International Journal of Plant & Soil Science
3(4): 408-418, 2014; Article no. IJPSS.2014.007

SCIENCEDOMAIN *international*
www.sciencedomain.org

Effect of TiO_2 and TiO_2 Nanoparticle on Germination, Root and Shoot Length and Photosynthetic Pigments of Mentha Piperita

Niloofar Samadi[1*], Sima Yahyaabadi[2] and Zahra Rezayatmand[2]

[1]Department of Plant Physiology, Islamic Azad University, Falavarjan Branch, Isfahan, Iran.
[2]Department of Biology, Islamic Azad University, Falavarjan Branch, Isfahan, Iran.

Authors' contributions

This work was a part of the M. Sc thesis of the lead author NS. Author SY was the supervisor and author ZR was the advisor. The authors designed the study, managed the experiment and analyzed the samples. The author NS conducted statistical analysis, wrote the protocol and first draft of the manuscript. All authors read and approved the final manuscript.

Short Communication

Received 31st October 2013
Accepted 1st February 2014
Published 15th February 2014

ABSTRACT

Aims: Today, the use of nanoparticles has been studied in various fields of science. One of the sectors that is perhaps less dealt with is the physiology of medicinal plants. The present study is aimed at investigating the phytotoxicity or beneficial effects of titanium dioxide (TiO_2) and titanium dioxide nanoparticle (NP-TiO_2) treatments on seed germination, root and shoot length, photosynthetic pigments and possible inhibitory effects particularly NP-TiO_2 on Mentha piperita.
This study was evaluated in 2012-2013 in the Islamic Azad University of Falavarjan, Isfahan, Iran, research Laboratory.
Methodology: Seeds were treated with TiO_2 and NP-TiO_2. Each treatment was conducted with four replicates, and the results were presented as mean ±SE (standard error of the mean). Germination percentage, root and shoot length and photosynthetic pigments were analysed using the SPSS 18.
Results: The different concentrations of NP-TiO_2 and TiO_2 had a negative significant effect on germination percentage and shoot length. However, root length was significantly influenced by 100mg L^{-1} concentration of NP-TiO_2 rather than nonNP-TiO_2 concentrations. Pronounced effect on photosynthetic pigments (chlorophyll a and b and carotenoids) was

**Corresponding author: E-mail: n.samadi67@gmail.com;*

found in 200mg L^{-1} concentration of TiO_2 and 100mg L^{-1} concentration of NP-TiO_2.
Conclusion: The results of this experiment showed that TiO_2 in higher concentration had pronounced effects on photosynthetic pigments while lower concentration of NP-TiO_2 had significantly increased root length.

Keywords: Mentha piperita; TiO_2; NP-TiO_2; germination percentage; root length; shoot length; photosynthetic pigments; medicinal plant.

1. INTRODUCTION

Nanoparticles are particulate with size between 1-1000nm. Nanotechnology has many applications in different research areas such as agriculture, medicine, etc. Today, NP-TiO_2 is widely used in industry [1]. NP-TiO_2 was the cause of changes in the industry and in research. This nanoparticle usually exists in crystal forms with three titles: rutile, anatase and brukite, and there are also noncrystallines. NP-TiO_2 is applied in coated surfaces, dipole electron tubes, optical module, disinfectant sprays, sporting goods, etc. It affects biological systems and physiologic parameter of plants. NP-TiO_2 has various effects on redox systems oxygen (ROS) in the presence of UV radiation [2]. NP-TiO_2 has increased effect on seed germination of fennel (Foeniculum vulgare Mill) [3]. NP-TiO_2 can stimulate antioxidant system, enhance abilities of absorbing and utilizing water, and hasten germination and growth in Glycine max [4].

Titanium is the ninth most galore element in the earth crust [3]. TiO_2 is the oxide of the metal titanium, and belongs to the family of transition metal oxides [5]. It is insoluble in water and thermally stable [6]. The research showed that the effects of TiO_2 on plants increase chlorophyll in paprika (*Capsicum annuum* L.) and green algae (*Chlorella pyrenoidosa*) [7].

Mentha piperita is a herbaceous, perennial, glabrous and strongly scented plant and belongs to the family Lamiaceae (Labiatae). The square stem is usually reddish-purple and smooth, and its leaves cross it over. They are short (2.5-5 cm), oblong-ovate and serrate [8,9] .*M. piperita* is a sterile hybrid mint, a cross between *Mentha aquatic* and *Mentha spicata* and it is sold through its transplants and cultivated species. It is found wild in central and southern Europe and native in Canada and US and has been naturalized in many parts of India [8,10].

Chlorophylls (Chl) and Carotenoids (Car) are essential pigments of higher plant assimilatory tissues and responsible for variations from dark-green to yellow. Car and Chl are the main pigments of green leaves. Chl can absorb light energy from the sun and change it into chemical energy by the carbon organic compounds existing in cells [11]. Leaf Chl content varies within wide limits. The highest Chl content in plants occurs at the outset of the flowering phase and Chl is part of the process of organogenesis [12].

Carotenoids (Car) are a class of natural fat-soluble pigments mainly in plants, algae and photosynthetic bacteria where they also play a critical role in the photosynthetic process. Carotenoids are usually represented by two (α and β) carotenes and xanthophylls (lutein, zeaxanthin, violaxanthin), which exhibit strong light absorption in the blue region of the spectrum and are non-uniformly distributed in photosystems and individual pigment-protein complexes of chloroplasts [13]. They play a role in attracting pollinators and in seed dispersal [11].

Some reports had demonstrated that TiO_2 and NP-TiO_2 have a negative impact on seed germination and growth and can induce phytotoxicity. Using TiO_2 can discourage fennel seed germination [14]. NP-TiO_2 could encourage and accelerate seed germination by helping water absorption in spinach seeds and inhibit photosynthesis in *Chlamydomonas reinhardtii* [15,16]. NP-TiO_2 at higher concentration decreased the shoot and root length of radish, rape, corn, lettuce and cucumber [17]. The authors relate that nanoparticle had inhibitory effects on seed germination and root length [18]. Some of the studies relate nanoparticle uptake by plant roots to shoot transfer. NP-TiO_2 was accumulated and translocated to the shoots of *Arabidopsis thaliana*. NP-TiO_2 promotes the photosynthesis and improves growth of spinach [18,19]. The soaking of flax seeds in the suspensions of anatase-nanoparticle has had an increased effect on seed germination and root growth [20].
A few study has been done on the effects of NP-TiO_2 and TiO_2 on various plants such as *Triticum aestivum,* Zea mays*,* Salvia officinalis [3,17,21] but *Mentha piperita* is one of the most important medicinal plants in the world. Unfortunately, although it is used in treatment of many diseases such as nausea, emphysema, gastric stimulations, etc., it has been less studied. In this study, we decided to find out the phytotoxicity or beneficial effects of different concentrations of NP-TiO_2 and TiO_2 on seed germination, root and shoot length and photosynthetic pigments of *Mentha piperita*. The reason for choosing the seed of this plant rather than other parts was that its seed is difficult to cultivate and if germination is accelerated [22], we can contribute to the increase of this plant. Moreover, their effect on root and shoot length and the amount of photosynthetic pigments is physiologically of much importance, because better growth of root will lead to the increase of plant access to water and food resources in the environment it lives in. Stem growth increase will also lead to better absorption of light when competing with other plants. As stated before, pigments are the most important factor for absorbing the light needed for photosynthesis; and photosynthesis is considered a vital process for any plant.

2. MATERIALS AND METHODS

In this study, we examined the effect of TiO_2 and NP-TiO_2 on seed germination, root and shoot length and photosynthetic pigments of Mentha piperita. An experiment was carried out in Laboratory Research of Islamic Azad University of Falavarjan, Isfahan, Iran.

2.1 Chemicals

Different concentrations of Titanium dioxide (TiO_2, 99.99%, Merck) and Titanium dioxide nanoparticle (NP-TiO_2, 99.5%, 10^{-10} A, Merck) were prepared by suspending 0, 100, 200 and 300 mg L^{-1} in double distilled water through ultrasonication (100W, 60 KHz) for 180 minutes.

2.2 Seeds

Seeds were collected from the Pakan Bazr institute, Isfahan, Iran, and were used in this study.

2.3 Seeds Germination

Mentha seeds were immersed in a 2.5% sodium hypochlorite solution for 15 minutes for sterilization. After rinsing for two times with double distilled water, 10 healthy and uniformly-sized seeds were selected and then sown at equal distance in petri dish (90mm x15mm) lined with filter paper (Whatman No.42, Ashless, England). Then 2ml of double distilled water with TiO_2 and NP-TiO_2 suspensions was added. In every experiment, control seeds were also taken for comparison with the treated ones. And for the control, only double distilled water was added to the petri dish. Petri dish was kept inside the culture chamber in a dark environment because light is one of the preventers of germination. Therefore, we supplied a dark condition for our seeds like the one under the soil; and after the germination process, we put them in light condition and soil to continue their normal growth.

During the experiment, germinated seeds were counted daily and were irrigated with TiO_2 and NP-TiO_2 suspensions (Fig.1), Following 10 days of treatment, germination percentage was calculated using the related formula. Additionally, root length and shoot length were measured using a ruler.

Fig. 1. Germination of seeds (control)

2.4 Measure of Pigments

Pigments were extracted by grinding 0.2g freshly sampled leaves in 80% acetone at room temperature. Chlorophyll and carotenoids were measured using absorbent recorded at 647nm, 663nm and 470nm for maximum absorption of chlorophyll-a, chlorophyll-b and carotenoid, respectively. The extinction coefficients were determined by a UV-Vis spectrophotometer (Unico-UV2100). Evaluations were made for the characters of chlorophyll (Chl) and Carotenoids (Car). The amounts of Chl (a, b) and Car were measured in accordance with the method cited in Lichtenthaler [23].

2.5 Statistical Analysis

This study was carried out as a factorial experiment in a complete randomized design with four replications. The results were presented as mean± SE (standard error of the mean).

The data were analysed using the SPSS18 software. The significant levels of difference for all measured traits were calculated and the means were compared by Duncan's multiple range tests at 5% level.

3. RESULTS AND DISCUSSION

Table 1 shows the effect of TiO_2 and NP-TiO_2 concentrations on seed germination, root length, shoot length and photosynthetic pigments.

Table 1. Calculated mean square values from the statistical analysis corresponding to data collected between TiO_2 and NP-TiO_2 of *Mentha piperita*

Mean square							
Sources of Variation		**%Germination**	**Root length**	**Shoot length**	**Chl a**	**Chl b**	**Carotenoid**
TiO_2 and Np-TiO_2(A)	1	22.781^{ns}	0.845**	0.217^{ns}	1.014E-02**	0.175**	4.623E-02**
Treatment(B)	3	1032.365*	8.850*	9.510*	8.005*	2.644*	0.356*
A*B	3	8.615^{ns}	1.549**	1.089**	3.062*	0.458*	0.152*
Error		97.365	0.189	0.349	1.675E-02	1.222E-02	3.099E-03

*Note: Ns: non-significant, * P < 0.01; ** P < 0.05*

3.1 Germination

The effect of different concentrations of TiO_2 and NP-TiO_2 on the germination compared to the control was not significant statistically and seed germination percentage decreased when exposed to concentrations of TiO_2 and NP-TiO_2 compared to control group. TiO_2 and NP-TiO_2 concentration in 300 mg L^{-1} caused to the inhibited germination, so we showed them just in Fig. 2 and ignored them in other figures. See Fig. 2. Other studies indicated that the germination index for NP-TiO_2 was consistently reduced with an increase in concentration. The negative effect on seed germination suggests that the seeds were likely stressed by the presence of TiO_2 and NP-TiO_2 [24].

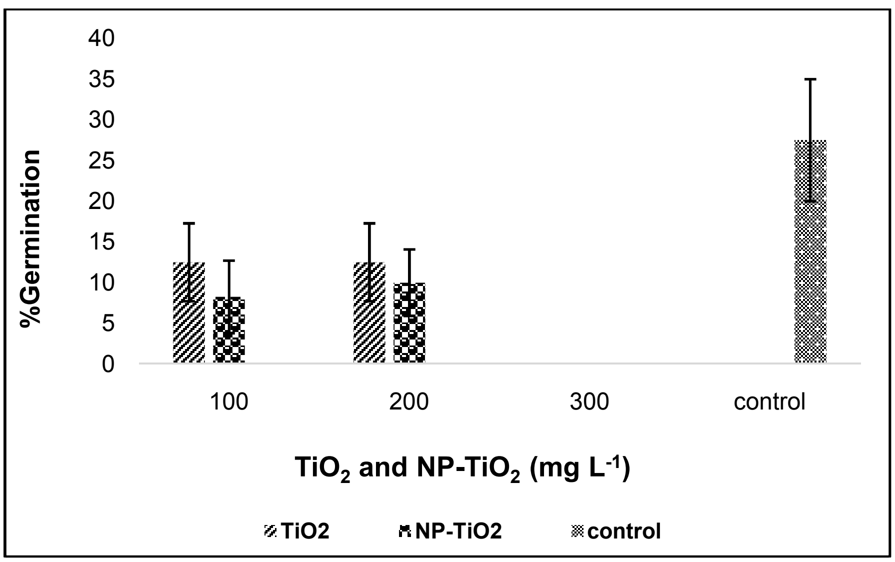

Fig. 2. Effect of different concentration of TiO_2 and NP-TiO_2 (0,100, 200, 300 mg L^{-1}) on germination

Mean ± S.E.M = Mean values ± Standard error of means of 4 replicates

3.2 Root Length

All of the concentrations of TiO_2 decreased the root length. The effect of 100 mg L^{-1} concentration of NP-TiO_2 on root length was significantly higher than the control Fig. 3. NP-TiO_2 at a suitable concentration had promotory effect and had an inhibitory effect in high concentrations on root length [17]. Increasing the concentration of TiO_2 and NP-TiO_2 induced aggregation of particles and clogging of root pores which interrupted water uptake by seeds [14]. The positive effect on root length could be due to the antimicrobial properties of the anatase crystalline structure of TiO_2 that increases plant resistance to stress [20].

3.3 Shoot Length

Shoot length of Mentha piperita can be seen in graphs presented in Fig. 4. TiO_2 concentrations in 200 mg L^{-1} saw the same effect as control group on shoot length. NP-TiO_2 had decreased the effect on shoot length. The reason could be due to the application of TiO_2 and NP-TiO_2 which has intensely decreased shoot biomass [3]. The same results were established by Chouychai et al. [25].

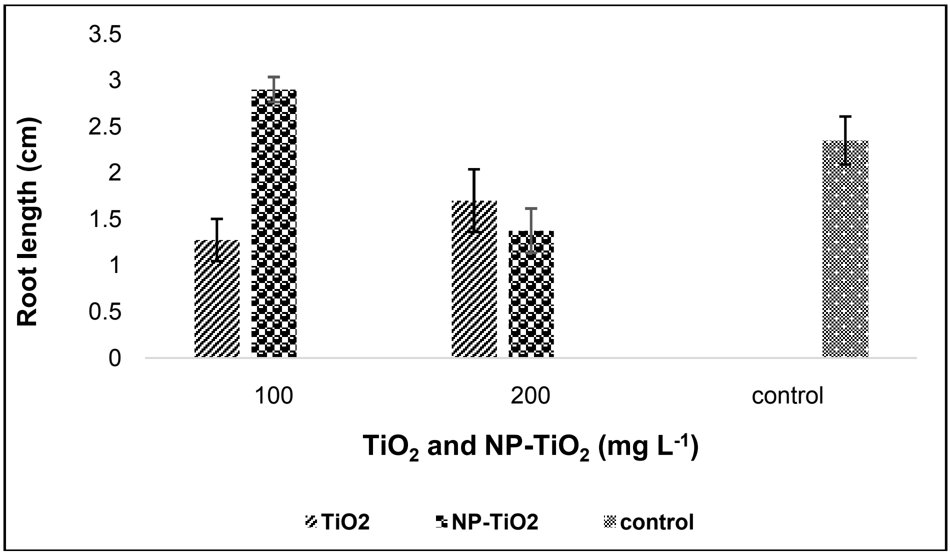

Fig. 3. Effect of different concentration of TiO_2 and NP-TiO_2 (0,100, 200, mg L^{-1} on Root length

Mean ± S.E.M = Mean values ± Standard error of means of 4 replicates

3.4 Photosynthetic Pigments

3.4.1 Chlorophylls

Results of measuring chlorophyll content in leaves of Mentha piperita can be seen in graphs presented in Fig. 5 and 6. TiO_2 concentration in 200mg L^{-1} and 100 mg L^{-1} concentration of NP-TiO_2 had significantly increased the amount of chlorophyll a and chlorophyll b. Other studies indicated that NP-TiO_2 and TiO_2 can raise the photosynthesis rate, chlorophyll formation and nitrogen metabolism at an optimum concentration. Results suggested that the physiological effects were related to the size of particles [15] and higher chlorophyll accumulation may be due to complementary effect of other inherent nutrients like Magnesium (Mg), Iron (Fe) and sulphur (S) [17]. NP-TiO_2 can improve the structure of chlorophyll, increase light absorbance, facilitate formation of pigments, better capture of sunlight and transfer of light energy to active electrons, chemical activities and having effect on photosynthesis [21]. The same results were established by [16,17,26].

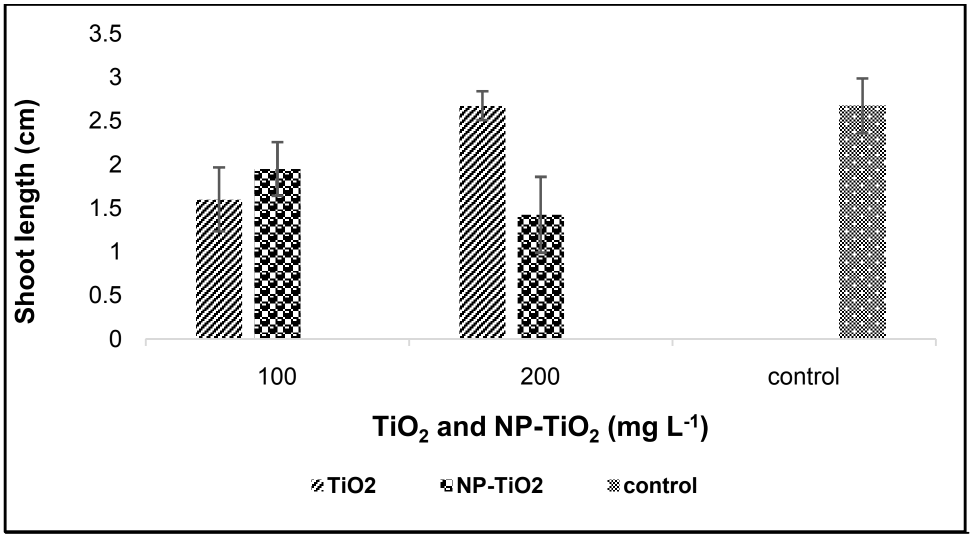

Fig. 4. Effect of different concentration of TiO_2 and NP-TiO_2 (0,100, 200 mg L^{-1}) on shoot length

Mean ± S.E.M = Mean values ± Standard error of means of 4 replicates

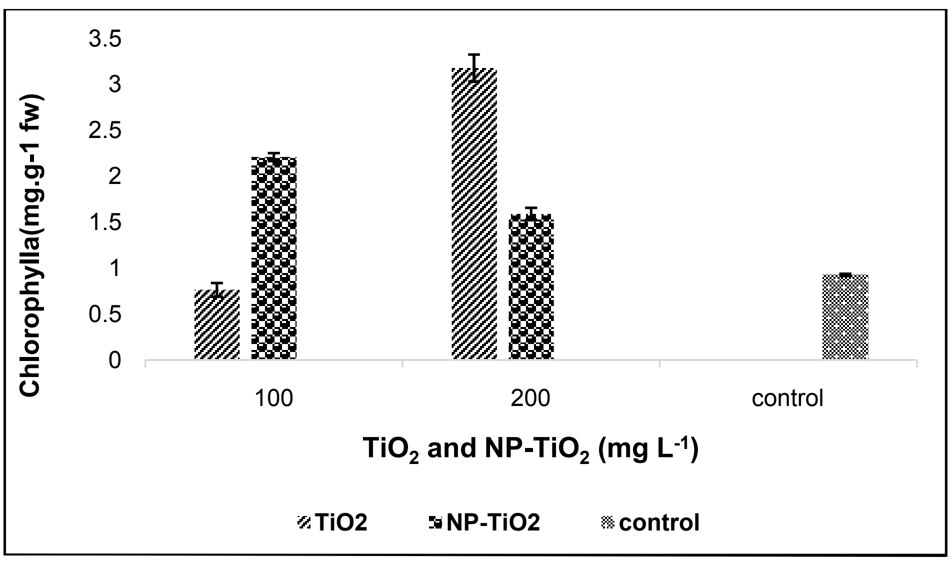

Fig. 5. Effect of different concentration of TiO_2 and NP-TiO_2 (0,100, 200 mg L^{-1}) on Chlorophyll a

Mean ± S.E.M = Mean values ± Standard error of means of 4 replicates

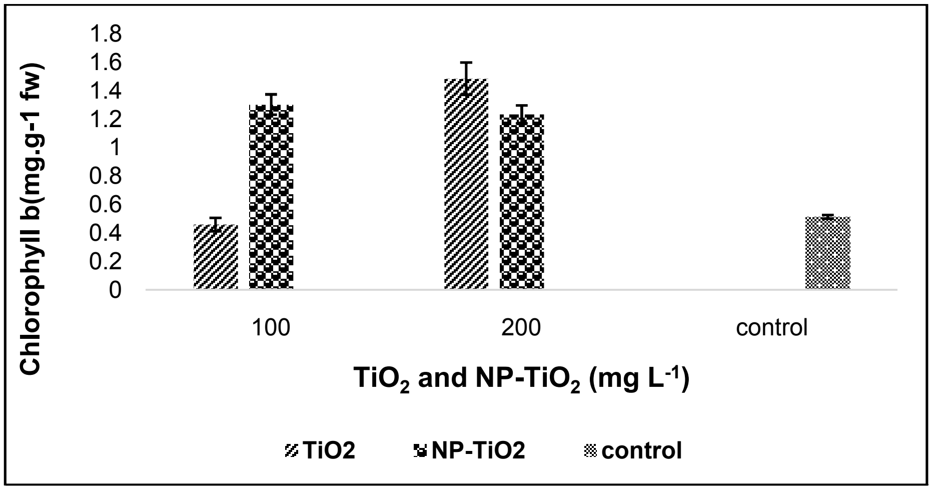

Fig. 6. Effect of different concentration of TiO_2 and NP-TiO_2 (0,100, 200 mg L^{-1}) on Chlorophyll b

Mean ± S.E.M = Mean values ± Standard error of means of 4 replicates

3.4.2 Carotenoids

TiO_2 and NP-TiO_2 concentrations in 200 mg L^{-1} and 100 mg L^{-1} had a significant stimulant effect on the amount of carotenoids in comparison with the control group Fig. 7.

Other studies reported that NP-TiO_2 had a significant increase in Zea mays pigments such as carotenoids and TiO_2 had decreased the effect on carotenoids of *Chlamydomonas reinhardtii* at first and then increased it significantly [16,21]. The carotenoid synthesis increases as a response to quenching ROS by heavy metals. Photosynthetic pigments are the most important internal factors. Decreased chlorophyll and carotenoids may be due to the fact that there is an oxidative [27]. In some cases, pigment loss is due to rising levels of toxic metals and metalloids [28].

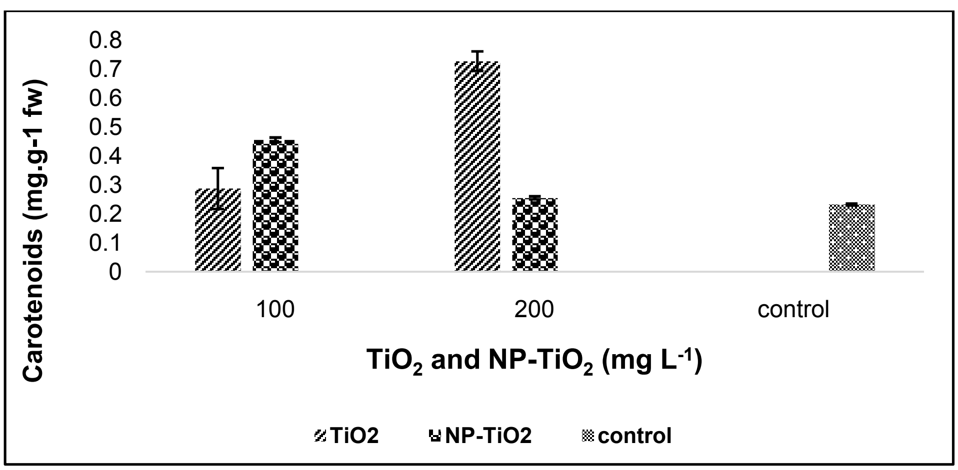

Fig. 7. Effect of different concentration of TiO_2 and NP-TiO_2 (0,100, 200 mg L^{-1}) on Carotenoids

Mean ± S.E.M = Mean values ± Standard error of means of 4 replicates

4. CONCLUSION

Mentha piperita L. is a medicinal plant and is used for food purposes. In order to understand the possible positive effects of applying nanoparticle in nutrient and medicinal industry, it is important to analyse penetration and transport of nanoparticles in plants. Results of our studies indicated that the applied NP-TiO_2 and TiO_2 were clearly toxic to seed germination, and experimental treatments had a negative effect on seed germination, while other studies reported that they had an increased effect on germination [15,29]. TiO_2 and NP-TiO_2 decreased the shoot length because they can decrease shoot biomass [3]. These results will help to understand inhibitory effects of TiO_2 and NP-TiO_2 and are important to the relationship between size and concentration of materials and their effects on germination and other parameters.

Although this study indicates the effects of TiO_2 and NP-TiO_2 in plant physiology, it seems necessary to study the effects of phytotoxicity mechanisms such as the size distribution of nanoparticles and possible positive effects of uptake and translocation of engineered nanoparticles by plants, because plants play an important role in human lives.

ACKNOWLEDGMENTS

Thanks of Miss Valivand (M.Sc.), and Lab Research of Islamic Azad University of Falavarjan, Isfahan, Iran.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mohanraj VJ, Chen Y. Nanoparticles– A Review. Tropical Journal of Pharmaceutical Research. 2006;5(1):561-573.
2. Kim E, Kim SH, Kim HC, Lee SG, Jeong SW. Growth inhibition of aquatic plant caused by silver and titanium oxide nanoparticles, Division of Nano & Bio Technology. Daegu Gyeongbuk Institute of Science and Technology (DGIST). 2010;3(1):1-6.
3. Feizi H, Kamali M, Jafari L, Rezvani Moghaddam P. Phytotoxicity and stimulatory impacts of Nano sized and bulk titanium dioxide on fennel (Foeniculum vulgare Mill). Chemosphere. 2013;91:506. DOI: 10.1016/j. chemosphere. 2012.12.012.
4. Lin D, Xing B. Phytotoxicity of nanoparticles: Inhibition of seed germination and growth. Environ Pollut. 2007;150(2):243-250. PMID. 17374428.
5. Li N, Ma L, Wang J, Zheng L, Dunan Y. Interaction between nano-anatase TiO_2 and liver DNA form Mice in vivo. Nanoscale Res Lett. DOI: 10.1007/s11671-009-9451-2. 2010;5:108-115.
6. About Titanium Dioxide. Cinkarna. 2012;9:14. Accessed 16 December 2013. Available: http://www.cinkarna.si/en/about-titanium-dioxide
7. Hurby M, Cigler P, Kuzel S. contribution to understanding the mechanism of titanium action in plant. Journal Plant Nutrition. 2002;25:577-598.
8. Alankar SH. A review on peppermint oil. Asian Journal of Pharmaceutical and Clinical Research. 2009;2(2):27-33.
9. Shah PP, Mello PMD. A review of medicinal uses and pharmacological effects of Mentha piperita. Natural Product Radiance. 2004;3(4):214-221.

10. Master data/monograph. Mentha piperita (Pfefferminze); 2007.
11. Uvalle Sauceda JI, Rodríguez G, Ramírez Lozano RG, Cantú Silva I, Gómez Meza MV. Seasonal Trends of Chlorophylls a and b and Carotenoids(x + c) in Native Trees and Shrubs of Northeastern Mexico. Conference on International Agricultural Research for Development. 2007;1-10.
12. Bojovic B, Stojanovic J. Chlorophyll carotenoid content in wheat cultivars as a function of mineral nutrition. Arch Biol Sci Belgrade. 2005;57(4):283-29.
13. Gitelson AA, Zur Y, Chivkunova OB, Merzlyak MN. Assessing carotenoid content in plant leaves with reflectance spectroscopy. Photochemistry and Photobiology. 2002;75(3):272–281.
14. Feizi H, Amirmoradi SH, Abdollahi F, Jahedi Pour S. Comparative effects of nanosized and bulk titanium dioxide concentrations on medicinal plant *Salvia officinalis* L. Annual Review & Research in Biology. 2013;3(4):814-829.
15. Zheng L, Hong F, Lu S, Liu C. Effect of nano-TiO_2 on strength of naturally aged seeds and growth of Spinach. Biological Trace Element Research. 2005;105:83-91.
16. Chen L, Zhou L, Liu Y, Deng S, Wu H, Wang G. Toxicological effects of nanometer titanium dioxide (nano-TiO_2) on Chlamydomonas reinhardtii. Ecotoxicology and Environmental Safety. 2012;84:155-162. DOI: 10.1016/j.ecoenv.2012.07.019.
17. Mahmoodzadeh H, Aghili R, Nabavi M. Physiological effects of TiO_2 nanoparticle on wheat (*Triticum aestivum*). Technical Journal of Engineering and Applied Sciences. 2013;3(14):1365-1370.
18. Laure C, Khodja H, Herlin-Boime N, Brisset F, Flank AM, Fayard B. Investigation of titanium dioxide nanoparticles toxicity and uptake by plants. Journal of Physics: Conference Series. 2010;1-7:304. DOI: 10.11088/1742-6596/304/1/012057.
19. Hong F, Zhou J, Liu C, Yang F Wu C, Zheng L. Effects of nano- TiO_2 on photochemical reaction of chloroplasts of spinach. Biol Trace Elem Res. 2005;105:269-279.
20. Clement L, Hurel C, Marmier N. Toxicity of TiO_2 nanoparticles to cladocerans, algae, rotifers and plants-Effects of size and crystalline structure. Chemosphere. 2013;90(3):1083-1090. DoI: 10.1016/j.chemosphere. 2012.09.013.
21. Morteza E, Moaveni P, Aiabadi Farahani H, Kiyani M. Study of photosynthetic pigments changes of maize (*Zea mays* L.) under nano TiO_2 spraying at various growth stages. Springer Plus. 2013;2:1-5.
22. Peppermint seeds.Gardenweb.2013;11:06. Accessed 15 December 2013. Available: http://forums.gardenweb.com/forums/load/herbs/msg041811379516.html.
23. Lichtenthaler HK, Wellburn AR. Determination of total carotenoids and chlorophyll a and b of leaf extract in different solvents. Biochemical Society Transactions. 1983;(11):591–592.
24. Mushtaq YK. Effect of nanoscale Fe_3O_4, TiO_2 and carbon particles on cucumber seed germination. Journal of Environmental Science and Health. 2011;46:1732-1735. DOI: 10.1080/10934529.2011.633403.
25. Chouychai W, Theerakarunwong C. Efficiency of titanium dioxide on Mungbean seed sterile and their nanotoxicity to Mungbean growth in vitro. International Journal of Agriculture & Biology. 2013;15(5):1039-1042. DOI: 12–1305/2013/15–5–1039–1042.
26. Skupin K, Oszmianski J. Influence of titanium treatment on antioxidants and antioxidant activity of strawberries. Acta Sci. Pol., Technol. Aliment. 2007;6(4):83-95.
27. Stoeva N, Tzvetanka B. Oxidative changes and photosynthesis in oat plants grown in as-contaminated soil. Bulg J Plant Physiol. 2003;29(1-2):87-95.
28. Bogoeva I, Pigment content in cereal crops growth on copper polluted soil. Bulg J Agr Sci. 1998;4(6):767–772.

29. Feizi H, Rezvani Moghaddam P. Impact of bulk and nanosized titanium dioxide (TiO_2) on wheat seed germination and seedling growth. Biol Trace Elem Res. 2012;146:101-106. DOI: 10.1007/s12011-011-92220-7.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=404&id=24&aid=3708