VOL. 12, NO. 5, MAY 2017 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science

www.arpnjournals.com

STUDY THE EFFECTS OF NANO TITANIUM DIOXIDE ON NON-ENZYMATIC MECHANISMS OF CUMIN

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ABSTRACT

Nanotechnology could open up new approaches in plant sciences and in agricultural researches. Therefore, the aim of this work was evaluation the effects of nano titanium dioxide on non-enzymatic mechanisms of cumin (*Cuminum cyminum* L.). This study was conducted at the personal field at the Garmsar, Iran. For field study, a factorial experiment based on a completely randomized block design with four replications was used. Evaluated traits in this experiment were hydrogen peroxide, cartenoids, flavonoids and anthocyanins. Treatments were concentrations of titanium dioxide nanoparticle (0, 0.01%, 0.03% and 0.05%) and times of spraying of this nano particle (vegetative and reproductive stages). Final results revealed that all evaluated traits affected by concentrations of nano-TiO_2 but treatment of spraying times only effected on the carotenoids' content of cumin. Results showed that the highest content of hydrogen peroxide, was achieved by control and the lowest amount of this trait was obtained by concentration of 0.05% nano-TiO_2 while the amounts of carotenoids, flavonoids and anthocyanins traits increased by increasing of concentration of this nanoparticle so that control treatment, had the lowest amount of these traits. The results showed that application of titanium dioxide nanoparticle had positive effects on the reduction of oxidative stress of cumin plant.

Keywords: Nano-TiO_2, cartenoids, flavonoids, anthocyanins, cumin.

1. INTRODUCTION

Cumin (*Cuminum cyminum* L.) is a significant seed spice and one of the earliest known minor spices. It is an aromatic plant included in the Apiaceae family and is used to flavor foods, added to fragrances, and used in medicinal preparations. Its fruit, known as cumin seed, is yellow to brownish-gray in color. Cumin seeds contain numerous phytochemicals that are known to have antioxidant, carminative and anti-flatulent properties. The active principles in the cumin may increase the motility of the gastro-intestinal tract as well as increase the digestion power by increasing gastro-intestinal enzyme secretions (Parashar *et al.*, 2014). Nanoparticles (NPs) are commonly accepted as materials with at least two dimensions between 1-100 nm. NPs fall in a transitional zone between individual molecules and the corresponding bulk materials and therefore, hold unique properties, which are peculiar from their molecular and bulk counterparts. Unique properties of NPs include very large specific surface area, high surface energy, and quantum confinement. The characteristic feature of NPs may result in different environmental fate and behaviors than their bulk counterparts (Singh *et al.*, 2015). Nowadays, many countries have identified the potential of nanotechnology in the food and agriculture sectors and are investing in its applications to food production (Taha, 2016). Also, this technology could open up new approaches in plant sciences and in agricultural researchers. In recent years, many scientists have studied the effects of nanomaterials on seed germination and plant growth with the aim to promote its use for agricultural productions (Ghorbanpour *et al.*, 2015). Titanium dioxide (also known as titanium (IV) oxide or Titania) is the naturally occurring oxide of titanium with chemical formula TiO_2. It has a wide range of applications, from paint to sunscreen to food colouring. Titanium dioxide (TiO_2) has attracted significant attention from researchers because of the many interesting physical and chemical properties that make it suitable for a variety of applications (Daniyan *et al.*, 2013). The nano-TiO_2 showed the following improvements after the crop or seedlings were treated with it: The enhancement of yield of various crops, 10% to 20%, an improvement of some essential element contents in plants, an increase in enzyme activity like peroxide, catalase and nitrate reductase activity in plant tissue and enhancement of chlorophyll pigment paprika (*Capsicum annuum* L.) and green alga (*Chlorella pyrenoidosa*) (Hruby *et al.*, 2002 and Husen & Siddiqi, 2014). Plant studies have addressed the uptake, translocation and cellular localization of NPs, as well as the physiological and toxicological effects of NP exposure [8]. It can also be stated that NPs might have promoted seed antioxidant system and reduced the oxidative stress by reducing reactive oxygen species (ROS), and increasing antioxidant so that Lei et al. (Wang *et al.*, 2011) in their experiment found that nano-anatase TiO_2 promotes antioxidant stress by decreasing the accumulation of superoxide radicals, hydrogen peroxide, malondialdehid content and enhances the activities of superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase and thereby increases the evolution oxygen rate in spinach chloroplasts under UV-B radiation. Some effects of TiO_2 NPs have also been analysed in plants (namely in spinach, maize, willow trees and Arabidopsis) (Gao *et al.*, 2006, Lei *et al.*, 2007, Seeger *et al.*, 2009 and Kurepa *et al.*, 2010). For example, Lu *et al.*, (2002) revealed that TiO_2 nanoparticles can increase soybean root activity and leaf nitrate reductase activity, enhance plant water and nitrogen use and increase antioxidant activities

(SOD, POD, and CAT). One experiment showed that photosynthetic pigment contents, carotenoids and anthocyanins were increased under spraying treatment of maize by nano TiO_2 (Morteza *et al*., 2013). In tests reported in Xuming et al., (2008), the protein expression of rubisco in spinach treated with nano TiO_2, was increased by 40% in comparison with the control, they indicated that rubisco activity in the nano TiO_2-treated spinach was significant. Most recently research revealed that the use of appropriate concentration of nano-TiO_2 increased the seed germination parameters and early growth of some medicinal and aromatic plants (Hatami and Ghorbanpour, 2014). Therefore, according to the positive effects of nano-TiO_2 on the traits of plants and importance of cumin as an important medicinal plant, this study was aimed to evaluation the effects nano titanium dioxide on hydrogen peroxide (H_2O_2), cartenoids, flavonoids and anthocyanins of cumin (*Cuminum cyminum* L.).

2. MATERIALS AND METHODS

In order to study the effect of nano titanium dioxide on non-enzymatic mechanisms of cumin (*Cuminum cyminum* L.), one experiment was conducted on spring season of 2015 at one farm in Garmsar, Iran. For field study, a factorial experiment based on a completely randomized block design with four replications was used. Treatments used in this experiment consisted of various concentrations of titanium dioxide nanoparticle (0, 0.01%, 0.03% and 0.05%) and times of spraying of this nanoparticle (vegetative and reproductive stages). Nano-TiO_2 with average particle size of 10-25 nm was supplied by US Research Nanomaterials, Inc. The sizes of the TiO_2 nanoparticles were determined by scanning electron microscopy (SEM) (Figure-1).

Seeds were planted on 25 October 2015 in 20 cm row distance, 1.5 cm sowing depth in 3×2 m^2 plots. All agricultural practices were performed in the same manner, as it is usually done in the cumin production areas. Foliar spraying of nano-TiO_2 was performed in two stages by hand sprayer.

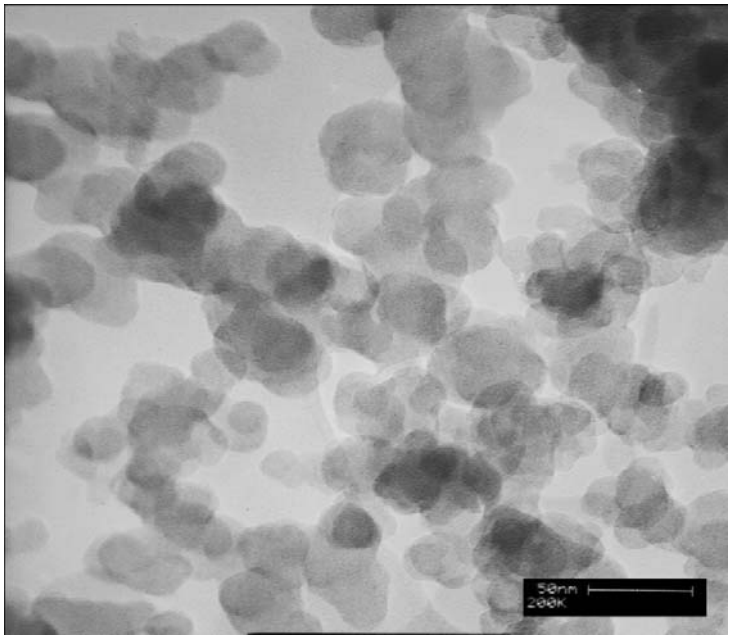

Figure-1. Image of nano-TiO_2 by scanning electron microscopy (SEM).

The characteristics measured were hydrogen peroxide (H_2O_2), cartenoids, flavonoids and anthocyanins. Sampling was conducted 72 h after the last spraying. Samples were randomly selected from each treatment. The samples were washed with water, were cut into small pieces and frozen in liquid nitrogen, then stored at -80ºC. At the end of growth season, samples were hand harvested. The obtained data was statistically using Statistical Analysis System (SAS Institute), and the treatments means were compared by using Duncan's multiple range tests.

2.1. Anthocyanins content assay

For determination of anthocyanins content, frozen tissue samples (100 mg) were soaked immediately in 10 ml of acidified methanol (methanol: HCl 99:1 (v/v). The tissue was crushed using a glass pestle and kept at 25 °C for 24 hours in the dark. The extract was then centrifuged at 4000 × g, for 5 min at room temperature (22 °C) and absorption at 550 nm of the supernatant was read by a UV-VIS spectrophotometer. Anthocyanin's content was calculated by the following formula for each sample (Wanger, 1979).

A_{Abs} 550= εbc
A= Read absorbance at 550 nm
ε= Extinction coefficient =33,000 mol^{-1} cm^{-1}
b= cell width=1cm
c= concentration of anthocyanin (μ $mol.g^{-1}$.fw)

2.2. Hydrogen peroxide (H_2O_2) assay

For determination of Hydrogen peroxide content, 0.5 g of leaf tissues from plants were homogenized with liquid nitrogen and the powders were suspended in 1.5 mL of 100 mM potassium phosphate buffer at pH 6.8. The suspensions were then centrifuged at 18,000 × *g* for 20 min at 4 °C. The enzymatic reaction was started with 0.25 mL of supernatant and a 1.25 mL peroxidase reagent consisting of 83 mM potassium phosphate buffer at pH 7.0, 0.005% (w/v) o-dianisidine, and 40 μg peroxidase mL^{-1} at 30 °C. The reaction was stopped after 10 min by adding 0.25 mL of 1 N perchloric acid, and the reaction mixture was centrifuged at 5000 × *g* for 5 min. The absorbance of the supernatant was measured at 436 nm, and the amount of hydrogen peroxide was determined by using an extinction coefficient of 39.4 mM^{-1} cm^{-1} (Bernt and Bergmeyer, 1974).

2.3. Carotenoids assay

The amount of carotenoids was determined according to the method of Lichtenthaler *et al*., (1987). The pigment extract was measured against a blank of 80% (V/V) acetone at wavelengths of 470 nm. Finally, evaluations for this trait was determined by the following formula:

Car = [(1000A470-1.8 Chl. A - 85.02 Chl. b) / 198]

2.4. Flavonoids assay

The amounts of flavonoids were measured in accordance with the method cited in Krizek *et al*., (1998). To determine the content of flavonoids, 0.1 g of leaf tissue was extracted in 15 ml glass centrifuge tubes containing 10 ml of acidified ethanol (ethanol: acetic acid, 99:1 (v/v). The samples were gently boiled for 10 min in a water bath at 80ºC and brought up to volume. Absorbance was measured at three wavelengths: 270 nm with UV-VIS spectrophotometer.

3. RESULTS

3.1. Hydrogen peroxide

According to the results of analysis of variance, the main effect of nano-TiO_2 concentrations treatment on the hydrogen peroxide content, was significant at the level of $P \leq 0.01$ and the effect of other treatments on this trait, were not significant.

The results for the means comparison of Duncan, (Figure-2), showed that the highest content of the hydrogen peroxide (0.27 μ.mol. $g.fw^{-1}$) was related to the non-application of this nanoparticle or control treatment that this treatment had not significant difference with the use of concentration of 0.01% with hydrogen peroxide content of 0.26 μ.mol. $g.fw^{-1}$ whereas the lowest amount of this trait (0.20 μ.mol. $g.fw^{-1}$) was obtained by spraying of 0.05% of nano-TiO_2.

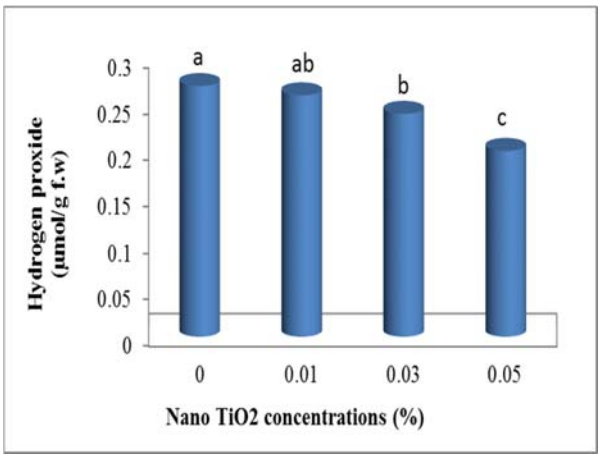

Figure-2. Effect of nano-TiO_2 concentrations on hydrogen peroxide content of cumin.

3.2. Cartenoids

According to the results, the simple effect of spraying time of nanoplarticle of TiO_2 on cartenoids content trait, was significant at $p \leq 0.05$ also the nano TiO_2 concentration's simple effect on this trait, was significant at $p \leq 0.01$ and the interaction effects of this nanoparticle concentration and spraying times, was not significant on the cartenoids amount.

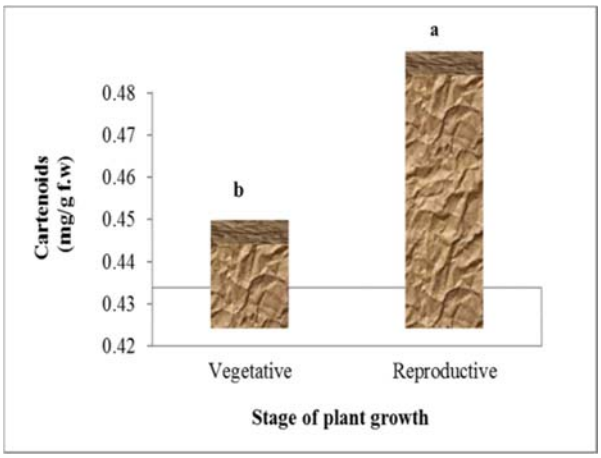

Figure-3. Effect of times of nano-TiO_2 spraying on cartenoids content of cumin.

The results for the means comparison of Figure-3, showed that the highest content of this trait (0.48 mg. g fw^{-1}) was related to the spraying of this at reproductive vegetative stage while the minimum level of this trait (0.44 mg.g fw-1) was obtained by application of TiO_2 nanoparticle at vegetative stage.

The comparison of the averages showed that cartenoids content by different concentration of nano-TiO_2 categorized into different statistical groups as well as the amount of carotenoids downtrend with increasing

concentrations of nanoparticles of titanium. So that the maximum amounts of this trait belong to the application of nano-TiO_2 at a concentration of 0.05% (0.50 mg.gfw^{-1}) and 0.03% (0.51 mg.gfw^{-1}) so that these treatments were in one group. The minimum amounts of cartenoids were for treatment of 0.01% (0.41 mg.gfw^{-1}) and control (0.44 mg.gfw^{-1}) treatments (Figure-4).

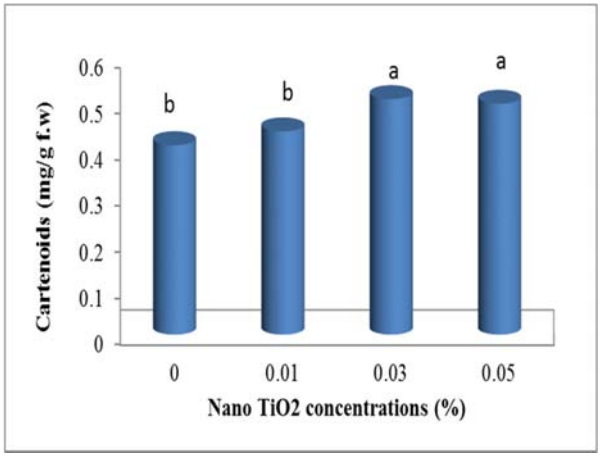

Figure-4. Effect of nano- TiO_2 concentrations on cartenoids content of cumin.

3.2. Flavonoids

Results showed that simple effect of concentrations of TiO_2 nanoplarticles on the flavonoids content trait was significant at $p \leq 0.05$ but the effects of interaction of nanoparticle concentration and spraying time's treatments and main effect of nano-TiO_2 spraying times treatment were not significant on the trait.

As the results showed that (Figure-5), the highest level of flavonoids content (0.59 %) was obtained by the foliar application of nano TiO_2 at a concentration of 0.05%, and the lowest content of this trait (0.48 %), was achieved by treatment of no-application of TiO_2 nanoparticle. Furthermore, the other treatments of this nanoparticle (0.01 and 0.03 %) were placed between the maximum and minimum treatments (Figure-4).

3.3. Anthocyanins

Results of analysis of variance showed that, the simple effect of nano-TiO_2 concentrations concentrations treatment on the content of anthocyanin, was significant ($p \leq 0.01$), while the effects of the other treatments on this trait, were not significant. According to the Figure-6, the results of means comparison showed that with increasing nano-TiO2 concentration, anthocyanins content of cumin plants increased. Treatments of 0.05%, 0.03% and 0.01% nano TiO_2 with amounts of 30.99, 30.07 and 29.53 µmol.g.fw^{-1} respectively, had the highest anthocyanins content so that these treatments have no significant difference with together and were placed in one superior statistical group. While the lowest content of anthocyanins with amount of 26.33 µmol.g.fw^{-1} was for the control treatment.

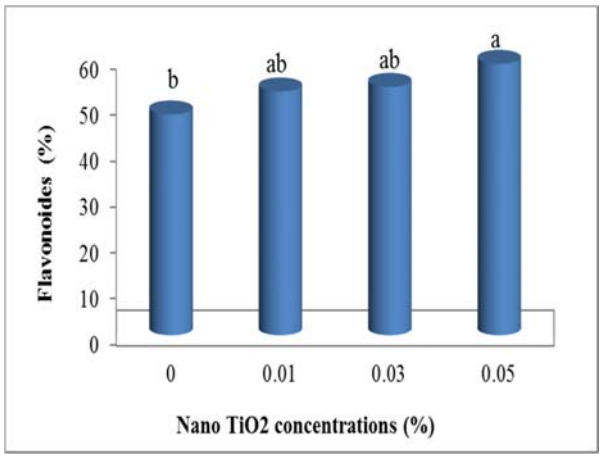

Figure-5. Effect of nano-TiO_2 concentrations on the flavonoids content of cumin.

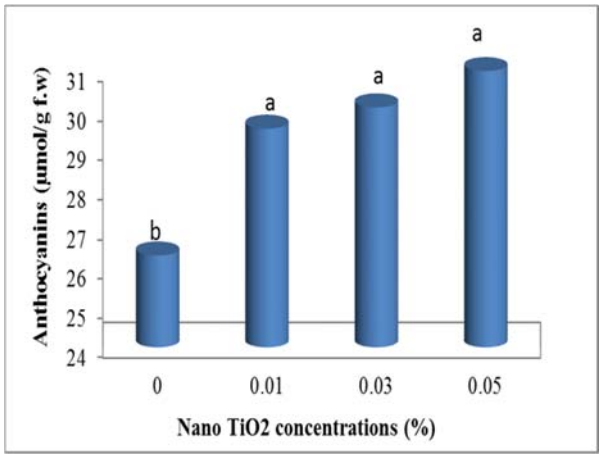

Figure-6. Effect of nano-TiO_2 concentrations on anthocyanins content of cumin.

4. DISCUSSIONS

In the present study, we observed that application of nano-TiO_2 in comparison with the no application of this nano particle or control treatment, increased cartenoids, flavonoids and anthocyanins content of cumin by reduction of hydrogen peroxide (H_2O_2). It seems that in accordance to the results of Singh *et al.*, (2015), the difference of nanoparticle of TiO_2 than the control treatment is because of special properties of nano particles include very large specific surface area, high surface energy, and quantum confinement. So that, Husen & Siddiqi (2014) suggested that the size of nanoparticles appears to be the critical factor. As the concentration of metal oxide nanoparticles increases, the growth increases and reaches an optimum value after which either it becomes constant or retardation in growth occurs. In this research, nanoparticles of titanium reduced amount of harmful hydrogen peroxide in the reproductive stage more than the vegetative growth phase. Considering that the critical reproductive stage has a significant effect on plant yield, thereby nanoparticles with reduction the harmful substance of hydrogen peroxide as a result of oxidative

stress, causes an improvement in growing condition for plant yield enhancement. As well as, in this study with increasing of nano-TiO_2 concentration, content of cartenoids, flavonoids and anthocyanins increased, whereas the harmful substance amount of hydrogen peroxide in cumin cells decreased. With regards to the finding of Bowler et al., (1992) and Yao *et al*., (2009), the production of various forms of active oxygen such as hydrogen peroxide (H_2O_2), can also damage cellular constituents such as lipids, carbohydrates, proteins and nucleic acids. The oxidative stress may also adversely affect the resistance of chlorophyll membrane and hence prevents the plant from photosynthetic and respiratory processes and the subsequent growth. Therefore nano-TiO_2 by controlling the production and adverse effects of active oxygen in plant, enzymatic and non-enzymatic mechanisms (such as cartenoids, flavonoids and anthocyanins) can enhance plant resistance.

Furthermore, in this case, Hong *et al*., (2005) emphasized that, nano TiO_2, promoted oxidative stress by decreasing accumulations of superoxide radicals, hydrogen peroxide, malondialdehid content and enhanced activities of antioxidant enzymes and thereby increased the rate of oxygen evolution in chloroplasts in tests on spinach under stress. Moreover, these results are in accordance with those reported by, Lei *et al*., (2007), so that they stated that nanoparticles (TiO_2) decreased oxidative damage in spinach chloroplast by increasing of antioxidant enzymes (APX, SOD, POX, and CAT activity). In confirmation of these results, Lu *et al*., (2002) in their study, showed that mixture of nano-TiO_2 increased nitrate reductase enzyme in soybean (*Glycine max* L.) and accelerated absorption and utilization of water and fertilizer, promoting the antioxidant system, and finally accelerated its germination and growth. Additionally, consistent with these results, Morteza et al., (2013) reported that application of nanoparticles of titanium increased the carotenoid's content of *Zea mays*.

5. CONCLUSIONS

In this study application of nano-TiO_2, by increasing of cartenoids, flavonoids and anthocyanin's content, and by decreasing the amount of hydrogen peroxide as a harmful substance in cumin cells, provide the better conditions for cumin growth. Therefore considering the importance of cumin as an important medicinal plant, the use of titanium nanoparticle to improve plant growth, especially in conditions oxtative stress, is important

REFERENCES

Bernt E, Bergmeyer HU 1974 Methods of Enzymatic Analysis. Academic Press. New York.

Bowler C, Van Montago M, Inze D. 1992 Superoxide dismutase and stress tolerance. Annual Review Plant Physiology and Plant Molecular Biology. 43: 83-116.

Gao F, Hong F, Liu C, Zheng L, Su M, Wu X, Yang F, Wu C and Yang P. 2006. Mechanism of nano-anatase TiO_2 on promoting photosynthetic carbon reaction of spinach: inducing complex of rubisco-rubisco activase. Biological Trace Element Research. 111: 239-253.

Hatami M, Ghorbanpour M. 2014. Defense enzymes activity and biochemical variations of *Pelargonium zonale* in response to nanosilver particles and dark storage. Turkish Journal Biology. 38: 130-139.

Hong FS, Yang F, Ma ZN, Zhou J, Liu C, Wu C, Yang P. 2005. Influences of nano-TiO_2 on the chloroplast ageing of spinach under light. Biological Trace Element Research. 104: 249-260.

Hruby M, Cigler P, Kuzel S. 2002. Contribution to understanding the mechanism of titanium action in plant. Journal of Plant Nutrition. 25: 577-598.

Husen A, Siddiqi KS. 2014. Phytosynthesis of nanoparticles: concept, controversy and application. Nanoscale Research Letters. 9: 229.

Kurepa J, Paunesku T, Vogt S, Arora H, Rabatic BM, Lu J, Wanzer MB, Woloschak GE, Smalle JA. 2010. Uptake and distribution of ultrasmall anatase TiO_2 alizarin red snanoconjugates in *Arabidopsis thaliana*. Nano Letters. 10: 2296-2302.

Lei Z, Su M, Xiao W, Chao L, Qu C, Liang C, Hao H, Liu X, Hong F. 2007. Effects of nano-anatase on spectral characteristics and distribution of LHCII on the thylakoid membranes of spinach. Biological Trace Element Research. 120: 273-283.

Lichtenthaler HK. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymology. 148: 350-382.

Lei Z, Mingyu S, Xiao W, Chao L, Chunxiang Q, Liang C, Hao H, Xiao- qing L, Fashui H. 2008. Antioxidant stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation. Biological Trace Elem Research. 121: 69-79.

Lu CM, Zhang CY, Wen JQ, Wu GR, Tao MX. 2002. Research of the effect of nanometer materials on germination and growth enhancement of *Glycine max* and its mechanism. Soybean Science. 21: 168- 171.

Morteza E, Moaveni P, Aliabadi Farahani H, Kiyani M. 2013. Study of photosynthetic pigments changes of maize *(Zea mays* L.) under nano TiO_2 spraying at various growth stages. Springer Plus. 2: 247.

Seeger EM, Baun A, Kästner M, Trapp S. 2009. Insignificant acute toxicity of TiO2 nanoparticles to willow trees. Journal of Soil and Sediment. 9: 46-53.

Yao X, Chu J, Wang G. 2009. Effects of drought stress and selenium supply on growth and physiological characteristics of wheat seedlings. Acta Physiologiae Plantarum. 31: 1031-1036.

Wang S, J Kurepa, JA Smalle. 2011. Ultra-small TiO_2 nanoparticles disrupt microtubular networks in *Arabidopsis thaliana*. Plant Cell and Environment Journal. 34: 811-820.

Wanger GJ. 1979. Content and vacuole/extra vacuole distribution of neutral sugars, free amino acids, and anthocyanis in protoplasts. Plant Physiology. 64: 88- 93.

Xuming W, Fengqing G, Linglan M, Jie L, Sitao Y, Ping Y, Fashui H. 2008. Effects of nano-anatase on ribulose-1, 5-bisphosphate carboxylase/oxygenase mRNA expression in spinach. Biological Trace Element Research. 126: 280-289.