Functional analyses of nanoparticle toxicity: A comparative study of the effects of TiO$_2$ and Ag on tomatoes (Lycopersicon esculentum)

Uhram Song $^a$, Heeju Jun $^a$, Bruce Waldman $^a$, Jinkyu Roh $^b$, Younghun Kim $^b$, Jongheop Yi $^c$, Eun Ju Lee $^{a,*}$

$^a$ School of Biological Sciences, Seoul National University, Seoul 151-742, Republic of Korea
$^b$ Department of Chemical Engineering, Kwangwoon University, Seoul 139-701, Republic of Korea
$^c$ School of Chemical and Biological Engineering, Seoul National University, Seoul 151-742, Republic of Korea

**A R T I C L E   I N F O**

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**A B S T R A C T**

Engineered nanoparticles (NPs), increasingly used in industry, enter and migrate through biological ecosystems. NPs may create some acute toxicity, but their overall effects on living organisms remain largely unknown. In particular, the behavior of NPs in natural conditions and their consequent ecological effects are still poorly understood. In this study, we developed methods to test the phytotoxicity of two distinctly different NPs, one aerosol (nano-TiO$_2$), and the other colloidal silver (AgNP), by specifically considering their tendencies to agglomerate and form precipitates. First we examined effects of these NPs on germination and root elongation. While exposure to neither of these NPs resulted in acute toxicity on germination, silver NPs caused significantly decreased root elongation at every concentration we tested. We found that the hydrodynamic diameters of AgNPs were much smaller than those of nano-TiO$_2$, which induced higher uptake and phytotoxicity. Based on the agglomeration behavior of the NPs, greenhouse trials were run using commercial soil, for nano-TiO$_2$, and Hoagland’s solution, for AgNP. Phytotoxicity of silver NPs in the mature plants was demonstrated by lower chlorophyll contents, higher superoxide dismutase activity at the highest concentration (5000 mg/kg). Both nano-TiO$_2$ and AgNPs were taken up into plant stems, leaves and fruits. Our results suggest that further studies of the ecological effects of nanoparticles and steps to mitigate appropriate management strategies are required.

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1. Introduction

With the development of nanotechnology, engineered nanoparticles (NPs) are increasingly found in a large variety of applications. Nanotechnologies, as well as being important industrial components, are now common use in commercial and consumer products. Therefore, the effects of NPs are increasingly drawing scrutiny from environmentalists as well as the scientific community. NPs may pose novel health and environmental risks that are not predictable by our current knowledge of the behavior of macroscopic particles (Franklin et al., 2007). The production, use, and disposal of NPs will inevitably lead to their release into the air, water, and soil (Lin and Xing, 2007) with certain amounts of exposure to humans. Risks posed by NPs currently are subject to robust debate (Ruffini Castiglione et al., 2011). Clearly, the safety of NPs must be established when considering further the development of further applications for nanotechnology.

Research into toxicity of NPs has been accelerating in recent years – using microbial (Jiang et al., 2009; Kang and Mauter, 2009), cellular (Lin et al., 2009), whole animal (Chen et al., 2009; Wang et al., 2006) and even neuronal (Pisanic et al., 2007) assays. Despite these efforts, the toxicity of NPs in higher plants remains largely unexplored (Ruffini Castiglione et al., 2011). The toxicity of NPs should in part be determined by their functional properties, which, in turn, affect how they are dispersed through the environment (Warheit, 2008). NPs typically are manufactured in powder form, or are packaged as aerosols, so their aggregation and agglomeration behavior, as well as their solubility, need to be examined. Agglomeration and aggregation patterns of NPs in solution should vary depending on properties of the media, whether culture solution, agar, or filter paper (Yang and Watts, 2005). Further, they will vary in response to the presence of ions that foster the aggregation of NPs, and thereby reduce their bioavailability (Navarro et al., 2009).

Previous research into the phytotoxicity of NPs has used culture solution (Yang et al., 2006; Zheng et al., 2005), agar medium (Khodakovskaya et al., 2009; Lee et al., 2008), filter paper on petri dish (Cañas et al., 2008; Lin and Xing, 2007) and soil (Doshi et al., 2008) for seed germination and root elongation (growth).
However, as the characteristics of NPs would be an important factor for nanotoxicity studies (Warheit, 2008), characteristics such as water solubility, agglomeration/aggregation in water should be considered during research.

Agglomeration/aggregation patterns of NPs in solution would be very different depending on the constituents of the media (Yang and Watts, 2005), thus altering their bioavailability (Navarro et al., 2009). Previous research (Lin and Xing, 2007; Ruffini Castiglione et al., 2011; Yang et al., 2006; Zheng et al., 2005) into root elongation had a tendency to apply nano-solutions only to seeds and measure germination and root elongation. Since the germination of seeds is often delayed (in our experiment, some seeds germinated after 9 days), unexpected errors in root elongation may occur during the experiment. Seeds with delayed germination will show low values of root elongation, especially in early measurement, even though they will eventually reach the same root length as other seeds. Hence the seed germination and root elongation tests should be made compatible, by using the filter paper on Petri dish method which is simple, reproducible and widely-used method.

Also, mature plants should be tested to confirm how NPs would work in the actual fields because they are not as fragile as seedlings. As mature plants would require nutrients in the culture solutions for healthy growth, DW (Distilled Water) would not be a preferred growing medium. Hydroponics systems with nutrient supply (such as Hoagland’s solution) would be useful for testing.

Special methods are required to study effects of NPs as they have characteristics that differ from those of other toxic pollutants such as heavy metals and pesticides. For example, assays of changes in chlorophyll contents of mature plants can reveal physiological stress (Kooten and Snel, 1990) resulting from exposure to NPs. In addition, stressors on plants can affect their antioxidant activities (Song and Lee, 2010), as induced, for example, by heavy metals (Patra and Panda, 1998), so NPs may affect these physiological measures in similar or different ways.

In this study, we developed and evaluated methods to detect the phytotoxicity of two distinctively different NPs, one an aerosol, the other a colloid. Nanometer titanium dioxide (nano-TiO2), in an aerosol form, is widely used in sunscreens and cosmetics, and widely used in sunscreens and cosmetics, and how these NPs are taken up into different plant tissues. We set out to develop methods to test the phytotoxicity of a wide range of NPs, which these affect mature crop plants is unknown.

2.1. Species selection and reagents

Tomato (Lycopersicon esculentum) has been recognized as a model plant for toxicity studies (OECD, 2003; USEPA, 1996). Further, it is a common crop of economic importance, so tests of its possible toxic effects of many nanoparticles, including nano-TiO2 and AgNP, are needed. We exposed tomatoes to Nano-TiO2 (AEROXIDE TiO2 P 25’, Evonik Industries, Germany, aerosol, ≥99.9%, anatase: rutile 80:20, 27 nm particle size) and AgNP (ABC Nanotech, Korea, 200,000 mg/L citrate capped, 10–15 nm particle size). NPs were diluted with distilled water for experimental concentrations.

2.2. Preparation of particles and cultures

Tomato seeds (Syngenta AG, Switzerland) were vernalized for two weeks and were sterilized for 10 min in 10% sodium hypochlorite solution (USEPA, 1996) before application.

To study the solutions’ effects on germination, we soaked seeds in nano-TiO2 solutions at concentrations of 0, 50, 100, 1000, 2500 and 5000 mg/L. For AgNP phytotoxicity, we used solutions of 0, 50, 100, 500, 1000 and 5000 mg/L (by diluting original AgNP solution with DW). The concentrations of nano-TiO2 solutions were chosen based on previous research that showed significant effects for 2000 up to 4000 mg/L treatment (Ruffini Castiglione et al., 2011). As AgNP was reported to be toxic even at low (100 mg/L) concentrations (Kumari et al., 2009), 50 mg/L treatments were also conducted for both particles. For AgNP, we substituted a 500 mg/L solution in place of the 2500 mg/L solution but also added a 5000 mg/L solution treatment. These modifications were made in light of reports that mature plants, rather than seedlings, can survive and take up AgNP at 10,000 mg/L of solution (Harris and Bali, 2008). We were particularly interested in examining possible inhibitory effects of these concentrations on root elongation.

The seeds were soaked in solutions for 48 h (Zheng et al., 2005) (dark condition, room temperature) with gentle shaking in an orbital shaker at 150 rpm. All samples were washed thoroughly with distilled water (DW) and transferred into 100 mm Petri dishes with one piece of filter paper (90 mm) and 5 mL of DW (Lin and Xing, 2007). The seeds were tested for germination in a growth chamber under a range of conditions established by OECD, 2003 guidelines: temperature: 24 °C, humidity: 70 ± 25%, photoperiod: 18 h light, light intensity: 300 μmol/m²/s with protection from drying. We placed five seeds into each Petri dish, with ten replicates, and measured germination rates four times, once every three days.

We used seedlings germinated within 36 h for root elongation examination. Each Petri dish contained five seedlings and 5 mL of the test medium. Experimental conditions were the same as those that we used for the germination studies above. The root lengths of the seedlings were measured every three days, five times in total. The seedlings were harvested after 15 days, washed thoroughly with DW and dried at 70°C for uptake analysis. We measured seedling masses after 15 days.

We also conducted greenhouse experiments, analyzing tomato growth in response to treatments with each NP. To study the effects of nano-TiO2, tomato seedlings were grown in pots (5 replicates) filled with 200 g of Sunshine Mix # 5 (Sun Gro, Canada). After 6 weeks, 200 mL of nano-TiO2 solution at 1000 and 5000 mg/L concentrations were pipetted into each pot. Solutions were administered in small increments using 10 mL pipettes to avoid leaching, thus ensuring that constant nano-TiO2 concentrations would be available to be absorbed from the growth media. Controls were also run without any added nano-TiO2. Antioxidant enzyme activities and chlorophyll contents of the plants were measured one week after treatment.

To study effects of AgNP, tomato seedlings were grown within nets, filled with surface-smooth vermiculite, two-thirds submerged in 10 L of Hoagland’s solution (Hoagland and Arnon, 1950), in a 23 × 23 × 25 cm polystyrene box. Within the hydroponic system, air was continuously supplied (0.8 L/min) into the nets which were covered. After six weeks, the solution was replaced with experimental treatments of Hoagland solution containing AgNP (0, 100, or 1000 mg/L). During the first seven days of experimental treatment, air supply was turned off to prevent agglomeration of AgNP. Antioxidant enzyme activities and chlorophyll contents of the plants were measured after one week. Five weeks after treatment, the plants were harvested for uptake analysis. The average temperature of the greenhouse during the experiment (from May to July) was 25.2 °C and average humidity was 66.8% (measured by HOB0 U10 temperature relative humidity data logger; Onset, Bourne, MA, USA).

2.3. Analytical methods

For antioxidant enzyme activities, plant samples (0.1 g) were frozen in liquid nitrogen and 50 mM phosphate buffer (pH 7.2) was used for extraction. Total Antioxidant Capacity (TAC) was measured by determining antioxidant activity of organic liquid using bathocuproine (Song and Lee, 2010). TAC values were determined by examining changes of spectrophotometric measurements at 490 nm, induced by copper sulfate solution introduced to the samples (Song and Lee, 2010). The superoxide dismutase activity (SOD) was measured using the protocols of the WST assay by Dojindo, Japan. Using a WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2Htetrazolium, monosodium salt) solution (Dojindo, Japan) and an enzyme working solution (Dojindo, Japan), SOD activities were determined by examining changes of spectrophotometric measurements at 450 nm (Song and Lee, 2010). Changes of spectrophotometric measurements were induced by release of superoxide by enzyme working solution.

Chlorophyll contents were determined by a DMSO extraction method (Hiscox and Israelstam, 1979). For Ti and Ag uptake analysis of seeds and seedlings, one
2.4. Statistical analysis

A one-way ANOVA was conducted to identify significant differences between treatments, and on detection of a significant difference, a post hoc Tukey’s Studentized Range (HSD) Test was carried out and assessed using SAS 9.1 (SAS Institute Inc., USA). Differences were considered significant when \( p < 0.05 \).

3. Results and discussion

3.1. Seed exposure

When preparing solutions for our experiments, we diluted NPs in powder form in DW to various concentrations. Although the solutions appeared to be uniform and dissolved rapidly, the particles agglomerated and began to form precipitates. In addition to nano-TiO\(_2\), other NPs frequently used for toxicity tests such as SiO\(_2\) NPs (Lee et al., 2010) and ZnO NPs (Lin and Xing, 2008; Shaymurat et al. 2011) also showed precipitates, even in low concentrations (100 mg/L), after several hours. Therefore, soaking the seeds in solution would have created spatial heterogeneity that seeds in the lower part of the solution might have come into contact with higher concentrations of NPs than others.

In our studies, we attempted to avoid this problem by gently shaking the solutions in an orbital shaker. A speed of 150 rpm was found to be the lowest that would accomplish total mixing in 50 mL and 200 mL tubes without causing damage to seed coats. Although this was sufficient for tomatoes, larger tubes probably will be required for larger seeds, such as mung beans (Phaseolus vulgaris). Even for colloidal forms of NPs, such as AgNP in this experiment, shaking achieved effective mixture of NPs with seeds as well as preventing air bubble gaps from forming between seeds.

3.2. Germination and root elongation

No significant differences in germination rates were found among plants treated with exposure to any concentration of either nano-TiO\(_2\) or AgNP (Fig. 1). Every treated plant showed almost full germination after 12 days. Table 1 shows that tomato seeds absorbed NPs as nanoparticulate. AgNP was taken up at relatively higher levels than nano-TiO\(_2\).

Possibly NPs did not fully penetrate the seed coat and endosperm and thus had limited effects on the embryos. In this case, seed coats and endosperm may have served as filters, absorbing metals but passing water. As there are no vessels in seeds, whether or not macromolecules move through cells remains poorly studied (Duke and Kakefuda, 1981). Therefore, NPs, which do not ionize in water, might find the seed coat and endosperm to be effective barriers, especially when they are agglomerated. This may explain why a certain amount of metals were detected but did not affect germination. Many seed hairs are observable in tomato seed coats. NPs might become easily attached to seed coats by adsorption and remain affixed even after repeated washings. To verify this, we considered measuring the metal contents of seeds with removed seed coats, but the seed coats of tomatoes were difficult to remove. Therefore, we exposed maize (Zea mays L.) to 5000 mg/L nano-TiO\(_2\) solution under the same conditions and removed the coats of

![Image](307x355 to 547x735)

**Table 1**

<table>
<thead>
<tr>
<th>Treatment (mg/L)</th>
<th>Metal uptake (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO(_2) 100</td>
<td>26.4 ± 4.6</td>
</tr>
<tr>
<td>TiO(_2) 1000</td>
<td>97.1 ± 18.2</td>
</tr>
<tr>
<td>TiO(_2) 5000</td>
<td>163.4 ± 53.5</td>
</tr>
<tr>
<td>AgNP 100</td>
<td>33.3 ± 9.3</td>
</tr>
<tr>
<td>AgNP 1000</td>
<td>111.7 ± 59.7</td>
</tr>
<tr>
<td>AgNP 5000</td>
<td>653.1 ± 54.5</td>
</tr>
</tbody>
</table>

Values represent mean ± SE of 3 replicates. Values having the same letter are not significantly different at the 0.05 level.
maize seeds before analysis of NPs. The maize seeds only absorbed $2.2 \pm 0.6$ mg/kg Ti (mean ± SE, $n=3$), providing indirect evidence that NPs are not penetrating into seed coats. Overall, neither NP noticeably affected seed germination.

Root elongation results are summarized in Fig. 2. Seedlings exposed to nano-TiO$_2$ showed no change in root elongation in response to concentration. However, those treated with AgNP showed significant decreases in root growth even at the lowest (50 mg/L) concentrations while those exposed to the highest concentration (5000 mg/L) failed to show significant increase in root growth throughout the experimental period. We presumed that these seedlings had died due to their lack of growth after six days, and consequently set 5000 mg/L as an upper limit of exposure. Even the 500 and 100 mg/L treated plants did not show significant increases in root length over the course of the experiment. Therefore, even though seedlings survived, their small increase in root size suggests that seedlings treated with concentrations as low as 100 mg/L would not subsequently survive to become mature plants.

Average biomass of tomato seedlings in the 500 mg/L treatment varied significantly from those in the 1000 and 5000 mg/L AgNP treatments (Fig. 3). Therefore, we considered 1000 mg/L to be a threshold concentration above which seedlings would not be likely to mature to adult plants. Even at the lowest AgNP concentration (50 mg/L), biomass showed a significant decrease, indicating that AgNP was phytotoxic at low levels. Other studies have revealed toxic effects of AgNP on plants at concentrations as low as 5 mg/L (Gubbins et al., 2011; also see Kumari et al., 2009), consistent with our findings. In contrast, responses of seedlings to nano-TiO$_2$ did not vary with concentration (Table 2).

Ag concentrations in the AgNP treated plants were much higher than Ti concentrations in nano-TiO$_2$ treated plants. As seedlings in the 5000 mg/L treatment of nano-TiO$_2$ took up less Ti than those in the 2500 mg/L treatment, severe agglomeration in the 5000 mg/L treatment may have interfered with absorption. Notably, Ag uptake was particularly high, even considering that values might have been slightly inflated by drying (Table 2).

Differences in the uptake of nano-TiO$_2$ and AgNP may be due to changes in their hydrodynamic diameters that resulted from variable experimental conditions. Table 3 shows the hydrodynamic diameters of NPs in a Petri dish 14 days after treatment. As NP concentrations rose, their hydrodynamic diameters increased but with certain differences for each NP. The original diameter of nano-TiO$_2$ was 27 nm and the original diameter AgNP was 10–15 nm. However, nano-TiO$_2$ showed a 100 times larger diameter even in the 100 mg/L treatment, while AgNP only showed a 2 to 3 fold increase in diameter. Nano-TiO$_2$ treatment caused particles to agglomerate, which would interrupt NP uptake.

![Fig. 2. Effect of (A) nano-TiO$_2$ and (B) AgNP 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.](image)

![Fig. 3. Effect of (A) nano-TiO$_2$ and (B) AgNP on 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.](image)
commercial soils that are globally available, so that results can be applied to allow us to examine specific attributes of NPs. Tomatoes were grown in Hoagland’s solution, comparable to a hydroponics system. Air was bubbled through the system except for a week after AgNP treatment. Rather than focusing on growth, we measured physiological changes, chlorophyll contents and antioxidant activities, in plants induced by NPs.

Tomatoes treated with Nano-TiO2 showed no differences in chlorophyll content due to NP treatments (Table 4). However, those treated with AgNP showed a significant decrease in chlorophyll content for every increase in AgNP concentration. The leaves of AgNP-treated tomatoes withered, especially those in the lower areas of the stems. These results indicate that exposure to AgNP stressed the plants.

Total Antioxidant Capacity (TAC) did not vary significantly among treatments exposed to either NP. For nano-TiO2 treated tomatoes, TAC values (mM/mg-protein) were 0.14 ± 0.01 in the control, 0.11 ± 0.01 in the 1000 mg/kg treatment, and 0.13 ± 0.02 in the 5000 mg/kg treatment (*SE of 4 replicates). For AgNP-treated tomatoes, TAC values (mM/mg-protein) were 0.10 ± 0.00 in the control, 0.12 ± 0.00 in the 100 mg/kg treatment, and 0.13 ± 0.02 in the 1000 mg/kg treatment (*SE of 4 replicates), also not significantly different among treatments.

However, superoxide dismutase activity (SOD) significantly differed among treatments in response both to nano-TiO2 and AgNP (Fig. 4). As SOD is a reliable indicator of stress and is known to increase in plants exposed to heavy metals (Song and Lee, 2010), these results provide evidence that NPs induced physiological stress. Although we found no evidence that nano-TiO2 affected germination, root elongation, chlorophyll contents, TAC and SOD of plants grown in 5000 mg/kg nano-TiO2 soil significantly differed from 1000 mg/kg and control treatments. Why SOD values of greenhouse-grown tomatoes showed evidence of an effect, while laboratory studies failed to reveal such an effect, defies simple explanation. However, since nano-TiO2 is well known for its unique reaction to UV such as oxidation (Bauer et al., 1999), sunlight might have changed the toxicity of nano-TiO2. Alternatively, high temperatures in the greenhouse (up to 46.5 °C) may have increased phytotoxicity. Overall, these results point to the possibility that toxic effects in natural conditions, subject to multiple potentially synergistic effects, may be much greater than those demonstrated in laboratory experiments. However, given the potential risks of NPs in the field, laboratory results should always serve as the basis for designing field trials.

Exposure to AgNP, but not nano-TiO2, decreased fruit production. Biomasses of plants exposed to nano-TiO2 did not significantly vary among treatments in the same growing media. Masses of tomatoes grown on soil were: 377.8 ± 13.0 g (0 mg/kg TiO2), 362.3 ± 21.4 g (1000 mg/kg TiO2), and 320.8 ± 9.5 g (5000 mg/kg TiO2; *SE, n = 4). Grown on Hoagland’s solution, comparable masses were: 205.0 ± 8.2 g (0 mg/kg AgNP), 203.8 ± 13.9 g (100 mg/L AgNP), and 187.5 ± 10.9 g (1000 mg/L AgNP; *SE, n = 4). However, the total fruit mass of tomatoes exposed to AgNP significantly different at the 0.05 level: 41.6 ± 5.0 g (0 mg/L AgNP)

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Metal uptake of tomato seedlings 14 days after exposure (mg/kg).</th>
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<tbody>
<tr>
<td>Treatment (mg/L)</td>
<td>Metal uptake (mg/kg)</td>
</tr>
<tr>
<td>TiO2 50</td>
<td>5.0</td>
</tr>
<tr>
<td>TiO2 100</td>
<td>5.7</td>
</tr>
<tr>
<td>TiO2 1000</td>
<td>8.4</td>
</tr>
<tr>
<td>TiO2 2500</td>
<td>193.0</td>
</tr>
<tr>
<td>TiO2 5000</td>
<td>25.5</td>
</tr>
<tr>
<td>AgNP 50</td>
<td>24.7</td>
</tr>
<tr>
<td>AgNP 100</td>
<td>197.0</td>
</tr>
<tr>
<td>AgNP 500</td>
<td>1274.4</td>
</tr>
<tr>
<td>AgNP 1000</td>
<td>4033.4</td>
</tr>
<tr>
<td>AgNP 5000</td>
<td>14681.8</td>
</tr>
</tbody>
</table>

Values of TiO2 treatments indicate concentrations of Ti and values of AgNP treatments indicate concentrations of Ag.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Hydrodynamic diameter of metal uptake of TiO2 and AgNP after 14 days in petri dish.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (mg/L)</td>
<td>Hydrodynamic diameter (nm)</td>
</tr>
<tr>
<td>TiO2 100</td>
<td>3422.9 ± 478.1</td>
</tr>
<tr>
<td>TiO2 1000</td>
<td>5834.3 ± 517.8</td>
</tr>
<tr>
<td>TiO2 5000</td>
<td>8869.7 ± 1036.3</td>
</tr>
<tr>
<td>AgNP 100</td>
<td>321 ± 2.7</td>
</tr>
<tr>
<td>AgNP 1000</td>
<td>1481 ± 34.0</td>
</tr>
<tr>
<td>AgNP 5000</td>
<td>10646 ± 180.8</td>
</tr>
</tbody>
</table>

Values represent mean ± SE of 50 replicates.

Hydrodynamic diameter of nanoparticles under 1000 nm were measured by field emission scanning electron microscope and for nanoparticles 1000 nm were measured by differential interference contrast microscope.

and eventually decrease their effects on plants. However, AgNP treatment was accompanied by a smaller diameter at every concentration, which allows seedlings to absorb it more easily. Although hydrodynamic diameters of AgNP in the 5000 mg/L treatment seem large, they are still much smaller than the diameters of nano-TiO2.

Understanding how NPs behave under actual experimental conditions is necessary to predicting their effects (Franklin et al., 2007). Solubility and agglomeration characteristics of NPs in solutions directly affect plant uptake. The fate of NPs, including their hydrodynamic diameters, need to be considered when analyzing NPs and their environmental effects. The toxicity of NPs is likely to differ depending on characteristics of the environment to which they disperse. Our results demonstrate that AgNP exposure induced phytotoxicity in root elongation, and validate the usefulness of our assay in measuring toxicity of NPs to plants.

3.3. Greenhouse experiment

We were interested not only in how NPs affect seedlings but also mature plants which are not as fragile as seedlings. We thus evaluated the toxicity of NPs on mature plants in a greenhouse experiment. Tomatoes were grown five extra weeks after treatment to allow us to examine specific uptake patterns of NP into plant tissues (e.g., Zhu et al., 2008). Based on the results of the seedling experiment, 1000 mg/L was selected as the treatment dose. For Nano-TiO2 treatments, we grew seedlings in standard commercial soils that are globally available, so that results can be compared with other studies. Although hydroponics systems more efficiently promote NP uptake, they are ineffective for those NPs that agglomerate and form precipitates such as TiO2. Thus, for AgNPs, which are in colloid form, Hoagland’s solution was used in a hydroponics system. Air was bubbled through the system except for a week after AgNP treatment. Rather than focusing on growth, we measured physiological changes, chlorophyll contents and antioxidant activities, in plants induced by NPs.

Table 4

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Chlorophyll contents of tomatoes 7 days after exposure (mg/L).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (mg/L)</td>
<td>Chl a</td>
</tr>
<tr>
<td>TiO2 0</td>
<td>5.82 ± 0.24</td>
</tr>
<tr>
<td>TiO2 1000</td>
<td>4.59 ± 0.38</td>
</tr>
<tr>
<td>TiO2 5000</td>
<td>3.88 ± 0.96</td>
</tr>
<tr>
<td>AgNP 0</td>
<td>5.03 ± 0.55a</td>
</tr>
<tr>
<td>AgNP 100</td>
<td>2.64 ± 0.08b</td>
</tr>
<tr>
<td>AgNP 1000</td>
<td>2.20 ± 0.45b</td>
</tr>
</tbody>
</table>

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

cantly decreased root elongation at

every concentration. Our analyses demonstrate that the hydro-
dynamic diameter of NPs, and their agglomeration behavior affects

Conclusions

Nano-TiO₂ showed no evidence of phytotoxicity in terms of germination and root elongation of tomatoes. In contrast, exposure to AgNP resulted in significantly decreased root elongation at every concentration. Our analyses demonstrate that the hydrodynamic diameter of NPs, and their agglomeration behavior affects how plant absorb them and their phytotoxicity. In greenhouse experiments, mature plants showed evidence of phytotoxicity due to AgNPs by exhibiting low chlorophyll contents, higher SOD, and less fruit production, but nano-TiO₂ treated plants only showed higher SOD values at the highest (5000 mg/kg) levels of treatment. As nano-TiO₂ only produced effects in sunlight, and our results only showed potential at day lengths beyond 14 hours. Hence, the results might not approximate the results that would happen in the field. Further research is urgently needed to examine the toxicity of NPs in real environmental settings.
under natural conditions, nano-TiO₂ might not be toxic to plants in most cases.

Our study suggests that NPs must be studied in the specific environments in which they occur, as their characteristics differ widely dependent on environmental conditions. The standard methods that we have developed may be useful in assessing the relative toxicity of NPs on plants that can be compared among laboratories and countries. Finally, since both nano-TiO₂ and AgNP clearly are absorbed by plants, further studies are needed to examine effects on plants as well as strategies to mitigate their effects or control their spread in the environment.

Acknowledgments

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References


