European Journal of Medicinal Plants
9(1): 1-11, 2015, Article no.EJMP.18055
ISSN: 2231-0894

SCIENCEDOMAIN *international*
www.sciencedomain.org

Stress Effects of TiO_2 and NP-TiO_2 on Catalase Enzyme and some Physiological Characteristics of *Melissa officinalis* L.

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

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

Authors' contributions

This work was carried out in collaboration between all authors. Authors NS and 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.

Article Information

DOI: 10.9734/EJMP/2015/18055
Editor(s):
(1) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Anonymous, Kastamonu University, Turkey.
(2) Anonymous, Universiti Malaya, Malaysia.
(3) Anonymous, USA.
(4) Anonymous, Malaysia Terengganu University, Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1190&id=13&aid=9403

Original Research Article

Received 1st April 2015
Accepted 5th May 2015
Published 25th May 2015

ABSTRACT

Aims: Today, detection of risk effects of metal and metal nanoparticles over environment has attracted much attention. Plants are always exposed to considerable nanoparticles. So we decided to study the stress effect of titanium dioxide (TiO_2) and titanium dioxide nanoparticles (NP-TiO_2) phytotoxicity on catalase (CAT) enzyme and some physiological characteristics of *Melissa officinalis L.* plant including germination, root length, shoot length, and photosynthetic pigments.
Place and Duration of Study: The experiment was conducted in 2013 in the Research Laboratory of Islamic Azad University of Falavarjan, Isfahan, Iran.
Methodology: Seeds were treated with solutions of TiO_2 and NP-TiO_2 in 4 replicates. The obtained results were the mean of 4 replicates±SD. Finally the results were analyzed using SPSS18 statistical software.
Results: The results indicated that different treatments of TiO_2 and NP-TiO_2 compared to the

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

control group had no significant effect on germination percentage. However, they had a positive effect on root length, shoot length, and photosynthetic pigments (chlorophylls and carotenoids). At low concentrations, TiO_2 and NP-TiO_2 had a stimulatory effect on all parameters except the germination. NP-TiO_2 had increase effect on CAT activity and TiO_2 had the decrease effect on it.
Conclusion: The ROS stress which produce by NPs, can stimulated the antioxidant enzymes in plants. In this article, the ROS stress had increased effect on CAT activity. To understand the positive and negative effects of nanoparticles on other physiological characteristics of *Melissa officinalis* L. requires further investigation.

Keywords: Melissa officinalis L; photosynthetic pigments; catalase enzyme.

1. INTRODUCTION

Nowadays with development use of nanotechnology, there has been a growth in the application of nanoparticles for different fields. With population growth increasingly, the need for food and medicine also increase. In order to meet their basic needs, human societies have turned to different plants and so far several studies have been conducted so as to get the most out of plants in different food and medical fields. On the other hand, with the advancement of different sciences, we witness the use of a variety of conditions for better growth of plants. One of these sciences which has had increasing developments in the last century is nanotechnology. In recent years, Nanotechnology has various application in industrial, medicine etc. [1]. One of the nanoparticles that used in studies, is NP-TiO_2. TiO_2 usually used as the most appropriate catalyst for photo catalytic reactions; upon exposure to ultraviolet light it mineralizes the organic chemicals in rivers to water and carbon dioxide with the potential to destroy microorganisms [2]. Researcher found that the NP-TiO_2 increase the photosynthesis and plant growth of spinach and enhanced the absorption and transmission of the sun's energy to electron energy and activates chemical energy [3]. It manufactured in large quantities for different applications [4].

Medicinal plants traditionally have played a major contribution in several studies. Therefore, the aim of this study was to investigate the inhibitory effects of TiO_2 and NP-TiO_2 on germination percentage, root length, shoot length and photosynthetic pigments of *Melissa officinalis* L. that is one of the most important medicinal plants. This plant is native of central and southern Europe and Asia [5]. It applies as food and beverage flavoring. It has medicinal properties for the state of nervousness, headaches, arthritis and digestive pain [6]. Seed germination is a process that plays an important role in coping with stress during the life cycle of plants. Seeds after the water uptake and then development of vegetative growth stages become very sensitive to the environmental stresses. Seeds by assessing external conditions such as light; temperature and nutrients as long as external factors prepare to growth and development stages has essential protective role for the plant [7,8]. Evaluation of germination percentage and root length is extensively used due to the toxicity of various materials on plants, especially root length that is considered as a criterion for determining the ability of metal tolerance in plants [9,10]. Research suggests that the NP-TiO2 could enhance germination in psyllium plant (*Plantago psyllium*) [11]. The researchers showed that the NP-TiO_2 have significant effects on germination and root length of tomato [12]. Also, it has been found that this nanoparticle had no effect on seed germination of rice and root length and also decreased the root number [1].

Photosynthetic pigments (chlorophylls and carotenoids) are very important primary metabolites. These pigments in addition to using in cosmetics and food industry, plays an important role in their antioxidant activities [13]. The NP-TiO_2 could increase the chlorophyll (Chl) pigments and RWC and WSD in wheat [14]. The inhibitory effect of PAS TiO_2 solution on pathogens affecting photosynthetic pigments of cucumber showed that this solution has inhibitory property of pathogen agents and photosynthesis improving [15]. It is well known that NP-TiO_2 inhibits growth of aquatic plant *Lemna paucicostata* [16]. Studies indicate that NP-TiO_2 has additive effects on photosynthesis, chlorophyll biosynthesis growth and germination rate of spinach [17]. Much research has been done on the toxicity of nanoparticles and their absorption in plants that most of them have been reported inhibit germination and growth of root length [18]. Some studies have emphasized that the nanoparticles are absorbed by plant roots and transported to the stem and causes decrease the stem biomass and in result reduce shoot growth [19,20]. However, studies are high, but as we are witnessing, perhaps discussion the physiology of medical plants has been less attention.

Common consequence of biotic and abiotic stress is an increase in ROS (reactive oxygen species such as peroxide hydrogen, superoxide radical and hydroxyl radicals. Some of these stress are heat, light and metal toxicity. ROS are very toxic and causing damage to photosynthetic or respiratory electron transport, DNA, lipids, proteins, etc. Other studies showed that the stresses like heat have a first target that it is plasmalemma. The accumulation of ROS can cause damage of photosynthetic enzymes and pigments, proteins (denaturation), nucleic acids (RNA, DNA) and peroxidation of membrane lipids and homeostasis [21]. Plant protect their systems from this oxidative stress by production or increase in ROS scavenging antioxidant enzymes such as catalase (CAT; EC 1.11.1.6) superoxide dismutase (SOD; EC 1.15.1.1) and peroxidase (POD; EC 1.11.1.7) etc. [22]. In addition, non-enzymatic antioxidative carotenoids (Xanthophylls and β carotene) can stabilize photosynthetic complexes by reduce the ROS [23].

Hence, in this study we decided to review the favorable and phytotoxic effects of different concentrations of TiO_2 and NP-TiO_2 on seed germination, root and shoot length and photosynthetic pigments of *Melissa officinalis* L.

2. MATERIALS AND METHODS

In this study, we examined the stress effects of TiO_2 and NP-TiO_2 on seed germination, root length, shoot length and photosynthetic pigments of *Melissa officinalis* L. An experiment was carried out in Laboratory Research of Islamic Azad University of Falavarjan, Isfahan, Iran.

2.1 Chemicals

Different concentrations of TiO_2 (9.99%, Merck) and NP-TiO_2 (Tetragonal 99.5%, 8.6nm, Merck) were prepared by suspending 0, 100, 200 and 300 mg L^{-1} in double distilled water through ultra-sonication (100W, 60 KHz) for 180 minutes.

2.2 Seed Preparation and Treatment

Seeds of *Melissa officinalis* L. were purchased from Pakan Bazr institute, Isfahan, Iran, for testing the effects of various concentration (0, 100, 200 and 300 mg L^{-1}) of TiO_2 and NP-TiO_2 on seed germination. After seeds surface sterilization with sodium hypochlorite solution (2.5%, for 15 minutes) and rinse immediately for two times with double distilled water, 10 healthy and uniformly-sized seeds were selected for each treatments and placed in petri dish (90mm x15mm) lined with filter paper (Whatman No.42, Ashless, England). Subsequently treated with solutions. The control was only double distilled water. Petri dish was kept inside the culture chamber in the dark. After the germination process, we put them in light and soil to continue their normal growth. During the experiment, germinated seeds were recorded daily and were irrigated with TiO_2 and NP-TiO_2 suspensions. After 10 days of treatment, the final germination percentage was calculated based on the related formula. Additionally, root length and shoot length were measured using a ruler.

2.3 Measure of Pigments

Pigments were extracted by grinding 0.2 g freshly sampled leaves in 80% acetone at room temperature. Pigments were measured using absorbents recorded at 647nm, 663nm and 470nm for maximum absorption of chlorophyll a, chlorophyll b and carotenoids, respectively. The extinction coefficients were determined by a UV-Vis spectrophotometer (Unico-UV2100). Evaluations were made for the characters of chlorophyll and carotenoids. The amounts of Chl (a, b) and Car were measured in accordance to the methods of Lichtenthaler [24].

2.4 Measure of Catalase Enzyme

The CAT activities were assayed according to Chance and Maehly [25]. The mixture comprised of 0.75 ml phosphate buffer pH=7 (100 mM), 2 ml enzyme extract and 0.75 ml (70 mM) H_2O_2. The activity of CAT was estimated by decreased in absorbance of H_2O_2 at 240 nm using an UV-Vis spectrophotometer (Unico-UV2100, America) and was expressed as µmole H_2O_2 destroyed min^{-1} g^{-1} FW.

2.5 Statistical Analysis

This study was carried out as a factorial experiment in a complete randomized design with 4 replications. The results were presented as mean±SE (standard error of the mean).

The data were analysed using SPSS18 software. The significant levels of difference for all measured traits were calculated and the means were compared by Duncan's multiple range test at 5% level (Table 1).

Data the CAT activity was evaluated by one-way analysis of the variance (ANOVA) to analyze data and determine differences (Table 2). To determine significant differences between means, Tukey HSD test at 5% significance, was used. Data the CAT activity was evaluated by Tukey HSD test at 5% significance (Table 3).

3. RESULTS AND DISCUSSION

Table 1 shows the effect of TiO_2 and NP-TiO_2 concentration on seed germination, root length, shoot length and photosynthesis pigments of *Melissa officinalis* L.

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. TiO_2 concentration in 300 mg L^{-1} cause inhibited % germination (Fig. 1).

The result of other studies showed that the use of TiO_2 and NP-TiO_2 on the germination of wheat at the proper and lower concentrations have additive effect and in high-concentration have reduction effect [19]. Inhibition caused by metallic titanium and titanium dioxide returns to the selective of metals uptake by plants. Because it has been proven that plants have the genetic potential for selective absorption of metals in nature [26]. Different degrees of permeability of seed hulls than on metal is leading to varied range of inhibitory effects on the germination. For example, ions Pb^{2+}, Cu^{2+} and Zn^{2+} in the seed germination stage haven't any toxicity effect, but at a later stage of growth as after breaking the shell of the seed will grow even in the least amount of influence on each other. While the cause of toxicity in Arabidopsis for germination is less than 50 mm [27]. Other studies indicated that the speed of germination of barley affected by NP-TiO_2 concluded that have no statistically significant effect and these nanoparticles had no effect on this trait [28]. The results obtained by other studies showed that the Seeds treated by concentrations increasing were observed reducing the germination rate [29].

That according to the results obtained in this study, the seeds of *Melissa officinalis* L. have absorption capability of TiO_2 and NP-TiO_2. This subject causes to decreasing of germination compared to control and with increasing concentration decreased germination rate.

3.2 Root Length

Root length of *Melissa officinalis* L. can be seen in graphs presented in Fig. 2. TiO_2 concentration in 100 and 200 mg L^{-1} and NP-TiO_2 in 100 mg L^{-1} had significantly effect on root length.

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

Sources of Variation	df	%GM	Root length	Shoot length	Chl a	Chl b	Carotenoids
TiO_2 and NP-TiO_2(A)	1	2112.500**	1.758**	2.761**	5.202E-04^{ns}	0.302*	5.053E-02*
Treatment(B)	3	5741.667*	9.234*	8.845*	42.034*	6.978*	2.220*
A*B	3	1137.500**	4.961*	1.795**	34.364*	7.264*	2.021*
Error		343.750	0.352	0.354	9.454E-03	2.515E-03	7.680E-04

ns: non-significant   * : $p<0.01$ ** : $p<0.05$

Table 2. Effect of TiO_2 and NP-TiO_2 on CAT enzyme

ANOVA					
Data					
	Sum of squares	df	Mean square	F	Sig.
Between Groups	.125	5	.025	469.269	.000
Within Groups	.008	144	.000		
Total	.132	149			

Table 3. Effect of TiO_2 and NP-TiO_2 on CAT enzyme

Tukey HSD[a]					
groups	N	Subset for alpha = 0.05			
		1	2	3	4
NP-TiO_2 100	25	.08088			
NP-TiO_2 200	25	.08488			
$TiO_2$200	25		.09888		
TiO_2 100	25		.10092		
control	25			.12088	
NP-TiO_2 300	25				.16648
Sig.		.382	.921	1.000	1.000

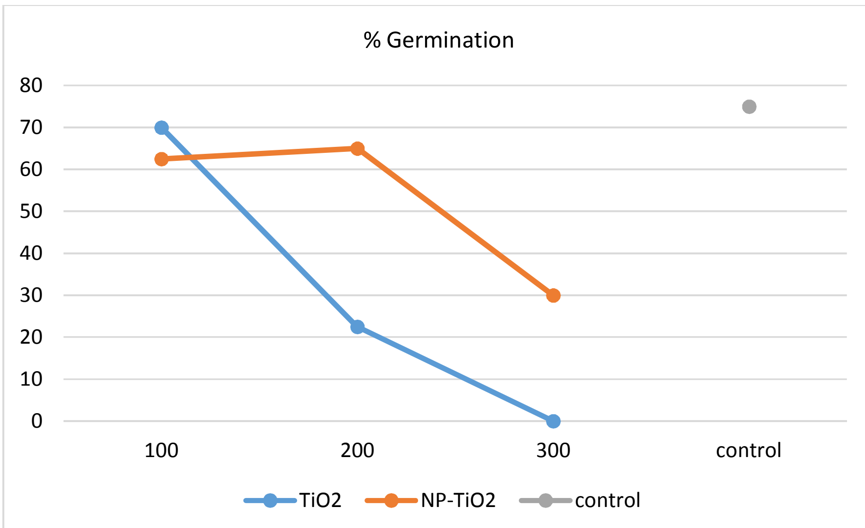

Fig. 1. Effect of TiO_2 and NP-TiO_2 on % germination
Mean±S.E.M = Mean values±Standard error of means of 4 replicates

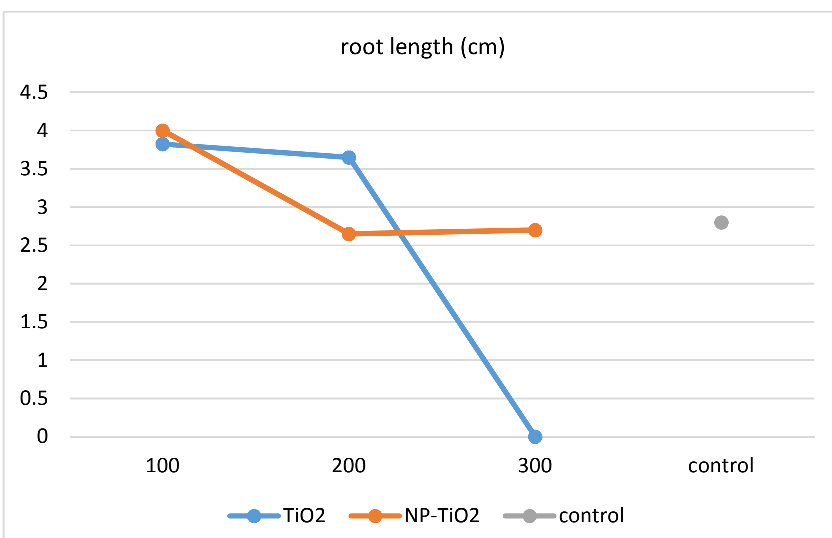

Fig. 2. Effect of TiO_2 and NP-TiO_2 on root length
Mean±S.E.M = Mean values±Standard error of means of 4 replicates

Other studies indicated that the nanoparticle causes to create large roots compared to metal and control [29]. However, growth increasing at low concentrations and growth slowdown at high concentrations of nanoparticles and metal correspond to the phenomenon hormesis.

A group of plants that have higher resistivity increase synthesis of some osmotic protector metabolites such as proline, betaine and reducing carbohydrates to protect their osmotic balance. They showed that, sunflower root antioxidant system in low concentration of heavy chromium metal have the ability to destroy free radicals and prevent oxidative damage to the plants, while the high accumulation of reactive species of oxygen in high concentrations of chromium overcome on plant antioxidant system and is increased the membrane lipids oxidation. Increasing the Malven De Aldeid (MDA) roots in the presence of low concentrations of chromium can cause a rapid protective response of sunflower against low stress of chromium, in this case released concentrations of MDA is low. Normally between the production of oxygen free radicals and the loss amount of plant cellular level, there is a balance that keeps plants in relatively stable. So, it seems *Melissa officinalis* L. at higher concentrations of metal and nanoparticles have oxidative stress [30].

3.3 Shoot Length

The effect of 100 mg L^{-1} concentration of TiO_2 and NP-TiO_2 on shoot length were significantly higher than control (Fig. 3).

Other studies showed that the plant growth means plant height at nanoparticle compared to control and metal concentrations are significant. Seeds treated with high concentrations of nanoparticle had the highest plant height [29]. Increasing the shoot length is due to the stimulatory effect of nanoparticles on it [11].

3.4 Photosynthetic Pigments

3.4.1 Chlorophyll a and b

Results of chlorophyll a and b of *Melissa officinalis* L. indicated that the TiO_2 at 100 and 200 mg L^{-1} concentration and all of the concentration at NP-TiO_2 increased the Chl a. (Fig. 4). Also, TiO_2 at 100 and 200 mg L^{-1} concentration and NP-TiO_2 at 200 and 300 mg L^{-1} concentration had significantly increased the amount of Chl b. (Fig. 5).

Nanoparticle compared to control and metal, increased the amount of leaf chlorophyll content [29]. Other research reported that the NP-TiO_2 has a positive effect on nitrogen metabolism, optical absorption and activation RuBisCo and consequently was a positive effect on chlorophyll content of spinach [14,20,31]

Also, it has been demonstrated that silver nanoparticles inhibit degradation of chlorophyll by reducing ethylene [32]. Hence it can be said that the NP-TiO_2 might also have the similar power and not only prevent chlorophyll degradation, but it also has increased it.

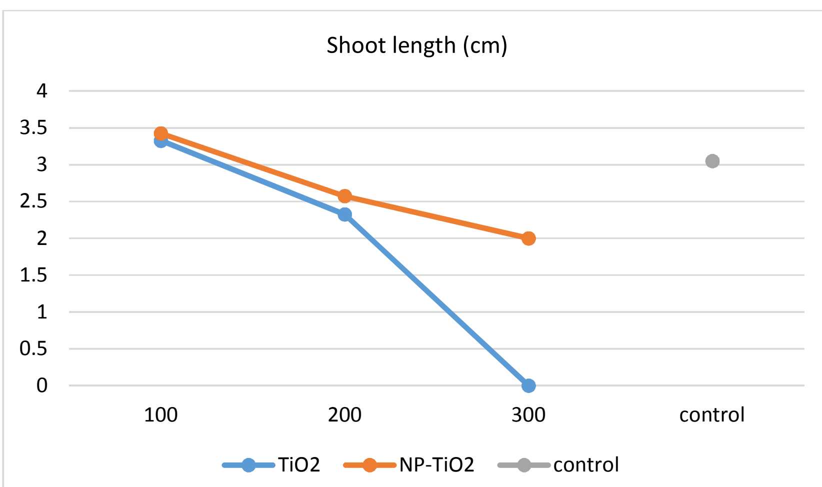

Fig. 3. Effect of TiO_2 and NP-TiO_2 on shoot length
Mean±S.E.M = Mean values±Standard error of means of 4 replicates

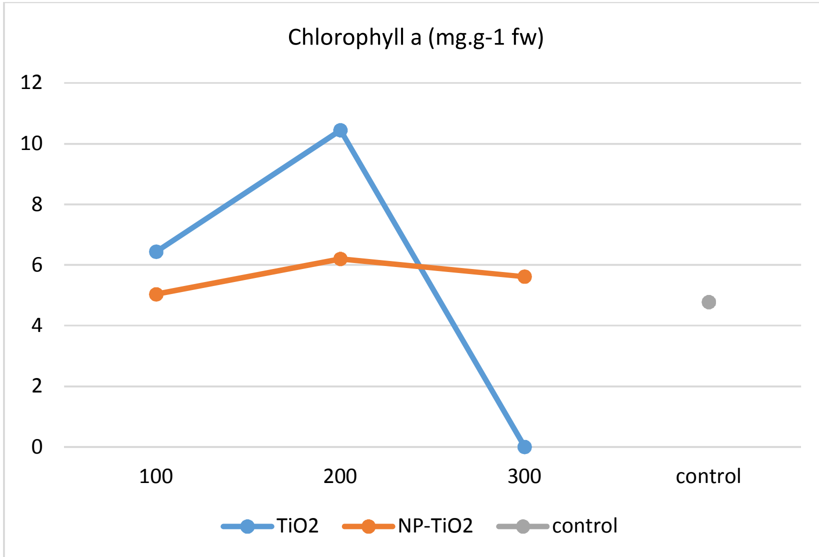

Fig. 4. Effect of TiO_2 and NP-TiO_2 on Chl a
Mean±S.E.M = Mean values±Standard error of means of 4 replicates

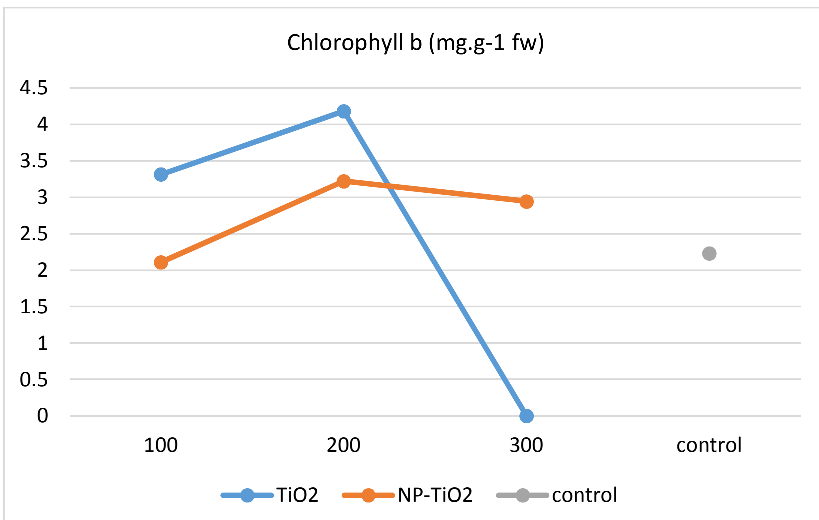

Fig. 5. Effect of TiO_2 and NP-TiO_2 on Chl b
Mean±S.E.M = Mean values±Standard error of means of 4 replicates

3.4.2 Carotenoids

The results showed that the concentration of TiO_2 and NP-TiO_2 at 200 and 100 mg L^{-1} had significantly increased effect on carotenoids, respectively (Fig 6).

The increase of carotenoids can be respond to suppressed ROS by heavy metals and reduction of them in high concentrations is due to oxidative stress [20].

3.5 CAT Activity

Our results with respect to CAT activity are displayed in Table 2 and 3. There was significant increase in the NP-TiO_2 at 100 and 200 mg L^{-1} and TiO_2 in 100 and 200 mg L^{-1} concentrations (Table 2). Furthermore, NP-TiO_2 at 300 mg L^{-1} had increased effect on CAT activity (Fig 7).

Antioxidant enzymes such as catalase are involved in scavenging the ROS like H_2O_2 and more active in the presence of stress. It is clear from data that the increase in CAT activity was due to NP-TiO_2 at 300 mg L^{-1} was more as compared to control and TiO_2 concentrations. Toxic effects of NP-TiO_2 on lemon balm are due to formation of ROS, following the NPs uptake. It seems that the activity of CAT enzyme which scavenge ROS is responsible for decreasing effects of these particles on *Melissa officinalis* L. [33].

TiO_2 at 100 and 200 mg L^{-1} concentration had decrease effect on CAT activity compared to control. The TiO_2 might have reduced ROS stress in treated lemon balm seedling by reducing H_2O_2 [34].

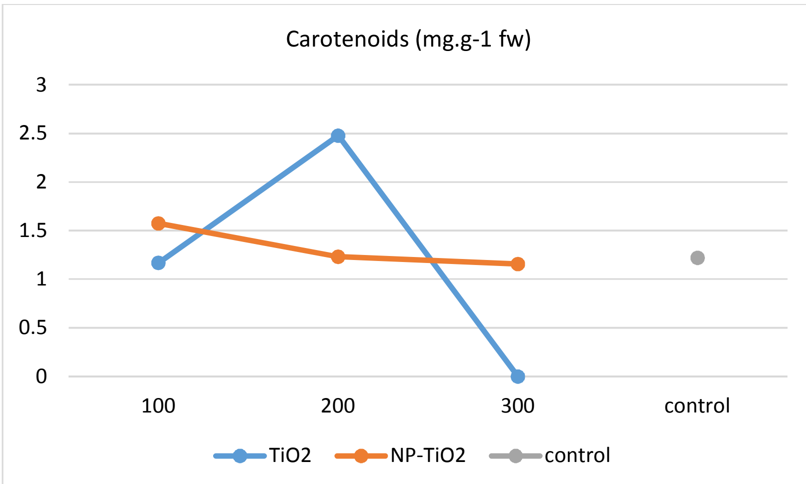

Fig. 6. Effect of TiO_2 and NP-TiO_2 on carotenoids
Mean±S.E.M = Mean values±Standard error of means of 4 replicates

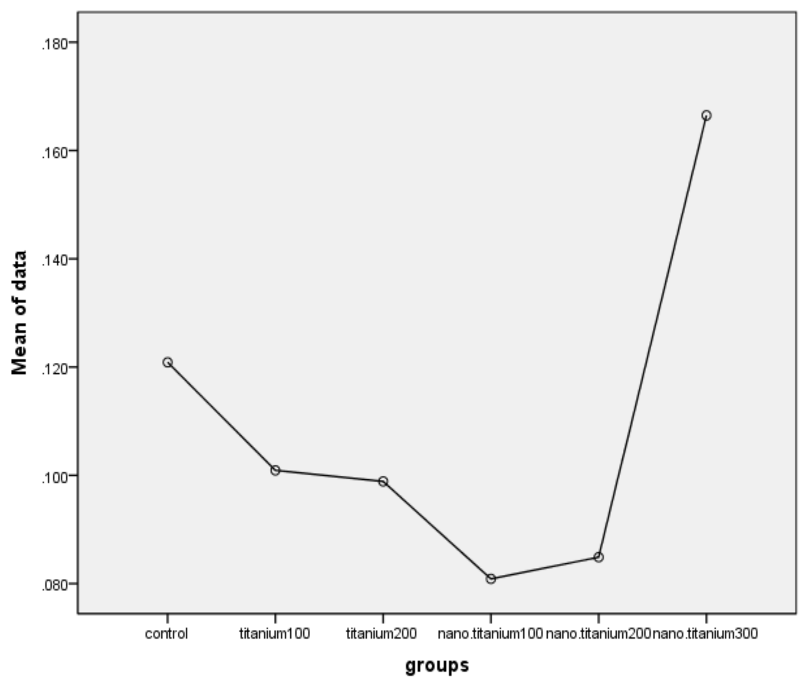

Fig. 7. Effect of different concentration of TiO_2 and NP-TiO_2 on CAT

Our results were supported by the previous studies who demonstrated that the ROS stress which produce by biotic and abiotic stress induced by MNPs (metal nanoparticles) over crop systems, can stimulated the antioxidant enzymes in plants. Reports indicated that the treatment of *Glycine max* with a mixture of NP-TiO_2 and SiO_2 stimulated its antioxidant activity enzymes and accelerated germination and growth [35]. Activity of specific antioxidant system was induced with silver nanoparticles in *brassica juncea* [36]. Also, the antioxidant enzymes of *Pelargonium zonale* had an increase response to nanosilver application [37]. Also some of studies showed that the NPs accumulated more chlorophyll and antioxidant enzymes activity of banana than the control [38].

4. CONCLUSION

In this study, experimental treatments have no significant effect on germination percentage than controls. The results of this study indicated that the TiO_2 and NP-TiO_2 in lowest concentration have additive effects on root length, shoot length, chlorophyll a and b and the carotenoids. Since both lemon balm and Peppermint are of Lamiaceae family can be concluded that TiO_2 and NP-TiO_2 have no effect on the germination of plants in this family and causes to reduce germination compared to control. But in both of these plants cause to increase in photosynthetic pigments, root length. Still, the study shows effects of TiO_2 and NP-TiO_2 on *Melissa officinalis* L., but to understand the positive and negative effects of nanoparticles on other physiological characteristics of *Melissa officinalis* L. it requires further investigation. It is necessary to examine aspects of the effect of nanoparticles prior to release of lemon balm plant to market.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Boonyanitipong P, Kumar P, Kositsup B, Baruah S, Dutta J. Effects of zinc oxide nanoparticles on roots of rice (*Oryza sativa* L.). International Conference on Environment and Bioscience. 2011;21:172-176.
2. Owolade OF, Ogunleti DO, Adenekan MO. Titanium dioxide affects diseases, development and yield of edible cowpea. EJEAFChe. 2008;7(5):2942–2947.
3. Lei Z, Mingyu S, Chao L, Liang C, Hao H, Xiao W, Xiaoqing L, Fan Y, Fengqing G, Fashui H. Effects of nanonatase TiO_2 on photosynthesis of spinach chloroplasts under different light illumination. Biol Trace Elem Res J. 2007;119:68–76.
4. Shi H, Magaye R, Castranova V, Zhao J. Titanium dioxide nanoparticles: a review of current toxicological data. Particle and Fibre Toxicology. 2013;10(15):1-33.
5. Hussain AI. Characterization and biological activities of essential oils of some species of Lamiaceae. A thesis submitted in partial fulfillment of the requirements for the degree of doctor of philosophy in chemistry. Department of chemistry & biochemistry faculty of Sciences University of Agriculture, Faisalabad, Pakistan; 2009.
6. Anon N. Iranian herbal pharmacopoeia. Tehran: Ministry of Health Publication. 2002;1:141–121.
7. Pritchard HW, Wood JA, Mjanger KR. Influence of temperature on seed germination and the nutritional requirements for embryo growth in *Arum maculatum* L. New phytology. 1993; 123:801-809.
8. Bungard RA, McNeil D, Morton JD. Effects of chilling, light and nitrogen-containing compounds on germinations, rate of germination and imbibitions of *Clematis vitalba* L. Ann. Bot.1997;79:643-645.
9. Zargari AI. Medicinal plants. Tehran: Tehran University Press. 1990;1:77-81. Persian.
10. Xiong ZT. Lead uptake and effects on seed germination and plant growth in a Pb hyper accumulator *Brassica pekinensis* Rupr. Bulletin of Environmental Contamination and Toxicology. 1998;60:285-291.
11. Aliabadi Farahani H Sani, B, Maroufi K. The Germination variations in Fleawort (*Plantago psyllium* L.) by Nano-Particle. International Conference on

Biotechnology, Nanotechnology and its Application. 2012;12:43-46.
12. Song U, Jun H, Waldman B, Roh J, Kim Y, Jongheop Y, Lee JE. Functional analyses of nanoparticle toxicity: A comparative study of the effects of TiO_2 and Ag on tomatoes (*Lycopersicon esculentum*). Ecotoxicology and Environmental Safety. 2013;93:60-67.
13. Sharafzadeh SH, Alizadeh O. Chlorophyll a, Chlorophyll b and carotenoids of garden thyme (*Thymus vulgaris* L.) as affected by nutrients. Advances in Environmental Biology. 2011;5(12):3725-3728.
14. Moaveni P, Aliabadi Farahani H, Maroufi K. Effect of nanoparticles Tio_2 spraying on deferent parameters of wheat (*Triticum aestivum* L.). Advances in Environmental Biology. 2011;5(8):2217-2219.
15. Cui H, Yang G, Jianfang J, Zahng P, Gu W. Biological effects of PAS TiO_2 sol on disease control and photosynthesis in cucumber (*Cucumis sativus* L.). Australian Journal of Crop Science. 2013;7(1):99-103.
16. 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.
17. Mandeh M, Omidi M, Rahaie M. *In vitro* influences of TiO_2 nanoparticles on barley (*Hordeum vulgare* I.) tissue culture. Biol Trace Elem Res. 2011;150(1-3):376-80.
18. 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.
19. 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.
20. Samadi N, Yahyaabadi S, Rezayatmand Z. Effect of TiO_2 and TiO_2 nanoparticle on germination, root and shoot Length and photosynthetic pigments of *Mentha piperita*. International Journal of Plant & Soil Science. 2014;3(4):408-418.
21. Myrene RD, Devaraj VR. Induction of thermotolerance through heat acclimation in lablab bean (*Dolichos lablab*). African Journal of Biotechnology. 2013;12(38): 5695-5704.
22. Mishra A, Jha B. Antioxidant response of the microalga *Dunaliella salina* under salt stress. Botanica Marina. 2011;54:195-199.
23. Ghasemzadeh A, Jaafar HZE. Interactive effect of salicylic acid on some physiological features and antioxidant enzymes activity in Ginger (*Zingiber officinale Roscoe*). Molecules. 2013;18: 5965-5979.
24. Lichtenthaler HK. Chlorophylls and carotenoids: Pigments of photosynthetic bio membranes. Methods Enzymol. 1987; 148:350–382.
25. Chance B, Maehly AC. Assay of catalase and peroxidases. Methods Enzymol. 1955;2:764-775.
26. Stefanov K, Seizova K, Yanishlieva N, Marinova E, Popov S. Accumulation of Pb, Zn and Cd in plant seeds growing in metalliferous habitats in Bulgaria. Food Chemistry. 1995;54:311-313.
27. Li W, Khan MA, Yamaguchi S, Kamiya Y. Effects of heavy metals on seed germination and early seedling growth of *Arabidopsis thaliana.* Plant Growth Regulation. 2005;46:45-50.
28. Takallu S, DavodI D, Omidi M, Ebrahimi MA, Rouzbeh F, Rasulnia AR. The effect of titanium dioxide nanoparticles on barley cytogenetical index, Journal of Agricultural Biotechnology. 2013;5(1):13-25.
29. Prasad TNVKV, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Raja Reddy K, Sreeprasad TS, Sajanlal PR, Pradeep T. Effect of nanoscale zinc oxide particles on the germination, growth and yield of Peanut. Journal of Plant Nutrition. 2012; 35:905–927.
30. Sadat Pirooz P, Manoochehri Kalantari KH, Nasibi FA. Physiological analysis of sunflower under chromium stress: impact on plant growth, bioaccumulation and oxidative stress induction on sunflower (*Helianthus annuus*). Iranian Journal of Plant Biology. 2012;4(11):86-73. Persian.
31. Yang F, Hong FS. Influence of nano-anatase TiO_2 on the nitrogen metabolism of growing spinach. Biol Trace Element Res. 2006;110:179-190.
32. Hatami M, Ghorbanpour M. Effect of nanosilver on physiological performance of pelargonium plants exposed to dark storage. Journal of Horticultural Research. 2013;21(1):15-20.
33. Rihai-Madvar A, Rezaee F, Jalali V. Effects of alumina nanoparticles on morphological properties and antioxidant

system of *Triticum aestivum*. Iranian Journal of Plant Physiology. 2012;3(1): 595-603.

34. Laware SL, Raskar Sh. Effect of titanium dioxide nanoparticles on hydrolytic and antioxidant enzymes during seed germination in onion. Int. J. Curr. Microbiol. App. Sc. 2014;3(7):749-760.
35. Lu CM, Zhang CY, Wen JQ, Wu GR. Research on the effect of nanometer materials on germination and growth enhancement of *Glycine max* and its mechanism. Soybean Sci. 2002;21:68–171.
36. Priyadarshini S, Deepesh B, Zaidi MGH, Pardha Saradhi P, Khanna PK, Arora S. Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. Appl Biochem Biotech. 2012;167:2225–2233.
37. Hatami M, Ghorbanpour M. Defense enzyme activities and biochemical variations of *Pelargonium zonalein* response to nanosilver application and dark storage. Turkish Journal of Biology. 2014;38:130-139.
38. Helay MN, El-Metwally MA, El-Hoseiny H, Omar SA, El-Sheery NI. Effect of nanoparticles on biological contamination of *in vitro* cultures and organogenic regeneration of banana. Australian Journal of Crop Science. 2014;8(4):612-624.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1190&id=13&aid=9403