

## Effect of Nanoscale Titanium Dioxide Particles on the Germination and Growth of Canola (*Brassica napus*)

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An investigation was initiated to examine the effects of nanoscale titanium dioxide particles on plant growth and development. In view of the widespread cultivation of canola in Iran and in other parts of the globe and in view of the potential influence of titanium on its growth, this plant was chosen as the model system. Canola seeds were separately treated with different concentrations of nanoscale titanium dioxide (10, 100, 1000, 1200, 1500, 1700 and 2000 mg l<sup>-1</sup> and the effect this treatment was studied on seed germination and seedling vigor. Treatment of nanoscale TiO<sub>2</sub> (20 nm mean particle size) at 2000 mg l<sup>-1</sup> concentration promoted both seed germination and seedling vigor. The lowest and the highest germination rate were obtained in 1500 and 2000 mg l<sup>-1</sup> treatments, respectively. Higher concentrations of nanoscale TiO<sub>2</sub> (1200 and 1500 mg l<sup>-1</sup>) showed large radicle and plumule growth of seedling compared to other concentrations and control. The inhibitory effect with lower nanoparticle concentration reveals the need for judicious usage of these particles in such applications. This is the first report on the effect of nanoscale particles on canola growth.

Abstract

**Keywords:** Canola, Nanoscale TiO<sub>2</sub>, Seedling growth, Seed germination, Seedling vigor.

## INTRODUCTION

Titanium dioxide (TiO<sub>2</sub>) is a non-toxic white pigment for use in manufacture of paints, plastics, paper, ink, rubber, textile, cosmetics, leather, and ceramics (Moore, 1997), and as adjuvant and additives in commercial formulations (Arthurs *et al.*, 2006). Photocatalytic degradation of pesticides with TiO<sub>2</sub> and other catalyst has shown promise as potential water remediation method (Lee *et al.*, 2003). TiO<sub>2</sub> is an artificial colour allowed for use in food products in Egypt (Gain Report, 2005). Barley (2003) also reported that TiO<sub>2</sub> is considered to be nontoxic and harmless, that is approved for use in food up to 1% of product final weight. In order to reduce the hazard from heavy metals and improved the whiteness of glass in glassmaking technology, Morse and Glover (2000) reported the use of TiO<sub>2</sub> to replace lead oxide and barium/zinc-based lithopone. It has been noted that titanium dioxide breaks down the ethylene gas produced in storage rooms into carbon dioxide and water. It is used to treat the air in fruit, vegetable, and cut flower storage areas to prevent spoilage and increase the product's shelf life (Fonseca, 2004). Korean researchers found that illuminated titanium dioxide photocatalysts are effective against food-borne bacteria such as *Vibrio* sp., *Salmonella* sp. and *Listeria* sp. suggesting that future use of this technique will likely target food safety issues (Fonseca, 2004). CETAC (2002) also reported the commercial use of titanium dioxide as the most suitable photocatalytic catalyst, which upon exposure to ultraviolet light mineralizes the organic chemicals in rivers to water and carbon dioxide with the potential to destroy microorganisms. However, the present invention (TiO<sub>2</sub>) relates to a liquid composition which contains TiO<sub>2</sub> nanoparticles. Application of titanium dioxide (TiO<sub>2</sub>) on food crops has been reported to promote plant growth, increase the photosynthetic rate, reduce disease severity and enhance yield by 30% (Chao and Choi, 2005). It is a potent photocatalyst that can break down organic matter when exposes to sunlight in presence of water vapour into carbon dioxide (CO<sub>2</sub>) and water (Frazer, 2001). Diluted application of TiO<sub>2</sub> had been proved beneficial to crops by increasing their photosynthetic ability. The application of TiO<sub>2</sub> has been found to show excellent efficacy in rice plant and Oliver cereal e.g. maize by reducing the effect of Curvularia leaf spot and bacteria leaf blight disease incidence and severity (Chao and Choi, 2005). They also reported that application of TiO<sub>2</sub> significantly reduced the incidence of rice blast and tomato spray mold with a correspondent 20% increase in grain weight due to the growth promoting effect of TiO<sub>2</sub> nano-particles (NPL, 2002). Bowen *et al.* (1992) demonstrated that, a combination of titanium dioxide, aluminum and silica was effective in controlling downy and powdery mildew of grapes through mechanism that may involve direct action on the hyphae, interference with recognition of plant surface, and stimulation of plant physiological defenses. Titanium dioxide does not deteriorate and it shows a longterm anti-bacterial effect. The has strong very strong oxidizing power of titanium dioxide can destroy bacteria's cell membrane, causing leakage, which inhibits cellular activity and ultimately results in the death and decomposition of the cell (Frazer, 2001). Disinfections with titanium dioxide is three times stronger than chlorine, and 1.5 times stronger than ozone (Fujishima *et al.*, 1999; Maness, *et al.*, 1999). It has also be reported that when TiO<sub>2</sub> is used at rates greater than recommended rate, there is no damage caused to plant (Frazer, 2001). It has also be reported there is no damage caused by the consumption of farm products sprayed with TiO<sub>2</sub> as a 250 cc bottle contains 7.2 g of TiO<sub>2</sub> (NPL, 2002). The objective of this study therefore, is to evaluate the response of canola plant to the material.

## MATERIALS AND METHODS

### TiO<sub>2</sub> Nanoparticles

Titanium dioxide nanoparticles (nano- TiO<sub>2</sub>) were prepared from commercial TiO<sub>2</sub> nanopowder (Sigma-Aldrich, USA) by dispersing nanoparticles in ethanol through ultrasonication (300 W, 40 kHz) for 30 minutes. Particle size distribution of the nanoparticles was determined through measurements carried out on Scanning Tunneling Microscopy (STM) (NANA – SS-5, Japan) images (Fig. 1). TiO<sub>2</sub> nanoparticles of mean size of 20 nm diameter were used in the study.

## Seeds

Canola seeds of variety 'RGS003' were procured from Agricultural Research Center of Khorasan Province, Mashhad, Iran. The average germination rate of the seeds was 85% as shown by a preliminary study. The seeds selected were of uniform size to minimize errors in seed germination and seedling vigor.

## Preparation of Particle Suspensions and Seed Treatment

Nanoscale TiO<sub>2</sub> suspensions were prepared at concentrations of 10, 100, 1000, 1200, 1500, 1700 and 2000 mg l<sup>-1</sup>. Canola seeds were surface-sterilized in 7% sodium hypochlorite for 10 min. then thoroughly rinsed with distilled water and ten seeds were placed in a Petri dish (100 mm x15 mm) with one piece of sterilized filter paper. Then 6 ml of the test medium were added to the Petri dishes (three replicates per treatment). Control seeds were only treated with 6 ml of distilled water. Petri dishes were placed in a germinator at 26 ± 1°C for seven days. Every day, the number of germinated seeds was recorded. A seed was considered germinated when the radicle was visible. Then length of the primary root and shoot of each seedling was measured.

In this study, we used following germination parameters: Germination percentage (GP, %), Germination rate (GR) and Mean germination time (MGT). These parameters were also calculated from the formulas proposed by Figueroa & Armesto (2001), Bu *et al.* (2007), and Wu & Du (2008):

GP = 100 × GN / SN; GN is the total number of germinated seed; SN is the total number of seeds tested.

GR =  $\sum G_i / I$ ; G<sub>i</sub> is the number of seeds germinated on day i

MGT =  $\sum G_i \times i / \sum G$ ; where i is the number of days since the day of sowing (day 0) and G<sub>i</sub> is the number of seeds germinated on day i. Only seeds that germinated were included in the calculation.

Seedling Vigor Index (SVI) was calculated by the formula described by Abdul-Baki and Anderson (1973).

Seed Vigor Index = Germination% × (root length + shoot length)

## Data Analysis

Significant differences for all statistical tests were evaluated at the level of P ≤ 0.05 with ANOVA. All data analyses were conducted using MINITAB for Windows, Version 16.0.

## RESULTS AND DISCUSSION

Canola seeds responded variably towards the treatment at various concentrations of nanoscale TiO<sub>2</sub> particles. Seed treated with 2000 mg l<sup>-1</sup> nanoscale TiO<sub>2</sub> recorded significant germination percentage (75%), germination rate and seedling vigor (Fig. 2). Among the different nanoscale TiO<sub>2</sub> concentrations, 2000 mg l<sup>-1</sup> showed the maximum and 1500 mg l<sup>-1</sup> showed decreased seedling vigor index. Such inhibitory effects of nanoparticles were also reported by Lin and Xing (2007) on radish, rape, and rye grass. Lin and Xing (2007) analysed phytotoxicity of five types of multiwalled nanoparticles at the level of seed germination and root growth in six higher plant species (*Raphanus sativus*, *Brassica napus*, *Lolium multiflorum*, *Lactuca sativa*, *Zea mays* and *Cucumis sativus*). Seed germination was not affected except for the inhibition of nanoscale zinc on *Lolium multiflorum* and nanoscale zinc oxide on *Zea mays*. Inhibition of root growth varied greatly among nanoparticles and plants and it is partially correlated to nanoparticles concentration. Also results indicated that among the canola germination indices only germination rate was affected by treatments. The lowest and the highest germination rate (9.6 vs. 15.2 No. day<sup>-1</sup>) were obtained in 1500 and 2000 mg l<sup>-1</sup> treatments, respectively. Similar results were observed by Zhang *et al.*, 2005 when *Spinacia oleracea* seeds were treated with nanoscale TiO<sub>2</sub> particles. An increase of germination rate and the vigor indices was noted at 0.25–4% nanoscale TiO<sub>2</sub> treatment.

Higher concentrations of Nanoscale TiO<sub>2</sub> (1200 and 1500 mg l<sup>-1</sup>) showed large plumule

growth of seedling compared to other (Fig. 6). Such promotory effect of nanoscale SiO<sub>2</sub> and TiO<sub>2</sub> on germination was reported in soya bean (Lu *et al.*, 2002), in which authors noticed increased nitrate reductase enzyme activity and enhanced antioxidant system.

## CONCLUSIONS

In order to understand the possible benefits of applying nanomaterials in agriculture, it is important to analyze penetration and transport of nanoparticles in the plants. Size plays an important role in behavior, in reactivity and in toxicity. Considering these aspects, both positive and negative effects of nanoparticles are observed in living plants. The results suggest that the be delivered into Canola seeds through TiO<sub>2</sub> nanoparticles. Higher concentrations of TiO<sub>2</sub> improve the germination, root growth the treated seeds. The results point to the use of nanomaterials in agriculture, especially in canola, one of the main sources of livelihood in certain parts of the world.

## ACKNOWLEDGMENTS

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**Figures**

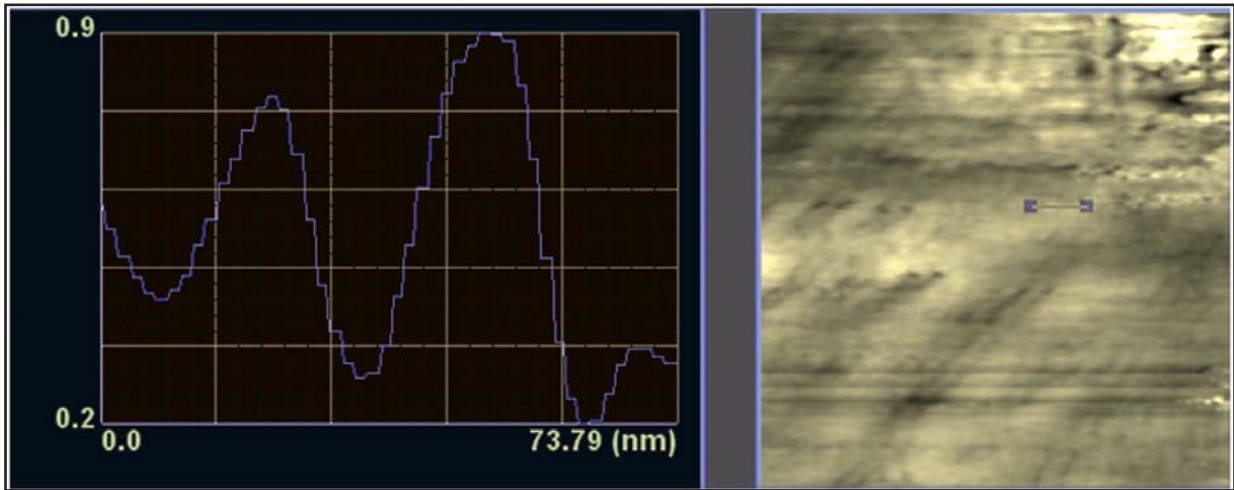


Fig.1. STM micrographs of TiO<sub>2</sub> nano- particles after dispersed in ethanol.

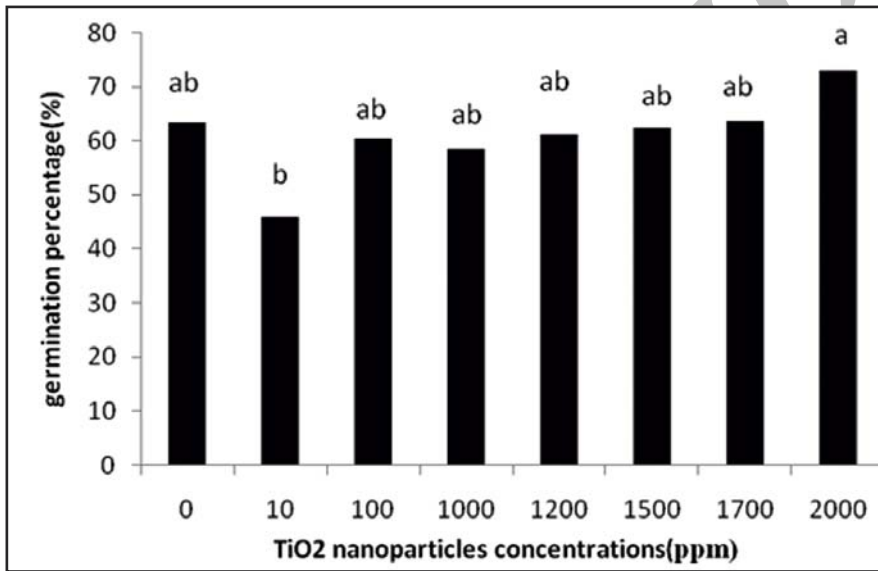


Fig.2. STM micrographs of TiO<sub>2</sub> nano- particles after dispersed in ethanol.

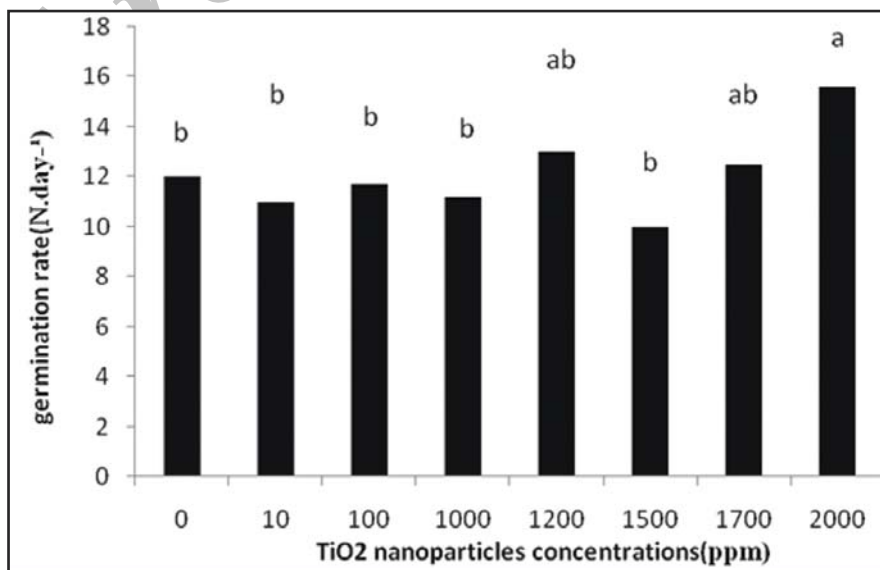


Fig.3. Effects of TiO<sub>2</sub> nanoparticles treatments on germination percentage of canola seeds.

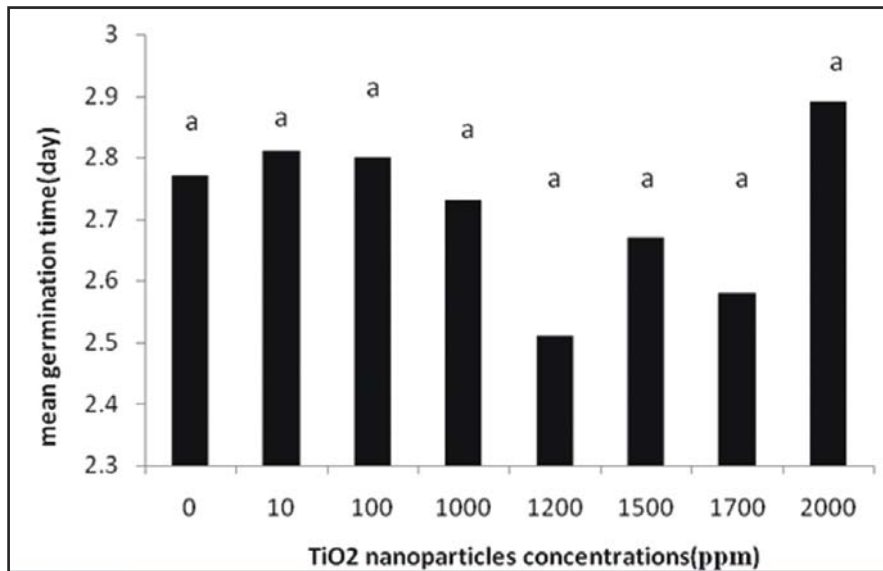


Fig.4. Effects of TiO<sub>2</sub> nanoparticles treatments on germination rate of canola seeds.

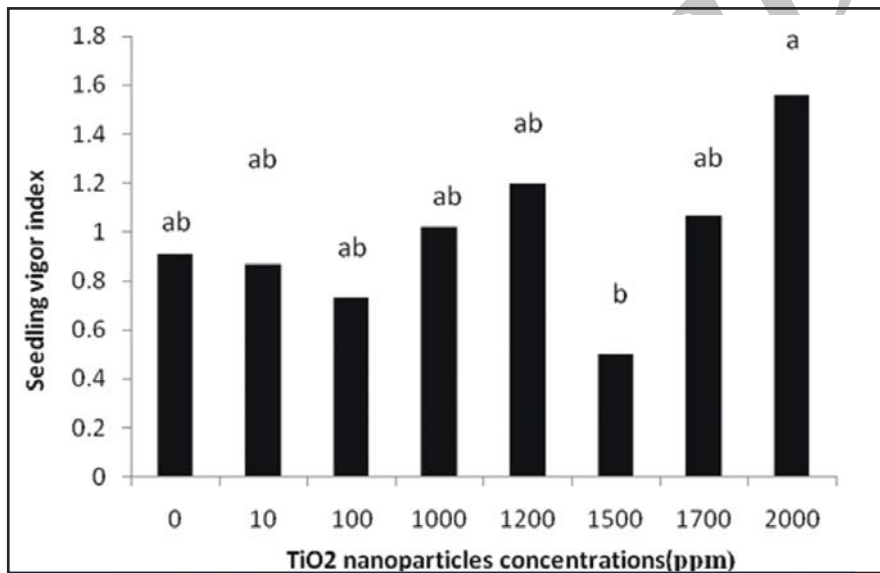


Fig.5. Effects of TiO<sub>2</sub> nanoparticles treatments on mean germination time of canola seeds.

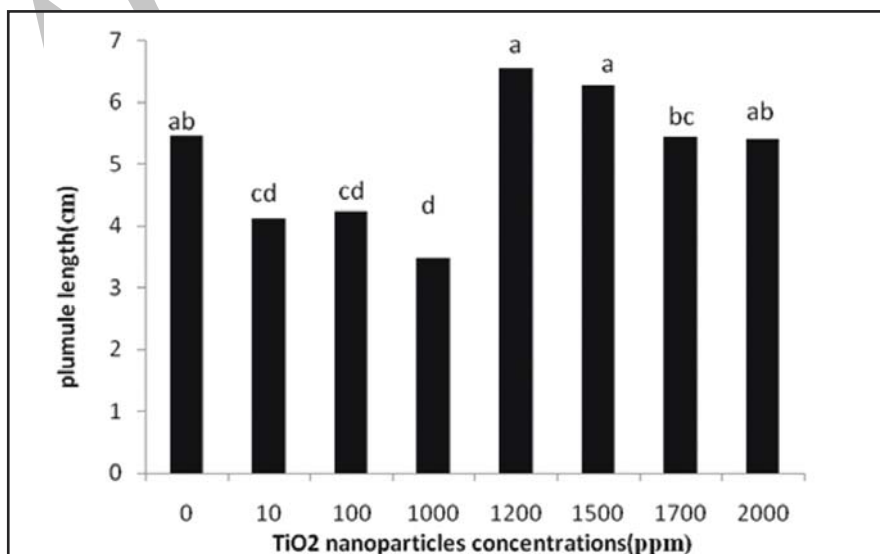


Fig.6. Effects of TiO<sub>2</sub> nanoparticles treatments on plumule length of canola seedlings.

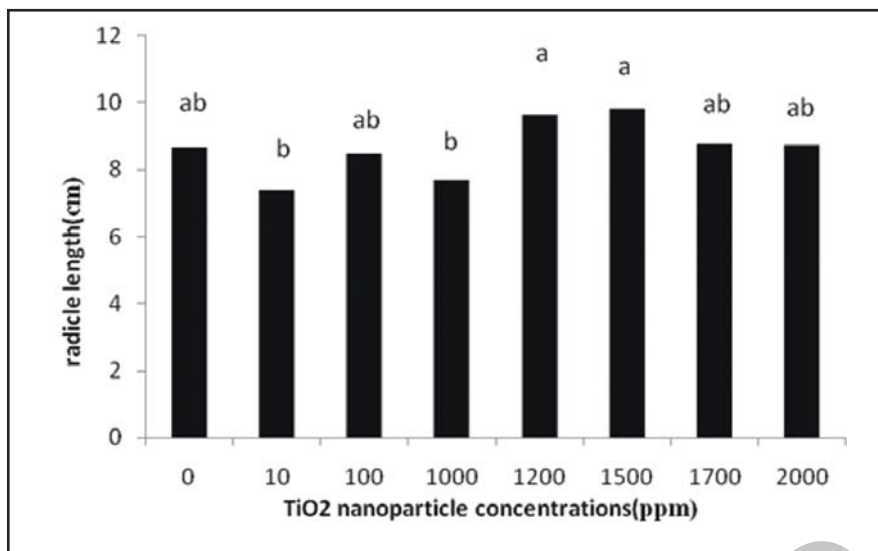


Fig.7. Effects of TiO<sub>2</sub> nanoparticles treatments on radical length of canola seedlings.

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