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Original Research Article

Nano-anatase TiO_2 Modulates the Germination Behavior and Seedling Vigority of some Commercially Important Medicinal and Aromatic Plants

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ABSTRACT

Nanoparticles have been applied worldwide, posing substantial effects on the environment and its living organisms. Plants as sessile organisms are always exposed to considerable fluctuations of nanoparticles concentrations. In this research, the influence of different concentrations (0, 10, 20, 40 and 80 mg.L^{-1}) of nanosized TiO_2 were studied on seed germination parameters including germination percentage (GP %), mean time germination (MTG), germination rate (GR), germination index (GI) and seedling vigor index (SVI) of five different medicinal plant species namely *Salvia mirzayanii*, *Alyssum homolocarpum*, *Sinapis alba*, *Carum copticum*, *Nigella sativa.* Results indicated that significant differences in examined traits were found among the plant species and also among the employed TiO_2 concentrations. Among the plant species, the highest GR (87%) was observed in *S. alba* seeds at the 20 mg.L^{-1} TiO_2 concentration, however, in *S. mirzayanii* plants it was obtained at 80 mg.L^{-1} TiO_2. The lowest MTG (1.55 day) was observed in *A. homolocarpum* seeds at 10 mg.L^{-1} TiO_2 concentration. Moreover, the highest SVI (102.6) was observed in the 20 mg.L^{-1} of the reference treatment for *S. alba* seeds. In *N. sativa* seeds, the highest GI value (9.29) was found at the anatase concentration of 20 mg.L^{-1}. In conclusion, application of TiO_2 stimulated the seed germination of all species. However, this response was dependent on the concentration of applied TiO_2 and plant species as well.

Key Words: Nano TiO_2, Anatase, Germination, Vigority, Medicinal Plants

INTRODUCTION

Nanotechnology application is now widely distributed throughout life, and especially in agricultural systems. Nanoparticles, because of their physicochemical characteristics, are among the potential candidates for modulating the redox status and changing the seed germination, growth, performance, and quality of plants (Mukherjee and Mahapatra, 2009).

One of the main problems that prevent sustainable use of medicinal and aromatic plants, native to the arid lands is that they readily germinate within the native environment, but fail to show good germination under laboratory conditions or when cultivation is attempted (Gupta, 2003).

The ninth most abundant element and the second most abundant transition metal in the earth's crust is titanium element (about 6.32 ppm). The most important effects of Ti compounds on plants are enhancement of the yield of various crops (about 10–20%); an improvement of some essential element contents in plant tissues; an increase in the peroxidase, catalase, and nitrate reductase activities in plant tissues; and an enhancement of the chlorophyll content in paprika (*Capsicum anuum* L.) and green alga (*Chlorella pyrenoidosa*) (Hruby *et al.,* 2002). It has been reported that the germination rate and the germination and vigor indexes of spinach at 250-4,000 mg.L^{-1} nanosized TiO_2 (<100 nm in diameter) treatments were improved in comparison with bulk-TiO_2 treatment (Zheng *et al.,* 2005). Lee *et al.* (2008) studied phytotoxicity and bioavailability of copper nanoparticles to the plants *Phaseolus radiatus* and *Triticum aestivum*, employing plant agar test as growth substrate. They concluded that the growth rates of both plants were constrained when exposure to nanoparticles and the seedling lengths were negatively related to the exposure concentration of nanoparticles. Contents of nanoparticles in plant tissues increased with increasing nanoparticle concentration in growth media; therefore, bioaccumulation was concentration dependent. Also, there is potential for expanding the range of TiO_2 nanoparticles use for improvement of physiological and morphological characteristics of crops (Mingyu *et al.,* 2007). However, some studies have reported negative effects of TiO_2 nanoparticles on higher plants that varied between plant tissues, growth stages, plant species, applied concentrations, and specific properties of nanoparticles (Castiglione *et al.,*

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2011). Thus, the exploration of their extensive application in agriculture and plant science is still in debate (Kurepa *et al.*, 2010).

However, a few studies have been done on the effects of nanoparticles on medicinal and aromatic plants. In this work, we aimed to find out the phytotoxicity or positive effects of different concentrations of nanosized TiO_2 on seed germination parameters and seedling growth of some commercially important medicinal plants including *Salvia mirzayanii, Alyssum homolocarpum, Sinapis alba, Carum copticum, Nigella sativa.*

MATERIALS AND METHODS

Plant materials

Seeds of five plant species from different families were used in this experiment (Table 1), as follows: *Salvia mirzayanii* Rech. f. & Esfand (Labiatae), *Alyssum homolocarpum* (Brassicaceae), *Sinapis alba* L. (Brassicaceae), *Carum copticum* L. (Umbelliferae), *Nigella sativa* (Ranunculaceae).

Table 1. Characteristics of different plant species and seeds tested in this experiment.

Species	Family	The part of the plant used	Seed 1000-weight (g)	Seed size (mm)	Seed color
Salvia mirzayanii	Labiatae	Seed	19	4	Grey
Alyssum homolocarpum	Brassicaceae	Seed	2	0.5	Yellow-Brown
Sinapis alba	Brassicaceae	Seed	6	2	Brown
Carum copticum	Umbelliferae	Seed	1.5	0.5	Grayish-Brown
Nigella sativa	Ranunculaceae	Seed	3	1	Black

Characterization of dioxide titanium (TiO_2)

TiO_2 nanoparticles were purchased from the Iranian Nanomaterials Pioneers Company, NANOSANY (Mashhad, Iran). The size of the TiO_2 nanoparticles was estimated to be 10-25 nm in diameter. A transmission electron microscopy (TEM) image of the TiO_2 particles is shown in figure 1. The crystal properties of TiO_2 nanoparticles were examined by X-ray diffraction (XRD). The XRD measurement showed that used TiO_2 nanoparticles were all present in the anatase form (Fig. 2). This form was considered to be more toxic than an equivalent sample of rutile TiO_2. Characteristics of the TiO_2 used in current experiment were presented in table 2.

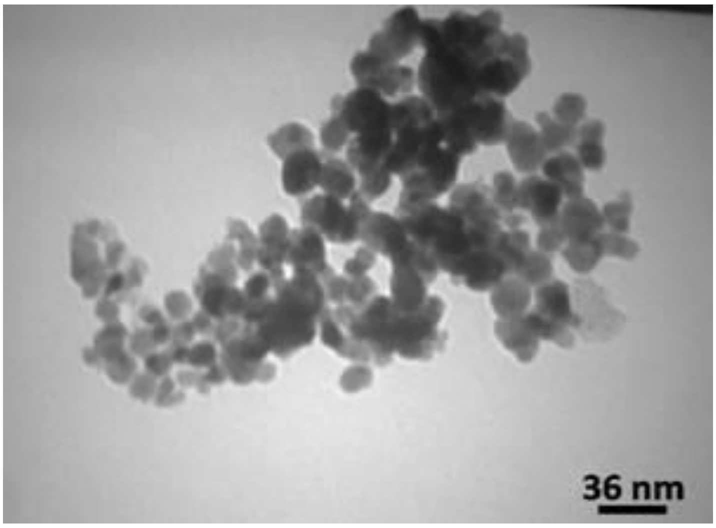

Figure 1. Transmission electron microscopy (TEM) image of TiO_2 nanoparticles. Distribution of particles size was estimated to be 10-15 nm (scale bar = 36 nm).

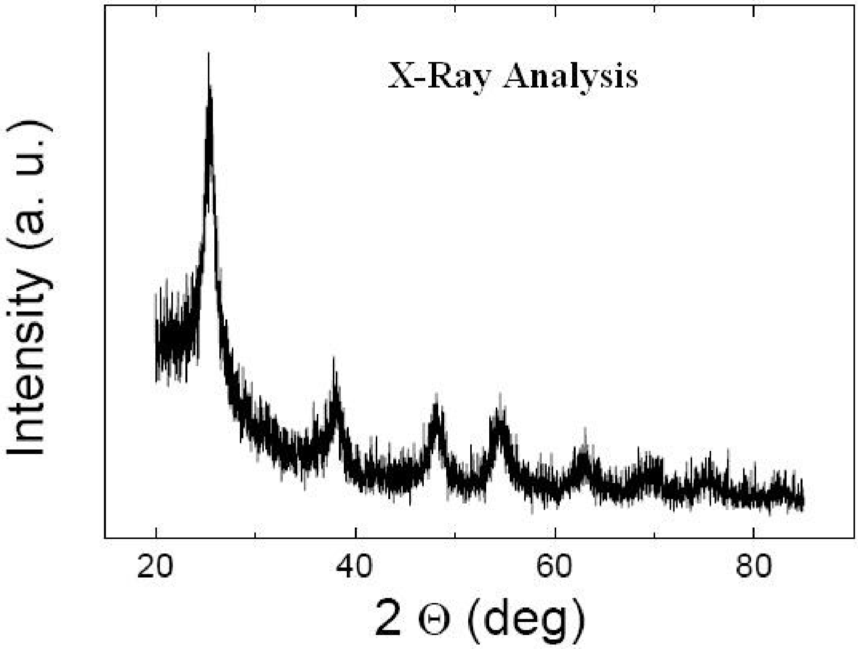

Figure 2. XRD pattern of TiO_2 nanoparticles. XRD measurement showed that used TiO_2 nanoparticles were in the anatase phase.

Table 2. Characteristics of nanosized TiO_2 used in current experiment.

Particel	Size (nm)	Purity (%)	Surface area ($m^2.g^{-1}$)	Color	Bulk density ($g.cm^{-3}$)	True density ($g.cm^{-3}$)	pH
Titanium Oxide Nanopowder (TiO_2, anatase)	10-25	> 99%	200-240	white	0.24	3.9	6-6.5

Seed preparation and treatment

Seeds of the plant species were collected from their natural habitats in Markazy province, Iran, for testing the effects of various concentrations (0, 10, 20, 40 and 80 $mg.L^{-1}$) of nanosized TiO_2 (anatase) on seed germination features including germination index (GI), germination percentage (GP), mean time germination (MTG), germination rate (GR) and seedling vigor index (SVI). After seed surface sterilization with sodium hypochlorite (1%, for 10 min) and rinse with sterilized water, they were placed on one layered Wathman No.1 filter paper moistened with 10 ml of employed solution in sterilized Petri dishes. Twenty seeds were selected for each treatment (in each plant species) and placed in Petri dishes and subsequently treated with reference solutions. In control, only distilled water in equal volume of anatase solution (10 ml) was used. After that, Petri dishes containing seeds were transferred to germinators with 16/8 h photoperiod, 20±2 ºC temperature and relative humidity of 75%. The seeds were considered to be germinating at the moment of radicle emergence (1-2 mm in length). The number of germinated seeds was recorded daily and the final percentage of germination was measured after 10 days. The germination characteristics were calculated based on their formula. The experiment was carried out using three replicates per each treatment in a randomized completely design (RCD).

Germination indices measurement

The final germination percentage (GP) was calculated based on total number of germinated seeds at the end of tenth day. The measurements were carried out according to International Rules for Seed Testing (ISTA, 1996). Germination indices as well as vigor index were calculated using the following equations (Alvarado *et al.*, 1987; Ruan *et al.*, 2002; Ellis and Roberts, 1981):

Germination percentage (GP %) = (Gf/n) × 100

Where, Gf is the total number of germinated seeds at the end of experiment and n is the total number of seed used in the test.

Mean Time Germination (MTG) = Σ NiDi/n

Where, Ni is number of germinated seeds till ith day and Di is number of days from start of experiment till ith counting and n is total germinated seeds.

Germination rate (GR) = Σ Ni/ΣTiNi

Where, Ni is the number of newly germinated seeds at the time of Ti.

Germination Index (GI) = Σ n/d

Where, n is the total germinated seeds at the time of d.

Vigor index (VI) = SL × GP%

Where, SL is seedling length at the end of test and GP% is the final germination percentage.

Statistical analysis

Analysis of variance was performed on a randomized completely design (CRD) with 3 replications. The data were analyzed using SAS software (version 9.1; CoHort Software). The significant levels of difference for all measured traits were calculated, and the means compared by multiple range Duncan's test ($P < 0.05$).

RESULTS AND DISCUSSION

Significant ($P < 0.05$) differences in examined traits were found among the five plant species and also among the employed TiO_2 concentrations (Table 3). In our current experiment, application of TiO_2 stimulated the seed germination of all species. However, this response was dependent on the concentration of applied TiO_2 and species as well.

In *Salvia mirzayanii* plants, results showed that the highest and the lowest GP (81% and 47%), were obtained in 80 mg.L^{-1} concentration of nano-sized TiO_2 and control, respectively. However, the lowest MTG (2.55 day) was observed in the highest TiO_2 concentration. Also, the highest and the lowest GR (0.39 and 0.28 seed/day), SVI (46.62 and 12.3) were observed at 80 mg.L^{-1} TiO_2 and control, respectively. Moreover, GI of the seeds was not significantly affected by treatments. Our findings possibly revealed the use of nano-TiO_2 (15-25 nm in diameter) increased the seed germination parameters of *S. mirzayanii* mucilage's seeds. Results of germination test on *Alyssum homolocarpum* plants indicated that the final GP was significantly affected by the employed treatment, however, increasing the concentration of nanosized dioxide titanium up to 40 caused an increase in seed germination and after that it declined. The lowest MTG (1.55 day) and the highest GR (0.62 seed/day) were obtained in 10 mg.L^{-1} concentration of treatment. However, the maximum seedling SVI (14.27) was observed in the medium concentration (40 mg.L^{-1}). However, in *Sinapis alba* plants the maximum GR (0.14 seed/day) with the lowest MTG (7.9 day) were obtained in 20 mg.L^{-1} of TiO_2 concentration. Moreover, the highest (102.6) and the lowest (22) SVI were observed in the 20 and 80 mg.L^{-1} of the reference treatment, respectively. However, variation of GI was not significantly ($P<0.05$) affected under all employed treatments. In our current study, nano-sized titanium oxide treatment especially in medium concentrations markedly improved the *S. alba* seed germination parameters and seedling vigor index (GP× seedling length). For *Carum copticum* seeds, the highest (75%) and lowest (42.5%) germination percentage were obtained at 10 and 80 mg.L^{-1} of TiO_2 concentration, respectively. Results also showed that the maximum GR (0.81 seed/day) and the lowest MTG (8.45 day) were obtained in 10 mg.L^{-1} concentration of employed treatment. However, the lowest SVI (4.19) and GI (1.3) were observed in seeds treated with the highest TiO_2. In our recent study, nano-sized titanium oxide treatment especially in low concentrations could markedly promote the *C. copticum* seed germination features and seedling vigor index. In *Nigella sativa* seeds, the lowest and the highest SVI (6.12 vs.9.29) were obtained in 20 mg.L^{-1} concentration of nanoanatase and control treatments, respectively. However, the maximum (84.6%) and the minimum GP (68%) were observed in 20 and in control treatments, respectively. Also, the lowest MTG (6.43 day) were obtained in 20 mg.L^{-1} of anatase concentration. Employing nanoanatase in appropriate concentration could promote the seed germination features and early growth of *N. sativa* in comparison to control. Generally, nanoparticles are materials that are small enough to fall within the nanometric range, with at least one of their dimensions being less than a few hundred nanometres. This reduction in size brings about significant changes in their physical properties with respect to those observed in bulk materials. Most of these changes are related to the appearance of quantum effects as the size decreases (Joseph and Morrison, 2006).

In a study with other type of nanomaterials, carbon nanotubes, results indicated that at the concentration range of 10-40 mg.L^{-1} dramatically enhanced the seed germination and growth of tomato plants (Khodakovskaya *et al.*, 2009). The researchers hypothesized that the positive effect of nanomaterials arose from the capability of them to penetrate seed coat and therefore promote water uptake. Water uptake in seed germination is critical because mature seeds are relatively dry and need a substantial amount of water to initiate cellular metabolism and growth. The measured water moisture content of seeds and the detection of nanomaterials inside seeds supported the hypothesis; however, the specific penetration mechanisms through the coat and the enhancement of water uptake by nanomaterials were not reported. As a photocatalyst, nano-TiO_2 under light could cause an oxidation-reduction reaction (Crabtree, 1998).

Table 3. Seed germination responses and seedling vigor index variation of the five plant species, *S. mirzayanii, A. homolocarpum*, *S. alba*, *C. copticum*, *N. sativa,* under different nanosized TiO_2 concentrations.

Plant species	Treatment	Traits				
	TiO_2 (mg.L^{-1})	GP (%)	MTG (day)	GR (seed/day)	SVI	GI
Salvia mirzayanii	0	47.0±1.2	4.58±0.3	0.28±0.01	12.30±0.1	5.26±0.9
	10	58.0±1.4	2.99±0.3	0.33±0.01	24.19±0.2	5.15±1.0
	20	68.0±1.9	2.85±0.2	0.36±0.02	34.43±0.3	5.53±0.8
	40	70.0±1.6	3.10±0.2	0.36±0.01	35.23±0.1	5.81±0.4
	80	81.0±2.1	2.55±0.1	0.39±0.03	46.62±0.1	6.08±0.5
LSD		**1.64**	**0.22**	**0.016**	**0.15**	**0.72**
Alyssum homolocarpum	0	68.3±1.4	2.36±0.4	0.44±0.02	9.15±0.3	5.62±0.2
	10	76.6±1.2	1.55±0.1	0.62±0.03	11.94±0.2	5.84±0.3
	20	77.6±1.6	1.62±0.2	0.54±0.03	11.98±0.4	6.62±0.1
	40	83.3±2.3	1.89±0.1	0.55±0.02	14.27±0.1	7.36±0.2
	80	71.6±1.3	2.25±0.2	0.57±0.04	14.0±0.5	6.38±0.3
LSD		**1.56**	**0.2**	**0.02**	**0.3**	**0.2**
Sinapis alba	0	71.3±2.3	8.10±0.2	0.11±0.05	45.0±1.5	5.47±0.2
	10	76.6±2.1	7.95±0.1	0.10±0.03	52.17±2.4	5.65±0.5
	20	87.0±2.5	7.90±0.4	0.14±0.02	102.67±5.7	5.68±0.4
	40	66.6±1.8	8.86±0.2	0.12±0.01	88.33±3.1	5.91±0.2
	80	48.3±1.5	9.46±0.2	0.10±0.03	22.0±1.8	5.71±0.2
LSD		**2.04**	**0.22**	**0.02**	**2.9**	**0.3**
Carum copticum	0	65.0 ±1.4	9.64±1.5	0.52±0.04	13.70±1.3	2.64±0.4
	10	75.0±2.2	8.45±1.2	0.81±0.07	29.12±1.5	3.82±0.3
	20	72.9± 1.9	7.07± 1.1	0.64±0.02	24.34±1.2	3.65±0.3
	40	51.0±1.3	9.84±1.6	0.46±0.04	12.70±0.9	2.0±0.2

	80	42.5±1.1	9.91±1.4	0.41±0.01	4.19±0.3	1.3± 0.1
LSD		**1.58**	**1.36**	**0.03**	**1.04**	**0.2**
Nigella sativa	0	68.0±1.5	8.47±0.9	0.72±0.03	22.73±0.3	6.12±0.2
	10	79.0±2.1	7.78±0.8	0.75±0.01	34.49±0.6	8.62±0.1
	20	84.6±2.2	6.43±0.5	0.83±0.04	53.20±0.2	9.29±0.6
	40	80.0±1.8	5.95±0.2	0.86±0.02	42.76±0.7	8.66±0.3
	80	70.0±1.3	9.46±1.1	0.77±0.01	23.45±0.1	6.21±0.2
LSD		**1.78**	**0.7**	**0.021**	**0.38**	**0.3**

Abbreviations: GP, MTG, GR, SVI and GI refer to germination percentage, mean time to germination, seedling vigor index and germination index, respectively. Values represent mean ± standard deviation (SD), n=3.

Research showed that nano-TiO_2 treatment could markedly promote aged seeds' vigor and chlorophyll biosynthesis of spinach, particularly, the ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity and the photosynthesis efficiency. The nano-TiO_2 treatment also has obvious effects on the improvement of growth and development in spinach. However, bulk TiO_2 treatment shows little effect (Zheng *et al.*, 2005).

Also, reported that a mixture of TiO_2 and SiO_2 nanoparticles at low concentrations increased nitrate reductase activity in the rhizosphere of soybean and consequently expedited soybean germination and growth (Lu *et al.*, 2002). According to Lin *et al.* (2004) nanoparticles SiO_2 enhanced the growth of Changbai larch (*Larix olgensis*) and the enhancement effect increased with concentration up to 500 $mg.L^{-1}$. Moreover, it is suggested that Al_2O_3 nanoparticles up to 4000 $mg.L^{-1}$ did not have any detectable effects on root elongation and development of Arabidopsis even though slight inhibition of seed germination was detected (Lee *et al.*, 2010). Metallic oxide nanoparticles such as ZnO were shown to be inhibitive at different developmental stages of plants such as seed germination and root elongation (Lin and Xing, 2007). Seed germination and root elongation are two standard indictors of phytotoxicity suggested by U.S. Environmental Protection Agency, yet several researches have indicated the insensitivity of seed germination for nanoparticles (Stampoulis *et al.*, 2009).

In our current work, different responses of the examined plant species to various nanosized TiO_2 dosages could be due to the following principal factors that previously reported by many researchers: concentration of nanoparticles, particle size and specific surface area, physicochemical properties of nanoparticles, plant species, plant age/life cycle stage, growth media conditions, nanoparticles stability, and dilution agent. The size of seeds could render more sensitivity to nanoparticles exposure. This is because a large seeded species (e.g., *S. mirzayanii*) has a lower surface to volume ratio than a small-seeded species (e.g., *S. alba*). However, a clear effect of the size of seeds on the toxicity of nanoparticles in plants cannot be confirmed at this time (Lee *et al.*, 2008; Lin and Xing, 2007). Lee *et al.* (2008) reported that mungbean was more sensitive than wheat to Cu nanoparticles toxicity, probably due to differences in root anatomy because xylem structures determine the speed of water transport and different xylem structures may demonstrate different uptake kinetics of nanoparticles. Mungbean is a dicot with one large primary root and several smaller lateral roots, whereas wheat is a monocot with numerous small roots without a primary root. However, generalization on whether the toxicity is based on dicot or monocot classification cannot be made. The concentration of nanoparticles is the major factor affecting toxicity in plants. The particle surface characteristic was also an important factor in nanoparticle toxicity (Yang and Watts, 2005). The works conducted in our laboratory also supported the more positive effects of nanosized TiO_2 than those of negative on seed germination parameters and vigority of tested medicinal plants particularly for mucilage's seeds.

In conclusion, here the seed germination and vigor index responses of five different medicinal plants, *Salvia mirzayanii, Alyssum homolocarpum, Sinapis alba, Carum copticum, Nigella sativa* were assessed with different concentration of TiO_2. The results indicated that the nanosized TiO_2 treatments in appropriate concentrations accelerates the germination characteristics of the reference plant seeds and increases their vigor. We found that nanosized TiO_2 in medium concentrations (20-40 $mg.L^{-1}$) has stimulatory effects on germination of most plants tested, except in *Salvia mirzayanii*, which the highest dosage (80 $mg.L^{-1}$) was found to be more efficient for this mucilage's seeds plant.

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