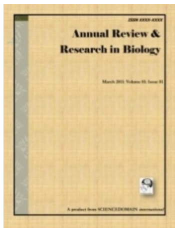

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Comparative Effects of Nanosized and Bulk Titanium Dioxide Concentrations on Medicinal Plant *Salvia officinalis* L.

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Authors' contributions

This work was carried out in collaboration between all authors. Author HF designed the study, wrote the protocol, and wrote the manuscript. Author SA performed the practical section in laboratory, Author FA performed statistical analysis and Author SJP managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The goal of the study was to evaluate concentrations of nanosized TiO_2 at 0, 5, 20, 40, 60 and 80 mg L^{-1} with same concentrations of bulk TiO_2 on sage (*Salvia officinalis* L.) seed germination and early growth stage.
Study Design: Experiment was performed in a completely randomized design with four replications.
Place and Duration of Study: The study was performed in a laboratory condition for 21 days at the College of Agriculture, Ferdowsi University of Mashhad, Iran.
Methodology: The treatments in the experiment were five concentrations (5, 20, 40, 60 and 80 mg L^{-1}) of bulk and five concentrations (5, 20, 40, 60 and 80 mg L^{-1}) of nanosized TiO_2 and an untreated control. The experiment was done in a germinator with an average temperature of 25 ±1ºC. The size of TiO_2 bulk and nanoparticles were determined through

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Scanning Tunneling Microscope (STM). Analysis of variance was performed between treatments samples. The data were subjected to analysis of variance using SAS software. Significant levels of difference for all measured traits were calculated and means were compared by the LSD test at 5% level.
Results: After 21 days of seed incubation, germination percentage improved following exposure to 60 mg L^{-1} bulk and nanosized TiO_2. Studied treatments had not significant effects on shoot, root and seedling elongation and biomass. Exposure of sage seeds to 60 mg L^{-1} bulk and nanosized TiO_2 obtained the lowest mean germination time (8.42 and 8.7 days, respectively) but higher concentrations did not improve mean germination time. Exposure of sage seeds to 60 mg L^{-1} concentrations of bulk and nano TiO_2 particles led to enhanced germination rate.
Conclusion: In general, there was a significant response by sage seed to nanosized TiO_2 presenting the possibility of a new approach to overcome problems with seed germination in some plant species, especially medicinal plants.

Keywords: Nanosized TiO_2; growth promotion; medicinal plant; mean germination time.

1. INTRODUCTION

Sage (*Salvia officinalis* L.) is cultivated in many countries around the world. Monoterpenoids of the sage essential oils exhibit antimicrobial, anti-inflammatory and antioxidant features. Beside the above actions, thujones and 1,8-cineole influence the central nervous system. Low doses of thujone or 1,8-cineole increased human activity in hard living situations [1]. Quick and uniform seedling emergence leads to successful establishment [2]. Usually most medicinal and aromatic plants have some problems in seed germination and seedling establishment stages in the field. Hence offer the solutions for improvement of seed germination will help to better performance in cultivation of medicinal plants.

The use of nanoparticles in commercial products and industrial applications have increased greatly in recent years although understanding of the interaction mechanisms at the molecular level between nanoparticles and biological systems is mainly lacking [3]. At the present time, various researchers have studied the effects of nanomaterials on plant germination and growth with the goal to promote its use for agricultural applications [4]. Nanosized TiO_2 is a commonly used nanoparticle, as a result there has been an exponential elevate 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 of effects of TiO_2 nanoparticles on plants provide information about the positive and stimulating effects as well as any negative impact [5,6]. Zheng et al. [7] have suggested that nanosized TiO_2 helped water absorption in spinach seeds and consequently accelerated seed germination. Foltete et al. [8] declared that altered TiO_2 nanocomposites were tested in the liquid phase on the plant model *Vicia faba*, which was exposed to three nominal concentrations: 5, 25 and 50 mg commercial sunscreen TiO_2 nanocomposites per liter for 48 h. Plant growth, photosystem II maximum quantum yield, genotoxicity (micronucleus test) and phytochelatins levels showed no alter compared to controls. Sunscreen nanocomposites seem not to exert deleterious effects on our plant model in 48 h, but the observed important clogging onto the roots [8]. Lu et al. [9] shown that a combination of nanosized SiO_2 and TiO_2 could increase nitrate reductase enzyme in soybean (*Glycine max*), increase its abilities of absorbing and utilizing water and fertilizer, encourage its antioxidant system, and actually hasten its germination and growth. In addition, it is stated that the positive effects of TiO_2

could be perhaps due to antimicrobial properties of engineered nanoparticles, which can increase strength and resistance of plants to stress [10].

Some reports have confirmed that nanoparticles can induce phytotoxicity and have a negative impact on seed germination and growth but the exclusive properties of nanoparticles can be used to improve seed germination and crop performance [6,11]. This use of the potentially positive effects of nanoparticles may be a helpful approach to reduce consumption of chemical substance in agriculture that would help to lower environmental pollution. In previous work it was demonstrated that using nanosized TiO_2 in low concentration (2 and 10 mg L^{-1}) could encourage seed germination of wheat in comparison to bulk TiO_2 and untreated control groups, but in high concentrations (100 and 500 mg L^{-1}) it had an inhibitory or no effect on wheat seed [12]. Although poor seed germination is a common occurrence in various medicinal plant species but there are no studies on the effects of nanoparticles on medicinal plants mainly sage, which is one of the most important medicinal plants cultivated in the world. This investigation was consequently performed to examine possible phytotoxicity and/or beneficial stimulatory effects of nanosized TiO_2 concentrations compared to bulk TiO_2 particles on features of seed germination and seedling growth of sage.

2. MATERIALS AND METHODS

2.1 Characteristics of Materials

The sage (*Salvia officinalis* L.) seeds were purchased from a commercial seed company located in Esfahan, Iran. 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.

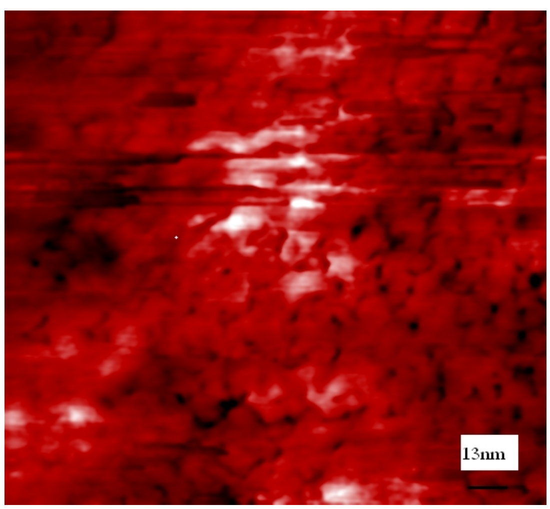

Fig. 1. Image of nanosized TiO_2 by Scanning Tunneling Microscope (STM)

In Fig. 2. was shown x-ray diffraction (XRD) pattern of TiO_2 nanoparticles. 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 [12].

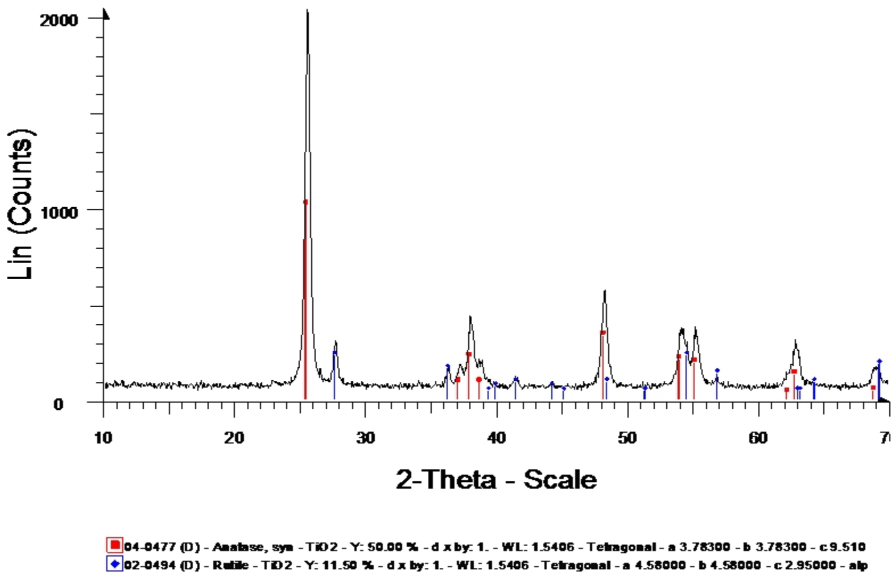

Fig. 2. X-ray diffraction (XRD) pattern of nano TiO_2 particles

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) [12]. XRD measurement showed that the bulk TiO_2 particles used in the study were made by 100% anatase. Analysis of particles in X-ray diffraction indicated tetragonal particles and the bulk TiO_2 particles had a crystalline nature (Fig. 4) [12].

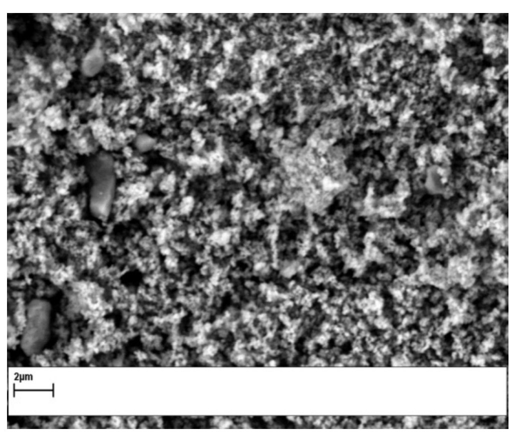

Fig. 3. Image of bulk TiO_2 particles by Scanning Electron Microscope (SEM)

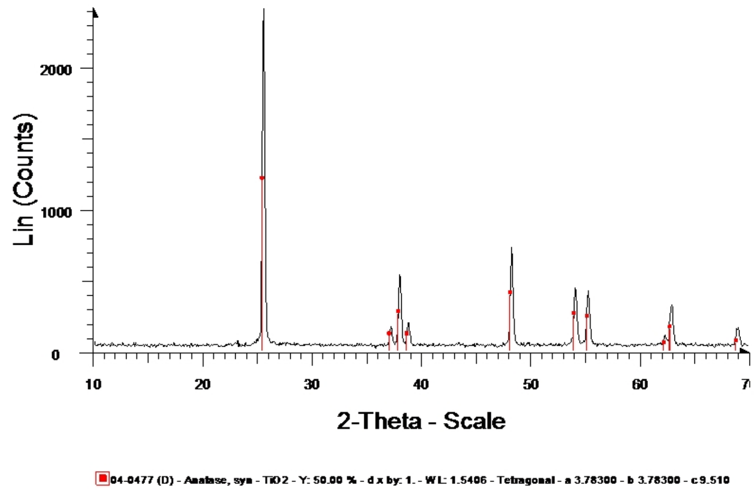

Fig. 4. X-ray diffraction (XRD) pattern of bulk TiO_2 particles

2.2 Seed Culture and Treatments

Experiment was carried out to assess the effect of different concentrations of bulk and nanosized TiO_2 on sage seed germination in a completely randomized design with four replications. The treatments in the experiment were five concentrations (5, 20, 40, 60 and 80 mg L^{-1}) of bulk and five concentrations (5, 20, 40, 60 and 80 mg L^{-1}) of nanosized TiO_2 and an untreated control. The experiment was performed in a germinator with an average temperature of 25 ±1ºC at the College of Agriculture, 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 get properly dispersed and stable TiO_2 suspensions of each concentration, an ultra-sonication action 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 3 ml of each concentration treatments was added. For the control, only distilled water was added to the Petri dishes. Distilled water was added to Petri dishes when needed. Germination tests were performed according to the rule issued by the International Seed Testing Association [13]. All concentrations of TiO_2 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 21 days [13]. 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 [14] (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 [15] (Eq. 2):

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

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

Seedling vigor was computed based on Vashisth and Nagarajan [16] (Eq. 3 and 4):

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

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

Evaluations of Mean Daily Germination (MDG), Pick Value (PV) and Germination Value (GV) were calculated by the following equations [17]:

$$MDG = Germination\% \,/\, Total\ experiment\ days \qquad \text{Eq. 5}$$

$$PV = Maximum\ germinated\ seed\ number\ at\ one\ day \,/\, day\ number \qquad \text{Eq. 6}$$

$$GV = PV \times MDG \qquad \text{Eq. 7}$$

2.3 Statistical Analysis

A one-way analysis of variance (ANOVA) was performed between treatment samples in a completely randomized design in four replications. The data were subjected to analysis of variance at 5% level of significance using SAS [18] software. Significant levels of difference for all measured traits were calculated and means were compared by the LSD test at 5% level.

3. RESULTS AND DISCUSSION

Table 1 show the effect of bulk and nanosized TiO_2 concentrations on seed germination, elongation and biomass of sage seedling. It was observed that use of TiO_2 nanoparticles with 60 mg L^{-1} enhanced sage seed germination by 9.2 percent in comparison to control. The seed germination of *S. officinalis* was adversely effected by exposure of 5 to 60 mg L^{-1} concentrations the bulk TiO_2 particles and 5 mg L^{-1} TiO_2 nanoparticles.

The main reason for this increased seed behavior could be the photo-sterilization and photo-generation of “active oxygen like superoxide and hydroxide anions” by nano-TiO_2 that encouraged capsule penetration for intakes of water and oxygen needed for quick germination [4]. Zheng et al. [7] reported that nanosized TiO_2 contributed to water absorption by spinach seeds and as result accelerated seed germination. An earlier study showed [12] that the highest germination percentage (98%) was in 2 mg L^{-1} of bulk and nanosized TiO_2 concentrations, but these treatments didn’t have significant effect on the seed germination percentage. Clément et al. [19] reported that the soaking of flax seeds in the suspensions of anatase nanoparticles at concentration 100 mg L^{-1} 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.

Although exposure of seeds to concentrations of nano TiO_2 were showed further elongation and biomass but shoot, root and seedling elongation and weights were not significantly affected by bulk and nanosized TiO_2 concentrations (Table 1). 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. Lin and Xing [20] 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 root 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 [3]. In an experiment, Barrena et al. [3] 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.

Several germination-related proceedings (gene transcription and translation, respiration and energy metabolism, early reserve mobilization and DNA repair) could also occur during seed priming [21], although often restricted due to reduced water supply compared to regular germination [22,23]. Khodakovskaya et al. [24] reported that multi wall carbon nano tubes (MWCNTs) can penetrate tomato seed and increase the germination rate by increasing water uptake. MWCNTs increased seed germination up to 90% (compared to 71% in control) in 20 days; it also increased plant biomass.

In most cases, lower mean germination time represents earlier seed germination. These results revealed that exposure of sage seeds to 60 mg L^{-1} bulk and nanosized TiO_2 obtained the lowest mean germination time (8.42 and 8.7 days, respectively) but higher concentrations did not improve mean germination time. Thus, 60 mg L^{-1} concentration of bulk TiO_2 treatments reduced mean germination time by 20.4% in comparison to the untreated control, whereas 60 mg L^{-1} concentration of nano TiO_2 contributed to a improved of mean germination time of about 17.5% in comparison with the control (Table 2). It is proposed activation of respiration and rapid ATP production appears to be the primary metabolic events induced by early seed germination [25]. Also, Zheng et al. [7] stated that the significant effect of nanosized TiO_2 on spinach germination in tests was maybe because of small particle size, which permitted nanoparticles to penetrate the seed during the treatment period, exerting its enhancing functions throughout growth.

Table 1. Influence of bulk and nanosized TiO_2 concentrations on seed elongation and biomass of sage seedling

TiO_2 concentration ($mg\ L^{-1}$)		Germination %	Shoot length (mm)	Root length (mm)	Seedling length (mm)	Shoot dry weight (mg)	Root dry weight (mg)	Seedling weight (mg)
Control	0	86.7bcd	138.07a	89.78a	227.85a	10.57a	4.310a	14.88a
Bulk TiO_2	5	88.0bcd	139.58a	93.50a	233.08a	10.67a	3.817a	14.49a
	20	85.3cd	139.18a	91.67a	197.52b	9.27a	4.640a	13.91a
	40	85.3 cd	138.7a	91.07a	229.77a	9.55a	4.073a	13.63a
	60	92.0ab	140.47a	91.08a	231.55a	10.75a	4.820a	15.57a
	80	86.7bc	140.78a	89.57a	230.35a	9.51a	4.647a	13.16a
Nano TiO_2	5	84.0d	140.80a	92.82a	233.62a	10.53a	4.370a	14.90a
	20	90.7abc	140.33a	92.49a	232.82a	10.09a	4.603a	14.69a
	40	89.3abc	141.82a	89.82a	231.64a	9.48a	4.447a	13.93a
	60	94.7a	140.33a	92.63a	232.96a	11.61a	4.437a	16.05a
	80	90.7abc	139.61a	92.10a	231.72a	10.16a	4.827a	14.98a
LSD		6.012	2.0739	3.9549	28.165	2.928	1.627	3.398

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

Table 2. Influence of bulk and nanosized TiO_2 concentrations on growth features of sage seedling

TiO_2 concentration ($mg\ L^{-1}$)		MGT (day)	Germination rate (seed day^{-1})	Germination value	MDG	PV	Vigour index I	Vigour index II
Control	0	10.58a	2.34e	2.19cd	5.78bcd	0.377ef	1975a	1.294a
Bulk TiO_2	5	9.59b-e	2.50cde	2.55bcd	5.87bcd	0.433c-f	2051a	1.271a
	20	10.07ab	2.28e	2.31def	5.69cd	0.406def	1679b	1.189a
	40	9.40c-f	2.46de	2.74bcd	5.69cd	0.481bcd	1961a	1.164b
	60	8.42h	3.36a	3.07ab	6.13ab	0.50bc	2130a	1.427a
	80	8.97fgh	2.84bcd	2.05f	5.78bcd	0.355f	1996a	1.142b
Nano TiO_2	5	9.77bcd	2.34e	2.93abc	5.60d	0.524ab	1963a	1.243a
	20	9.34def	2.58cde	2.68b-e	6.04abc	0.444b-e	2108a	1.333a
	40	9.99abc	2.57cde	2.65b-e	5.96a-d	0.444cde	2070a	1.245a
	60	8.70gh	3.17ab	3.54a	6.31a	0.581a	2206a	1.522a
	80	9.05efg	2.92bc	2.67c-e	6.04abc	0.407def	2161a	1.359a
LSD		0.616	0.4301	0.54	0.4008	0.0792	263.6	0.3055

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

Response of germination rate of sage to treatments was similar to MGT results. Exposure of sage seeds to 60 mg L^{-1} concentrations of bulk and nano TiO_2 particles led to enhanced germination rate (Table 2). The maximum germination rate was found in 60 mg L^{-1} bulk and nano-TiO_2 particles treatments (3.36 and 3.17 seed day^{-1}, respectively) and increasing concentration decreased the germination rate. The untreated group, 20 mg L^{-1} bulk-TiO_2 and 5 mg L^{-1} nano TiO_2 treatments showed the lowest germination rate. Among the bulk-TiO_2 treatments only 60 and 80 mg L^{-1} concentrations showed more values in germination rate in comparing to the control (Table 2). Using bulk TiO_2 particles significantly decreased germination value of seeds except in 60 and 80 mg L^{-1} concentrations while nanosized TiO_2 had a more positive effect than bulk TiO_2 treatments on germination value. It is most probable that nanoparticles could penetrate into the seed coat and exert a beneficial effect on the process of seed germination but bulk particles, having a larger size, cannot easily enter the same pathway, consequently may accumulate in the pores of a seed coat and clog up water and oxygen transition. Based on studies on nanoparticles effect on seed germination mechanism it could state nanoparticles might helped the water absorption by the seeds [7], increase nitrate reductase enzyme, increase seed abilities of absorbing and utilizing water and fertilizer, promote seed antioxidant system [9], 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 following UV-B radiation [26] result in improve seed germination in some plant species.

Exposure of seeds to majority of concentrations of bulk TiO_2 significantly diminished MDG compared to the nano TiO_2 treatments. Use of nanosized TiO_2 not only had negative effect on MDG, but also the greatest MDG was found in 60 mg L^{-1} nanosized TiO_2 treatment. However, the highest pick value (PV) of sage seedlings was observed in 60 mg L^{-1} nanosized TiO_2 treatment. Application of bulk-TiO_2 concentrations had a negative effect on vigor index I but the stimulating effect of nanoparticle treatments was seen on vigor index I of sage seeds. Exposure of seeds to 20 mg L^{-1} bulk TiO_2 decreased vigor index I by 15% and 20% comparing to control and 20 mg L^{-1} nano TiO_2. Additionally, the lowest vigor index II value was showed in bulk group treatments (Table 2). Applying of 40 and 80 mg L^{-1} bulk TiO_2 showed 10 and 12% lesser value in vigor index II than control, respectively. It has been declared 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 [27]. Zheng et al. [7] showed that the growth of spinach plants was greatly improved at concentrations of 250–4,000 mg L^{-1} nano TiO_2 than concentrations of bulk-TiO_2. Ghosh et al. [28] 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 of TiO_2 nanoparticles (319 mg L^{-1}).

4. CONCLUSIONS

Similar to other technologies, low-cost nanomaterials and field application technologies are needed for their applications in agriculture. Nanotechnology is leading to the development 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 effort is the first report related to the effects of bulk and nanosized TiO_2 particles on Sage (*Salvia officinalis* L.). Using bulk and nanosized TiO_2 nanoparticles at 60 mg L^{-1} promoted sage seed germination percentage. Exposure of sage seeds to 60 mg L^{-1} bulk and nanosized TiO_2 obtained the lowest mean germination time but higher concentrations did not improve mean germination time. Application of seeds to nano TiO_2 increased vigor index of sage comparing to control and bulk TiO_2 treatments. Nanomaterial can improve seed germination in certain plants but can have adverse affects on others. In such cases, studies on nanomaterials aspects for instance the nanoparticles dosage in the different mediums, their physical and chemical characterization, the mechanisms allowing them to pass through cellular membranes and cell walls, the particular properties that are related to positive and toxic effects of nanoparticles, and the mechanism underlying nanoparticles trophic transports are necessary to conducted. Also, the existing application methods need to be reviewed for improved efficiency of nanomaterials on future targets. Moreover, the necessity of further studies on the possible risks related to the use of nanomaterials and their potential adverse effects are needed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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