



Effect of Different Concentrations of Titanium Dioxide Nanoparticles on Germination and Early Growth of Five Desert Plant Species

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ABSTRACT

Aims Studying the effects of nanoparticles on living organisms seems to be necessary, especially in plants as the first trophic level. Thus the phytotoxicity of different concentrations of nano-TiO₂ on five desert plant species was investigated in the present study.

Materials & Methods The phytotoxicity of different concentrations (0, 10, 100, 500, 1500mg l⁻¹) of nano-TiO₂ on five desert plant species of *Halothamnus glaucus* Botsch, *Haloxylon aphyllum* L., *Nitraria schoberi* L., *Zygophyllum eurypterum* Boiss. & Buhse, *Halocnemum strobilaceum* were investigated using seed germination percentage, radicle, and plumule elongation measurement. Experiments were conducted based on a completely randomized design with four replications.

Findings Outcomes of the study demonstrated that the application of nano-TiO₂ had no adverse effect on germination at low concentrations (up to 500mg l⁻¹), it also increased the germination of *H. aphyllum* (72 to 88%). The concentration of 1500mg l⁻¹ had a negative effect on germination and radicle growth of three species of *N. schoberi* (decrease in germination from 32 to 20% and radicle length from 13.85 to 10.68cm), *H. aphyllum* (decrease in germination from 72 to 44% and radicle length from 6.105 to 4.03cm).

Conclusion Generally, in most plants, low concentrations of nano-TiO₂ did not significantly affect germination and seedling growth, but in high concentrations (1500mg l⁻¹) due to toxicity effect, germination and seedling growth were reduced. Therefore, in using nanoparticles, attention to dosage, which is useful and not causes toxicity, is significant.

Keywords Desert Plant; Germination Properties; Priming; Phytotoxicity; Nano-TiO₂

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[1] Safety and risk associated with ... [2] Effects of acute and subchronic exposure of topically applied fullerene extracts on ... [3] Comparison of acute and chronic toxicity ... [4] Long-term effects of carbon ... [5] FDA. Nanotechnology programs ... [6] Safer Chemicals ... [7] Plant nanotechnology: Principles ... [8] Nanotoxicology: An emerging discipline ... [9] Toxic potential of materials at ... [10] Safe handling of ... [11] Effects of rare earth oxide nanoparticles ... [12] Uptake, translocation, and accumulation ... [13] Effects of nano-ferric oxide on the growth and ... [14] Influence of nano-priming on *Festuca ovina* seed ... [15] Can bulk and nanosized titanium dioxide ... [16] In vitro cytotoxicity of oxide ... [17] In vitro toxicity of nanoparticles ... [18] Cytotoxicity of carbon nanomaterials: Single-wall ... [19] A review of carbon nanotube toxicity and ... [20] Biological effects of nanoparticulate ... [21] Evaluation of spray-dried lignin formulations and adjuvant ... [22] The effect of titanium dioxide alumina beads on the ... [23] Characterization of size, surface charge, and agglomeration state ... [24] Protocol for nanoparticle dispersion of niobium ... [25] Ecological effects test guidelines ... [26] Phytotoxicity of nanoparticles: inhibition ... [27] effect of rare earth elements on growth and nutrition of coconut palm ... [28] The effect of cerium (III) on the chlorophyll ... [29] effect of lanthanum on rice production, nutrient ... [30] Nanoparticles and the ... [31] The potential environmental impact of engineered ... [32] Health and environmental impact of nanotechnology ... [33] Assessing the risks of manufactured ... [34] Root uptake and phytotoxicity of ZnO ... [35] Impact of bulk and nanosized titanium dioxide ... [36] Defining ... [37] Sunflower chlorophyll levels after magnetic nanoparticle ... [38] Effect of nano-ZnO particle suspension on growth of Mung ... [39] Particle surface characteristics may play an important ... [40] Carbon nanotubes are able to penetrate plant seed coat and dramatically ... [41] Nanoparticles and higher ... [42] Effect of nano-TiO₂ on strength ... [43] effect of nano-TiO₂ on photochemical ... [44] Influences of nano-anatase TiO₂ on the nitrogen ... [45] Was improvement of spinach growth ...

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Introduction

Nanoparticles (NPs) have been increasingly used in industrial applications, with industry market segments being in the main demand. These particles are smoothly introduced to nature and have affected their organisms due to their unique properties, such as higher specific areas and levels. The current rise of the nanotechnological industry and variety of nanobiotechnological applications are raising legitimate questions about the evaluation of risks associated with the production of different NPs and products containing nanosized compounds such as industrial and agricultural products. The interaction of nanosized materials with organisms is dramatically different compared with identical regular-sized materials [1]. Additionally, NPs can easily overcome cellular barriers, enter organisms, and stay in cells for a long time [2-4]. In the recent past, worldwide governmental organizations and major nanotechnological companies made significant efforts for the regulation of flow of nanoproducts and the creation of the first nanopolicies. Such efforts were based on new scientific discoveries of the possible toxicity of NPs for humans, animals, plants, microbes, and other organisms [1]. In the United States, several government organizations, including the US Department of Agriculture (USDA-NIFA), US Environmental Protection Agency (EPA), US Food and Drug Administration (FDA), Consumer Product Safety Commission, and National Institute for Occupational Safety and Health (NIOSH) are guiding the safety of nanotechnological products and produce recommendations for regulatory aspects. On August 5, 2015, FDA published one final guidance paper associated with nanotechnological food for animals [5]. EPA is making available information about environmental fate, transport, transformation, biodistribution, exposure, and toxicity of NPs and nanoproducts to humans and other species [6]. Both organizations (FDA and EPA) indicated that current law is sufficient for the regulation of nano-based-products [7]. Accordingly, further research is required to understand how better, and by what magnitude, NPs impact the living things to establish and facilitate an environmentally-sustainable framework for future applications [8-11]. This is particularly relevant to plants as significant in the trophic level since they play a critical role in

transporting nanoparticles from water and soil towards higher trophic levels in the ecosystem [12]. However, studies on nanoparticles strongly raised recently, but their effects on plants are considered less. Based upon which, negative and/or positive effects of nanoparticles on seed germination, plant growth and development have been generally observed. For example, the simultaneous use of SiO₂ (nano-SiO₂) and TiO₂ (nano-TiO₂) have led to an increase in the level of nitrate reductase, the ability to absorb fertilizers, and water use efficiency in soybean (*Glycine max*) [13]. Abbasi *et al.* [14] investigated the effects of silver nanoparticles as priming on germination of *Festuca ovina* under drought stress. They stated an increase of silver nanoparticles concentration could improve *F. ovina* seed germination and seedling traits. They also reported that nano-priming 75% had the most significant influence in the most studied indices. Azimi *et al.* [15] reported the germination percentage of *Agropyron desertorum* improved by 9% following exposure to 5 ppm nanosized TiO₂ treatment comparing to control. Similar positive effects occurred in terms of germination value and meant daily germination. Their results indicated that the application of bulk TiO₂ particles in 80 ppm concentration significantly decreased the most studied traits. Brunner *et al.* [16], Hussain *et al.* [17], Jia *et al.* [18], Lam *et al.* [19], and Soto *et al.* [20] reported the negative effect of utilization of NPs at relatively low dosage on some plants such as corn, cucumber, soybean, cabbage, and carrot.

Titanium dioxide has been widely applied in commercial and industrial products, examples of which are paints, adhesives, plastic materials, inks, cosmetic industries, pottery and ceramics, and more [21]. These particles have also been utilized as artificial colors in food industries, pesticides, and photocatalysts and in filtering groundwater contamination. Therefore, such an extensive application of the nanoparticles in agriculture necessitates acquiring a more precise knowledge on the mechanisms through which the particles influence living things, with particular emphasis on plant growth and development as being involved in the initial steps of the trophic level [22].

Materials and Methods

Nanoparticles

Titanium dioxide (TiO₂) nanoparticles were used in this study. Titanium dioxide

Nanoparticles were purchased from Nano Pars Lima Co.; their characteristics are listed in Table 1. The TEM images were obtained using a Hitachi Transmission-Electron Microscope, as shown in Figure 1. To calculate the average particle size of nano-TiO₂, 600 particles were selected randomly and measured at a magnification of 100,000 using Axio Vision digital Figure processing software (Release 4.8.2.0, GmbH Carl Zeiss Micro Imaging-German).

Table 1) Characteristics of nanoparticles used for the experiments

Nanoparticles	Size (nm)	Purity (%)	Surface area (m ² /g)
TiO ₂	<30	99%	>60

Seeds

Seeds of five desert plant species (*H. glaucus*, *H. aphyllum*, *N. schoberi*, *Z. eurypterum*, *H. strobilaceum*) were taken from Kazem Abad sand stabilization station and kept in the dark at 4°C. Seed viability was determined using the tetrazolium chloride test before initiating the main experiments.

Preparation of particle suspensions

The suspension of NPs was prepared using the procedure described by Ma *et al.* [11]. The milky white and uniformly of nano-TiO₂ suspension was obtained. The suspension was placed in a dark place because the dilute suspension of nano-TiO₂ has high light sensitivity [23, 24].

Seed germination and exposure

Seeds were surface-disinfected using 5% sodium hypochlorite solution for 10 min [25], before rinsing with deionized water. Germination tests were done in 9cm-diameter Petri dishes containing a Whatman No-1 filter paper, moistened with 5 ml of suspension or distilled water. Ten seeds were placed on each petri dish in the way similar to what Lin and Xing [26] did. After covering and sealing the Petri dishes with tape, they were placed in the dark at 25°C at germinator. Germination assays finished at seven days. The seed germination percentage was calculated using the below equation.

$$Gp = n/N * 100$$

Where Gp represents germination percent, n is the number of germinated seeds, N is the total number of seeds. The length of seedling, rootlet,

and plumule was measured with a millimeter ruler.

Data analysis

Each treatment had four replications, and the mean±SD (standard deviation) of each of the experimental values was presented as a result, comparing with its corresponding control. The results were analyzed by one way ANOVA using SPSS 21 software.

Findings

Characterization of NPs

Transmission electron microscope (TEM) images of nano-TiO₂ are shown in Figure 1. Particle size measurements showed that 83.66% of particles have the diameters of 4 to 30nm, 7.83% of the particles were more massive than 50nm, and the maximum particle diameter was measured 64nm (Diagram 1).

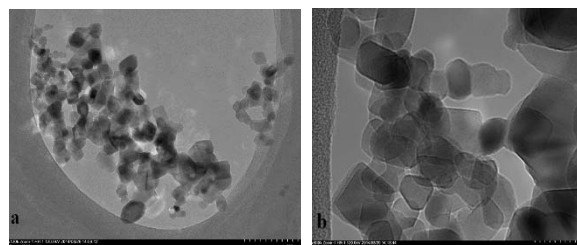


Figure 1) TEM images of nano- TiO₂ particles

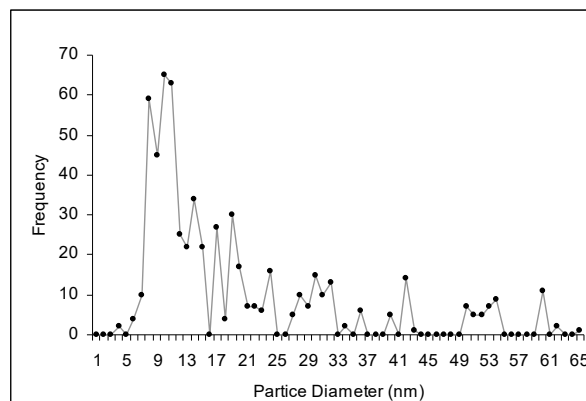


Diagram 1) The size distribution of Titanium dioxide nanoparticles

Effect of Nano-Titanium dioxide suspensions on seeds germination and seedling growth

Impact of different concentrations of TiO₂ nanoparticles on germination percentage, plumule, and rootlet length, also seedling growth was dissimilar at understudy plant species (Table 2).

Table 2) Effect of different concentrations of Nano-TiO₂ on the plant's characteristics

Plant species	MS. germination	MS. radicle length	MS. Plumule length	MS. seedling length	F germination	F radicle length	F plumule length	F seedling length
<i>H. glaucus</i>	356.80	2.59	0.35	7.64	23.06**	3.29*	1.15	5.17**
<i>H. aphyllum</i>	1011.20	3.72	0.86	3.86	122.32**	4.33**	1.59	2.38*
<i>N. schoberi</i>	117.20	9.34	1.15	12.87	9.56**	9.74*	1.64	9.65**
<i>Z. eurypterum</i>	169.20	0.80	0.05	0.914	7.37**	1.80	0.10	0.79
<i>H. strobilaceum</i>	6.80	0.00	0.002	0.003	0.48 ^{ns}	0.008	0.007	0.009 ^{ns}

*Significant difference at 5% level; **Significant difference at 1% level

H. strobilaceum

Different concentrations of TiO₂ nanoparticles were ineffective on the studied characteristics of this species.

H. aphyllum

Different concentrations of TiO₂ nanoparticles were significant on the germination, rootlet growth, and seedling growth in this species, but were ineffective on plumule growth (Table 2). The concentration of 500mg l⁻¹ of this nanoparticle not only did not reduce the germination percentage in this species but also increased the germination percentage of 72 in control to 88%. The concentration of 1500mg l⁻¹ reduces the germination percentage of 72 (control) to 44%, at lower concentrations; there was no significant effect on seed germination. The concentration of 1500mg l⁻¹ reduced rootlet and seedling growth from 6.1 to 4.2cm and 13.64 to 10.75cm, respectively (Diagram 2-5).

N. schoberi

As well as *H. aphyllum*, different concentrations of TiO₂ nanoparticles were influential on the germination percentage, rootlet growth, and seedling growth in this species, as the concentration of 1500mg l⁻¹ reduced the germination percentage from 32% in control to 20% (Diagram 2). The concentration of 1500mg l⁻¹ reduced rootlet and seedling growth from 13.85 to 10.68cm and 26.52 to 22.87cm, respectively (Diagram 3 and 5). Lower concentrations did not influence the studied factors.

Z. eurypterum

The concentration of 1500mg l⁻¹ reduced the germination percentage from 59 to 44%; other concentrations did not affect any of the factors (Diagram 2).

H. glaucus

The concentration of 500mg l⁻¹ increased the germination percentage (from 32 to 50%), rootlet growth (from 8.32 to 10.17cm), and seedling growth (from 17.2 to 19.75cm) (Diagram 2-5).

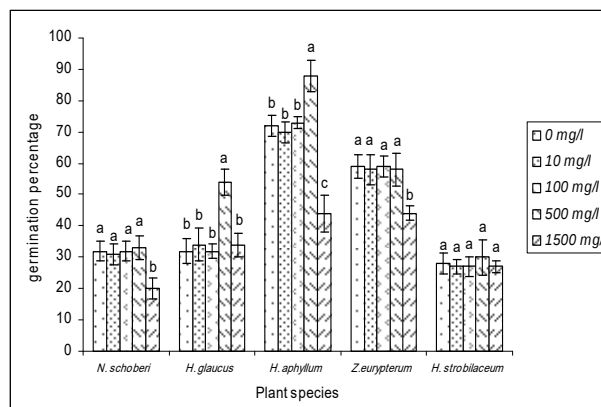


Diagram 2) Effect of different concentrations of Nano-TiO₂ on germination percentage of plants

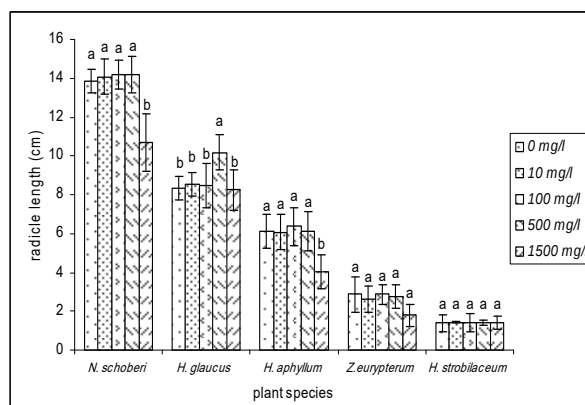


Diagram 3) Effect of different concentrations of Nano-TiO₂ on radicle length of plants

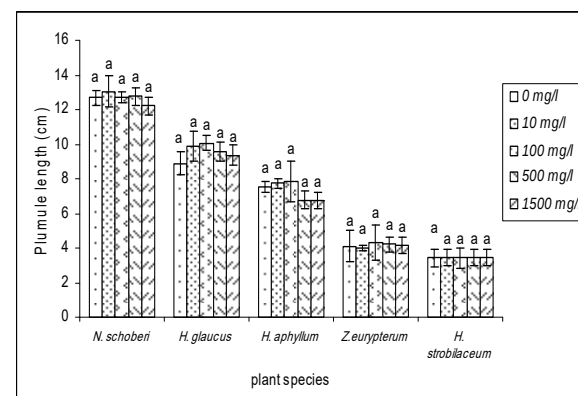


Diagram 4) Effect of different concentrations of Nano-TiO₂ on plumule length of plants

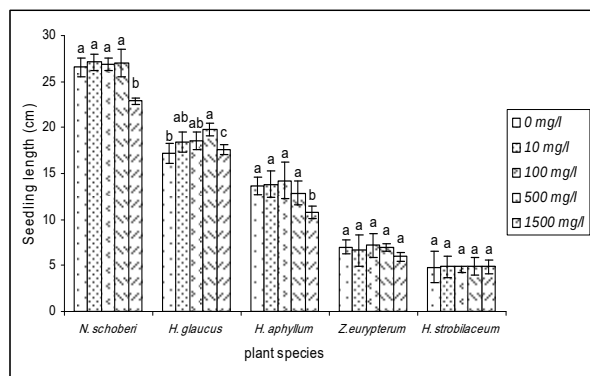


Diagram 5) Effect of different concentrations of Nano-TiO₂ on seedling length of plants

Discussion

In recent years, attention to the possible toxicity of nanoparticles on living organisms, including plants has increased [8, 11, 25, 27-35]. In the current study, the positive and negative effects of TiO₂ nanoparticles were investigated via an increase and decrease in germination percentage and seedling length. Different plants and their different parts showed different responses to different concentrations of TiO₂ nanoparticles. Studies about the impact of nanoparticles on plants represent different conclusions depending on the plant species, stage of development, type of nanoparticle, the nanoparticle concentration, and unknown aspects of the mechanism of nanoparticles in plants [11, 36]. Studies on the toxicity of nanoparticles (multi-walled carbon nanotube, aluminum, alumina, zinc, and zinc oxide) on germination and radicle growth of 6 species (radish, rape, rye grass, lettuce, corn, and cucumber) have revealed that nano-Zn, at a concentration of 2000 mg/l⁻¹ reduced rye grass germination; the same concentration of nano-ZnO reduced the germination of the corn too. It is also reported that different nanoparticles have variously affected radicle growth of the different species [34]. Oprisan *et al.* [37] observed that magnetic nanoparticles had affected sunflower seedlings' chlorophyll contents. Likewise, Mahajan *et al.* [38] noticed the effects of nano-ZnO particles on seedlings of mung (*Vigna radiate*) and gram (*Cicer arietinum*); the authors found that the seedlings grew at the highest rate when exposed to a lower level of nanoparticle concentrations, yet and by contrast, the seedlings' growth delayed at higher concentrations. A similar effect was also observed, where nano-iron oxide particles were

exposed to soy (*Glycine max* (L.) Merr.) plants. In the latter study, using nano-iron oxide particles at 0.75gl⁻¹ concentration has led to elevated leaf, pad, and dry seed weights. The ineffectiveness of TiO₂ nanoparticles on *H. strobilaceum*, may be due to the size and shape of seed cells, which prevent penetration of nanoparticles into the seed. Young and Watts [39] suggested that the level of toxicity of nanoparticles is affected by their surface properties. The positive effect of Nano-TiO₂ on seed germination and seedling growth of some understudy species like *H. aphyllum* & *H. glaucus*, can be due to the facilitating role of this nanoparticle in water and nutrient absorption, also improving nitrogen metabolism and photosynthesis. Because the seed needs water to start the metabolism and cell division, imbibition is an important factor in seed germination. Khodakovskaya *et al.* [40] stated that CNTs increase imbibition, increase metabolism, and improve seed germination. Ruffini and Cremonini [41] stated that the impact of nanoparticles on plants could be dependent on the chemical composition and particle size and/or shape. Zhang *et al.* [42] stated that the cause of the impact of TiO₂ nanoparticles on seed germination of *Spinacia oleracea* is probably their tiny size, which allows them to penetrate the seed. Some studies had shown that Titanium dioxide would increase enzyme activities, germination rates, and nitrate uptake in Spinach when TiO₂ was sprayed at the nanoscale. Besides, those particles would also accelerate the mineralization of organic nitrogen. [42-45].

Conclusion

The results showed a concentration of 500mg/l⁻¹ TiO₂ nanoparticles increased the germination percentage of *H. glaucus* and *H. aphyllum*; these two species are of important plants of desert areas of Iran, also are essential for afforestation in these areas. According to recent droughts in many arid and semiarid areas of Iran, the seeds of these species have lost their ability to germinate. The application of 500mg/l⁻¹ nanoparticles at these two plants can increase germination, enhancing the chance of land restoration success.

Furthermore, due to the widespread use of TiO₂ nanoparticles in different industries, the effect of different concentrations of these particles on plants should be studied to prevent the entrance of its excessive concentrations to nature.

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References

- 1- Vishwakarma V, Samal SS, Manoharan N. Safety and risk associated with nanoparticles-a review. *J Miner Mater Charact Eng*. 2010;9(5):455-9.
- 2- Nelson MA, Domann FE, Bowden GT, Hooser SB, Fernando Q, Carter DE. Effects of acute and subchronic exposure of topically applied fullerene extracts on the mouse skin. *Toxicol Ind Health*. 1993;9(4):623-30.
- 3- Zhao CM, Wang WX. Comparison of acute and chronic toxicity of silver nanoparticles and silver nitrate to *Daphnia magna*. *Environ Toxicol Chem*. 2011;30(4):885-92.
- 4- Shvedova AA, Yanamala N, Kisin ER, Tkach AV, Murray AR, Hubbs A, et al. Long-term effects of carbon containing engineered nanomaterials and asbestos in the lung: one year postexposure comparisons. *Am J Physiol Lung Cell Mol Physiol*. 2014;306(2):170-82.
- 5- FDA. Nanotechnology programs at FDA [Internet]. Washington: Food and Drug Administration; 2020 [Unknown Cited]. Available from: <https://www.fda.gov/science-research/science-and-research-special-topics/nanotechnology-programs-fda>
- 6- Unites states environmental protection agency. Safer Chemicals Research [Internet]. Washington: Unites States Environmental Protection Agency; 2020 [Unknown Cited]. Available from: [epa.gov/chemical-research](https://www.epa.gov/chemical-research)
- 7- Kole C, Kumar DS, Khodakovskaya MV. Plant nanotechnology: Principles and practices. Berlin: Springer; 2016.
- 8- Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect*. 2005;113(7):823-39.
- 9- Nel A, Xia T, Madler L, Li N. Toxic potential of materials at the nanolevel. *Science*. 2006;311(5761):622-7.
- 10- Maynard AD, Aitken RJ, Butz T, Colvin V, Donaldson K, Oberdorster G, et al. Safe handling of nanotechnology. *Nature*. 2006;444:267-9.
- 11- Ma Y, Kuang L, He X, Bai W, Ding Y, Zhang Z, et al. Effects of rare earth oxide nanoparticles on root elongation of plants. *Chemosphere*. 2010;78(3):273-9.
- 12- Zhu H, Han J, Xiao JQ, Jin Y. Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. *J Environ Monitor*. 2008;10(6):713-7.
- 13- Liu XM, Zhang FD, Zhang SQ, He XS, Fang R, Feng Z, et al. Effects of nano-ferric oxide on the growth and nutrients absorption of peanut. *Plant Nutr Fertil Sci*. 2005;11:14-8.
- 14- Abbasi Khalaki M, Ghorbani A, Dadjou F. Influence of nano-priming on *Festuca ovina* seed germination and early seedling traits under drought stress, in laboratory condition. *Ecopersia*. 2019;7(3):133-9. [Persian]
- 15- Azimi R, Feizi H, Khaje Hosseini M. Can bulk and nanosized titanium dioxide particles improve seed germination features of wheatgrass (*agropyron desertorum*). *Not Sci Biol*. 2013;5(3):325-31.
- 16- Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limbach LK, et al. In vitro cytotoxicity of oxide nanoparticles: Comparison to asbestos, silica, and the effect of particle solubility. *Environ Sci Technol*. 2006;40(14):4374-81.
- 17- Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicol Vitro*. 2005;19(7):975-83.
- 18- Jia G, Wang H, Yan L, Wang X, Pei R, Yan T, et al. Cytotoxicity of carbon nanomaterials: Single-wall nanotube, multiwall nanotube, and fullerene. *Environ Sci Technol*. 2005;39(5):1378-83.
- 19- Lam CW, James JT, McCluskey R, Arepalli S, Hunter RL. A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks. *Crit Rev Toxicol*. 2006;36(3):189-217.
- 20- Soto KF, Carrasco A, Powell TG, Murr LE, Garza KM. Biological effects of nanoparticulate materials. *Mater Sci Eng*. 2006;26(8):1421-7.
- 21- Arthurs SP, Lacey LA, Bechle RW. Evaluation of spray-dried lignin formulations and adjuvant as solar protectant for granulovirus of colding moths *Cydic pomonella*. *J Invertebr Pathol*. 2006;93(2):88-95.
- 22- Lee DJ, Senseman SA, Sciumbato AS, Jung SC, Krutz LJ. The effect of titanium dioxide alumina beads on the photocatalytic degradation of picloram in water. *J Agric Food Chem*. 2003;51(9):2659-64.
- 23- Jiang J, Oberdorster G, Biswas P. Characterization of size, surface charge, and agglomeration state of nano particle dispersions for toxicological studies. *J Nanopart Res*. 2008;11:77-89.
- 24- OECD WPMN. Protocol for nanoparticle dispersion of niobium as a precursor for the hydrothermal preparation of cellulose acetate Nb₂O₅ photocatalyst. *J Molecular Catalysis*. 2010;237:115-9.
- 25- US environmental protection agency. Ecological effects test guidelines (OPPTS 850.4200): Seed germination/root elongation toxicity test [Internet]. Washington: US Environmental Protection Agency; 1996 [Unknown Cited]. Available from: [shorturl.at/mqHNY](https://www.epa.gov/shorturl.at/mqHNY)
- 26- Lin D, Xing B. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. *Environ Pollut*. 2007;150(2):243-50.
- 27- Wahid PA, Valiathan MS, Kamalam NV, Eapen JT, Vijayalakshmi S, Prabhu RK, et al. effect of rare earth elements on growth and nutrition of coconut palm and root competition for these elements between the palm and *Calotropis gigantea*. *J Plant Nutr*. 2000;23(3):329-38.
- 28- Hong F, Wang L, Meng X, Wei Z, Zhao G. The effect of cerium (III) on the chlorophyll formation in Spinach. *Biol Trace Elem Res*. 2002;89(3):263-76.
- 29- Xie ZB, Zhu JG, Chu HY, Zhang YL, Zeng Q, Ma HL, et al. effect of lanthanum on rice production, nutrient uptake, and distribution. *J Plant Nutr*. 2002;25(10):2315-31.
- 30- Biswas P, Wu CY. Nanoparticles and the environment. *J Air Waste Manag Assoc*. 2005;55(6):708-46.

- 31- Colvin VL. The potential environmental impact of engineered nanomaterials. *Nat Biotechnol.* 2003;21:1166-70.
- 32- Dreher KL. Health and environmental impact of nanotechnology: Toxicological assessment of manufactured nanoparticles. *Toxicol Sci.* 2004;77(1):3-5.
- 33- Wiesner MR, Lowry GV, Alvarez P, Dionysiou D, Biswas P. Assessing the risks of manufactured nanomaterials. *Environ Sci Technol.* 2006;40(14):4336-45.
- 34- Lin D, Xing B. Root uptake and phytotoxicity of ZnO nanoparticles. *Environ Sci Technol.* 2008;42(15):5580-5.
- 35- Feizi H, Moghaddam RP. Impact of bulk and nanosized titanium dioxide (TiO₂) on wheat seed germination and seedling growth. *Biol Trace Elem Res.* 2012;146(1):101-6.
- 36- Calabrese EJ, Baldwin LA. Defining hormesis. *Hum Exp Toxicol.* 2002;21(2):91-7.
- 37- Oprisan MU, Focanici E, Creanga D, Caltun OF. Sunflower chlorophyll levels after magnetic nanoparticle supply. *Afr J Biotechnol.* 2011;10(36):7092-8.
- 38- Mahajan P, Dhoke SK, Khanna AS. Effect of nano-ZnO particle suspension on growth of Mung (*Vigna radiata*) and Gram (*Cicer arietinum*) seedling using plant agar method. *J Nanotechnol.* 2011;1(1):1-7.
- 39- Young L, Watts DJ. Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. *Toxicol Lett.* 2005;158(2):122-32.
- 40- Khodakovskaya M, Dervishi E, Mahmood M, Xu Y, Li Z, Watanabe F, et al. Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. *ACS Nano.* 2009;3(10):3221-7.
- 41- Ruffini CM, Cremonini R. Nanoparticles and higher plants. *Caryologia.* 2009;62(2):161-5.
- 42- Zheng L, Hong F, Lu S, Liu C. Effect of nano-TiO₂ on strength of naturally aged seeds and growth of Spinach. *Biol Trace Elem Res.* 2005;104(1):83-92.
- 43- Hong F, Zhou J, Liu C, Yang F, Wu C, Zheng L, et al. effect of nano-TiO₂ on photochemical reaction of chloroplasts of Spinach. *Biol Trace Elem Res.* 2005;105(1-3):269-79.
- 44- Yang F, Hong F, You W, Liu C, Gao F, Cheng W, et al. Influences of nano-anatase TiO₂ on the nitrogen metabolism of growing Spinach. *Biol Trace Elem Res.* 2006;110(2):179-90.
- 45- Gao F, Liu C, Qu C, Zheng L, Yang F, Su M, et al. Was improvement of spinach growth by nano-TiO₂ treatment related to the changes of Rubisco activase?. *Biometals.* 2008;21(2):211-7.