Journal of Chemical Health Risks

Journal of Chemical Health Risks (2014) 4(1), 29–36

ORIGINAL ARTICLE

Effect on Germination and Early Growth Characteristics in Wheat Plants (*Triticumaestivum L.*) Seeds Exposed to TiO_2 Nanoparticles

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(Received: 4 August 2013 Accepted: 17 October 2013)

KEYWORDS

Seedling growth

Germination indices

Nano-TiO_2

Biomass

ABSTRACT: The present study was aimed to investigate the effects of titanium dioxide nanoparticles (nano-TiO_2) on wheat (*Triticumaestivum* L.) plants cv. Parsi. The experimental treatments included seven concentrations of nano- TiO_2 (10, 100, 1000, 1200, 1500 and 1700 ppm nano-TiO_2 and control (without any TiO_2). The results showed that among the wheat germination indices, germination rate and weighted germination index were affected by TiO_2 nanoparticles treatments. The lowest and the highest germination rate (16.7 vs. 11.1n.day^{-1}) were obtained in control and 1000 and 1200 ppm concentration of nano- TiO_2 treatments, respectively. These values for weighted germination index were (2.4 vs. 2.08) in 2000 ppm and control treatments respectively. In addition, plumule and radicle length, seedling fresh weight and seedling vigor index were affected by nano- TiO_2 concentrations, significantly. Plumule and radicle lengths at 1200 ppm concentration of nano-TiO_2 were higher than untreated control. This study shows that using of TiO_2nanoparticles in suitable concentration caused increasing of seed germination of wheat cv. Parsiin comparison to control plants, otherwise low concentrations had inhibitory effects on wheat germination characteristics.

INTRODUCTION

Nanoparticles are particles sized less than 100 nanometres (nm), up to 10,000 times smaller than the diameter of a humanhair. They have powerful properties due to unique physical and chemical characteristics and large surface area relative to size, which give them the potential to improve quality of life and contribute to industrial competitiveness. Currently, nanoparticles are produced from a large variety of bulk materials[1], with broad industrial applications including biomedicine and biotechnology;[2, 7] hence it is to be expected that these particles will find their way into various ecosystems [7].

However, some NPs have also been shown to be toxic to many species, including bacteria, algae, invertebrates and vertebrates. According to the EU's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), NPs are not per se dangerous, but there are many uncertainties about their safety.

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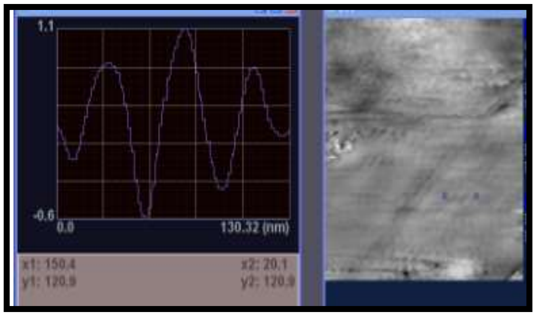
Therefore, safety assessment of NPs must be conducted on a case-by-case basis. Most studies have investigated the toxicity of NPs in aquatic environments and the effects of NPs on terrestrial plants and ecosystems remain largely unknown. NPs are being considered or are already used in a large number of commercial and consumer applications, such as batteries, cosmetics, coatings and anti-bacterial clothing, as well as biocides and pesticides, which are very likely to enter the soil environment. Consequently, this raises concerns about potential health risks associated with food contamination through agricultural products. The researchers examined the toxicological impacts of titanium dioxide the, common industrial additive with various applications, on wheat growth and soil health in a field in China. To assess the health of soil, the researchers focused on the activity of enzymes in the soil, which are highly sensitive to change and provide a good indicator of soil quality and health. Data on the wheat's biomass (overall size), uptake of NPs, the elements' contents in harvested wheat, and the activities of soil enzymes were collected and statistically analysed. The results revealed that both NPs reduced the wheat's biomass, and thus were harmful to the plant. The titanium dioxide NPs, considered to have low solubility, remained in the soil for long periods and stuck to the plants' cell walls, which might create potential environmental risks for deeper soil layers. Additionally, a few individual small-sized titanium dioxide NPs (around 20nm) were able to penetrate the cell wall [8]. Also positive effects of this oxide nanoparticle on higher plants have been observed [9]. TiO_2-NP exposure induced increased root elongation but did not affect germination, evapotranspiration, and plant biomass of wheat and rapeseed. Lower amount (<2.5mM) of nano-TiO_2 is beneficial for the growth, germination, and biochemical parameters, and overall health of the *Brassica oleracea* var. *capitata* plants [11]. Highly colloidal stability of laser synthesized anatase TiO_2 NPs has facilitated their disparity and availability in the plant growth media for longer time. However, majority of the reports available in the literature indicate phytotoxicity of ENPs. Nano-aluminum oxide(Al_2O_3) could inhibit root elongation of corn, cucumber, soybean, cabbage, and carrot [12] whereas nano-ZnO was reported to be one of the most toxic nanoparticles that could terminate root growth of test plants (radish, rape, ryegrass, lettuce, corn, and cucumber) [9]. Similar research was undertaken on the toxicology of nano-Al_2O_3, nano-SiO_2, nano-magnetite (Fe_3O_4) and nano-ZnO on *Arabidopsis thaliana*, with the results showing that nano-ZnO at 400 mg/L could inhibit germination so root elongation was not measured [13]. The aims of this study were to investigate whether seed germination and seedling growth in *Triticumaestivum* were altered by titatium oxide nano particles.

MATERIALS AND METHODS

Chemicals

Nano-TiO_2 was prepared from commercial TiO_2 nanopowder (Sigma-Aldrich, USA) by dispersing nanoparticles in Milli-Q water through ultra-sonication (300 W, 40 kHz) for 30 minutes. The size and topography of TiO_2 nanoparticles (Figures1 and 2) were determined by scanning tunneling microscope (STM) (Nama-SS-6 model) in the Central Laboratory of Ferdowsi University of Mashhad, Iran. XRD measurement showed that the used TiO_2 nanoparticles were made by 80% anatase and 20% rutile. Analysis of particles in X-ray diffraction indicated tetragonal particles and crystalline nature of TiO_2 particles. (Figure 3).



Figuer 1. STM micrograph of nano-TiO_2 particles

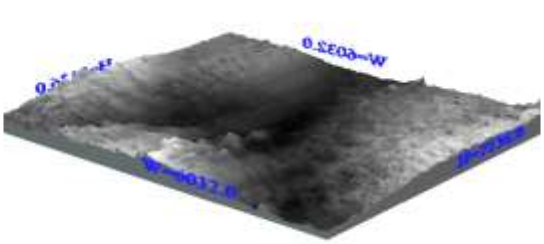


Figure 2. Topographic image of nanosized TiO2

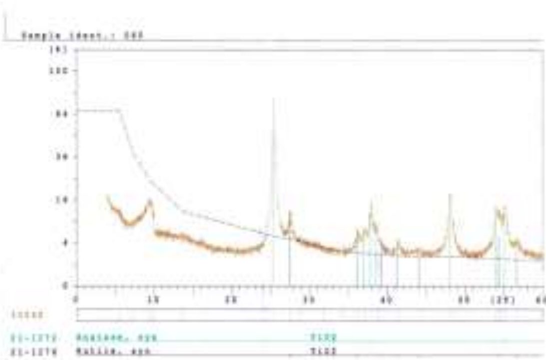


Figure 3. X-ray diffraction (XRD) pattern of nano-anatase TiO_2

Seeds of wheat cv. Parsi were supplied from Agricultural Research Center of Mashhad, Khorasan province, Iran. The seeds were surface sterilized with 5% sodium hypochloride for 10 min then rinsed with the distilled water for several times to remove excess of chemical. Nano-TiO_2 was prepared by dispersing 10 mg of the TiO_2 powder in 100 mL of ultrapure water. The stock suspension was stored at 4°C and sonicated with ultrasound (Ultrasonic Cleaners, Bronson, USA) twice for 30 min prior to further dilution and exposure experiments. One piece of filter paper was put into each 100 mm× 15 mm Petri dish, and 5 ml of nano-TiO_2 concentrations(0,10, 100, 1000, 1200, 1500, 1700 and 2000 ppm) was added. Seeds were then transferred onto the filter paper, with 10 seeds per dish and 1 cm or larger distance between each seed [12]. Petri dishes were covered and sealed with tape, and placed in an incubator. Every day, the number of germinated seeds was recorded. Seeds were regularly watered with distilled water. A seed was considered germinated when the radicle was visible.

In this study, we used following germination parameters: Weighted germination index (WGI), Final percentage germination (GP) for each treatment was calculated after seven days. These parameters were also calculated from the below formulas [14, 15,16,17,18, 19].

Germination rate= N1/D1+N2/D2+…….+Ni/Di

A weighted germination index (WGI) as described by Bu and colleagues [18] was calculated with maximum weight given to the seeds germinating early and less to those germinating late.

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

where n1, n2, …, n60 are the number of seeds that germinated on first, second, and subsequent days until the 60th day, respectively; N is total days of experiment; N´ is the total number of seeds placed in incubation.

Vigor index = germination% × seedling length (root + shoot) [20]   (5)

After an incubation period of 7 days, plumule and radical length of seedlings were measured using a ruler.

STATISTICAL ANALYSIS

To detect the significant differences of variables, statistical analysis was performed employing one way ANOVA test using MINITAB software.

RESULTS AND DISCUSSION

In this study, we assessed the impact of TiO_2 nanoparticles on the most sensitive stages of plant development, i.e. seed germination and seedling growth. Among wheat germination indices, seed germination rate and weighted germination index were affected by applied treatments. The highest germination rate (16.6) was shown in control and higher concentrations of nano-TiO_2 (1500-2000) and the lowest value was found in 1000 ppm (Figure 4). Weighted germination index (WGI) was calculated with maximum weight given to the seeds germinating early and less to those germinating late[18].The weighted germination index in 2000 ppm concentration nano-TiO_2 was more than the other treatments (Figure 5).

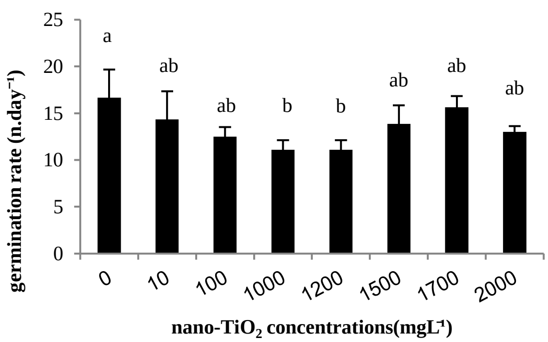


Figure 4.Effect of nano- TiO_2 concentrations on seed germination rate of wheat (cv.parsi)

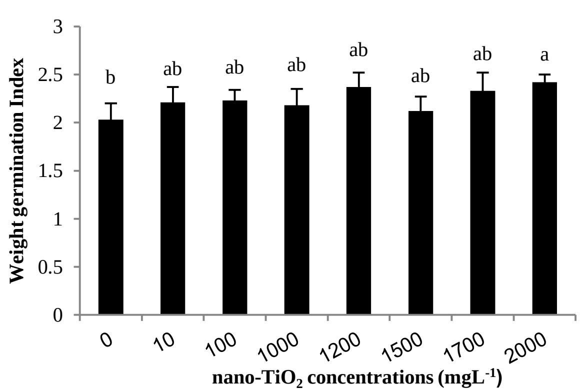


Figure 5.Effect of nano- TiO_2 concentrations on weight germination index of wheat (cv.parsi)

Seed germination is the beginning of a physiological process that needs water imbibitions. Our results indicated that nanosized TiO_2 in higher concentration could promote the seed germination and seedling growth of wheat in comparison to control. Nanoparticles can explain their actions depending on both the chemical compound and on the size and/or shape of the particles [21]. Seed soaking with nano-TiO_2 could increase the germination capacity of *Tibetan capillary* [22]. The effects of TiO_2-NP on wheat and rapeseed plantlets in hydroponics conditions were studied either through root or leaf exposure. It was found that TiO_2-NP exposure induced increased root elongation but did not affect germination and plant biomass. Their results confirm that TiO_2-NP may be accumulated in plant crops but may only moderately impact plant development.

Seedlings length and biomass were measured after a 7-day growth in this nanoparticle suspension. Radicle length increased in plantlets exposed to 1200-2000 ppm, as compared to plantlets germinated and grown in 10-1000 ppm TiO_2 nanoparticles (Figure 6). It was also significantly decreased in plantlets exposed to 10-1000 ppm as compared to control. Plumule length at 1200 and 1700 ppm concentrations of nano-TiO_2 were 8.09 and 8.04, respectively which was significantly different from 10-1000 treatments (Figure 7).

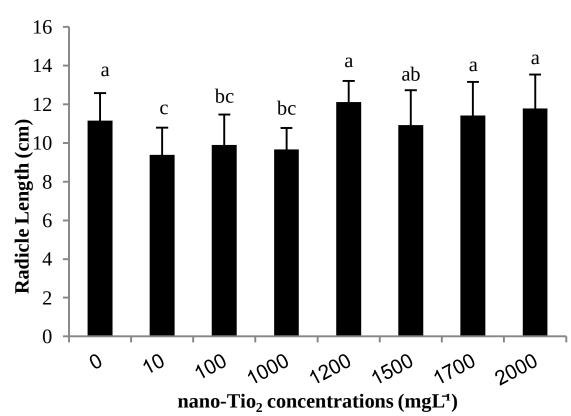


Figure 6.Effect of nano- TiO_2 concentrations on radical length of wheat(cv.parsi)

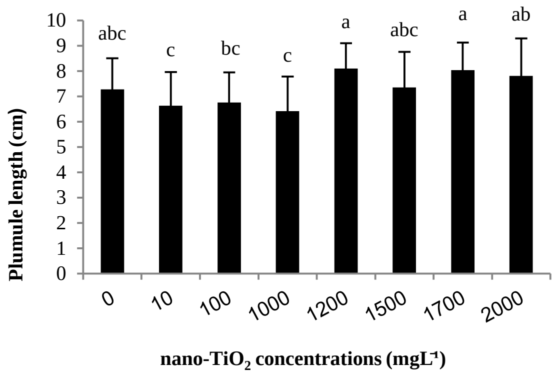


Figure 7. Effect of nano- TiO_2 concentrations on plumule length of wheat (cv.parsi)

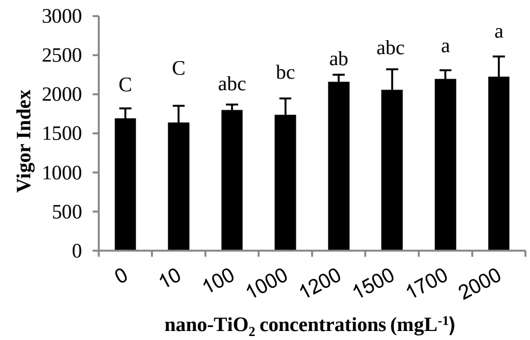


Figure 9. Effect of nano- TiO_2 concentrations on vigor index of wheat (cv.parsi)

The results showed that treatments of 1200 and 2000 ppm of nano-TiO_2 increased seedlings fresh weight significantly (Figure 8). The highest value of fresh weight was shown in 1200 ppm and the lowest fresh weight was found in control.

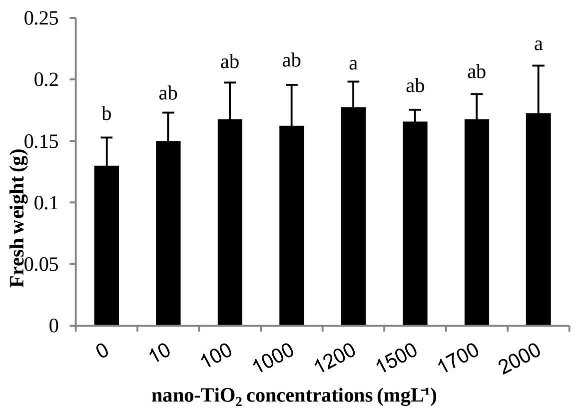


Figure 8. Effect of nano- TiO_2 concentrations on fresh weight seedlings of wheat (cv.parsi)

As shown in Figure 9 vigor index was affected by nano-TiO_2. Vigor index in the presence of 1200-2000 ppm nanoparticles were significantly larger than in control ($p<0.05$) while it was slightly (not significantly) increased when compared to control in both 100,1000 and 1500 ppm treatments (Figure 9).

Seed coat plays a very important role in protecting the embryo from harmful external factors. Seed coats can have selective permeability. Pollutants, though having obviously inhibitory effect on root growth, may not affect germination if they cannot pass through seed coats. This may explain that some seed germination indices in this study were not greatly altered by nanoparticles [23]. The selective permeability by seed coat is supported by an observation that root growth of radish and rape incubated in DI-water after being soaked in the nano-Zn suspension was significantly inhibited, but not while being soaked in the nano-ZnO suspension [9]. Significant retardation of ryegrass root was also observed when the seed soaking process was done in either nano-Zn or nano-ZnO suspensions. Radicles, after penetrating the seed coats, could contact the nanoparticles directly. Therefore, root elongation of sensitive plant species would have a dose-dependent response. Since roots are the first target tissue to confront with excess concentrations of pollutants, toxic symptoms seem to appear more in roots rather than in shoots [24]. In our study, shoots could grow to a certain degree even though root elongation was halted in the presence of nano-TiO_2.

The effect of studied treatments on root length was significant, but they had not a significant effect on shoot length. Radicle lengths at 10, 100 and 1000 ppm concentrations of nanosized TiO_2 were lower than those of the untreated control (15.9%, 11.2 and 13.9% respectively) treatments. Increasing concentrations of nanosized TiO_2 after 1500 ppm increased radical lengths. The lowest radical length was achieved at 10 ppm nanosized TiO_2. Toxicity and bioavailability of copper nanoparticles to the bean (*Phaseolusradiatus*) and wheat (*T. aestivum*) plants were analyzed [9]. They stated that the exposure of bean and wheat plants to copper nanoparticles decreased growth and seedling lengths; and mentioned criteria were negatively related to the exposure concentration of nanoparticles. X-ray fluorescence microspectroscopy showed that nano-TiO_2 can attach to the *Vicia faba* root surface in 48 h, thus resulting in the inhibition of plant growth [26]. The results of an investigation showed that the growth of spinach plants was greatly improved at 250–4,000 ppm nano TiO_2 concentrations, but there was no improvement at higher concentrations. They reported that the significant effect of nanosized TiO_2 on spinach germination is probably attributed to the small particle size, which allows its penetration into the seed during the treatment period, exerting its enhancing functions during growth [27].

Our results indicated that nanosized TiO_2 in an appropriate concentration (more than 1200 ppm) could promote the seed germination and seedling growth of wheat in comparison to control plants. TiO_2 nanoparticles could penetrate wheat root only below a threshold of diameter, which was 36 nm [10]. The effects of nano-TiO_2 on *Gymnodiniumbreve* is more significant, as shown by the LC50 (median lethal concentration) of 9.7mg L^{-1} in 72 h. The activities of superoxide dismutase (SOD), catalase (CAT), and MDA reached their maximum in12h, whereas that of the hydroxyl radical (OH^-) significantly increased in 48 h. The disruption of the free radical and antioxidant system is the mechanism of suppression of *G. breve* growth [28]. The effects of nanomaterials on plant growth were reviwed and concluded that nanomaterials exert a positive promotion effect as well as a negative inhibitory effect on plant growth, in addition to the different physiological effects, depending on the nanomaterial type, particle size, concentration, and plant species [29].

CONCLUSION

In conclusion, the results of our studies under laboratory conditions indicated that the nanosized TiO_2 treatments in proper concentration accelerated the germination of the wheat seeds and increased its vigor. Nano TiO_2 improved the weight germination index and growth of wheat seedling in comparison to untreated control. We found that nanosized TiO_2 in higher concentration had stimulatory effect on wheat seedling. Low concentrations of nanosized TiO_2 had inhibitory effects on studied criteria.

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