Physiological effects of TiO$_2$ nanoparticles on wheat (Triticum aestivum)

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ABSTRACT: Nanoparticles (NP) are introduced in a growing number of commercial products and their production may lead to their release in the environment. Plants may be a potential entry point for NP in the food chain. Up to now, results describing NP phytotoxic effects and plant accumulation are scarce and contradictory. To increase knowledge on titanium dioxide NP (TiO$_2$-NPs) accumulation and impact on plants, we designed a study on wheat (Triticum aestivum). This plant was exposed to TiO$_2$-NPs, with diameter 20 nm, either anatase or rutile. Green house trials were conducted to evaluate the influence of TiO$_2$ on physiological characters. Seven parameters are examined in this study: shoot and root length, fresh and dry biomass of shoot and root and leaf chlorophyll content. The results showed that there is no significant effect on shoot and root length of wheat under nano-TiO$_2$ spraying, however nano- TiO$_2$ is observed to have detrimental effects on wheat shoot and root biomass. TiO$_2$ nanoparticles has no effect on chlorophyll content compare to control except treatment of 1000 mgL$^{-1}$. This study shows that direct exposure to specific types of nanoparticles causes significant phytotoxicity, emphasizes the need for ecologically responsible disposal of wastes containing nanoparticles and also highlights the necessity for further study on the impacts of nanoparticles on agricultural and environmental systems.

Keywords: Chlorophyll content, Caretenoides, Nano scale particles, Plant biomass, phytotoxicity

INTRODUCTION

Among Nanoparticles (NP), TiO$_2$ anatase nanoparticles (TiO$_2$-NPs) are one of the most produced NPs in the world (www.nanowerk.com). They are used as a pigment in paint, paper, ink and plastic. They are also introduced in cosmetics such as sunscreens for their UV protective properties (see Nanowerk database, www.nanowerk.com). Finally, they are presently used in pilot water-purification reactors, because of their ultraviolet irradiation-induced bactericidal effects (photocatalysis). For these reasons, these NPs will surely be released in the environment (Gottschalk et al., 2009). The knowledge of their potential effects on human health is rapidly increasing, however little is known on their potential toxicological effects on environment, i.e. destabilization of the ecosystems and trophic transfer, but also on their potential transfer to the food chain via plant ingestion. The literature relating NP toxicity and accumulation in plants is scarce; authors mostly relate NP inhibitory effects on seed germination and root elongation (Lin et al., 2007; Lin et al., 2008; Yang et al., 2006; Zhu et al., 2008). A few studies relate NP uptake by plant roots (Lin et al., 2008; Zhu et al., 2008), and root to shoot transfer (Zhu et al., 2008). Concerning TiO$_2$-NPs, TiO$_2$-based Nano composites have recently been shown to be accumulated and translocate to the shoots of Arabidopsis thaliana (Kurepa et al., 2010). These translocation studies are based on elemental analysis of the ionic form of metal in plants, but neither address the presence of metal as NPs, nor the spatial distribution of NPs throughout the plant. Actually, TiO$_2$-NP dissolution has been demonstrated to be low in aqueous solution (Schmidt and Vogelsberger, 2009), however its occurrence has never been tested in plant exposure solution and/or during NP transfer to the roots or from the roots to the shoots. Increasing numbers of publications are emerged recently concerning the interactions of ENPs ith plant (Ravel and Newville, 2005; Wu et al., 2002). Most of these studies are focused on the potential toxicity of ENPs to plants and
both positive and negative or inconsequential effects have been reported (Luca et al., 1998). Among the positive effect reports on plants, nano-TiO$_2$ was observed to promote the growth of Spinach through an increase in photosynthetic rate and nitrogen metabolism (Hong et al., 2005; Yang et al., 2006). Carbon nanotubes (CNTs) could enhance root growth of onion (Allium cepa) and cucumber (Cucumis sativa) and nanotubes sheets were formed by both functionalized single-walled carbon nanotubes (fCNTs) and non-functionalized (CNTs) on root surfaces but none entered into the roots (Canas et al., 2008). Although CNTs were found to decrease root growth in tomato plants, a recent work reported that CNTs can penetrate tomato seed coat and dramatically increase seed germination rate and seedling growth (Khodakovskaya et al., 2009). However, majority of the reports available in the literature indicate phytotoxicity of ENPs. Nano-aluminum oxide (Al$_2$O$_3$) could inhibit root elongation of corn, cucumber, soybean, cabbage, and carrot (Yang and Watts, 2005) 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). Similar research was undertaken on the toxicology of nano-Al$_2$O$_3$, nano-SiO$_2$, nano-magnetite (Fe$_3$O$_4$) and nano-ZnO on Arabidopsis thaliana, with the results showing that nano-ZnO at 400mg/L could inhibit germination so root elongation was not measured (Lee et al., 2010). Evidences that ENPs penetrate into plant cell were also reported, with or without showing adverse effects (Khodakovskaya et al., 2009; Birbaum et al., 2010; Cifuentes et al., 2010). In the present study, we examined the effects of photo catalyst nanoparticle, nano-TiO$_2$, on one of the most important food plant (wheat). Nano-TiO$_2$ has widespread usage, as scussed before, in a number of applications, and it is likely to find it’s way into the agricultural environment. This study provides new information on nano particles effects on plants. This approach enhances our understanding of the effects of the ENPs on this plant.

**MATERIALS AND METHODS**

Nano-TiO$_2$ was prepared from commercial TiO$_2$ nanopowder (Sigma-Aldrich, USA) by dispersing nanoparticles in Milli-Q water through ultrasonication for 30 minutes. Nano- Particle size distribution of the nanoparticle was determined through measurements carried out on Scanning Tunneling Microscopy (STM) (NANA – SS-5, Japan) images (Figure 1). TiO$_2$ nanoparticles of mean size of 20 nm diameter were used in the study.

![Figure 1. Tridimensional figure of TiO$_2$ nanoparticle (x5000)](image-url)

**Pot Culture Experiments**

The experiment was conducted in green house of the faculty of Science, Islamic Azad University, Mashad, Iran. The design of the experiment was a randomized complete block with four replicates. Wheat seeds were sown in pots(30 cm × 40 cm) filled with equal quantity of soil, watered to field capacity and placed in a greenhouse under controlled conditions: day/night photoperiod: 16/8 h; temperature (day/night), 24/20 ± 1°C. Leaf spraying in eight levels of Tio2 (0, 10, 100, 1000, 1200, 1500, 1700 and 2000 ppm) was carried out two times. The characters measured were: shoot and root length, fresh and dry biolass of shoot and root and chlorophyll content. After 30 days from sowing, plants were uprooted gently along with the whole soil mass. Roots and shoots were separated and used for recording the parameters. Total length of root and shoot was measured and expressed in cm. Their fresh weights were measured and then were dried for two days at 80°C in an oven and dry weight was taken and expressed in grams. Leaf chlorophyll content was measured by Arnon method (1956).
Statistical Analysis
Each treatment was conducted with four replicates. The statistical analysis of experimental data utilized the ANOVA program. Each experimental value was compared to its corresponding control. Statistical significance was accepted when the probability of the result assuming the null hypothesis (p) is less than 0.05 (level of probability).

RESULTS AND DISCUSSION
The results revealed the promotory effect of Nano scale TiO$_2$ at optimum concentrations and inhibitory effect at high concentrations on root and shoot growth (Figure 2,3). Nano scale TiO$_2$ at 100 mgL$^{-1}$ proved to be effective in improving both shoot and root length. At higher concentration of nan scale TiO$_2$ (more than 100 mgL$^{-1}$), shoot and root length decreased and these results were in accordance with the reports on radish, rape, corn, lettuce and cucumber by Lin and Xing (2007).

![Figure 2. Effects of TiO2 nanoparticle treatments on shoot height of wheat plants.](image)

![Figure 3. Effects of TiO2 nanoparticle treatments on root length of wheat plants.](image)
Fresh weight of shoots was greatly affected by nano-TiO₂ concentrations (Fig. 4) However there was no significant effect on dry weight of shoots from nano-TiO₂ treatments (Fig. 5). The result corroborate the earlier reported work by Seeger et al. (2009) who found no significant differences in growth of willow trees in the range of 1 - 100 mg/L nano-TiO₂.

Fresh weight of roots was greatly affected by nano-TiO₂ concentration (Fig. 6) similar to root dry weight (Fig. 7). The highest value of fresh and dry weight of wheat roots recorded in 100 mgL⁻¹ of nano-TiO₂, however the treatments higher than 100 mgL⁻¹ showed inhibitory effects on both fresh and dry weights of roots.

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Higher concentrations of nanoscale TiO$_2$ (1700 and 2000 mgL$^{-1}$) increased leaf chlorophyll content compared to control (Fig. 8). Nanoscale TiO$_2$ at 2000 ppm recorded the highest chlorophyll content (1.557 mg/gFW). Higher chlorophyll accumulation may be due to complementary effect of other inherent nutrients like magnesium, iron and sulfur. Similar results were observed by Zheng et al., 2005 when Spinacia oleracea seeds were treated with nanoscale TiO$_2$ particles. An increase of germination rate and the vigor indices was noted at 0.25–4% nanoscale TiO$_2$ treatment. During the growth period, the plant dry weight increased.
These results confirmed that the physiological effects were related to the nanometer sized particles.

REFERENCES


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