*) after exposure of 5 weeks (mg/L).

Treatment	root	stem	leaf	fruit
TiO_2 1000 mg/L	94.64 ± 17.81	10.76 ± 1.29[a]	5.82 ± 0.66	2.63 ± 0.17
TiO_2 5000 mg/L	52.36 ± 11.86	5.76 ± 0.49[b]	4.40 ± 0.82	3.10 ± 0.43

Values represent mean ± SE of 3 replicates.
Values having the same letter are not significantly different at the 0.05 level.

Table 10. nano-ZnO uptake of mature tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) after exposure of 5 weeks (mg/L).

Treatment	mature plant
tomato 1000 mg/L	7.41 ± 1.44[b]
tomato 5000 mg/L	20.98 ± 3.25[a]
kidney bean 1000 mg/L	2.68 ± 0.06
Kidney bean 5000 mg/L	4.37 ± 0.88

Values represent mean ± SE of 3 replicates.
Values having the same letter are not significantly different at the 0.05 level.

3.8.2. Effect of nano-ZnO on the height of mature kidney bean (*P. vulgaris*)

Fig. 13. shows the 15 days after treatment. Values represent mean ± SE of 10 replicates. Bars having the same letter are not significantly different at the 0.05 level. The concentration levels were setted as 0, 100, 500, 1000 and 5000 mg/L. It shows that the effect of nano-ZnO on the height of mature kidney bean (*P. vulgaris*) is not significantly different with concentration gradients.

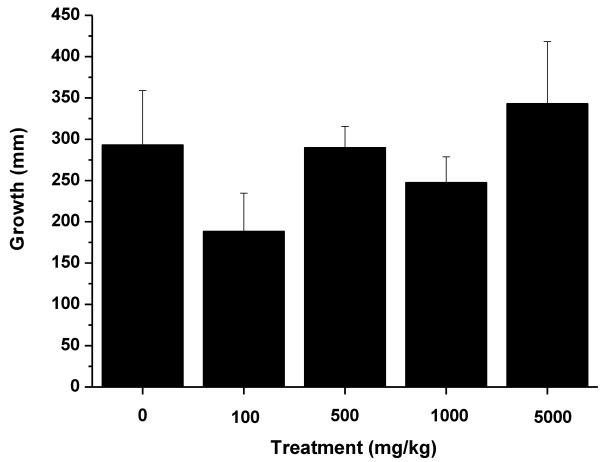


Fig. 13. Effect of nano-ZnO on the height of mature kidney bean (*P. vulgaris*) 15 days after treatment. Values represent mean ± SE of 10 replicates. Bars having the same letter are not significantly different at the 0.05 level.

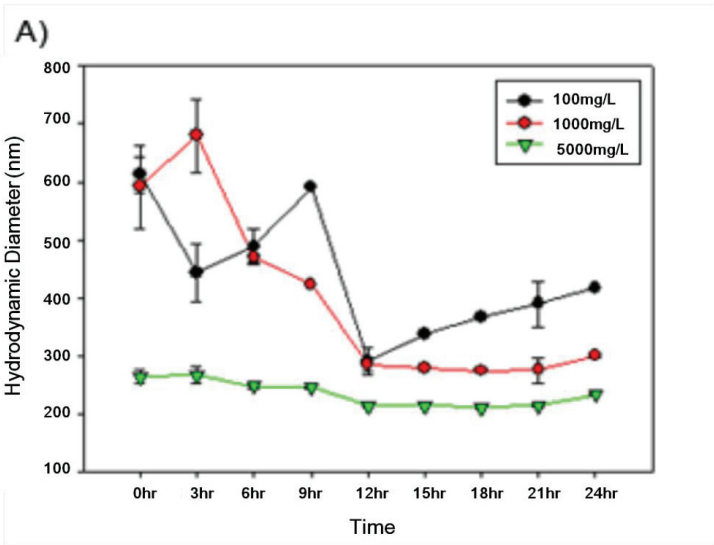
3.9. Pchem results of nano-TiO_2 and nano-ZnO

3.9.1. Pchem results (Hydrodynamic diameter) of A) nano-TiO_2 in DW and B) nano- TiO_2 in Hoagland solution

Fig. 14. shows the Pchem results (Hydrodynamic diameter) of A) nano-TiO_2 in DW and B) nano- TiO_2 in Hoagland solution. Values represent mean ± SE of 150 replicates for Hydrodynamic diameter.

In Fig. 14, A), the hydrodynamic diameter of nano-TiO_2 in DW showed a pattern of fluctuation with concentrations of 100, 1000 and 5000 mg/L for 24 hours. (It was calculated every 3 hours.) In the case of 100 mg/L of nano-TiO_2, the hydrodynamic diameter changed between 300 nm and 600 nm and it reached the highest peak of 600 nm at 0 hour. And in the case of 1000 mg/L of nano-TiO_2, the hydrodynamic diameter also changed between 300 nm and 700 nm so it reached the highest peak of 700 nm at 3 hour. Finally, at 5000 mg/L of nano-TiO_2, the hydrodynamic diameter changed between 200 nm and 300 nm having small fluctuations. In summary, comparison to 100 mg/L and 1000 mg/L of nano-TiO_2, 5000 mg/L of nano-TiO_2 showed small hydrodynamic diameters.

In Fig. 14, B), the hydrodynamic diameter of nano-TiO_2 in Hoagland solution showed a pattern of fluctuation with concentrations of 100 mg/L and 1000 mg/L for 24 hours. (It was calculated every 3 hours.) In the case of 100 mg/L of nano-TiO_2, the hydrodynamic diameter changed between 0 nm and 5000 nm and it reached the highest peaks at 6 hour and 18 hour. At 1000 mg/L of nano-TiO_2, the hydrodynamic diameter changed between 5000 nm and 10000 nm and it reached the highest peak at 9 hour. These results indicate that when the concentration of nano-TiO_2 in Hoagland solution increases, the hydrodynamic diameter of nano-TiO_2 also increases.



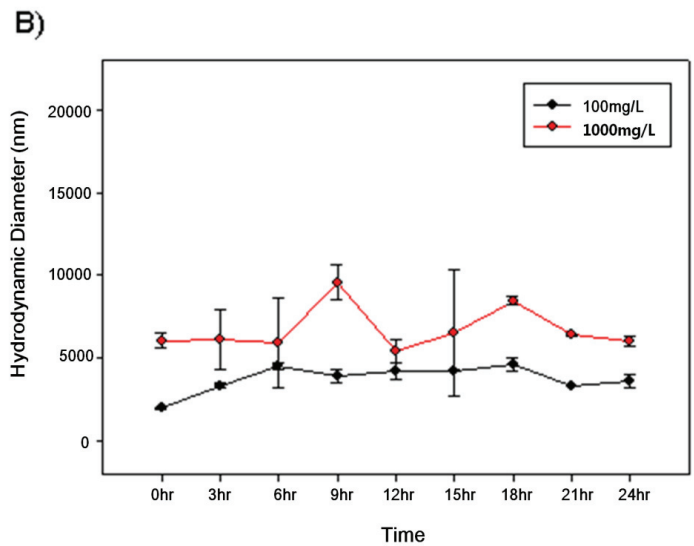


Fig. 14. Pchem results (Hydrodynamic diameter) of A) nano-TiO_2 in DW and B) nano- TiO_2 in Hoagland solution. Values represent mean ± SE of 150 replicates for Hydrodynamic diameter.

3.9.2. Pchem results of A) nano-ZnO (Hydrodynamic diameter) in DW and B) nano-ZnO (Zeta Potential) in DW

Fig. 15. shows the Pchem results of A) nano-ZnO (Hydrodynamic diameter) in DW and B) nano-ZnO (Zeta Potential) in DW. Values represent mean ± SE of 150 replicates for Hydrodynamic diameter and 9 replicates for Zeta Potential.

In Fig. 15, A), the hydrodynamic diameter of nano-ZnO in DW was tested with concentrations of 100, 500, 1000, 2500 and 5000 mg/L. At 100 mg/L , the hydrodynamic diameter of nano-ZnO was 500 nm and at 500 mg/L, the hydrodynamic diameter of nano-ZnO was between 400 nm and 500 nm. Also, at 1000 and 2500 mg/L, the hydrodynamic diameter of nano-ZnO was between 200 nm and 300 nm. Finally, at 5000 mg/L, the hydrodynamic diameter of nano-ZnO was between 100 nm and 200 nm. From the above results, we can conclude that as the concentration of nano-ZnO increases, the hydrodynamic diameter of nano-ZnO decreases.

In Fig. 15, B), the zeta potential of nano-ZnO in DW was tested with concentrations of 100, 500, 1000, 2500 and 5000 mg/L. At 100 mg/L, the zeta potential of nano-ZnO was between 20 nm and 25 nm and at 500 mg/L, the zeta potential of nano-ZnO was 30 nm. And then, at 1000 mg/L , the zeta potential of nano-ZnO was between 30 nm and 35 nm and at 2500 mg/L, the zeta potential of nano-ZnO was between 35 nm and 40 nm. Finally, at 5000 mg/L, the zeta potential of nano-ZnO was between 45 nm and 50 nm. From the above results, we can conclude that as the concentration of nano-ZnO increases, the zeta potential of nano-ZnO also increases.

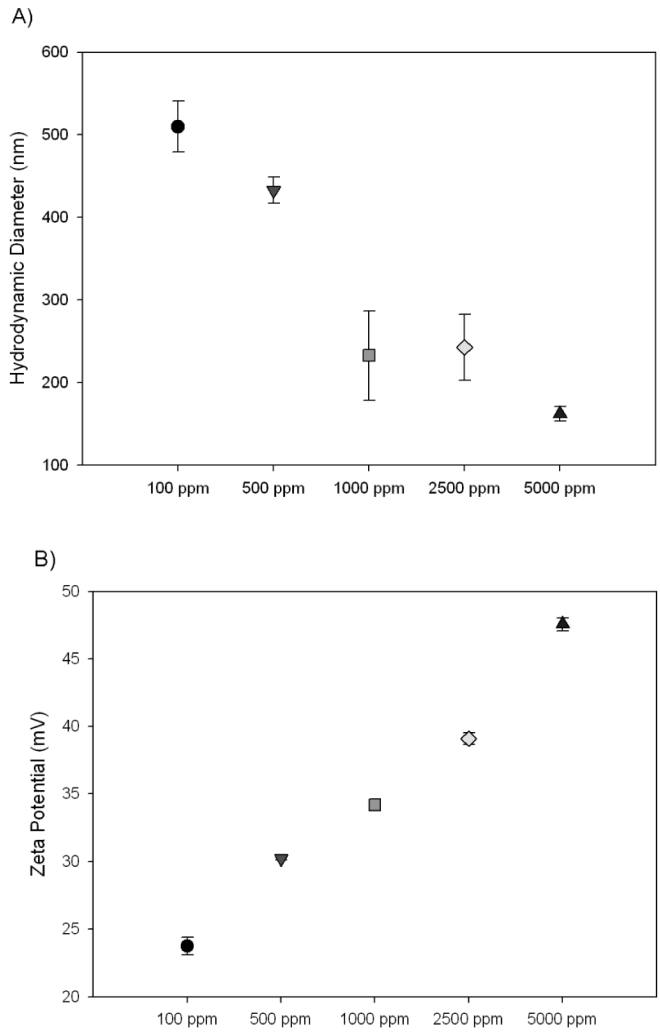


Fig. 15. Pchem results of A) nano-ZnO (Hydrodynamic diameter) in DW and B) nano-ZnO (Zeta Potential) in DW. Values represent mean ± SE of 150 replicates for Hydrodynamic diameter and 9 replicates for Zeta Potential.

Ⅳ. Discussion

Observing seed germination and root elongation is a rapid and widely used acute test used for the measurement of plant growth (Wang et al. 2001; Munzuroglu and Geckil 2002). Due to its several advantages—sensitivity, simplicity, low cost and suitability for unstable chemicals (Wang et al. 2001; Munzuroglu and Geckil 2002)—it can be readily applied to nanoparticles (NPs). From this experiment's result, nano-TiO_2 showed no effect on the tomato (*Lycopersicon esculentum*) and kidney bean (*Phaseolus vulgaris*)'s seed germination and root elongation (Fig. 3, 4, 5, 6), while nano-ZnO similarly showed no effect on the seed germination of tomato (*L. esculentum*) and kidney bean (*P. vulgaris*). However, it did show negative effect on the root elongation of tomato (*L. esculentum*) at all concentration levels and kidney bean (*P. vulgaris*) at high levels (1000, 2500 and 5000 mg/L) (Fig. 3, 4, 5, 6). It is also important to note that unlike the germination data, the root elongation data begins 3 days from the origin of the X axis because newly germinated seed roots at day 0 are hard to measure and overall had the same lengths.

From the above results, it can be deduced that nano-ZnO is considered to be more toxic than nano-TiO_2 at the early seedling stage. This negative effect of nano-ZnO is consistent with several previous studies as discussed previously (Lin and Xing 2007; Lin and Xing 2008; Lee et al. 2010; López-Moreno et al. 2010). Furthermore, there is a study that in Arabidopsis thaliana, seedling growth is more sensitive than seed germination to heavy metals (Li et al. 2005).

During the uptake experiment of nano-TiO_2 and nano-ZnO by the seeds, the kidney beans' (*P. vulgaris*) thick seed coat was of note. During the uptake experiment with nano-TiO_2 or nano-ZnO and the seeds, I thought the coat might affect the uptake amount of nano-TiO_2 and nano-ZnO by the seeds. Therefore, some kidney bean (*P. vulgaris*) seeds had the coating removed and were compared to kidney bean (*P. vulgaris*) seeds without the coating removed. However, both seeds did not show an uptake of nano-TiO_2. In the case of tomato (L. esculentum), when the concentration of nano-TiO_2 increased, the uptake amount by seeds also increased (no significant difference with

concentration gradients). Likewise, nano-ZnO uptake by kidney bean (*P. vulgaris*) similarly increased with raised concentration levels.

Moreover, through the Axio Zeiss Imager A1 with a differential interference contrast (DIC) microscope (Carl Zeiss, Oberkochen, Germany), I observed many hairs on the surface of the tomato (*L. esculentum*) seed. It is possible that these hairs induced adsorption of nano-TiO_2. In the case of kidney bean (*P. vulgaris*), the uptake amount of nano-ZnO was larger than nano-TiO_2, so it is assumed that nano-ZnO was less affected by aggregation than nano-TiO_2.

During the uptake experiments of nano-TiO_2 and nano-ZnO with the seedlings, when the concentration of nano-TiO_2 and nano-ZnO increased, the uptake by the tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) also increased. There was no significant difference in the uptake of nano-TiO_2 by the tomato (*L. esculentum*).

From the above results, we can see that the concentration and uptake of NPs (nano-TiO_2 and nano-ZnO) have a positive correlation. This relation can be explained by aggregation degrees with concentration levels.

Nano-TiO_2 on mature tomato (*L. esculentum*) showed the significantly highest SOD at 1000 mg/L (Fig. 12, A), while nano-ZnO showed no significant differences in chlorophyll contents, TAC and SOD on the mature tomato (*L. esculentum*) and mature kidney bean (*P. vulgaris*) plants. Heavy metals like nano-TiO_2 and nano-ZnO induce the generation of ROS and many other antioxidant enzymes such as SOD, CAT (Catalase) and GPX (Glutathione peroxide). In these enzymes, SOD is the most efficient in scavenging superoxide radicals, (Boominathan and Doran 2002) so SOD was used as a method of measuring antioxidant stress. The higher the SOD value, the more superoxide radicals need to be reacted (Pan et al. 2003). In addition, Total antioxidant status (TAS) is an overall indicator of the antioxidant status of an individual (Pan et al. 2003). As the value increases, the antioxidant defense against free radical reaction increases (Pan et al. 2003). Because TAC can be measured by TAS (Nemec et al. 2000; Fingerova et al. 2007), we used TAC as a parameter for measuring antioxidant stress, as well.

To the author's knowledge, the properties of nano-TiO_2 and nano-ZnO in soil and other conditions of the experiment are considered to be the cause of

the highest SOD (1000 mg/L) of the mature tomato (*L. esculentum*) in the pot with nano-TiO_2. Generally, if they are exposed to soil, through the presence of diverse organic and inorganic materials in soil, NPs are aggregated. The size of these organic and inorganic materials are in the nanometer range (Asli and Neumann 2009) and also can be aggregated with NPs. If the aggregation strongly occurs, NPs lose many of their properties (국립환경과학원 2011). So in the case of this experiment, it is assumed that nano-ZnO is aggregates and precipitates more than nano-TiO_2. Thus nano-TiO_2 may have more NP properties than nano-ZnO. Also, because nano-TiO_2's experiment on the tomato (*L. esculentum*) was conducted in a greenhouse, it is possible that UV rays increased nano-TiO_2's toxicity because the rays induce the generation of ROS. In fact, mature plants don't seem to be affected by NPs much when we see the results.

After harvesting the mature tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) treated by nano-TiO_2 and nano-ZnO, the uptake of nano-TiO_2 and nano-ZnO was analyzed (Table 9, 10). In the uptake of nano-TiO_2 by the tomato (*L. esculentum*), only the stem showed a significant decrease of NP when the concentration increased from 1000 to 5000 mg/L. In addition except fruit, the uptake by the root and leaf also decreased when concentration increased from 1000 to 5000 mg/L. Also, when we transition from root to fruit, we find that the uptake of nano-TiO_2 decreased. In contrast, in the uptake of nano-ZnO by the tomato (*L. esculentum*), It showed a significant increase when the concentration increased from 1000 to 5000 mg/L.

From the uptake results of nano-TiO_2 by the mature tomato (*L. esculentum*) if the concentration of nano-TiO_2 increased, uptake of nano-TiO_2 decreased in the stem area. From this, we can assume that the soil's properties increased the aggregation of nano-TiO_2 when concentration increased. Also, because nano-TiO_2 was initially absorbed from the soil to the root, as it flowed to the leaf, the uptake amount would gradually decreased. Nano-TiO_2 and nano-ZnO's uptake patterns with the tomato (*L. esculentum*) can be explained by their uptake amount difference. Although nano-ZnO's uptake by tomato increased with higher concentrations, its amount was smaller than the amount of nano-TiO_2 uptake by the tomato (*L. esculentum*). The uptake and transport of some

heavy metal species (Howden et al. 1995; Blaudez et al. 2003; Song et al. 2003) and related transporter proteins (Clemens 2001) have previously been studied, but the mechanism is not clarified. There are many research papers about phytoremediation (phytoextraction and phytostabilization) of metal by plants (Dahmani-Muller et al. 2000; Weis and Weis 2004).

In the case of the hydrodynamic diameter by SEM, the size of nano-TiO_2 and nano-ZnO significantly increased from the original size, growing significantly when the concentration of nano-TiO_2 and nano-ZnO increased (Table 5, 6). Also, the size of nano-ZnO was larger than the size of nano-TiO_2 at 1000 and 5000 mg/L.

The similar steady rise of concentration and size of nano-TiO_2 and nano-ZnO can be explained by the process of preparation for the SEM analysis. When we experimented the solution of nano-TiO_2 and nano-ZnO in a petri dish for several days, the solution usually dried and lost volume. Therefore then, we added DW which is considered to have activated the agglomeration and aggregation of NPs. Also, for the SEM analysis, NPs were laid on slide glasses. Then, the NPs from the water solution would show aggregations due to their drying on slide glasses or aggregations in the suspension (Lin and Xing 2008). Thus, it is assumed that SEM was additionally affected by aggregations of nano solutions on slide glasses compared to nano used solutions directly. In fact, SEM can measure the primary diameter that is the shortest of the nanoparticles in the solution. However, when we measured the sizes of NPs on the slide glasses, they were so dense and formed layers. This complicated the discovery of the primary diameter of the NPs.

Due to this complication, another experiment was needed to confirm the relationship between the size of NPs (nano-TiO_2 and nano-ZnO) and the concentration of NPs (nano-TiO_2 and nano-ZnO). Therefore, a Pchem analysis was used. Pchem results indicate that both nano-TiO_2 and nano-ZnO's hydrodynamic diameters decrease when concentrations of nano solutions increase in DW (Fig. 14 A, Fig. 15 A). Conversely, in the Hoagland solution (not DW), when the concentration of nano-TiO_2 increased, the hydrodynamic diameter of nano-TiO_2 also increased (Fig. 14 B), and when concentrations of nano-ZnO increased, the Zeta potential also increased (Fig. 15 B).

From this, we can conclude that when the concentration of NPs increases, the surface potential of the NPs increases, thereby decreasing the aggregation of NPs. The reverse behavior of nano-TiO_2 in the Hoagland solution can be explained because many ions in solution cause the aggregation of NPs (Navarro et al. 2008).

From the above results, we can conclude that the size of the NPs (nano-TiO_2 and nano-ZnO) and the uptake of the NPs (nano-TiO_2 and nano-ZnO) affect the effect of NPs (nano-TiO_2 and nano-ZnO) on the tomato (*L. esculentum*) and kidney bean (*P. vulgaris*) plants. In addition, we can see that when we use different measuring conditions for one purpose, many factors affecting the results should be considered. Our results also indicate that characteristic-dependent research is required for the study of NPs, since NPs have very different characteristics in different environmental conditions.

V. References

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국문초록

나노물질은 생활속에서 광범위하게 이용되고 있다. 그러나 그들이 고농도로 생태계에 축적되었을 경우, 인간과 환경에 어떠한 영향을 미치는가에 대해서는 아직까지도 많은 연구가 필요한 실정이다. 현재까지 연구자들은 미생물과 동물, 식물 외에도 세포와 심지어는 뉴런 수준에 이르기까지 나노물질의 독성에 관한 연구를 수행하고 있으나, 특히 식물을 대상으로 한 연구는 아직까지 부족하다. 연구조건은 culture solution, agar medium, filter paper in petri dish, soil 등 다양하게 이루어지고 있으나 현재로는 나노물질의 응집과 침전을 최소화 할 수 있는 petri dish 법이 가장 널리 사용되고 있다. 본 실험에서는 몇개의 선행연구가 존재하지만, 그 양이 적고 서로 다른 연구결과를 보이는 나노물질인 nano-TiO_2 와 AgNP 과 같은 강한 독성을 보이지는 않으나, 식물의 생장을 저해시키는 연구결과들이 보고된 nano-ZnO 를 선정하여 인간과 밀접한 관련성을 가지는 농작물인 토마토와 강낭콩 2 종을 대상으로 연구를 진행하였다. 실험은 발아와 유근생장을 기본으로, 식물이 성체 단계에서는 나노물질에 비교적 저항성을 가질 것이라는 판단하에 물리적 방법이 아닌 화학적 스트레스 측정 지표인 TAC 와 SOD 를 사용하여 나노물질의 독성을 간접적으로 측정하였다. 또한 나노물질이 농도에 따라 어떠한 거동을 보이는지 확인하기 위해 SEM 과 Pchem 방법을 이용하였다. 실험 결과, nano-TiO_2 와 nano-ZnO 모두 식물의 발아에는 영향을 미치지 못했으나, nano-ZnO 의 경우 유식물의 생장을 저해하였고, nano-TiO_2 의 경우, 토마토 성체에서 SOD 에 대한 유의한 반응을 보였다. 또한 나노물질은 농도가 높아질수록 DW 에서 그 크기가 감소, Zeta potential 은 증가하는 경향을 보였으며, 유식물의 나노물질 uptake 결과에서도 그러한 경향을 확인할 수 있었다. 그러나, 나노물질은 그 종류에 따라 각각의 특성이 다르고, 식물의 특성과 주변환경도 고려되어야 하므로, 더 많은 연구가 이루어져야 할 것이다.

주요어 : 나노 산화티타늄 , 나노 산화아연, 토마토 (*Lycopersicon esculentum*), 강낭콩 (*Phaseolus vulgaris*), 항산화 활성, Pchem

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