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INVESTIGATION OF THE TiO_2 NANOPARTICLES EFFECT ON SEED GERMINATION CHARACTERISTICS OF *ZIZIPHORA CLINOPODIOIDES* LAM.

ABSTRACT

Improvement in the rate and amount of germination of seeds has a very important effect on the establishment of primary seedlings and the increase of rangeland production. The rapid and uniform germination of seeds leads to the successful establishment of plants. The use of nanoscale materials can help germinate faster seeds. Therefore, in this study, the effects of TiO_2 nanoparticles in concentrations of 0, 10, 20, 30, 40, 60 and 80 mg / l on the rate and speed of seed germination of *Ziziphora clinopodioides* Lam. paid. This design was carried out in a completely randomized design with four replications for 20 days at a constant temperature of 20°C under 12 hours of light and 12 hours of darkness at the Germinator of Natural Resources Faculty of the Ferdowsi University of Mashhad. The results showed that germination percentage of treated seeds with TiO_2 nanoparticles increased to 23% ppm compared to control treatment. Also, in other concentrations of other nanoparticles, there was a positive effect on speed and germination percentage, so that the effect of different concentrations of nanoparticles on germination characteristics of *Ziziphora clinopodioides* Lam. seeds was significant. The highest germination percentage was observed in the concentration of 30 ppm and the lowest germination rate at 30 and 20 ppm concentrations. In high concentrations of TiO_2 nanoparticles, no positive effects were observed on the germination characteristics of seed *Ziziphora clinopodioides* Lam. To conclude the use of TiO_2 nanoparticles can be improved by improving the seed germination properties of the medicinal plant *Ziziphora clinopodioides* Lam. that cause increases plant's establishment in natural areas.

Key words: germination, Iran, nanoparticles, *Ziziphora clinopodioides* Lam.

INTRODUCTION

Nanotechnology research is the background of advanced technology, which has led to the rapid development of electronic science, biotechnology, medicine, space science and defense industries. So far, few studies have

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been done on the effects and mechanisms of nanoparticles on plant growth (Zhang *et al.*, 2005). Examples of the unique properties of nanoparticles include a very high specific surface area, high surface energy, and quantum imprisonment. These unusual properties may even affect their fate and environmental behavior in both mass and non-nanoscale materials (Thakkar *et al.*, 2009). Plants, except for the foundation of all ecosystems, play a crucial role in the fate and transfer of nanoparticles in the environment through absorption and bioaccumulation. Silicon oxide and titanium dioxide are the most commonly used nanoparticles used in the industry. Of course, some of them are also used in agriculture and natural resources. Below we will investigate several studies on the role of these nanoparticles in the germination of different plant species.

Among different methods, nanoparticles (NPs) of different metal oxides by absorbing water, oxygen and nutrients and having the antimicrobial properties can affect seed germination percentage, improve growth, dry weight, photosynthesis, chlorophyll biosynthesis, and plant metabolism (Rezaei *et al.* 2015). Thus, soaking seeds in NP solution can be used as an option to increase seed germination percentage. The application of 100% TiO_2-NP treatment increased seed germination percentage, germination index, germination energy, vigour index, seedling height and fresh weight of *Pinus tabulaeformis* Carrière (Yinfeng, Xiaohua 2009). The effect of silver NPs on *Boswellia ovalifoliolata* N.P. Balakrishnan & A.N. Henry caused an increase and acceleration of the seed germination percentage (Savithramma *et al.* 2012).

Abdel Latef *et al.* (2017) in a research titled " Titanium Dioxide Nanoparticles Improve Growth and Enhance Tolerance of Broad Bean Plants under Saline Soil Conditions" compared the effects of three different $nTiO_2$ concentrations (0·01%, 0·02% and 0·03%) with respect to plant growth and stress responses. The 0·01% $nTiO_2$ application significantly increased shoot length, leaf area and root dry weight of plants under normal conditions.

Feizi *et al.* (2012) concluded in a study titled " Effects of various concentrations of nanoparticles and non-nano-titanium dioxide on seed germination and seedling growth of fennel", which showed that germination percentage of seeds was significantly increased in treatment Application of concentration of 60 mg/l titanium oxide nanoparticles (76%) increased compared to other treatments and control (54%). The average germination time improved by application of nanoparticles with a concentration of 40 mg / L compared to the control by about 31%, while non-nano particles improved the mean germination time by only 21%. In general, the use of titanium dioxide nanoparticles as an option for seed that has a germination problem and a low germination percentage can be used. Also, Feizi *et al.* (2013), in another study titled "The effects of plant and native titanium dioxide on the fennel," showed that with the concentration of TiO_2 nanoparticles at 0, 5, 20, 40, 60 and 80 After 14 days of inoculation, the germination percentage increased to 31.8% (60 ppm TiO2 nanoparticles) with non-nano TiO_2 after 14 days of inoculation. The same positive effects were observed for stem dry weight and germination. Behnam *et al.* (2012), in a study titled

"The effect of titanium nanoparticles and non-nanodioxid on germination performance of *Echinacea purpurea* under drought stress", concluded that the application of titanium dioxide treatment on the seeds of *Echinacea purpurea* in conditions without The stress on roots and length of seedling length and root length, rootstock and seedlings, seed vigor index, I and II, and mean germination time at 1% were very significant in drought stress conditions. Drought stress at 3-bar intensity did not negatively affect the most traits such as percentage and speed of germination, average daily germination and seed vigor index I and II in Sardinia. The concentration of 159 mg / L of non-nano titanium dioxide increased the length of stem, root, and seedlings compared to the control 3 times. In the face of drought stress, the application of 100 milligrams per liter of nanoparticles and non-nano particles often improves the germination characteristics of *Echinacea purpurea* and can be recommended in areas with drought stress. Feizi *et al.* (2013) in a study titled "Comparative effects of different levels of titanium dioxide in nano and non-nano plants of Salvia officinalis L." concluded that after 21 days of inoculation, Germination increased with 60 mg/l nano-titanium dioxide and non-nano, but did not affect root and shoot length and biomass. Agheli *et al.* (2016), in a study titled "*Silybum marianum* L. seed germination induction using titanium dioxide nanoparticles and magnetic field", which was tested as a factorial experiment in a completely randomized design with three Repeated experiments were carried out and the first factor was seed treatment at different concentrations of titanium dioxide nanoparticles containing 50, 10, 0 and 100 mg × l^{-1}, and the second factor of the treatment of seeds with a magnetic field at 0.30, 60 and 90 millitesla for 30 minutes showed that the highest percentage of seed germination in the interaction of nanoparticles of TiO_2 nanoparticles with a concentration of 100 ppm and intensity The magnetic field intensity was 30 millitesla and the application of titanium dioxide nanoparticles with a concentration of 50 ppm and magnetic field intensity of 60 milliseconds. These treatments improved the germination percentage by 5.6 and 5.7 times the control treatment. Feizi *et al.* (2012) showed in a study titled "The reaction of wheat seed to different concentrations of titanium dioxide nanoparticles (TiO_2) compared to non-nano particles".

Experimental treatments were based on seeds' germination percentage and germination rate did not have a significant effect but had a significant effect on average germination time. The lowest mean germination time (MGT) was 0.89 days at a concentration of 10 ppm nanoparticles of TiO_2 and the highest was observed in the control treatment with 1.35 days (Table 2).

Therefore, the concentration of 10 ppm nanoparticles of non-nano TiO_2 reduced the MGT content by 34% compared to the control, while the concentration of 10 ppm TiO_2 in non-nano, MGT did not changed against the control. One-year-old seedlings of Pine (*Larix elgensis*) were placed in concentrations of 62, 125, 250, 500, 1000, 2000 µl × l^{-1} of nano silicon dioxide (SiO_2) for about 6 hours. Nano treatment greatly improved the growth and quality of seedlings. Treatment with 500 µl × l^{-1} had the best result, with an increased average height of 42.5%, a root diameter of 30.7%, a root length of 14%, and the number of lateral roots of seedlings of 31.6% in comparison with control. Also, treatment with 500 μl × l^{-1} showed the highest concentration of chlorophyll (Lin *et al.*, 2004). Lee *et al.* (2008) examined the toxicity of copper nanoparticles on beans and wheat, and stated that TiO_2 concentrations had a significant effect on root dry weight but did not have a significant effect on stem and seedling dry weight. Takallo *et al.* (2012), in a study titled "The effect of TiO2 nanoparticles on germination and cytogenetic indices of barley plant, showed that the comparison of nanoparticle treatments compared to control increased the index of chromosomal deviations of barley, whereas on other cytogenetic indices and germination traits had no significant effect.

The mixing of SiO_2 and TiO_2 nanoparticles with the low concentration, increased nitrate reductase activity in soybean rhizosphere, resulting in increasing of soybean germination and growth (Lu *et al.*, 2002). It has been reported that nanoparticles can accelerate soybean germination and growth and prevent rotting by molds. Nanoparticles can enhance roots power and nitrate reductase activity and improve the root ability to absorb water and fertilizer and increase the activity of antioxidants, dismutase and catalase enzymes and improve soybean resistance to stress. (Liu *et al.*, 2005). The remarkable effect of nanoparticles is probably due to the small size of the particles, which allows its penetration into the seed. In a study by Ivani *et al.* (2012), the effect of nano silica and non-nano silicon dioxide particles on stress tolerance of *Trigonella foenum-graecum* seedlings, the results of the analysis of variance showed that the levels of water stress and nano and non-nano silicon dioxide treatments had a significant effect on the studied traits.

The results of Zhang *et al.* (2005) showed that spinach (*Spinacia oleracea*) old seeds germination rate was very low. Treatment with TiO_2 increased the rate by 23%, but treatment with nano TiO_2 significantly increased germination rate, germination index, seedling dry weight and Vigor index of older seeds. In spinach (*Spinacia oleracea*), the best treatment was 5.2 per 1000 of TiO_2 nanoparticles, with the fresh and dry weight of each plant increasing by 63% and 76%, respectively against the control (Zhang *et al.*, 2005). All treatments significantly affected germination percentage and germination rate. The highest germination percentage was observed in the seeds of titanium nanoparticles with concentrations of 30 (48%), 40 and 20 mg × l^{-1} (35%), and the lowest rate of germination in the seeds impregnated with titanium nanoparticles with concentrations of 30 (29.9 days) and 20 (6.9 days) were observed (Zhang *et al.*, 2005). The germination percentage of *Arabidopsis thaliana* seeds was affected by SiO2. A significant positive effect on rootlet length was observed on all concentrations of nano Al_2O_3 and 400 nano SiO_2 concentrations, while other concentrations, as well as nano Fe_3O_4 and ZnO, showed inhibitory effects on root length. All concentrations of ZnO contained fewer leaves (Lee *et al.*, 2005). Khodakovskaya *et al.* (2009) showed that carbon nanotubes with concentrations of 10-40 mg × l^{-1} increased germination and tomato growth, which is probably due to the ability of carbon nanotubes to penetrate the seed crust and stimulation of water absorption.

MATERIALS AND METHODS

Introducing the plant used in the experiment

Mountain *Ziziphora clinopodioides* Lam. belongs to the genus Ziziphora, the plant has a height of 10 to 40 centimeters. Booty and stable, a woody base, and sometimes it has a stem of pubes with different densities. The aerial parts of the *Ziziphora clinopodioides* Lam. are widely used in traditional medicine and pharmaceutical industries of Iran (Kheirkhah, 2011). The resistance of the mountain *Ziziphora clinopodioides* Lam. to drought is suitable and can be used for cultivation in arid and semi-arid regions (Azizi *et al.*, 2004). Also, since plants have essential oils such as peppermint plants (*Ziziphora clinopodioides* Lam. and etc.) have a good function in semi-arid and Mediterranean regions, these plants can be considered as a commercial product for Iran and an alternative surplus product (Naghdabadi *et al.*, 2004). The geographical distribution of *Ziziphora clinopodioides* Lam. in the world is in the Eastern Balkan Peninsula, Southwest Asia and Central Asia to the Pamir Himalaya Mountains (Iran, Iraq, and the central and eastern parts of Turkey) and Africa (Jamzadeh, 2009). *Ziziphora clinopodioides* Lam. grows in Iran in mountainous areas, rocky and deposit slopes, and steppe areas at altitudes between 800 and 3700 m above sea level (Jamzadeh, 2009).

Laboratory operations

Germination test was performed in two stages. In the first step, in order to study the quality and percentage of germination of the *Ziziphora clinopodioides* Lam., 25 healthy seeds of *Ziziphora clinopodioides* Lam. were placed in petri dish in four replicates and germination test was done with distilled water for 20 days. After 20 days of the experiment, the germination percentage of the *Ziziphora clinopodioides* Lam. seed was about 25% and very low. Therefore, different concentrations of nanoparticles were used to improve the seed germination characteristics of *Ziziphora clinopodioides* Lam. This experiment was carried out to evaluate the different concentrations of titanium nanoparticles on the germination traits of *Ziziphora clinopodioides* Lam. seed. For this purpose, 7 treatments containing different concentrations of 0, 1000, 2000, 3000, 4000, 6000 and 8000 mg/l nanoparticles of titanium particles were performed in a completely randomized design with four replications. The seeds of *Ziziphora clinopodioides* Lam. were obtained from Khorasan Razavi Natural Resources Office. The titanium dioxide nanoparticle powder was as AEROXIDE® TiO2 P25, that prepared from Evonik DegussaGmbH company in Germany. The purity of nanoparticle powder was 99.8%, the average particle size was 21 nm and its specific surface area was 50 $m^2 \times g^{-1}$ (Figs 1 to 4). Non-nano iron oxide was prepared from AppliChem GmbH Germany with a purity of 99% and a particle size of about 1 micrometer. Before the experiment, the size of the nanoparticles was determined by an STM tunnel microscope (STM) at the Central Laboratory of Ferdowsi University of Mashhad. Also, purity and their compounds were determined by X-ray diffraction (XRD) at Damghan University of Science.

In order to obtain the desired concentrations, we first weighed the nanoscale materials in distilled water. Ultrasound bath was used for 20 minutes to prepare a uniform suspension. 2 ml of prepared suspension was placed in per petri dish along with 25 seeds. In the control group, 2 ml of distilled water was added. In the next steps, if necessary, only distilled water was added to the dishes. Seed scratching treatment was performed at 4°C for one week (ISTA, 2009). This experiment was carried out at germinator of the Faculty of Natural Resources and Environment of Ferdowsi University of Mashhad at a temperature of 20 centimeters under 12 hours of light and 12 hours of darkness. To prevent evaporation of the extract and loss of moisture, Petri dishes port were laid and placed in plastic. The germinated seeds were counted and recorded daily. Seed counting continued until 20 days after starting germination. Data and daily measurements were entered into Excel spreadsheet software and after processing, statistical analysis of data was done by SPSS18 and Minitab16 softwares and the meanings were compared by Duncan's multiple domain tests at 5% probability level.

Data analysis

The following equations were used to determine the rate of germination from the formula of Maguire (1982) and the mean germination time (MGT) (Matthews and Khajeh-Hosseini, 1990):

$$GR = a + \frac{b-a}{2} + \frac{c-b}{3} + \cdots + \frac{n-(n-1)}{N}$$

where, *GR* – the germination rate according to germinating seed per day, *a*, *b*, *c*, and *n* – represent the numbers of germinated seeds after 1, 2, 3 and *N* – days after starting to absorb water.

$$MGT = \frac{\sum F(X)}{\sum F}$$

where, *MGT* – the average germination time [days], *F* – the number of new seeds germinated on the day of counting and X_i and *X* – the day of counting.

Mean daily germination, pick value and germination value were calculated using the following equations (Hartman *et al.*, 1990):

$$MDG = \frac{GP}{TDE}$$

where *MDG* = mean daily germination, *GP* = percentage of germination and *TDE* = total days of experiment

$$PV = \frac{G_h}{N_{gd}}$$

where *PV* – pick value (maximum value), G_h – the highest germination seed number and N_{gd} – germination day

$$GV = PV \times MDG$$

where *GV* – germination value, *PV* – pick value and *MDG* – mean daily germination

RESULTS AND DISCUSSION

In figures 1 to 4, it can be seen an image of the size of the titanium dioxide nanoparticles, using a tunneling scanning microscope (STM), a topographic image of the titanium dioxide nanoparticles, using a tunnel scanning microscope (STM), illustration of the size of the non-nano titanium dioxide particles using Scanning electron microscopy (SEM) and X-ray diffraction spectrum (XRD) of titanium dioxide nanoparticles.

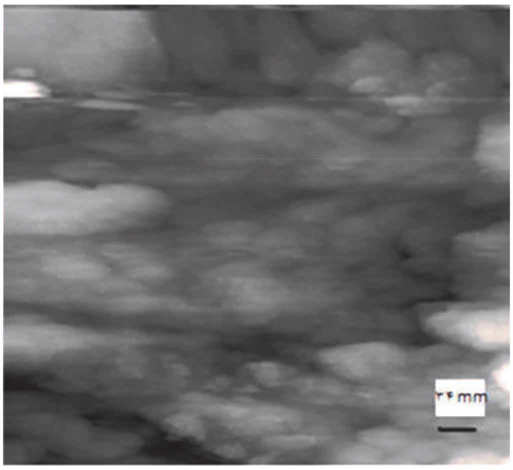

Fig. 1. TiO_2 nanoparticle size image by using tunneling scanning microscopy (STM)

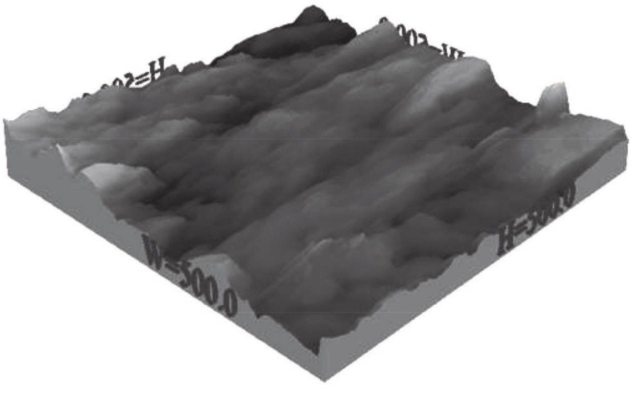

Fig. 2. The topographic image of TiO2 nanoparticles using a tunneling scanning microscope (STM)

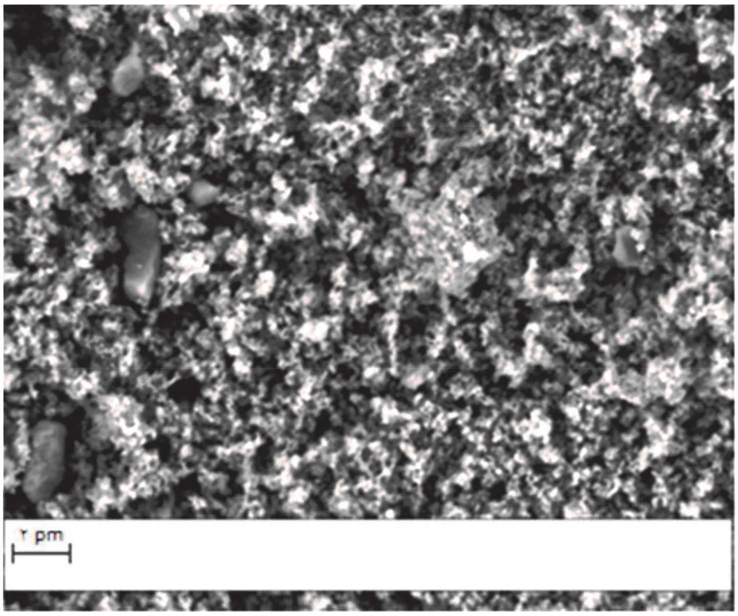

Fig. 3. Non-nano TiO_2 Particle Size Using Electron Scanning Microscopy (SEM)

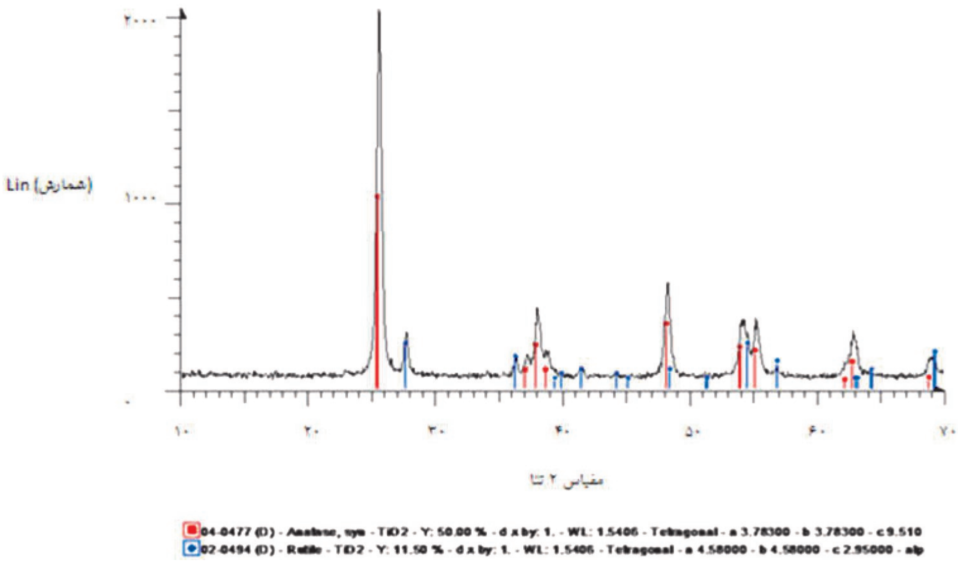

Fig. 4. X-ray Spectrum (XRD) of TiO_2 Nanoparticles

The results of the analysis of variance showed that different concentrations of titanium nanoparticles had a significant effect on seed germination characteristics of *Ziziphora clinopodioides* Lam. Based on the results, different concentrations of titanium nanoparticles showed a significant effect on germination percentage, germination rate at 1% and 5% probability levels (Table 1). Also, the comparison of the mean of different concentrations of titanium nanoparticles on the characteristics of germination of *Ziziphora clinopodioides* Lam. seed is given in Table 2.

Table 1

Effect of different concentrations of titanium nanoparticles on seed germination characteristics of *Ziziphora clinopodioides Lam.*

Coefficient of variations	Degrees of freedom	Average germination time	Average daily germination rate	Maximum value	Germination value	Germination percentage
Nanoparticles	6	4.59*	0.62**	0.03**	0.25**	247.81**
Error	21	1.56	0.02	0.004	0.01	5.9

ns, *, and ** indicate a none-significant and significant difference at the probability level of 5% and 1% respectively. In order to reduce the amount of data in the table of analysis of variance, only the mean square of the data and significant levels were used at the probability level of 1 and 5%

Table 2

Mean comparison of different concentrations of titanium nanoparticles on seed germination characteristics of *Ziziphora clinopodioides* Lam.

Germination percentage	Average germination time	Average daily germination rate	Maximum value	Germination value	Amount of TiO_2 [ppm]
25d	12.23a	1.25d	0.23bc	0.28c	0
27cd	11.42ab	1.35cd	0.19c	0.25c	10
35b	9.6bc	1.75b	0.3b	0.53b	20
48a	9.29c	2.4a	0.4a	0.95a	30
35b	10.51abc	1.75b	0.24bc	0.42bc	40
28cd	11.75ad	1.4cd	0.17c	0.24c	60
29c	11.03abc	1.45c	0.21bc	0.3c	80

The numbers with the same letters in each column are not statistically significant (according to Duncan's multiple range test at 5% probability level)

The best rate of germination was observed at concentrations of 20, 30 and 40 mg (Table 2). The concentration of 30 ppm of nanoparticles had the greatest effect on improving seed germination properties of *Ziziphora clinopodioides* Lam. Therefore, it seems that titanium nanoparticles can be one of the treatments to improve germination properties. Seed germination percentage of the control treatment was about 25%, while seed germination percentage reached 48% in the seeds treated with the nanoparticles solution (Table 2). The seeds that were treated with nanoparticles had the best results from the observations. The maximum germination percentage and the minimum germination time were about 48 and 29.9%, respectively, compared to the control. Germination speed improved from 12.23 days in control treatment to 9.29 days in nano-treated seeds (30 ppm). Germination percentage increased with increasing concentrations up to 30 mg × l^{-1}, and then from 40 mg, germination percentage had decreasing trend (Table 2), while Feizi *et al.* (2013), in a study titled "Effects of different concentrations of nano and non-nano titanium dioxide on seed germination and *Foeniculum vulgare* seedling growth", concluded that seed germination percentage was significantly increased in the application of 60 mg × l^{-1} (76%) than other treatments and control (54%).

Also Feizi *et al.* (2013), in another study titled "Effects of stimulant and toxicity of nano and non-nano titanium dioxide on *Foeniculum vulgare*" showed that with a concentration of TiO2 nanoparticles, the germination percentage increased to 31.8%. The same positive effects were observed for stem dry weight and germination. Feizi *et al.* (2013) in another study titled "Comparative effects of various amounts of nano and non-native titanium dioxide in *Salvia officinalis* L." concluded that germination percentage increased with 60 mg × l^{-1} nano and non-nano TiO_2, but did not affect root and shoot length and biomass. Also, Behnam *et al.* (2013), in a study titled "The effect of nanoparticles and non-nano titanium dioxide on germination performance of *Echinacea purpurea* under drought stress", concluded that the concentration of 159 mg × l^{-1} of non-Titanium dioxide nanoparticles increased the length of stem, root, and seedlings by 3 times against the control.

Feizi *et al.* (2012 in a study titled "Wheat seed reaction to various concentrations of TiO_2 nanoparticles compared to non-nanoparticles", showed which a concentration of 10 ppm nanoparticles of TiO_2, decreased MGT until 34% relative to the control, while the concentration of 10 ppm of TiO_2 non- nanoparticles did not change the MGT value compared with the control. The results of Lee *et al.* (2008) showed that toxicity of copper nanoparticles in beans and wheat that TiO_2 concentration had a significant effect on root dry weight, but no significant effect was observed on stem and seedling dry weight.

Studies by Zhang *et al.* (2005) show that increased germination and dry weight are probably due to increased absorption of mineral nutrients and the photosynthesis process catalyzed by TiO_2 nanoparticles. The remarkable effect of nanoparticles is probably due to the small size of the particles, which allows it to penetrate into the seed during the treatment period (Zhang *et al.*, 2005) Ley *et al.* (2010) showed that there was a significant positive effect on root length by all concentrations of nano Al_2O_3 and 400 nano SiO_2 concentrations, while other concentrations, as well as Fe_3O_4 nano and ZnO had inhibitory effects on rootlet length. All concentrations of ZnO contained fewer leaves (Lee *et al.*, 2010). Khodakovskaya *et al.* (2009) showed that carbon nanotubes at a concentration of 10- 40 mg/l increased germination and tomato growth, probably due to the ability of carbon nanotubes to penetrate the seeds crust and stimulation of water absorption.

Also, Takallo *et al.* (2014), in a study titled "The effect of TiO_2 nanoparticles on germination traits and cytogenetic indices of barley plants, concluded that the comparison of nanoparticle treatments compared to control increased the index of chromosomal deviations of barley, while there was no significant effect on other cytogenetic indices and germination traits. Le *et al.* (2002) reported that the mixture of SiO_2 and TiO_2 nanoparticles increased at low concentration of nitrate reductase in soybean rhizosphere, resulting in increased germination and soybean growth. According to Zhang *et al.* (2005), nano TiO2 treatment significantly increased germination rate, germination index, seedling dry weight and Vigor index of older seeds. It is possible that superoxide and hydroxide ions increase the permeability of the seed and facilitate the entry of water and oxygen into the cell, and thus aggravate the seed germination metabolism. In addition, the entry of TiO2 nanoparticles into cells can produce oxidation-reduction reactions through radical superoxide ions during germination in the dark, leading to the release of free radicals in the germinating seeds. The oxygen produced in such a process can be used for breathing, which will further stimulate germination.

All treatments of this study significantly affected germination percentage and germination rate. The highest germination percentage was observed in the seeds of titanium nanoparticles with concentrations of 30 (48%), 40 and 20 mg × l^{-1} (35%), and the lowest germination rate were observed in the seeds impregnated with titanium nanoparticles with concentrations of 30 (29.9 days) and 20 (6.9 days) (Table 2), While, Aghelie and *et al.* (2015), in a study titled Germination Stimulation "*Silybum marianum* L. using titanium dioxide nanoparticles and magnetic field", showed that the applied treatments improved germination percentage against the control treatment by 5.7 and 5.6 times. In a study by Ivani *et al.* (2012), by titled The effect of nano silicon and non-nano silicon dioxide particles on tolerance to water deficit stress of *Trigonella foenum-graecum* seedlings showed that levels of water deficit and nano and non-nano silicon dioxide treatments had a significant effect on the traits.

CONCLUSION

Improvement in the rate and rate of seed germination has a very important effect on the establishment of primary seedlings and the increase of rangeland production. Rapid and uniform germination of seeds leads to the successful establishment of plants. The use of nanomaterials can help seeds to germinate faster. Therefore, in this research, the effects of TiO2 nanoparticles in concentrations of 0, 10, 20, 30, 40, 60 and 80 mg × l^{-1} on the rate and speed of seed germination of *Ziziphora clinopodioides* Lam were studied. The effect of different concentrations of titanium nanoparticles on germination percentage and germination rate of *Ziziphora clinopodioides* Lam. seed was significant at 1% and 5% probability level. Titanium nanoparticles in the concentration of 30 and 20 mg × l^{-1}, stimulating effect and in higher concentrations had an inhibitory or neutral effect on seed germination of *Ziziphora clinopodioides* Lam. High concentrations of titanium nanoparticles in the germination stage had a negative effect on MGT, and the best and suitable concentration was used to stimulate growth and germination of 30 mg × l^{-1} of nanoparticles. These results indicate that the use of TiO_2 nanoparticles can increase the establishment of this plant in natural areas by improving the seed germination properties of the *Ziziphora clinopodioides* Lam. medicinal plant.

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