Can Bulk and Nanosized Titanium Dioxide Particles Improve Seed Germination Features of Wheatgrass (*Agropyron desertorum*)

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Abstract

The goal of this study was to evaluate concentrations of nanosized TiO$_2$ at 0, 5, 20, 40, 60 and 80 mg L$^{-1}$ with bulk TiO$_2$ for possible stimulatory effects on wheatgrass seed germination and early growth stage. After 14 days of seed incubation, germination percentage improved by 9% following exposure to 5 ppm nanosized TiO$_2$ treatment comparing to control. Similar positive effects occurred in terms of germination value and mean daily germination. Application of bulk TiO$_2$ particles in 80 ppm concentration greatly decreased the majority of studied traits. Therefore phytotoxicity effect observed on wheatgrass seedling by application of bulk TiO$_2$ particles in 80 ppm concentration. Exposure of wheatgrass seeds to 5 ppm nanosized TiO$_2$ and bulk and nanosized TiO$_2$ at 60 ppm obtained the lowest mean germination time but higher concentrations did not improve mean germination time. In general, there was a positive response by wheatgrass seed to some concentrations of nanosized TiO$_2$. Usage of nanoparticles in order to improve germination and establishment of range plant in adverse environments similar to rangeland could be possible.

Keywords: mean germination time, nanosized TiO$_2$, seed germination

Introduction

Nowadays, various researchers have studied the effects of nanomaterials on plant germination and growth with the objective to promote its use for agricultural applications (Khot *et al.*, 2012). Nanosized TiO$_2$ is a frequently used nanoparticle, consequently there has been an exponential increase in data collection on the effects of TiO$_2$ nanoparticles on different species but there is much less information on the effects of nanoparticles on plants compared to animals. Studies the effects of TiO$_2$ nanoparticles on plants provide information about the positive and stimulating effects as well as any negative impact (Klancnik *et al.*, 2011). Despite the low availability of Ti element to plants, its beneficial effects on plants have already been proved. In oats (*Avena sativa*), Ti uptake as a nutrient solution by roots was more effective than spraying it on the leaves, benefiting various plant physiological parameters such as biomass yield, chlorophyll content, and growth (Kuzel *et al.*, 2003). Kiss *et al.* (1985) and Daood *et al.* (1998) also showed that Ti could activate photosynthesis, probably by changing the redox state of specific regulatory proteins and eliciting an alteration in enzyme activity, the most important enzyme being fructose-1,6-bisphosphatase (F-1,6BP), which participates in the Calvin cycle, gluconeogenic, and oxidative pentose phosphate pathways of carbohydrate metabolism which are assumed to be associated with Ti.

Haghighi *et al.* (2012) demonstrated that 1 mg L$^{-1}$ Ti can almost fully compensate the fresh and dry weight of shoots and roots, flowering time, number of flowers, chlorophyll content, and photosynthetic capacity of tomato when nitrogen is reduced by 50%. This has very practical applications for tomato hydroponic culture by maximizing macronutrient and micronutrient absorption while lowering N concentration by half, thus making the system more cost-effective.

In arid and semiarid regions with low and unreliable rainfall, germinating seeds are often experience different durations of drought stress. Seed germination of various plant species may respond differently to dry conditions (Wilson, 1973).

Crested wheatgrass (*Agropyron desertorum*) is a perennial bunchgrass and one of the important species of the Poacea family. It is widely used for rangeland rehabilitation on light-textured soils of both shrub lands and grasslands where the rainfall is low (Basiri *et al.*, 1988). Under rangeland conditions, seeds are often exposed to high and low temperatures and severe drought. Hence, choosing suitable techniques for increase seed tolerance to adverse conditions would serve to increase the probability of success (Wilson, 1973).

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The key reason for the increased growth rate by TiO$_2$ nanoparticles could have been the photo-sterilization and photo-generation of "active oxygen like superoxide and hydroxide anions" by nano TiO$_2$ that can increase the seed stress resistance and promote capsule penetration for intake of water and oxygen needed for fast germination. (Khot et al., 2012). The key to increased seed germination rate is the penetration of nanomaterials into the seed. Khodakovskaya et al. (2009) reported that multi wall carbon nanotubes can penetrate into the tomato seeds and increase the germination rate by increasing water uptake. The Multi wall carbon nanotubes (MWCNT) increased the seed germination, up to 90% (compared to 71% in control) in 20 days, and the plant biomass.

Foltete et al. (2011) stated that altered TiO$_2$ nanocomposites and phytochelatins levels showed no modify on plant growth, photosystem II maximum quantum yield, genotoxicity (micronucleus test) compared to controls (Foltete et al., 2011). Zheng et al. (2005) confirmed that nanosized TiO$_2$ helped water absorption in spinach seeds and as result enhanced seed germination. Lu et al. (2002) shown that a combination of nanosized SiO$_2$ and TiO$_2$ could increase nitrate reductase enzyme in soybean (Glycine max) and its abilities of absorbing and utilizing water and fertilizer, promoting antioxidant system, and finally accelerate its germination and growth. Also, the positive effects of TiO$_2$ could be probably due to the antimicrobial properties of engineered nanoparticles, which can enhance resistance of plants to stress (Navarro et al., 2008).

Some reports have demonstrated that nanoparticles can induce phytotoxicity and have a negative impact on seed germination and growth while using unique properties of some nanoparticles it will possible to improve seed germination and crop performance. This use of the potentially positive effects of nanoparticles may be a helpful approach to decrease consumption of chemical agents in agriculture that would help to lower environmental pollution. Results of our earlier work demonstrated that using nanosized TiO$_2$ in low concentration (2 and 10 ppm) could encourage seed germination of wheat in comparison to bulk TiO$_2$ and untreated control groups, but in high concentrations (100 and 500 ppm) it indicated an inhibitory or no effect on wheat seed (Feizi et al., 2012). However weak seed germination is a common occurrence in some rangeland plants and there are limited studies on the effects of nanoparticles on rangeland plants particularly wheatgrass. This study was consequently performed to explore possible phytotoxicity and/or stimulatory effects of nanosized TiO$_2$ concentrations compared to the bulk TiO$_2$ particles on wheatgrass seed and seedling growth.

**Materials and methods**

**Description of materials**

Wheatgrass (Agropyron desertorum) seeds were taken from Karnakh Research Station, Natural Resources Organization of Mashhad. Nanosized TiO$_2$ powder was AEROXIDE TiO$_2$ P25, supplied by Degussa GmbH Company. Specific surface area of nanosized TiO$_2$ was 50 m$^2$ g$^{-1}$, average primary particle size was 21 nm and purity was >99.5%. The size of TiO$_2$ nanoparticles (Fig. 1) was determined through Scanning Tunneling Microscope (STM) in Central Laboratory of Ferdowsi University of Mashhad.

Analysis of x-ray diffraction (XRD) of nanoparticles TiO$_2$ was shown in Fig. 2. XRD measurement showed that the TiO$_2$ nanoparticles used in the study were made by 80% anatase and 20% rutile. Analysis of particles in X-ray diffraction indicates Tetragonal particles and the crystalline nature of TiO$_2$ particles.

Bulk TiO$_2$ particles were supplied by AppliChem GmbH Company, they had 99% purity and particle size was measured by Scanning Electron Microscope (SEM) in Central Laboratory of Ferdowsi University of Mashhad (Fig. 3). XRD measurement showed that the bulk TiO$_2$ particles used in the research were made by 100% anatase. Analysis of particles in X-ray diffraction indicated particles with tetragonal shape and the bulk TiO$_2$ particles had a crystalline nature (Fig. 4).

![Fig. 1. Images of nanosized TiO$_2$ by Scanning Tunneling Microscope (STM)]](image-url)
Seed culture and exposure

Experiment was made to evaluate the effect of different concentrations of bulk and nanosized TiO$_2$ on wheatgrass seed germination in a completely randomized design with four replications. The treatments in the experiment were five concentrations (5, 20, 40, 60 and 80 ppm) of bulk and five concentrations (5, 20, 40, 60 and 80 ppm) of nanosized TiO$_2$ and an untreated control (without any TiO$_2$ types). Prechilling treatment on seeds exert at 4°C for 7 days before beginning of germination test (ISTA, 2009). The Experiment was performed in a germinator with an average temperature of 25/15 ±1°C for 16/8 hours (day/night) at the College of Natural Resources, Ferdowsi University of Mashhad, Iran in 2012.

Seeds of similar size were randomly selected and sterilized using NaClO (5%) for 3 minutes and then carefully washed with distilled water three times. In order to obtain properly dispersed and stable TiO$_2$ suspensions of each concentration, an ultra-sonication treatment was applied to bulk and nanoparticles TiO$_2$ powders dispersed in water for 15 minutes. The seeds were placed on paper in four groups of 25 seeds in Petri dishes, and after that 2 ml of each concentration treatments was added. For the control, only distilled water was added to the Petri dishes. Germination tests were performed according to the rule issued by the International Seed Testing Association (ISTA, 2009). All concentrations of TiO$_2$ and the control were tested at the same time to make sure uniform conditions of light and temperature across all tests. Number of germinated seeds was noted daily during 14 days. Seeds were considered germinated when the radicle showed at least 2 mm in length (ISTA, 2009). Mean germination time was calculated based on Matthews and Khajeh-Hosseini (2007) (Eq. 1):

$$MGT = \frac{\sum F \times X}{\sum F}$$  
Eq. 1

Where F is the number of seeds newly germinated at the time of X, and X is the number of days from sowing.

Germination rate was determined based on Maguire (1982) (Eq. 2):

$$Germination\ rate = (a/1)+(b-a/2)+(c-b/3)+...+(n-n-1/N)$$  
Eq. 2

Where a, b, c, ..., n are numbers of germinated seeds after 1, 2, 3, ..., N days from the start of imbibition.

Seedling vigors were computed based on Vashisth and Nagarajan (2010) (Eq. 3 and 4):

$$Vigor\ index\ I = Germination\% \times Seedling\ length\ (cm)$$  
Eq. 3

$$Vigor\ index\ II = Germination\% \times Seedling\ weight\ (g)$$  
Eq. 4

Evaluations of Mean Daily Germination (MDG), Pick Value (PV) and Germination Value (GV) were calculated by the following equations (Hartmann et al., 1990):

$$MDG = \frac{Germination\%}{total\ experiment\ days}$$  
Eq. 5

$$PV = Maximum\ germinated\ seed\ number\ at\ one\ day\ /\ day\ number$$  
Eq. 6

$$GV = PV \times MDG$$  
Eq. 7

Data analysis

Analysis of variance (ANOVA) was performed between treatment samples in a completely randomized design in four replications. Records were analyzed using MSTAT-C computer software. Significant levels of difference for all studied traits were calculated and means were compared by the multiple ranges Duncan test at 5% level.
Results and discussion

Results demonstrated that treatments in this experiment had significant effects on most of studied traits. Employment of TiO$_2$ nanoparticle with 5 ppm enhanced wheatgrass seed germination, while seed germination percentages decreased from exposure to 80 ppm concentrations of bulk and nano TiO$_2$ particles compared to the control group (Tab. 1). It seems that the highest concentration of bulk and nano TiO$_2$ particles had phytotoxicity effect on seed germination.

Wheatgrass seeds exposed to 5 ppm TiO$_2$ nanoparticles exhibited improvements in seed germination percentage by 9% compared to the control. The main reason for this increased growth rate could have been the photosterilization and photo-generation of "active oxygen like superoxide and hydroxide anions" by nano-TiO$_2$ that enhanced seed stress resistance and encouraged capsule penetration for intakes of water and oxygen needed for quick germination (Khot et al., 2012). An earlier study was done (Feizi et al., 2012) demonstrating that although the highest germination percentage (98%) was in both 2 ppm bulk and nanosized TiO$_2$ concentrations, the two treatments had no significant effect on the seed germination percentage. Zheng et al. (2005) reported that nanosized TiO$_2$ contributed to water absorption by spinach seeds and as result accelerated seed germination. Clément et al. (2012) reported that soaking of flax seeds in the suspensions of TiO$_2$ nanoparticles at 100 mg L$^{-1}$ concentration had positive effects on seed germination and root growth. These positive effects could be due to antimicrobial properties of anatase crystalline structure of TiO$_2$ that increase plant resistance to stress (Clément et al., 2012).

Shoot, root and seedling elongation were significantly affected by bulk and nanosized TiO$_2$ treatments. Application of bulk TiO$_2$ at 5 ppm, nano TiO$_2$ at 40 ppm and bulk TiO$_2$ at 60 ppm demonstrated the highest shoot and seedling length, but bulk TiO$_2$ at 80 ppm showed the lowest shoot and seedling length. Treatments had no significant effect on root length. Use of bulk TiO$_2$ particles in 80 ppm concentration greatly decreased shoot dry weight up to 42% compared to the control seeds while at the concentration of 80 ppm nano TiO$_2$ did not demonstrate such reduction in shoot biomass (Tab. 1). The greatest shoot biomass was found in 5 ppm bulk particles (10.4 mg) of titanium dioxide. The highest root biomass was achieved from concentrations of 5 ppm bulk-TiO$_2$ and 40 ppm nano-TiO$_2$ but an increased concentration of bulk particles of 80 ppm significantly reduced root dry weight. It is probable that increasing the concentration of bulk-TiO$_2$ induced aggregation of particles and resulted in clogging of root pores that interrupted water uptake by seeds. It seems that nano TiO$_2$ could stimulate process of seed germination like water and oxygen uptake led to improve seed germination percentage but in later growth stages, seedling might respond as different. Lin and Xing (2007) confirmed the phytotoxicity of nano-Al and Al$_2$O$_3$ significantly affected root elongation of ryegrass and corn, respectively whereas, nano-Al facilitated root growth in radish and rape. Although root length and weight are not standardized in toxicity tests, they may be helpful to compare the toxicity effects after seeds exposure to nanoparticles since low values can be related to non-acute toxicological or stress effects (Barrena et al., 2009). In an experiment, Barrena et al. (2009) stated that it seems that in the case of Fe- nanoparticles treatment, the development of thicker roots was favored, whereas in the case of Au, root growth was mainly due to elongation. The root growth in length but not in width might be an avoidance mechanism of the seed to a stress issue produced by the presence of some nanoparticles (Barrena et al., 2009).

A lot of germination-related occurrences (gene transcription and translation, respiration and energy metabolism, early reserve mobilization and DNA repair) could also happen during seed treatment (Varier et al., 2010), although often restricted due to reduced water supply.

Tab. 1. Influence of bulk and nanosized TiO$_2$ concentrations on seed germination, elongation and biomass of wheatgrass seedling

<table>
<thead>
<tr>
<th>TiO$_2$ concentration (ppm)</th>
<th>Germination (%)</th>
<th>Shoot length (cm)</th>
<th>Root length (cm)</th>
<th>Seedling length (cm)</th>
<th>Shoot dry weight (mg)</th>
<th>Root dry weight (mg)</th>
<th>Seedling weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>79 ab</td>
<td>7.51 abc</td>
<td>6.55 a</td>
<td>14.06 bc</td>
<td>9.47 ab</td>
<td>3.45 bc</td>
<td>12.92 abc</td>
</tr>
<tr>
<td>5 bulk</td>
<td>83 a</td>
<td>8.97 a</td>
<td>7.97 a</td>
<td>16.95 a</td>
<td>10.4 a</td>
<td>5.57 a</td>
<td>15.97 a</td>
</tr>
<tr>
<td>5 nano</td>
<td>86 a</td>
<td>7.56 abc</td>
<td>7.61 a</td>
<td>15.18 b</td>
<td>6.9 bcd</td>
<td>4.52 ab</td>
<td>11.42 bcd</td>
</tr>
<tr>
<td>20 bulk</td>
<td>73 ab</td>
<td>7.24 abc</td>
<td>8.52 a</td>
<td>15.76 ab</td>
<td>7.02 bcd</td>
<td>4.47 ab</td>
<td>11.5 bcd</td>
</tr>
<tr>
<td>20 nano</td>
<td>74 ab</td>
<td>6.08 bc</td>
<td>7.96 a</td>
<td>14.05 bc</td>
<td>6.45 cd</td>
<td>4.5 ab</td>
<td>10.95 cd</td>
</tr>
<tr>
<td>40 bulk</td>
<td>76 ab</td>
<td>7.77 ab</td>
<td>7.63 a</td>
<td>15.45 ab</td>
<td>8.42 abc</td>
<td>4.37 ab</td>
<td>12.8 abc</td>
</tr>
<tr>
<td>40 nano</td>
<td>84 ab</td>
<td>8.63 a</td>
<td>8.51 a</td>
<td>17.14 a</td>
<td>8.95 abc</td>
<td>5.72 a</td>
<td>14.67 ab</td>
</tr>
<tr>
<td>60 bulk</td>
<td>84 ab</td>
<td>8.61 a</td>
<td>8.73 a</td>
<td>17.34 a</td>
<td>9.15 abc</td>
<td>4.57 ab</td>
<td>13.72 abc</td>
</tr>
<tr>
<td>60 nano</td>
<td>78 ab</td>
<td>7.65 abc</td>
<td>8.4 a</td>
<td>16.65 ab</td>
<td>8.42 abc</td>
<td>4.52 ab</td>
<td>12.95 abc</td>
</tr>
<tr>
<td>80 bulk</td>
<td>58 c</td>
<td>5.78 c</td>
<td>6.72 a</td>
<td>12.51 c</td>
<td>5.47 d</td>
<td>2.65 c</td>
<td>8.12 d</td>
</tr>
<tr>
<td>80 nano</td>
<td>70 bc</td>
<td>7.50 abc</td>
<td>7.79 a</td>
<td>15.29 ab</td>
<td>9.1 abc</td>
<td>4.35 ab</td>
<td>13.45 abc</td>
</tr>
</tbody>
</table>

*Means, in each column, followed by similar letter are not significantly different at the 5% probability level- using Duncan’s Multiple Range Test
Influence of bulk and nanosized TiO$_2$ having a larger size, cannot easily enter the same pathway, effect on the process of seed germination but bulk particles, could penetrate into the seed coat and exert a helpful effect on germination value. It is most probable that nanoparticles of seeds while nanosized TiO$_2$ can penetrate tomato seed and increase the germination rate by rising water uptake. That multi wall carbon nanotubes increased seed germination up to 90% (compared to 71% in control) in 20 days; it also increased plant biomass.

On the whole, lower mean germination time represents earlier germination. These results revealed that exposure of wheatgrass seeds to 5 ppm nanosized TiO$_2$ and bulk and nanosized TiO$_2$ at 60 ppm obtained the lowest mean germination time but higher concentrations did not improve mean germination time. Thus, use of nanosized TiO$_2$ at 5 and 60 ppm reduced mean germination time by 14 and 11% in comparison to the untreated control respectively, whereas 5 ppm concentration of bulk TiO$_2$ did not contribute to a reduction of mean germination time in comparison with the control (Tab. 2). Zheng et al. (2005) stated that the significant effect of nanosized TiO$_2$ on spinach germination in tests was probably because of small particle size, which allowed nanoparticles to penetrate the seed during the treatment period, exerting its enhancing functions during growth.

Exposure of wheatgrass seeds to high concentrations of nano TiO$_2$ particles (80 ppm) led to diminished germination rate (Tab. 2). The highest germination rate was found in 60 ppm bulk TiO$_2$ particles (5.26 seed day$^{-1}$) and increasing concentration decreased the germination rate. Bulk-TiO$_2$ treatment at 80 ppm showed the lowest germination rate (2.91 seed day$^{-1}$) (Tab. 2). In most cases (Zheng et al., 2005; Feizi et al., 2009, 2012, 2013), using bulk TiO$_2$ particles significantly decreased germination value of seeds while nanosized TiO$_2$ had a positive effect on germination value. It is most probable that nanoparticles could penetrate into the seed coat and exert a helpful effect on the process of seed germination but bulk particles, having a larger size, cannot easily enter the same pathway, therefore may accumulate in the pores of a seed coat and clog up water and oxygen transition. Based on studies on nanoparticles effects on mechanism of seed germination it could have stated that the nanoparticles might help water absorption by the seeds (Zheng et al., 2005), increase nitrate reductase enzyme, increase seed abilities of absorbing and utilizing water and fertilizer, promote seed antioxidant system (Lu et al., 2002), reduced anti oxidant stress by reducing H$_2$O$_2$, superoxide radicals, and malonyldialdehyde content, and increasing some enzymes such as superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and catalase activities (Lei et al., 2008) result in improve seed germination in some plant species. Foltete et al. (2011) examined altered TiO$_2$ nanocomposites (ATN) in the liquid phase on Vicia faba, exposed to three nominal concentrations 5, 25 and 50 mg ATN/L for 48 h. They concluded plant growth, photosystem II maximum quantum yield, genotoxicity (micronucleus test) and phytochelatins levels showed no change compared to controls.

Exposure of seeds to nano and bulk TiO$_2$ particles at 80 ppm significantly diminished MDG compared to the untreated control. However, the pick value (PV) of wheatgrass seedlings was not affected by different concentrations of bulk and nanoparticles. Application of bulk-TiO$_2$ at 80 ppm concentration had a negative effect on vigor index I and II (Tab. 2). It has been stated that the biological activity and biokinetics of nanoparticles depends on parameters such as size, shape, chemistry, crystallinity, surface properties (area, porosity, charge, surface modifications, coating), agglomeration state, biopersistence, and dose (Casals et al., 2008). Zheng et al. (2005) showed that the growth of spinach plants was greatly improved at concentrations of 250 - 4,000 ppm nano TiO$_2$ than concentrations of bulk-TiO$_2$. Ghosh et al. (2010) observed adverse effect of TiO$_2$ nanoparticles for another plant species, Nicotina tabacum. They reported that TiO$_2$ nanoparticles induced DNA injury in N. tabacum simply at high concentration (319 mg L$^{-1}$) of TiO$_2$ nanoparticles.

Tab. 2. Influence of bulk and nanosized TiO$_2$ concentrations on growth features of wheatgrass seedling

<table>
<thead>
<tr>
<th>TiO$_2$ concentration (ppm)</th>
<th>MGT (day)</th>
<th>Germination rate (seed day$^{-1}$)</th>
<th>Germination value</th>
<th>MDG</th>
<th>PV</th>
<th>Vigour index I</th>
<th>Vigour index II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.38 a</td>
<td>4.51 ab</td>
<td>13.83 ab</td>
<td>6.07 ab</td>
<td>2.26 a</td>
<td>1111 abc</td>
<td>1016 abc</td>
</tr>
<tr>
<td>5 bulk</td>
<td>5.88 a</td>
<td>4.36 ab</td>
<td>14.36 ab</td>
<td>6.38 ab</td>
<td>2.24 a</td>
<td>1142 ab</td>
<td>1343 a</td>
</tr>
<tr>
<td>5 nano</td>
<td>4.64 b</td>
<td>4.55 ab</td>
<td>18.86 a</td>
<td>6.61 a</td>
<td>2.80 a</td>
<td>1307 abc</td>
<td>978 bcd</td>
</tr>
<tr>
<td>20 bulk</td>
<td>5.84 a</td>
<td>4.00 b</td>
<td>11.98 bc</td>
<td>5.61 a</td>
<td>2.10 a</td>
<td>1161 ab</td>
<td>838 ede</td>
</tr>
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<td>20 nano</td>
<td>5.44 a</td>
<td>3.70 bc</td>
<td>12.99 bc</td>
<td>5.69 ab</td>
<td>2.27 a</td>
<td>1039 ed</td>
<td>812 de</td>
</tr>
<tr>
<td>40 bulk</td>
<td>5.16 ab</td>
<td>3.97 b</td>
<td>12.87 bc</td>
<td>5.84 ab</td>
<td>2.16 a</td>
<td>1186 ab</td>
<td>966 bcd</td>
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<td>5.09 ab</td>
<td>4.60 ab</td>
<td>16.12 ab</td>
<td>6.46 ab</td>
<td>2.47 a</td>
<td>1439 a</td>
<td>1227 ab</td>
</tr>
<tr>
<td>60 bulk</td>
<td>4.78 b</td>
<td>5.26 a</td>
<td>15.10 ab</td>
<td>6.46 ab</td>
<td>2.34 a</td>
<td>1457 a</td>
<td>1158 abc</td>
</tr>
<tr>
<td>60 nano</td>
<td>4.77 b</td>
<td>5.02 b</td>
<td>13.52 bc</td>
<td>6.00 ab</td>
<td>2.25 a</td>
<td>1260 abc</td>
<td>1025 abcd</td>
</tr>
<tr>
<td>80 bulk</td>
<td>5.42 a</td>
<td>2.91 c</td>
<td>8.63 c</td>
<td>4.46 c</td>
<td>1.81 a</td>
<td>773 d</td>
<td>526 c</td>
</tr>
<tr>
<td>80 nano</td>
<td>5.35 a</td>
<td>3.95 b</td>
<td>11.18 bc</td>
<td>5.38 bc</td>
<td>2.05 a</td>
<td>1070 bcd</td>
<td>950 bcd</td>
</tr>
</tbody>
</table>

*Means, in each column, followed by similar letter are not significantly different at the 5% probability level- using Duncan’s Multiple Range Test.
Conclusions

Nanotechnology is leading to the progress of a range of inexpensive applications for enhanced plant growth. Applications of nanomaterial can encourage earlier plant germination and improve plant production. To our knowledge, this work is the first publication related to the effects of bulk and nanosized TiO$_2$ particles in wheatgrass (*Agropyron desertorum*). Usually seed germination and establishment of wheatgrass is difficult in detrimental conditions of natural rangeland. Using TiO$_2$ nanoparticles at low dosage could promote wheatgrass seed germination percentage, while seed germination percentages decreased following exposure to high concentrations of bulk and nano TiO$_2$ particles compared to the control group. Exposure of wheatgrass seeds to 5 and 60 ppm nanosized TiO$_2$ obtained the lowest mean germination time but higher concentrations did not improve mean germination time. Low and intermediate concentrations of nanosized TiO$_2$ improved seedling growth indices but higher concentration (80 ppm) had an inhibitory effect on seed and seedling. Application of bulk TiO$_2$ at 80 ppm reduced some traits in the study such as germination rate, GMD, GV and vigor index compared to nanosized TiO$_2$ and the control. Nanomaterial can improve plant germination in certain plants but can have adverse affects on others. In such cases, nanomaterial can be applied for pretreatment of rangeland seeds and then cultivate in the natural rangelands to promote germination and establishment of seedling. Nevertheless, on the basis of these results it is highly recommended that the influence of low dose nanomaterial be assessed in order to encourage seed germination and seedling growth of different range plant species. Although this study demonstrated the potential of nanomaterial for natural resources application, additional exploration and research is needed to elucidate and develop these potential.

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