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Assessment of Concentrations of Nano and Bulk Iron Oxide Particles on Early Growth of Wheat (*Triticum aestivum* L.)

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Authors' contributions

This work was carried out in collaboration between all authors. Author HF worked in the practical part, analyses of the study and wrote the first draft of the manuscript, author PRM supervised the work in all its aspects, author NS supported in nanoscience section and providing the microscopic images. Author AF designed the study and treatments. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: In this work we assessed Fe_2O_3 nanoparticles with bulk Fe_2O_3 for possible phytotoxicity and stimulative effects on wheat seed germination and early growth stage.
Methodology: The treatments in the experiment were five concentrations of bulk (100, 500, 1000, 5000 and 10000 ppm) and five concentrations of nanosized Fe_2O_3 (100, 500, 1000, 5000 and 10000 ppm) and an untreated control. Germination tests were performed according to the rule issued by ISTA. Analysis of variance (ANOVA) was performed between treatment samples. The information was analyzed using MSTAT-C computer software. Means compared by multiple range Duncan test and a 95% significance level ($p < 0.05$) was employed for all comparisons.
Results: Results showed that exposure of seeds to 100 ppm iron oxide nanoparticles indicated the greatest germination rate (by 41% more than control group) related to other

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treatments. Increasing nanoparticles concentration above 100 ppm reduced seed germination rate. It has not found any significant effects by bulk and nanoparticles on elongation of shoot, root and seedling of wheat. Application of 100 ppm concentration of nanosized Fe_2O_3 reduced mean germination time (MGT) by 38.5% in comparison to the control, while 100 ppm concentration of bulk Fe_2O_3 did not decrease MGT in comparison with the control. The highest root biomass was achieved from concentration of 100 ppm nano- Fe_2O_3, but an increased concentrations of nanoparticles Fe_2O_3 significantly reduced root weight. 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.

Keywords: Nanosized Fe_2O_3; phytotoxicity; wheat seed; germination; growth of wheat.

ABBREVIATIONS

MGT: Mean Germination Time; ISTA: International Seed Testing Association; ANOVA: Analysis of Variation; VI: Vigour Index; STM: Scanning Tunneling Microscope; SEM: Scanning Electron Microscope.

1. INTRODUCTION

Promising applications in an extensive variety of areas using recently engineered or improved materials in the form of nanosized particles are being developed rapidly [1]. Nanoscale engineering delivers accurate potential to control and fabricate matters at this scale to provide novel and helpful properties therefore leading to many new applications [2]. Iron is one of the essential elements for plant growth and plays an important role in the photosynthetic reactions. Iron activates several enzymes and contributes in RNA synthesis and improves the performance of photosystems in plants [3].

A relatively little number of studies are devoted to the influence of iron oxide on seed germination revealing the stimulatory effect on the seed germination indices and seedling growth. Some reports regarding influence of iron oxide upon the plant growth evidenced a positive influence in cereals, explained on the basis of importance of iron in the vegetal organism. The iron oxides can be a source of iron for the plant development. The biosynthesis of siderophores by plant was assumed to be stimulated with the iron from iron oxides [4].

An assessment showed that low concentrations of aqueous ferrofluid stimulated maize (*Zea mays*) plant growth whilst high concentrations induced inhibitory or toxic effects [5]. Lee et al. [6] reported that the seedling growth of *Phaseolus radiatus* and *Sorghum bicolor* was adversely affected by exposure to silver nanoparticles.

After 20 days of growth, there were no apparent visual differences in the pumpkin plants grown with and without Fe_3O_4 particles, indicating that the particles did not pose any toxicological effects to the plants at the concentration level tested. [1]. In earlier work it was 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 had an inhibitory or no effect on wheat seed [7]. Sheykhbaglo et al. [3] showed that foliar application of iron oxide nanoparticles at the concentration of 0.75 g L^{-1} was increased leaf + pod dry weight and pod dry weight in soybean. They added the highest grain yield was observed with using 0.5 g l^{-1} nano-iron oxide that showed 48% increase in grain yield in comparison with control. It has been reported that the application of nano-iron oxide significantly affects peanut and causes increasing growth and photosynthesis. Nano-iron oxide compared to other treatments such as organic materials and iron citrate facilitated the photosynthate and iron transferring to the leaves of peanut [8]. It has been proved that nanosized TiO_2 helped water absorption in spinach seeds and as result accelerated seed germination [9]. Iron deficiency occurs not because of Fe insufficiency in soil or plants, but because various soil and plant factors affect Fe availability, inhibit its absorption, or impair its metabolic use [10]. Much less is known about the effect of bulk and nano iron oxide on wheat. Hence, we studied possible advantageous and comparative effects of nanosized and bulk iron oxide particles on seed germination and seedling growth of wheat.

2. MATERIALS AND METHODS

2.1 Study Site

Dry seeds of wheat (*Triticum aestivum* L.) were provided from Agricultural Research Center of Khorasan Razavi province, Iran. Nanosized Fe_2O_3 powder was supplied by Chinese Company. According to the manufacturer, the particle sizes ranged from 20 to 30 nm. Specific surface area of nanosized Fe_2O_3 was 80-90 m^2 g^{-1}, and purity was >99.8%. The size of Fe_2O_3 nanoparticles (Fig.1 and 2) was determined through Scanning Tunneling Microscope (STM) in Central Laboratory of Ferdowsi University of Mashhad.

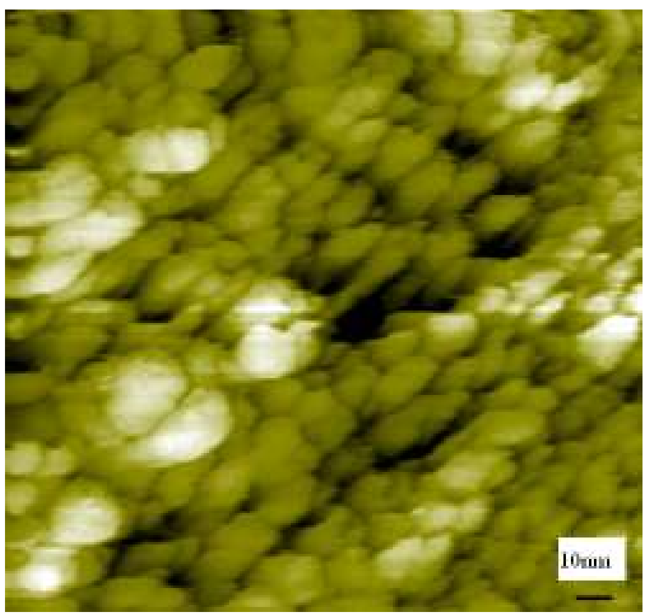

Fig. 1. Images of nanosized Fe_2O_3 by Scanning Tunneling Microscope (STM)

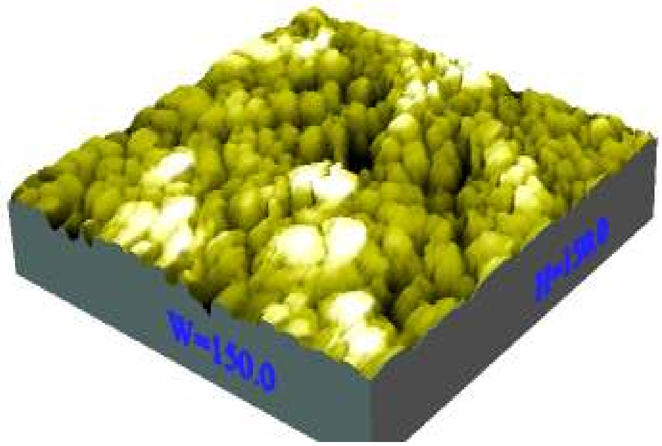

Fig. 2. Topographic image of nanosized Fe_2O_3 by Scanning Tunneling Microscope (STM)

Bulk Fe_2O_3 particles were supplied by Across company, it had >99% purity and particle size was measured by Scanning Electron Microscope (SEM) in Central Laboratory of Ferdowsi University of Mashhad (Fig. 3).

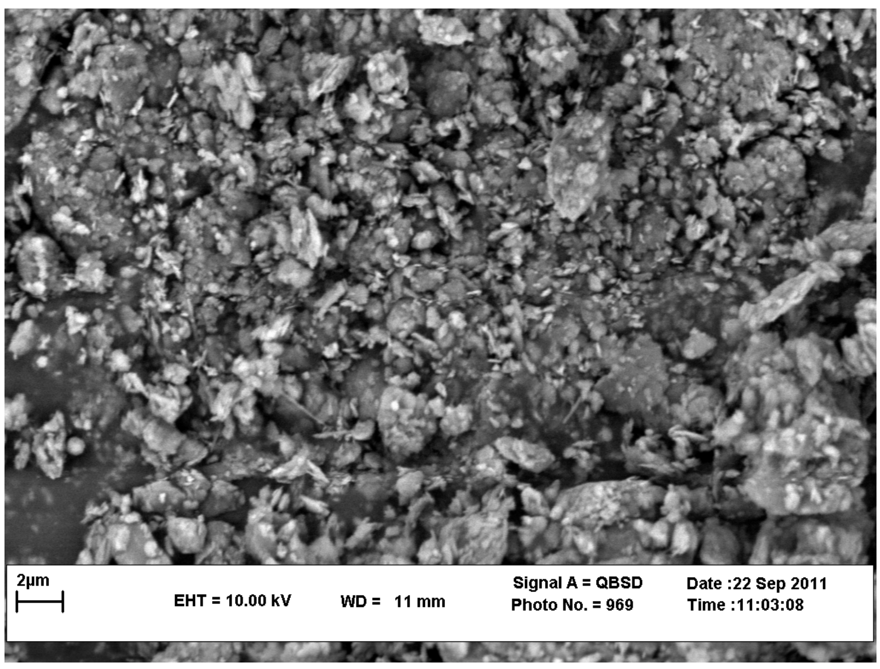

Fig. 3. Image of bulk Fe_2O_3 particles by Scanning Electron Microscope (SEM)

2.2 Experimental Procedures

Experiment was performed to evaluate the effect of different concentrations of bulk and nanosized Fe_2O_3 on wheat seed germination in a completely randomized design with four replications. The treatments in the experiment were five concentrations of bulk (100, 500, 1000, 5000 and 10000 ppm) and five concentrations of nanosized Fe_2O_3 (100, 500, 1000, 5000 and 10000 ppm) and an untreated control. The experiment was performed in a germinator with an average temperature of 20 ±1ºC at the College of Agriculture, Ferdowsi University of Mashhad, Iran.

Seeds with similar size were randomly selected and sterilized in a 5% sodium hypochlorite solution for 3 min then rinsed thoroughly several times with distilled water. In order to obtain properly dispersed and stable Fe_2O_3 suspensions of each concentration, an ultra-sonication treatment was applied to bulk and nanoparticles Fe_2O_3 powders dispersed in distilled water for 15 minutes. The seeds were placed on paper in four groups of 25 seeds in Petri dishes, and after that 3 ml of each concentration treatments was added. For the control treatment, only distilled water was added to the Petri dishes.

Germination tests were performed according to the rule issued by the International Seed Testing Association [11]. All concentrations of Fe_2O_3 and the control were tested at the same time to ensure uniform conditions of light and temperature across all tests. Number of germinated seeds was noted daily for 8 days. Seeds were considered germinated when the radicle showed at least 2 mm in length [11]. Mean germination time was calculated based on Matthews and Khajeh-Hosseini [12]. (Eq. 1):

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

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 [13] (Eq. 2):

$$\textit{Germination rate} = (a/1) + (b - a/2) + (c - b/3) + \ldots .. + (n - n - 1/N) \qquad \text{Eq. 2}$$

Where a,b,c,…,n are number of germinated seeds after 1,2,3,…, N days from starting of their imbibitions.

Seedling vigor computed based on Vashisth and Nagarajan [14] (Eq. 3 and 4):

$$\textit{Vigor index I} = \textit{Germination\%} \times \textit{Seedling length (Root + Shoot)} \qquad \text{Eq. 3}$$
$$\textit{Vigor index II} = \textit{Germination\%} \times \textit{Seedling weight (Root + Shoot)} \qquad \text{Eq. 4}$$

2.3 Statistical Analysis

A one way analysis of variance (ANOVA) was performed between treatments samples as a randomized completely design. The information was analyzed using MSTAT-C computer software. The significant levels of difference for all measured traits were calculated and the means compared by multiple range Duncan test and a 95% significance level ($p < 0.05$) was employed for all comparisons.

3. RESULTS AND DISCUSSION

As shown in Table 1, among the studied traits, germination rate only was impacted by the bulk and nano particles of iron oxide. But exposure of seeds to 100 ppm iron oxide nanoparticles had the greatest germination rate related to other treatments. This treatment enhanced germination rate around 41% in comparison to the control group. Increasing nanoparticles concentration more than 100 ppm reduced germination rate. After 8 d of growth, there were no apparent visual differences in the plants grown with and without bulk and nano Fe_2O_3 particles, indicating that the particles did not pose any toxicological effects to the plants at the concentration level tested. Additionally, this result is expectable because of applied wheat seeds in this study normally had high germination percentage (92% in control group). Also it has not found any significant effects by bulk and nanoparticles on elongation of shoot, root and seedling of wheat. Our earlier investigation demonstrated that although the highest germination percent (98%) was shown in both 2 ppm bulk and nanosized TiO_2 concentrations but the treatments had no significant effect on seed germination percentage of wheat [9]. Adversely, the authors found after 14 days of fennel (*Foeniculum vulgare* Mill) seed incubation, germination percentage highly improved following exposure to 60 ppm TiO_2 nanoparticles [15]. These results may be because of difference in plant species and nanoparticles type. The authors concluded that nanosized TiO_2 presenting the possibility of a new scheme to overcome problems with seed germination in some plant species which have low seed germination [15].

Table 1. Influence of bulk and nanosized Fe_2O_3 concentrations on seed Germination and seedling elongation of fennel

Fe_2O_3 concentration (ppm)		**Germination %**	**Germination rate(seed day^{-1})**	**Shoot length (cm)**	**Root length (cm)**	**Seedling length (cm)**
Control	0	92 a	15.42 b	13.17a	14.51a	27.64a
Bulk Fe_2O_3	100	95 a	16.65 b	14.18a	13.41a	27.60a
	500	91 a	16.25 b	13.78a	12.79a	26.58a
	1000	91 a	17.21 b	12.98a	13.43a	26.40a
	5000	92 a	16.21 b	14.10a	12.20a	26.31a
	10000	95 a	16.67 b	13.01a	11.78a	24.80a
Nano Fe_2O_3	100	94 a	21.77 a	13.52a	13.45a	26.97a
	500	91 a	13.37 b	13.41a	12.98a	26.39a
	1000	90 a	15.02 b	14.10a	12.59a	26.68a
	5000	90 a	14.19 b	14.81a	12.95a	27.75a
	10000	91 a	15.57 b	14.32a	11.80a	26.17a
Sd		2.818	2.060	0.2460	0.6950	0.7247

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

As a general rule, lower mean germination time represents earlier seed germination. These results show that exposure of wheat seeds to 100 ppm Fe_2O_3 nanoparticles obtained the lowest mean germination time (0.824 days) but higher concentrations did not improve mean germination time. Consequently, 100 ppm concentration of nanosized Fe_2O_3 treatment reduced mean germination time by 38.5% in comparison to the untreated control, while 100 ppm concentration of bulk Fe_2O_3 did not decrease the mean germination time in comparison with the control (Fig. 4). Zheng et al. [9] reported that nanosized TiO_2 contributed to water absorption by spinach seeds and as result accelerated seed germination. Based on studies on nanoparticles effects on seed germination mechanism it could conclude nanoparticles might helped the water absorption by the seeds [9], increase nitrate reductase enzyme concentrations, 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 [16] result in improve seed germination in some plant species.

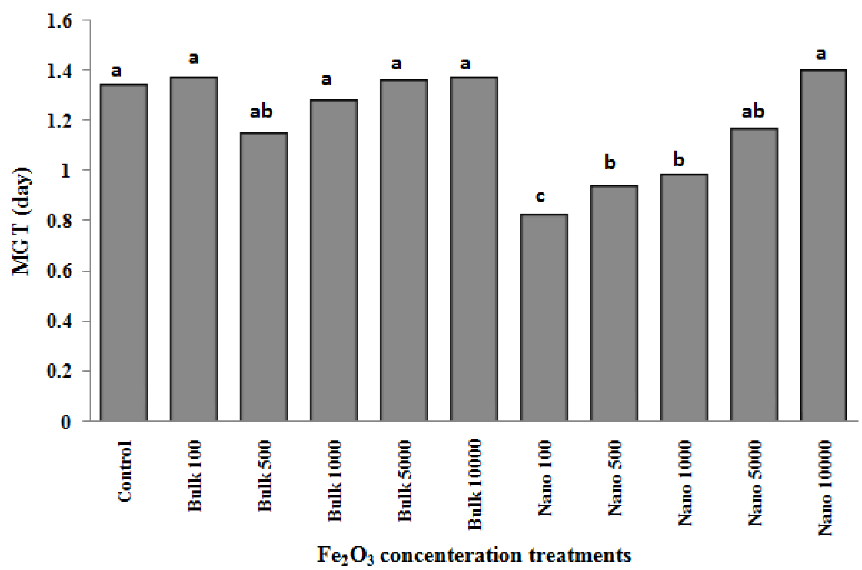

Fig. 4. Effect of bulk and nano Fe_2O_3 concentrations on mean germination time of wheat seed (Sd=0.1466)

Although shoot weight of wheat seedlings did not affect by Fe_2O_3 bulk and nanoparticles, but these treatments significantly influenced on root weight (Table 2).

The highest root biomass was achieved from concentrations of 100 ppm nano- Fe_2O_3, but an increased concentrations of nanoparticles Fe_2O_3 significantly reduced root weight. It is probable that increasing the concentration of bulk- Fe_2O_3 induced aggregation of particles and resulted in clogging of root pores that interrupted water uptake by seeds. Raccuciu and Creanga [5] found that low concentrations of aqueous ferrofluid stimulated growth of young maize (*Zea mays*) plants while high concentrations induced inhibitory or toxic effect. It seems that nano Fe_2O_3 could stimulate process of seed germination like water and oxygen uptake result in improves seedling growth. Lin and Xing [17] 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 root 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 [18]. In an experiment, Barrena et al. [18] 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 nanoparticles [18].

The highest seedling weight (16.227 g) was shown at 100 ppm nano-Fe_2O_3 which was 23.6% more than untreated control whereas bulk Fe_2O_3 increased seedling weight only by 8% in comparison to the control. Higher concentrations of iron oxide exhibited phytotoxicity effect on seedling growth of wheat. It has been reported the iron excess treatment generate oxidative stress in cells. But the ferrophase nanoparticles may have not only a chemical but also a magnetic influence on the enzymatic structures implied in the different stages of the photosynthesis reactions. Racuciua and Creanga [5] revealed stimulatory effects of ferrofluid and magnetic exposure upon the studied cereals plant species. Begum et al. [19] reported that multi-walled carbon nanotubes (MWNTs) showed no detrimental effects on seed germinations of seven elected species of plants. Induction in root and shoot growth of red spinach, lettuce, rice and cucumber were observed after the 15-days exposure to MWNTs. Also MWNTs induced phytotoxicity at the seedling stage at above 1000 mg L^{-1}.

Application of Fe_2O_3 bulk and nanoparticles concentrations did not have any significant effect on vigor index I but the stimulating effect of nanoparticle treatments was seen on vigor index II of wheat seedlings. Additionally, use of 100 ppm nanosized Fe_2O_3 showed the maximum vigor index II value compared to other doses (Table 2). 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 [20]. Sheykhbaglo et al. [3] showed that nano-iron oxide at the concentration of 0.75 g l^{-1} was increased leaf + pod dry weight and pod dry weight of soybean. They stated that the highest grain yield was observed with using 0.5 g l^{-1} nano-iron oxide that showed 48% increase in grain yield in comparison with control. Clement et al. [21] revealed that TiO_2 nanoparticles in anatase crystal structure are toxic in the entire set of tests conducted. However, at high concentration, through their antimicrobial properties, they significantly promoted growth of plant roots.

Table 2. Effect of bulk and nanosized Fe_2O_3 concentrations on growth features of wheat seedling

Fe_2O_3 concentration (ppm)		Shoot weight (mg)	Root weight (mg)	Seedling weight (mg)	Vigour index I	Vigour index II
Control	0	6.28a	6.85b	13.13b	2467a	1207c
Bulk Fe_2O_3	100	7.32a	6.91b	14.18b	2570a	1345ab
	500	6.89a	7.37ab	14.26b	2417a	1298bc
	1000	7.65a	7.72ab	15.37ab	2400a	1398ab
	5000	7.97a	7.08ab	15.05ab	2420a	1385ab
	10000	6.68a	7.15ab	13.84ab	2365a	1314b
Nano Fe_2O_3	100	7.31a	8.92a	16.23a	2529a	1525a
	500	6.87a	7.18ab	14.04b	2403a	1277b
	1000	6.43a	7.12ab	13.55b	2401a	1219c
	5000	6.91a	7.14ab	13.99b	2507a	1259bc
	10000	7.60a	6.65b	13.64b	2384a	1241bc
Sd		0.3626	0.4508	0.7187	104.7	71.16

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

4. CONCLUSIONS

Applications of nanomaterial can promote earlier plant germination and improve plant production. The laboratory study was conducted to determine the phytotoxicity and or stimulatory effect of bulk and nanosized Fe_2O_3 on wheat. Another goal was to compare and determine the suitable concentrations of these treatments. Exposure of seeds to 100 ppm iron oxide nanoparticles had the greatest germination rate related to other treatments. This treatment enhanced germination rate around 41% in comparison to the control group. It has not found any significant effects by bulk and nanoparticles on elongation of shoot, root and seedling of wheat. Application of 100 ppm concentration of nanosized Fe_2O_3 treatment reduced mean germination time by 38.5% in comparison to the untreated control, while 100 ppm concentration of bulk Fe_2O_3 did not decrease the mean germination time in comparison with the control. Shoot weight of wheat seedlings did not affect by Fe_2O_3 bulk and nanoparticles, but nanoparticles in low concentrations significantly improved root weight. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zhu H, Han J, Xiao JQ, Jin Y. Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. J Environ Monit. 2008;10: 713–717.
2. Chen H, Yada R. Nanotechnologies in agriculture: New tools for sustainable development. Trends in Food Sci Technol. 2011;22:585:594
3. Sheykhbaglou R, Sedghi M, Tajbakhsh Shisvan M, and Seyed Sharifi R. Effects of nano-iron oxide particles on agronomic traits of soybean. Notulae Sci Biol. 2010;2(2):112-113.
4. Racuciu M, Creanga D. TMA-OH coated magnetic nanoparticles internalized in vegetal tissue. Romanian J Physics. 2007;52:395–402.
5. Racuciua, D, Creanga E. Influence of water-based ferrofluid upon chlorophylls in cereals. J Magn Mag Materials. 2007;311:291–294.
6. Lee W, Kwak J, An Y. Effect of silver nanoparticles in crop plants *Phaseolus radiatus* and *Sorghum bicolor*: Media effect on phytotoxicity. Chemosphere. 2012;86:5491–499.
7. Feizi H, Rezvani Moghaddam P, Shahtahmassebi N, Fotovat A. Impact of bulk and nanosized titanium dioxide (TiO_2) on wheat seed germination and seedling growth. Biol Trace Elem Res. 2012;146:101–106.
8. Liu XM, Zhang FD, Zhang SQ, He XS, Fang R, Feng Z, Wang Y. Effects of nano-ferric oxide on the growth and nutrients absorption of peanut. Plant Nutr Fert Sci. 2005;11:14-18.
9. Zheng L, Hong F, Lu S, Liu C. Effect of nano-TiO_2 on strength of naturally aged seeds and growth of Spinach. Biol Trace Elem Res. 2005;105:83-91.
10. Fageria NK, Moreira A. The role of mineral nutrition on root growth of crop plants. Adv in Agron. 2011;110.
11. ISTA. ISTA rules. International Seed Testing Association. Zurich, Switzerland; 2009.

12. Matthews S, Khajeh-Hosseini M. Length of the lag period of germination and metabolic repair explain vigor differences in seed lots of maize (*Zea mays*). Seed Sci Technol. 2007;35:200-212.
13. Maguire ID. Speed of germination- Aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 1982;22:176-177.
14. Vashisth A, Nagarajan S. Effect on germination and early growth characteristics in sunflower (*Helianthus annuus*) seeds exposed to static magnetic field. J Plant Physiol 2010;167:149-156.
15. Feizi H, Kamali M, Jafari L, Rezvani Moghaddam P. Phytotoxicity and stimulatory impacts of nanosized and bulk titanium dioxide on fennel (*Foeniculum vulgare* Mill). Chemosphere. 2013;91:506–511.
16. Lei Z, Mingyu S, Xiao W, Chao L, Chunxiang Q, Liang C, Hao H, Xiao-qing L, Fashui H. Antioxidant stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation. Biol Trace Elem Res. 2008;121:69–79.
17. Lin D, Xing B. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environ Pollution. 2007;150:243-250.
18. Barrena R, Casals E, Colon J, Font X, Sanchez A, Puntes V. Evaluation of the ecotoxicity of model nanoparticles. Chemosphere. 2009;75:850–857
19. Begum P, Ikhtiari R, Fugetsu B, Matsuoka M, Akasaka T, Watari F. Phytotoxicity of multi-walled carbon nanotubes assessed by selected plant species in the seedling stage. Appl Surface Sci. 2012;262:120–124.
20. Casals E, Vazquez-Campos S, Bastus NG, Puntes V. Distribution and potential toxicity of engineered inorganic nanoparticles and carbon nanostructures in biological systems. Trends in Anal Chem. 2008;27(8).
21. Clément L, Hurel C, Marmier N. Toxicity of TiO_2 nanoparticles to cladocerans, algae, rotifers and plants – Effects of size and crystalline structure. Chemosphere. 2013;90:1083-1090.

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