AcademicPres

Available online at www.notulaebiologicae.ro

Print ISSN 2067-3205; Electronic 2067-3264

Not Sci Biol, 2013, 5(3):325-331

Notulae Scientia Biologicae

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 nanoparicles 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 (Bassiri *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).

Received 25 April 2013; accepted 30 June 2013

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).

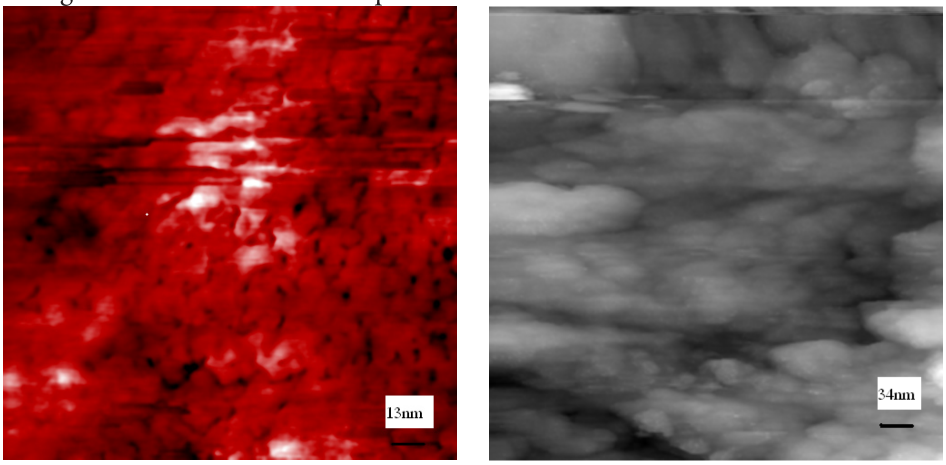

Fig. 1. Images of nanosized TiO_2 by Scanning Tunneling Microscope (STM)

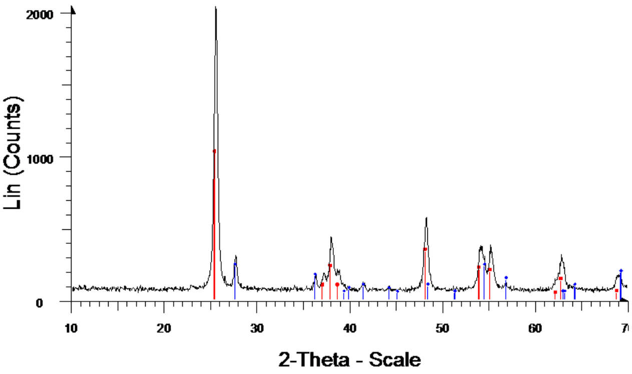

Fig. 2. X-ray diffraction (XRD) pattern of nano TiO_2 particles

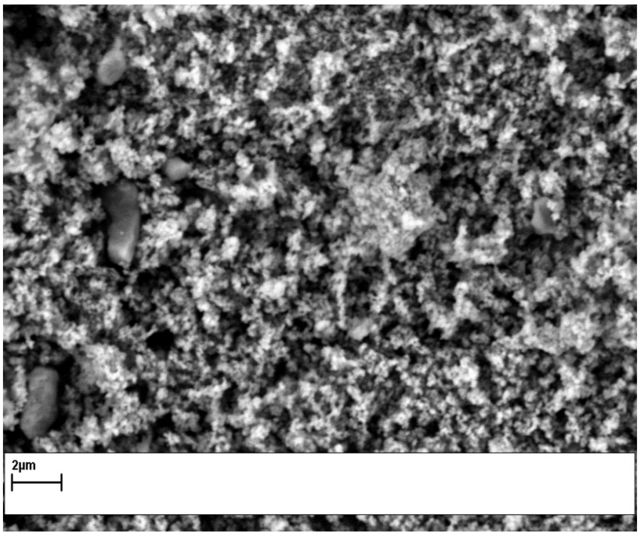

Fig. 3. Image of bulk TiO_2 particles by Scanning Electron Microscope (SEM)

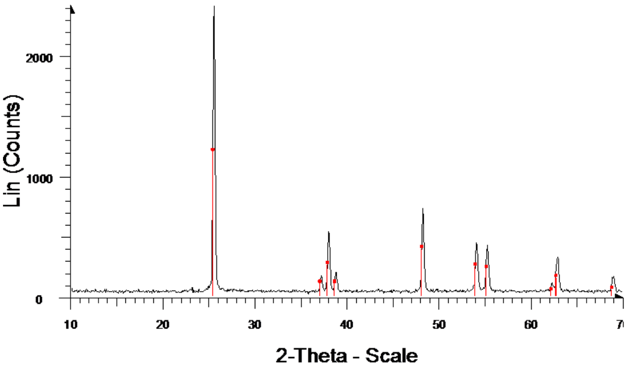

Fig. 4. X-ray diffraction (XRD) pattern of bulk TiO_2 particles

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):

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

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% × Seedling length (cm) Eq. 3

Vigor index II = Germination% × 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 = Germination% / total experiment days Eq. 5

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

GV= PV × 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 phytotoxity 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 not 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_2O_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

TiO_2 concentration (ppm)	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Shoot dry weight (mg)	Root dry weight (mg)	Seedling weight (mg)
Control	79 ab	7.51 abc	6.55 a	14.06 bc	9.47 ab	3.45 bc	12.92 abc
5 bulk	83 ab	8.97 a	7.97 a	16.95 a	10.4 a	5.57 a	15.97 a
5 nano	86 a	7.56 abc	7.61 a	15.18 b	6.9 bcd	4.52 ab	11.42 bcd
20 bulk	73 ab	7.24 abc	8.52 a	15.76 ab	7.02 bcd	4.47 ab	11.5 bcd
20 nano	74 ab	6.08 bc	7.96 a	14.05 bc	6.45 cd	4.5 ab	10.95 cd
40 bulk	76 ab	7.77 ab	7.63 a	15.45 ab	8.42 abc	4.37 ab	12.8 abc
40 nano	84 ab	8.63 a	8.51 a	17.14 a	8.95 abc	5.72 a	14.67 ab
60 bulk	84 ab	8.61 a	8.73 a	17.34 a	9.15 abc	4.57 ab	13.72 abc
60 nano	78 ab	7.65 abc	8.4 a	16.05 ab	8.42 abc	4.52 ab	12.95 abc
80 bulk	58 c	5.78 c	6.72 a	12.51 c	5.47 d	2.65 c	8.12 d
80 nano	70 bc	7.50 abc	7.79 a	15.29 ab	9.1 abc	4.35 ab	13.45 abc

*Means, in each column, followed by similar letter are not significantly different at the 5% probability level- using Duncan´s Multiple Range Test compared to regular germination (Chen and Bradford, 2000; Li *et al.,* 2005). The key to increased seed germination rate is the penetration of nanomaterial into the seed. Khodakovskaya *et al.* (2009) reported that multi wall carbon nanotubes 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.,* 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_2O_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 injure 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

TiO_2 concentration (ppm)	MGT (day)	Germination rate (seed day^{-1})	Germination value	MDG	PV	Vigour index I	Vigour index II
Control	5.38 a	4.51 ab	13.83 ab	6.07 ab	2.26 a	1111 abcd	1016 abcd
5 bulk	5.88 a	4.36 ab	14.36 ab	6.38 ab	2.24 a	1412 ab	1343 a
5 nano	4.64 b	4.55 ab	18.86 a	6.61 a	2.80 a	1307 abc	978 bcd
20 bulk	5.84 a	4.00 b	11.98 bc	5.61 ab	2.10 a	1161 abc	838 cde
20 nano	5.44 a	3.70 bc	12.99 bc	5.69 ab	2.27 a	1039 cd	812 de
40 bulk	5.16 ab	3.97 b	12.87 bc	5.84 ab	2.16 a	1186 abc	966 bcd
40 nano	5.09 ab	4.60 ab	16.12 ab	6.46 ab	2.47 a	1439 a	1227 ab
60 bulk	4.78 b	5.26 a	15.10 ab	6.46 ab	2.34 a	1457 a	1158 abc
60 nano	4.77 b	5.02 b	13.52 bc	6.00 ab	2.25 a	1260 abc	1025 abcd
80 bulk	5.42 a	2.91 c	8.63 c	4.46 c	1.81 a	773 d	526 e
80 nano	5.35 a	3.95 b	11.18 bc	5.38 bc	2.05 a	1070 bcd	950 bcd

*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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