RESEARCH ARTICLE

Effects of titanium dioxide nanoparticles (TiO_2) on germination and seedling growth of Vitex plants (*Vitex agnus-castus* L.)

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ABSTRACT

Different concentrations of nanoparticles impose impact different effects on germination and plant growth. The purpose of this study was to investigate the effects of 11 different concentrations of titanium dioxide nanoparticles on germination and seedling growth characteristics of Vitex plant (*Vitex agnus-castus* L.). This experiment was conducted in the laboratory of the Islamic Azad University of Tehran in 2018 as a completely randomized design with 4 replications. The measured traits included germination percentage, relative germination percentage, germination rate, mean germination time, germination index, weighted germination index, vigor seedling index, fresh and dry weight of seedling and root and shoot length. XRD measurements showed that the titanium dioxide nanoparticles used in the anatase form were present. The results showed that the highest germination percentage (80%), relative germination percentage (115%), germination rate (22.37), germination index (190), weighted germination index (2.30), vigor seedling index (541) and fresh weight (0.52) were observed through application of 200 $\mu g.ml^{-1}$ titanium nitric oxide. Higher concentrations of nano titanium dioxide had no positive effect on germination indices. The response index also showed that nano-titanium dioxide at all concentrations had a stimulatory effect on mean germination time, root length, shoot length and dry weight but only at 200 $mg.l^{-1}$ treatment had a stimulatory effect on germination percentage and germination rate. The results of this study indicated that the treatment of 200 $\mu g.ml^{-1}$ titanium dioxide nanoparticles had the most positive effect on germination and seedling growth factors of Vitex plantlets.

Key words: Nanoparticles, Vitex (*Vitex agnus-castus* L.), Titanium, Germination indices, Response index

Introduction

Nanotechnology is one of the most extensive and attractive sciences that has many applications in various fields such as agriculture, industry, biotechnology, electronics, medicine, energy, and life sciences. (Nair & Chung, 2014; Tripathi et al., 2017; Tiwari et al., 2019). Research on nanoparticles is increasing rapidly due to distinct physical and chemical properties such as particle morphology, larger surface area, pore size, high reactivity, and physical and chemical properties related to particle size (Siddiqui et al., 2015). Today, large amounts of nanoparticles for use in various disciplines and industry research have led to an increase in their applications. These nanoparticles and their intermediate compounds interact with plants, animals, and the other living systems and expose positive and negative effects (Tripathi et al., 2017). Most nanoparticles have been reported to have toxic effects even at very low concentrations and affect the morphophysiological, biochemical, and molecular properties of living systems. However, some nanoparticles also have positive effects on plants by increasing their growth and protecting them against various stresses (Syu et al., 2014; Rico et al., 2015; Tripathi et al., 2016). In recent years, the use of titanium dioxide nanoparticles due to their biological properties has attracted much attention from plant physiologists (Qi et al., 2013). Dioxide nanoparticles have high soluble cyanides and can serve as water-soluble carriers, iron oxides, ammonium salts, and energy-soluble kinases (Kenanakis & Katsarakis, 2014). TiO_2 nanoparticles are available in three crystalline forms of anatase (a quadrilateral with a density of 0.8 $g.cm^{-3}$), rutile (a quadrilateral with a density of 2.9 $g.cm^{-3}$) and brukite (amorphous with a density of 0.5 $g.cm^{-3}$) (Afzali & Maghsoodlou, 2016). Titanium is the ninth most abundant element in the earth's crust, albeit secondarily in a transition metal, and evidence suggests that it can increase crop yields by about 10 to 20 percent (Feizi et al., 2013). It can also stimulate the absorption of some elements such as nitrogen, phosphorus, calcium, magnesium, iron, manganese and zinc (Marschner, 2011). Nano-TiO_2 is capable of enhancing plant fresh and dry weight by improving light absorption and activity of Rubisco (Mingyu et al., 2007) as well as by increasing nitrate uptake and accelerating the conversion of inorganic matter (Nair et al., 2010). Du et al. (2011) showed that due to nanoparticles the growth rate of wheat (*Triticum aestivum*) roots were more than shoots, also compared to control, both roots and shoots showed significantly higher growth. Jaberzadeh et al. (2013) reported that Nano titanium dioxide improved wheat growth and its yield components under drought stress. Combination of Tio_2 and Sio_2 nanoparticles in mungbean (*Vigna radiate*) and chickpea (*Cicer arietinum*) increased nitrate reductase activity, germination and growth (Mahajan et al., 2011). Studies have shown that germination of tomato (*Solanum lycopersicum*), lettuce (*Lactuca sativa*) and onion (*Allium cepa* L.) seeds increased in the presence of TiO_2 and after exposure to light (Elghniji et al., 2014). Despite research on crops, there is still limited information on the effects and physiological changes induced by these nanoparticles on germination and growth of plants, especially medicinal and forest plants.

Vitex is the largest genus in the Lamiaceae family, comprising of 250 species worldwide (Rani & Sharma, 2013). Species of this genus are shrubs. One of the most important species used in medicine is the Vitex or Chaste tree (*Vitex agnus-castus* L.) and grown in natural ecosystems in the Mediterranean regions of southern Europe and Central Asia by the riversides and along the waterways (Rani & Sharma, 2013). The fruit of this plant is used to treat menstrual problems in women, including premenstrual syndrome, disruption of estrogen and progesterone hormone balance, menopause, menopausal complications, hyperprolactinemia (Carmichael, 2008; Dugoua et al., 2008). In Iranian traditional medicine, its leaves and fruits are also used to increase human milk production (Azadbakht et al., 2005). One of the major problems in the cultivation and operation of the Vitex is the lack of proper germination and consequently lack of proper establishment in the field conditions. Therefore, it is necessary to apply treatments to improve its germination. The effect of nanoparticles on Vitex seed germination indices has not been studied so far, and due to applications and use of nanoparticles in agronomy and the end-production and cultivation of different crops, it is the goals of this study to investigate the effects of different concentrations of nanoparticles on germination characteristics of Vitex seeds.

Materials and Methods

Plant Material and Treatment Levels

Vitex seeds were obtained from Dashtyar Isfahan Company in 2018. Seeds were then sterilized with 7% sodium hypochlorite for 10-15 minutes and rinsed three times with distilled water. Then, 25 seeds were located in each 10 cm diameter Petri-sterile. After adding 10 ml of each treatment solution to the Petri, the prepared petri was transferred to a germinator at 25±1°C. Seeds were counted on a daily basis. The germination criterion was when roots of 2 mm length. Distilled water used as control and concentrations of titanium dioxide nanoparticles of 10, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 $mg.l^{-1}$ titanium dioxide nanoparticles were applied as treatments with four replications. Titanium dioxide nanoparticles were obtained from Nutirino Co., Tehran. To provide a uniform solution of titanium dioxide, in addition to the usual shaker, the solution was placed in an ultrasonic bath for 30 minutes and then added to the Petri dishes.

Study factors

Characterization of nanoparticles including spectroscopy, purity and particle size was performed by nanoparticle sizer and XRD device at Mashhad Central Laboratory, Ferdowsi University of Mashhad. At the end of the experiment, after measuring the root and shoot length by the ruler, the weights of the plant parts were measured with digital scales at the accuracy level of mg. These organs were then placed in an oven at 72 °C for 48 hours and weighed to determine the dry weight (Yang et al., 2007). The following equations were used to calculate germination parameters.

Equation 1) Germination percentage

$$GP = \frac{GN}{SN} \times 100$$

Where *GN* is the total number of germinated seed on the last day of counting and *GN* is the total number of germinated seed (Panwar & Bhardwaj, 2005).

Equation 2) Relative germination percentage

$$RGP = \frac{GP(treatment)}{GP(control)} \times 100$$

Where *GP* (treatment) is the germination percentage of the treatment and *GP* (control) the germination percentage (Panwar & Bhardwaj, 2005).

Equation 3) Germination rate

$$GR = \sum_{n}^{i} \frac{Si}{Gi}$$

Where *Si* is the number of germinated seeds per desired day and *Gi* is the number of days elapsed since the start of the experiment (Panwar & Bhardwaj, 2005).

Equation 4) Mean germination time

$$MGT = \sum_{i} \frac{Gi \times i}{\sum_i Gi}$$

Where *Gi* is the number of germinated seeds per day and *i* is the number of days since planting (Kulkarni et al., 2007).

Equation 5) germination index

$$GI = \frac{(\sum(N - i) \times Gi) \times 100)}{N \times GN}$$

Where *Gi* is the number of germinated seeds on day *i*, *i* the number of days sown, *N* the duration of the experiment or the number of study days, and *GN* is the total number of germinated seeds (Wu & Du, 2007).

Equation 6) Weighted germination index

$$WGI = \frac{[(N \times n1 + (N-1) \times n2 + (N-2) \times n3 + \cdots]}{N \times N'}$$

Where *N* is the total number of study days, N' is the total number of seeds, *n1*, *n2*, *n3*, etc. The number of germinated seeds on the first, second, third day, etc. (Wu & Du, 2007).

Equation 7) Vigour Seedling Index

(Kandil et al., 2015)

$$SVI = GP \times Seedling\ Length\ (Root + Shoot)$$

Equation 8) Optimal Seedling Index

(Vashisth & Nagarajan, 2010)

$$SOI = GP \times Dry\ Weight$$

Equation 9) Response Index

(Kandil et al., 2015) If control (*C*) exceeds treatment (*T*) then

$$RI = \frac{T}{C} - 1$$

If treatment (*T*) is greater than control (*C*) then

$$RI = 1 - C/T$$

Treatments RL (root length), PL (shoot length) and WW (fresh weight of seedling) and DW (dry weight of seedling) was measured.

Statistical analysis was performed using SPSS software version 16 for analysis of variance with the assumption of normality of data. Comparisons were made by Duncan's multiple range test at 5% probability level.

Results

Characterization of titanium dioxide nanoparticles

The crystalline properties of titanium dioxide nanoparticles were investigated by X-ray diffraction (XRD) and the results are presented in (Fig. 1). XRD measurements showed that the titanium dioxide nanoparticles used are in the form of anatase (Fig. 1). According to the size of titanium dioxide nanoparticles which was measured by Nanoparticle size analyzer and shown in (Fig. 2), mean diameter of nanoparticles was 98.87, (D mean), particle hydrodynamic diameter: 197.01 (Zaverage), and particle dispersion index was 0.2050 (PDI).

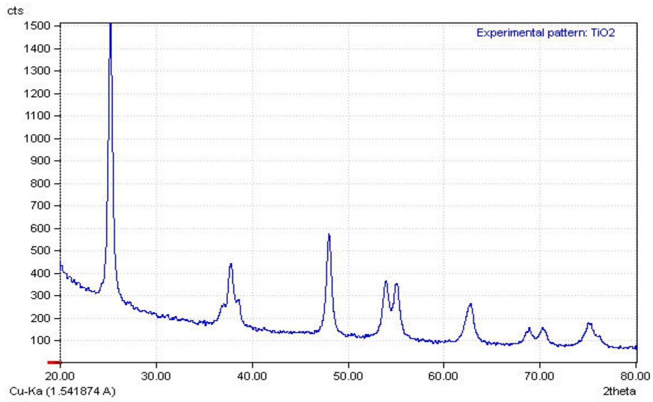

Figure 1. *XRD pattern of TiO2 nanoparticles.*

Influence of titanium dioxide nanoparticles on germination indices

Analysis of variance showed that treatment with titanium dioxide nanoparticles showed statistically significant changes in most germination and seedling growth traits and the results are summarized in (Table 1).

A comparison of mean germination percentage at different concentrations of titanium dioxide nanoparticles showed that only 100 and 200 µg.ml-1 nanoparticles applied, increased germination percentage of Vitex. The highest germination percentage (80%) was obtained from 200 $\mu g.ml^{-1}$ titanium dioxide treatment, which was 15% more than control (Table 2).

However, higher concentrations decreased germination percentage and the lowest germination percentage was observed in 800, 900 and 1000 $\mu g.ml^{-1}$ titanium dioxide treatments which decreased by 42, 51 and 51% compared to control, respectively (Table 2).

A comparison of the mean relative germination percentage at different levels of titanium dioxide showed that 100 and 200 $\mu g.ml^{-1}$ treatment showed the highest relative germination percentage (80%) and relative germination percentage increased by 65% (Table 2). The lowest relative germination percentage (50%) was observed at concentrations of 900 and 1000 $\mu g.ml^{-1}$ (Table 2).

Table 1. *Results of ANOVA tests for the effects of TiO_2 NPs different concentrations on (Vitex agnus-castus L.) studied parameters.*

GP	RGP	MGT	GR	GI	WGI	SVI	RL	PL	WW	DW	SOI
.00	.001	.584	.021	.046	.039	.006	.008	.995	.01	.041	0.04

GP: Germination percentage, RGP: Relative germination percentage, MGT: Mean germination time, GR: Germination rate, GI: Germination index, WGI: Weighted germination index, SVI: Vigor seedling index, RL: Root length, PL: Shoot length, WW: Seedling fresh weight, DW: Seedling dry weight, SOI: Optimal Seedling Index.

Table2. *Mean value of Germination Percent (GP%), Relative Germination Percent (RGP%), Mean Germination Time (MGT), Germination Rate (GR), Germination Index (GI) and Weighted Germination Index (WGI) for seeds of (Vitex agnus-castus L.) under TiO2 NPs different concentrations.*

TiO_2 Concentration (ppm)	GP%	RGP%	GR (N/day)	MGT (day)	GI (day)	WGI
Control	69.33ab	69.33c	17.48ab	64.19ab	145ab	1.78ab
10	36.00c	51.91c	8.17bc	68.60ab	76.85bc	0.94bc
100	68.00ab	98.07ab	16.39ab	69.89ab	149ab	1.84ab
200	80.00a	115a	22.37a	66.23ab	190a	2.30a
300	52.00bc	75.00c	10.74bc	65.54ab	96.38bc	1.20bc
400	52.00bc	75.00bc	10.92bc	65.96ab	100bc	1.25bc
500	52.00bc	75.00bc	15.28abc	70.50ab	132abc	1.61abc
600	48.00bc	69.23c	6.30c	65.56b	54.85c	0.72c
700	48.00bc	69.23c	10.83bc	69.77ab	98.47bc	1.22bc
800	40.00c	57.69c	9.84bc	66.74ab	84.90bc	1.04bc
900	34.00c	50.00c	9.20bc	72.29a	83.90bc	1.02bc
1000	34.00c	50.00c	7.77bc	68.90ab	72.19bc	0.89bc

Means followed by different letter(s) show significant difference at ($P<0.05$) significance level according to the Duncan's multiple ranges test

Treatments that could have a higher germination percentage in less time would also have a higher germination rate. A comparison of the mean germination rate showed that different levels of TiO_2 had different effects on the germination rate. Some treatments decreased the germination rate and some increased it compared to control. The lowest germination rate (3.6 seeds/day) was obtained in 600 $\mu g.ml^{-1}$ treatment, which was 63% lower than control (Table 2). The highest germination rate was also observed in 200 $\mu g.ml^{-1}$ treatment with anincrease of 27% compared to control (Table 2). The positive effect of Nano titanium dioxide on seed germination rate has also been reported in other studies (Feizi et al., 2013).

Undoubtedly, with the introduction of nanoparticles into the seeds, permeability, water and oxygen entry pathway would increase as well therefore germination changes would be expected (Fathi et al., 2017). The positive effects of titanium dioxide on the percentage and rate of germination of sage (Hatami et al., 2014), bitter myrtle (Hatami et al., 2014), onion (Laware & Raskar, 2014) and fennel (Feizi et al., 2013) have also been reported. Titanium dioxide improves seed germination by increasing seed water uptake and oxygen uptake (Zheng et al., 2005). As Nano-titanium particles enter the seed cells, the redox oxidation reactions are intensified by superoxide ion radicals, leading to the release of free radicals into the germinating seeds, as well as the oxygen produced in such a young process. (Zheng et al., 2005; Pais, 2008). Some studies have found that improving the activity of nitrate reductase and other antioxidants is an effective factor in improving the germination properties of Nano-titanium dioxide-treated seeds (Lu et al., 2002). Clement et al. (2013) showed that soaking the seeds in anatase titanium oxide nanoparticles at 100 $mg.L^{-1}$ had a positive effect on germination and root growth of flax plants. These positive effects could be due to the antimicrobial properties of the TiO_2 crystal structure, which increases the plant's resistance to stress. However, there are also reports pointing at adverse effects of titanium nitric oxide on the germination of some seeds such as tobacco (Frazier et al., 2014) and grass (Azimi et al., 2014). In this experiment, high concentrations of TiO_2 decreased germination indices. This result indicated that as the concentration of nanoparticles increased, their uptake by the seed coat probably increased and caused toxicity in the seed, which decreased with water absorption and decreased germination. Yang and Watts (2005) reported that the effect of Nano titanium dioxide on studied plants (radish, ryegrass, lettuce, maize and cucumber) caused toxicity in the plant and even inhibited root growth. The effects of nanocomposites on plants appear to be different depending on the plant type, growth stage, type of nanoparticle, physicochemical properties of the particle and its amount under different conditions (Aghdam et al., 2016). There was no statistically significant difference between the mean of different levels of titanium dioxide for the mean germination time (Table 1).

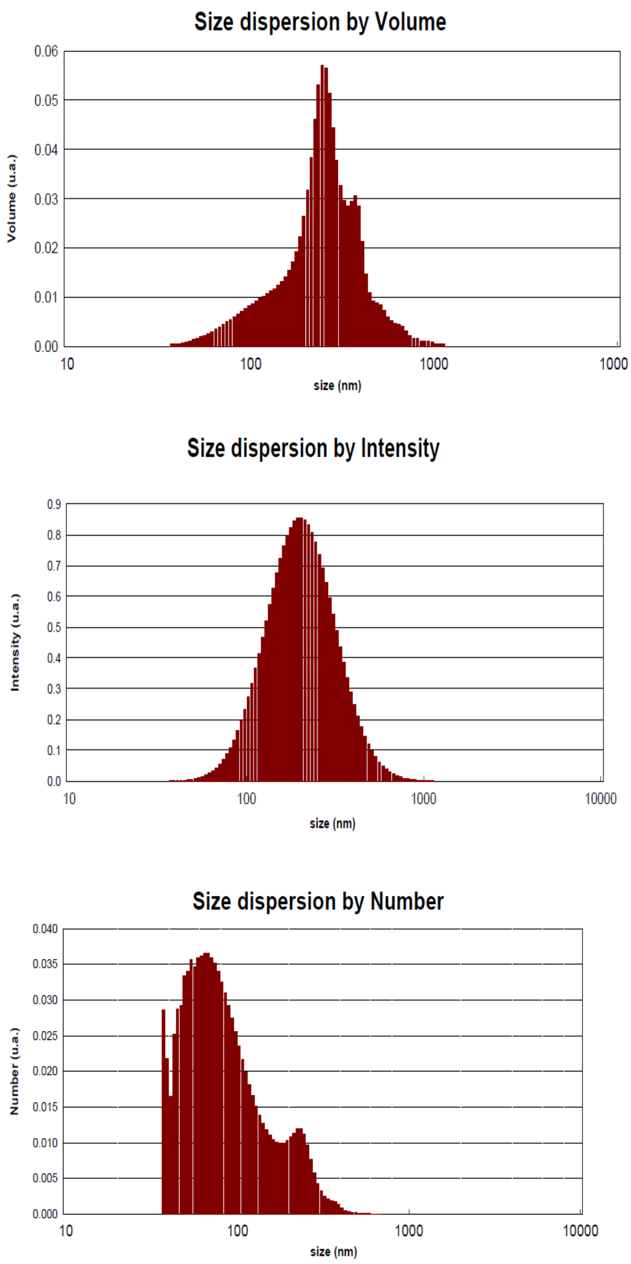

Figure 2. *Size of titanium dioxide nanoparticles measured by nanoparticle sizer with: a) scattered intensity, b) scattered volume, and c) scattered number*

The highest germination time was obtained at a concentration of 900µg.ml-1, which was 8% more than control (Table 2).

In general, the germination index is one of the important parameters in determining seed germination and is directly related to quality and bioenergy of seeds.

A comparison of the mean germination index showed that among different levels of titanium nitric oxide, the germination index increased only at two concentrations of 100 and 200 µg.ml^{-1}, (Table 2). The highest germination index of 190 was obtained under 200 µg.ml^{-1} with 31% higher compared to control.

The maximum germination index was observed under 600 µg.ml^{-1} treatment. The maximum and minimum were 2.3 and 0.72 under concentrations of 200 and 600 µg.ml^{-1}, respectively. It should be noted that the weighted germination index also increased only at concentrations of 100 and 200 µg.ml^{-1} compared to the control treatment. The vigor seedling index represents the germination percentage and germination potential. This index is one of the most important traits whose increase is a sign of increased quality and germination energy of the seed. Quantitatively this trait is the product of seedling length by germination percentage therefore and treatment which increases seedling length and germination percentage is able to enhance the seeds vigor. The lowest value of Vigour Seedling Index was obtained at concentrations of 900 and 1000 µg.ml^{-1} which showed a 20% decrease compared to the control (Table 3).

The highest value of this index was observed at 200 µg.ml^{-1} with an increase of 186% compared to control (Table 3). Zhang et al. (2008) examined the effect of Nano titanium dioxide on spinach seed germination and showed that Nano-particle treatment significantly increased the germination index and seedling vigor index. Feizi et al. (2013) suggested that increasing the vigor index of the seed was beneficial and could improve seedling establishment by nanoparticles, which is due to biological activity and biological mobility of nanoparticles influenced by factors such as their size, shape, chemical nature, stability and bioavailability.

Impact of Titanium Dioxide Nanoparticles on Seedling Growth and Biomass

Titanium is a beneficial element for plants and stimulates their growth (Nair et al., 2010). The subsequent effects of these nanoparticles on seedling growth and biomass were also investigated. By comparison, the lowest root length (3.3 mm) was obtained in control plants and the highest root length (26.0 mm) was obtained in 10 µg.ml^{-1} titanium dioxide treatment (Table 3). There was no statistically significant difference in shoot length between different concentrations of nano titanium dioxide (Table 1). The shortest shoot length (24.32 mm) was observed in control and the longest shoot length (36.6 mm) was observed in 1000 µg.ml^{-1} titanium dioxide (Table 3).

A comparison of the mean seedling fresh weight showed that the highest seedling fresh weight as 0.53 mg was observed under 200 µg.ml^{-1} Nano titanium dioxide, which was 32% more than control (Table 3). The lowest seedling fresh weight (0.17 mg) was obtained under 600 µg.ml^{-1} treatment, which was 57% lower than the control (Table 3). According to the analysis of variance, no significant differences were observed between seed treatments for the seedling dry weight (Table 1). The highest seedling dry weight (0.5 µg.ml^{-1}) was observed in 1000 µg.ml^{-1} titanium dioxide and the lowest was observed in the control treatment

Table 3. *Mean value of Seedling Vigor Index (SVI), Radicle Length (RL), Plumule Length (PL), Wet Weight (WW) and Dry Weight (DW), Seedling Optimum Index (SOI) for Seedling of (Vitex agnus-castus L.) under TiO_2 NPs different concentrations.*

TiO_2 Concentration (ppm)	SVI	RL(mm)	PL(mm)	WW(mg)	DW(mg)	SOI
Control	189[bc]	3.66[d]	24.33 [a]	0.406[abc]	0.0087[d]	1.51[a]
10	196[bc]	26.66[a]	26.33 [a]	0.333[bcd]	0.0140[cd]	0.86[a]
100	340[bc]	17.66[ab]	27.33 [a]	0.439[ab]	0.0207[bc]	3.33[a]
200	541[a]	11.00[bc]	28.33 [a]	0.529[a]	0.0223[bc]	0.69[a]
300	378[ab]	12.66[bc]	29.33 [a]	0.324[bcd]	0.0290[bc]	0.75[a]
400	233[bc]	18.33[ab]	31.67 [a]	0.198[d]	0.0323[bc]	1.68[a]
500	287[bc]	25.00[a]	31.67[a]	0.239[bcd]	0.0413[b]	6.60[a]
600	188[bc]	11.00[bc]	31.67 [a]	0.171[d]	0.0483[b]	1.37[a]
700	170[c]	7.66[c]	32.67 [a]	0.217[cd]	0.0553[b]	7.58[a]
800	271[bc]	14.33[bc]	35.00 [a]	0.258[bcd]	0.0560[b]	1.68[a]
900	151[c]	14.66[bc]	35.00 [a]	0.250[bcd]	0.1400[a]	1.92[a]
1000	154[c]	11.66[bc]	36.67 [a]	0.284[bcd]	0.1413[a]	1.93[a]

Means followed by different letter(s) show significant difference at ($P<0.05$) significance level according to the Duncan's multiple ranges test.

Table 4. *Inhibition index value (RI) of TiO_2 NPs different concentrations on seed Germination Percent (GP), Mean Germination Time (MGT), Germination Rate (GR), Germination Index (GI) and Weight Germination Index (WGI) of (Vitex agnus-castus L.)*

Tio_2 Concentration (ppm)	GP	MGT	GR	GI	WGI
10	-0.94	+0.06	-0.42	-0.47	-0.99
100	-0.01	+0.08	-0.06	+0.02	+0.03
200	+0.13	+0.03	+0.22	+0.22	+0.22
300	-0.24	+0.02	-0.38	-0.33	-0.32
400	-0.24	+0.02	-0.38	-0.31	-0.29
500	-0.24	+0.08	-0.12	-0.08	-0.09
600	-0.30	+0.02	-0.63	-0.62	-0.99
700	-0.30	+0.07	-0.38	-0.32	-0.31
800	-0.42	+0.03	-0.43	-0.41	-0.41
900	-0.50	+0.11	-0.47	-0.42	-0.42
1000	-0.50	+0.06	-0.55	-0.50	-0.99

(Table 3). The lowest optimal seedling index was observed at 10 $\mu g.ml^{-1}$ nitric oxide, which was lower than the control, and the highest optimum index was observed under 700 $\mu g.ml^{-1}$.

Titanium dioxide elongates plant organs such as root and shoot by increasing cell division (Farahani et al., 2012). It seems that titanium dioxide nanoparticles act similarly to cytokinin and gibberellin and stimulate cell division and increase cell volume (Mandeh et al., 2012). Gao et al. (2008) reported that titanium nanoparticles can enhance biomass growth in spinach leaves by increasing Rubisco enzyme activity. According to Khodakovskaya et al. (2009), titanium dioxide nanoparticles improved germination and root growth by increasing uptake by the thick shell of tomato seed and increasing water uptake around the seed. Water absorption is important in seed germination because mature seeds are relatively dry and require significant amounts of water to initiate cell metabolism and seed growth. Determining the moisture content of seed and nano-materials inside the seeds supports this hypothesis; however, the mechanism of specific penetration through the seed shell and enhancing water uptake by nano-materials is not completely clear. Positive effects of titanium on plant growth such as cowpea by increasing number and length of pods and the increasing number of seeds per pod (Owolade et al., 2008), increasing the fresh and dry weight of spinach seedlings by increasing nitrate uptake and leaf biomass (Mingyu et al., 2007; Yang et al., 2007), increasing seedling length and vigor of maize seed

Table 5. *Inhibition index value (RI) of TiO_2 NPs different concentrations on Seedling Vigor Index (SVI), Seedling Optimum Index (SOI), Radicle Length (RL), Plumule Length (PL), Wet Weight (WW) and Dry Weight (DW) on Seedling of (Vitex agnus-castus L.).*

TiO_2 Concentration (ppm)	SVI	SOI	RL	PL	WW	DW
10	0.03	-1.64	0.86	0.07	-0.17	0.42
100	0.44	0.54	0.79	0.10	0.06	0.96
200	0.65	-0.53	0.66	0.14	0.23	0.63
300	0.5	-0.50	0.71	0.17	-0.2	0.72
400	0.18	0.10	0.80	0.23	-0.52	0.75
500	0.34	0.77	0.85	0.23	-0.42	0.80
600	-0.005	-0.09	0.66	0.23	-0.57	0.83
700	-0.10	0.80	0.52	0.96	-0.47	0.85
800	0.30	0.10	0.74	0.30	-0.37	0.85
900	-0.20	0.21	0.75	0.30	-0.37	0.94
1000	-0.18	0.21	0.68	0.33	-0.3	0.94

RL (root length), PL (shoot length) and WW (fresh weight of seedling) and DW (dry weight of seedling).

(Buzea et al., 2007), increasing tomato seedling weight (Khodakovskaya et al., 2009), increasing fresh seedling weight of wheat (Mahmoodzadeh & Aghili, 2014), increasing fresh and dry weight of stem (Zhu et al., 2008), and an increasing number of apple leaves (Wojcik & Klamkowski, 2004) have been reported. Zhang et al. (2008) have reported that spraying titanium nanoparticles on cucumber leaves after several hours increased photosynthesis, which in turn increased root growth. Increased metabolism and growth of rice crop due to the use of Nano titanium dioxide have also been reported (Chutipaijit, 2015). In contrast, (Klancnik et al., 2011) reported that titanium dioxide nanoparticles did not affect the average root length and number of roots per tuber in onion. Thus, the uptake efficiency and the effect of nanoparticles on the growth and metabolic activities of different plants appear to be very different (Nair et al., 2010).

Response Index

Response index (RI) to different concentrations of titanium dioxide nanoparticles on germination and seedling growth indices are presented in (Tables 4 and 5).

The RI value is in the range of -1 and +1. Positive values indicate stimulation by treatment and negative values indicate inhibition compared to control. Titanium dioxide at all concentrations had a stimulating effect on mean germination time, root length, shoot length and dry weight (Tables 4 and 5). Also, titanium dioxide only had a stimulatory effect on germination percentage and germination rate in 200 $mg.L^{-1}$ treatment only. Treatments of 100 and 200 $mg.L^{-1}$ Nano titanium dioxide had a stimulating effect on the germination index and weighted germination index (Table 4). In general, titanium oxide nanoparticles had a stimulatory effect on most of the studied traits. However, its high concentrations also showed an inhibitory effect on some traits as shown in (Tables 4 and 5).

Conclusion

The Finally, it can be concluded that treatment with Nano titanium dioxide promoted germination and seedling growth, but this effect was not positive at all concentrations. Treatment of 200 $\mu g.L^{-1}$ titanium dioxide nanoparticles had the most stimulatory effect on germination and seedling growth factors of Vitex plantlets. The highest germination percentage, germination index, relative germination percentage, germination rate, germination index by weight, Vigor seedling index and seedling fresh weight were observed under 200 $\mu g.ml^{-1}$. As this concentration is relatively low, in addition to reducing the risk of adverse effects of the nanoparticles, it is also economically cost-effective and can be considered as a successful treatment to improve germination and early establishment of this valuable plant.

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