Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 6, No. 6, p. 300-306, 2015
http://www.innspub.net

INNSPUB
RESEARCH PAPER OPEN ACCESS

Changes in biochemical traits and grain yield of cumin (*Cuminum cyminum* L.) affected by titanium nanoparticle spraying at different stages of plant growth

Elham Morteza[1], Payam Moaveni[2], Mohammad-Reza Bihamta[3], Tayebeh Morteza[4*], Ali Joorabloo[5], Hadi Saemi[6]

[1]*Agronomy and Plant Breeding Department, Aburaihan Campus University of Tehran , Tehran, Iran*

[2]*Agronomy Department and Islamic Azad University, Shahr-e-Qods Branch, Shahr-e-Qods, Iran*

[3]*Agronomy and Plant Breeding Department, Agriculture and Natural Resources Campus, University of Tehran, Karaj, PO. Box: 4111, Iran*

[4]*Graduate from the Islamic Azad University, Plant Protection Department, Damghan Branch, Damghan, Dmghan, Iran*

[5]*Agronomy Department, Agriculture Jahad Office of Garmsar, Garmsar, Iran*

[6]*Graduate from the Rasht University, Horticulture Department, Rasht, Iran*

Article published on June 17, 2015

Key words: Catalase, Nanoparticle of titanium, Soluble sugars, Soluble proteins.

Abstract

In order to investigate the changes of biochemical traits and seed yield of cumin (*Cuminum cyminum* L.) by spraying of titanium nanoparticle in different growth stages of the plant, a factorial experiment based on a complete randomized block design with four replications was done, in the years of 2013 and 2014, at Garmsar- Iran. Treatments used in this experiment consisted of various concentrations of titanium dioxide nanoparticle (0, 0.02%, 0.04% and 0.06%) and time of spraying of this nano particle (foliar application in vegetative and reproductive stages). In this test characteristics of soluble proteins, soluble sugars, catalase enzyme activity and seed yield were evaluated. Analysis of variance showed that the effects of concentrations of titanium dioxide nanoparticles were significant on all traits and simple effect of spraying times on the characteristics of soluble sugars was significant. However, the interaction's effect of titanium dioxide concentrations and times of spraying on all characteristics was not significant. In general, the results of this experiment showed that spraying of cumin plants with titanium doxide nanparticle had a positive effect on the biochemical traits of the plant in comparison with the control (no foliar application) treatment, therefore the use of this nanoparticle increased the yield of this plant.

***Corresponding Author:** Tayebeh Morteza ✉ eli_morteza @yahoo.com

Introduction

Cumin (*Cuminum cyminum*) is a small annual and herbaceous plant belonging to the Apiaceae (Umbelliferae) family. The fruit is a lateral fusiform or ovoid achene 4-5 mm long, containing a single seed. It is one of the popular spices regularly used as a flavoring agent. It is cultivated in arid and semi-arid regions such as countries bordering the Mediterranean Sea, Iran, Saudi Arabia, India and China (Thippeswamy and Naidu, 2005).

Nanotechnology permits broad advances in agricultural research, such as reproductive science and technology, conversion of agricultural and food wastes to energy and other useful byproducts through enzymatic nanobioprocessing, disease prevention and treatment in plants using various nanocides (Carmen *et al.*, 2003). Having a photocatalyzed characteristic, nano-anatase TiO_2 under light could cause an oxidation–reduction reaction. Therefore, nano-anatase TiO_2 might be closely related to the photosynthesis of plants (Gao *et al.*, 2008). Tiny, nano-size substances can be found everywhere in nature, as single atoms or as macromolecules, such as hemoglobin, or DNA. It has been shown that lotus leafs can keep its surface clean simply because of the complicated, multiple, microscopic ultrastructure at the nanometer and micron scale. Such a structure also exists in other plants, protecting the plant from bacterial and parasitic invasions. In addition, the fine structure of plant leaves increase the efficiency of light absorption and improves the photosynthetic process (Zheng *et al.*, 2005).

Previous researches has shown that photocatalytic activity of nano TiO_2 done in the visible-light spectrum, wavelength of 437 and 675 nm and ultraviolet wavelength of 400 to 800 (Buchanan *et al.*, 2002).This nanoparticle strengthens the nitrogen metabolism in plants so that, increases the nitrogen and protein content and glutamine, glutamate, nitrate reductase, Glutamate dehydrogenase (GLDH) and thereby increases the formation of chlorophyll in plants (Hong *et al.*, 2005; Zhng *et al.*, 2005; Yang *et al.*, 2006). In fact, in plants, increasing in protein and chlorophyll content by this nanoparticle performed through the conversion of inorganic nitrate to organic nitrogen (e.g., protein and chlorophyll) (Yang *et al.*, 2006) as well as nano particle of TiO_2 caused an increasing in activity of catalase, peroxidase, superoxide dismutase enzymes (Hong *et al.*, 2005) and this nanoparticle by increasing of light energy absorption by photosystem I and transfer of it into the photosystem II, transfer of electrons and also by development of photolysis of water and releasing of oxygen, caused an increasing in the yield of plant (Mingyu *et al.*, 2007).

It should be noted that the nano particles of TiO_2 due to smaller size, catalyzes the reduction and oxidation reactions and accelerate the release of the high-energy electrons in comparison to the type of TiO_2 of bulk (Yang *et al.*, 2006) and by the control of plant diseases and strengthen of plant immune system, thereby increasing the yield of the plant. However, in one study, nano titanium dioxide increased activity of antioxidant enzymes and nitrate reductase of soybean seeds (Lu *et al.*, 2002). Moaveni *et al.* (2011a) also found that spraying of titanium nano particle on the wheat plants increased harvest index, grain and biological yield, so that the highest values of these characteristics, was reported by use of these nanoparticle at concentrations of 0.01% and 0.03% concentrations, respectively. Gao *et al.*, (2008), in one study about the effectiveness of nano TiO_2, concluded that application of this nanoparticle increased amounts of soluble proteins as well as rubisco enzyme.

Jaberzadeh *et al.* (2013), studied the effect of drought stress and time and concentrations of nano TiO_2 spraying on wheat plant, results of their research showed that simple effect of spraying time was not significant on the height, panicle weight, panicle number, seed number, seed weight, biomass, gluten and starch but its effect was significant on the chlorophyll, grain yield and harvest index traits. Moreover, findings of Hruby *et al.* (2002) showed that the TiO_2 nanoparticles had a positive effect of the characteristics of the green algae and pepper plants so that this nanoparticle increased nitrat reductase, peroxidase and catalase enzyme's activity and by increasing of chlorophyll content and elements uptake, increased yield of these plants. Hong *et al.* (2005) as well stated that photosynthesis rate, chlorophyll content, growth and development, and finally, fresh and dry weight of spinach plants that sprayed by this nanoparticle, increased because of increasing of antioxidant enzyme's activity, thereby protect of the plant chloroplasts from the adverse effects of the malondialdehyde and hydrogen peroxide produced by the light tension as well as Li *et al.* (1978) in one test showed that application of nano TiO_2, significantly increased protein content of spinach.

In accordance to this note, cumin is a very important medicinal plant for its essential oil, and on the other hand, due to the positive effect of nanoparticle of titanium dioxide to increasing of plant yield. This research was done to evaluate the effect of nanoparticle titanium concentrations on the amount of soluble proteins, catalase enzyme activity, soluble sugars and grain yield of cumin.

Materials and methods

Preparation of materils and cultivation of cumin

This study was carried out at one farm in Garmsar, Iran during the winter and spring seasons of 2013-2014. Soil in the test was sandy loam. A field experiment was conducted as a factorial experiment based on a completely randomized block design with four replications. Treatments used in this experiment consisted of various concentrations of titanium dioxide nanoparticle (0, 0.02%, 0.04% and 0.06%) and time of spraying of this nano particle (foliar application at vegetative and reproductive stages). Nano-TiO_2 with a primary particle size of 44.17 nm was supplied by chemical synthesis (Plasma Chem, Germany). The size of the TiO_2 nanoparticles were determined by scanning electron microscopy (SEM) at the Rezaei laboratory at Tehran, Iran. (Fig. 1).

Seeds were planted on 15 November 2013 in 20 cm row distance, 1.5 cm sowing depth in 2×2 m^2 plots. Foliar spraying of plant growth was performed in two stages by hand sprayer in this test characteristics of soluble proteins, soluble sugars, catalase enzyme activity and grain yield was evaluated. Plants of 1 m^2 in each plot were harvested on 15 May 2014 and dried at the end of growth season and after harvesting, branches were dried in the shade and grain yield was measured using a carriage scale using standard moisture at 14%.

Measurement of soluble sugar

Soluble sugar contents were determined based on the method of Dubois *et al.* (1956) (phenol-sulfuric acid). 0.5 g dry weight of cumin leaves was homogenized with deionized water, extract was filtered and treated with 5% phenol and 98% sulfuric acid. Mixture remained for 1 h and then absorbance at 485 nm was determined by spectrophotometer. Content of soluble sugar was expressed as mg. g dry weight.

Measurement of soluble protein

Protein content was estimated following the method of Bradford (1976) at 595 nm wavelength was determined by spectrophotometer using serum albumin as standard protein and amount of it was recorded mg.fw^{-1}.

Catalase activity assay

Catalase activity determination was carried out according to Chance and Maehly (1995). Samples containing 100 µg of protein were suspended in 1 mL of 50 mM Tris-HCl suspension solution at pH 7.8. The assay medium consisted of 50 mM of potassium phosphate buffer at pH 7.0 and 10 mM of H_2O_2. The decrease in H_2O_2 absorbance was followed for 90 s at 240 nm at room temperature.

Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS Institute 1988) and Duncan was used to compare means at a $P < 0.05$ probability.

Results

Soluble proteins

According to the results of analysis of variance (Table 1), the effect of nano TiO_2 concentrations treatment on soluble protein's content of cumin plant, was significant at $p \leq 0.01$. The highest amount of this trait (1.93 mg.g fw^{-1}) was obtained from the foliar application of nano TiO_2 at a concentration of 0.06%, and the least amount of it was achieved from the use of no-application of this nanoparticle. So that the other concentrations of this nanoparticle (0.02 and 0.04 %) were between the maximum and minimum treatments (Fig 2).

Table 1. Analysis of variance results of the cumin traits under different concentrations of nanoTiO_2 spraying in two stages of plant growth.

		Means square			
Sources of variation	df	Soluble proteins	Soluble sugar	CAT enzyme activity	Grain yield
Replication	3	0.01^{ns}	2837.82^{**}	57.70^{ns}	16464.46^{**}
Times of spraying	1	0.01^{ns}	16803.40^{*}	41.72^{ns}	1548.18^{ns}
of nanoTiO_2 Concentrations	3	0.16^{**}	16312.08^{*}	5147.63^{**}	13965.73^{**}
Times×Concentrations	3	0.02^{ns}	403.87^{ns}	14.54^{ns}	342.69^{ns}
Error	21	0.03	3529.08	59.98	645.85

* and **: Significant at 5 and 1% levels respectively.

Soluble sugar

Simple effects of nano TiO_2 concentrations and time of spraying of this nanoplarticle on soluble sugar trait was significant at $p \leq 0.05$ (Table 1). The results for the means comparison of Duncan, (Fig. 3), demonstrated that the highest content of this attribute (521.44 mg.g dw^{-1}) was related to the non-application of this nanoparticle or control treatment while the least amount of this trait (455.83 mg.g dw^{-1}) was achieved by application of 0.06% of nano TiO_2 that this treatment had not significant difference with the use of concentration of 0.04% with soluble sugar's amount of 491.09 mg.g dw^{-1}.

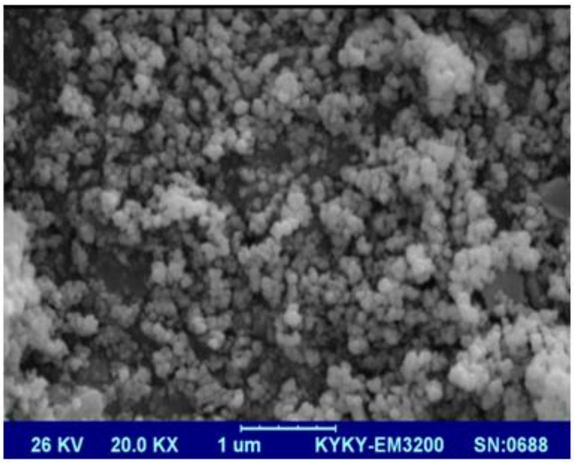

Fig. 1. Image of nano TiO_2 by scanning electron microscopy (SEM).

According to the Fig. 4, the maximum (491.09 mg. g dw^{-1}) and minimum (445.26 mg. g dw^{-1}) amounts of soluble sugar's content of cumin, were obtained by spraying of nanoTiO_2 at vegetative and reproductive stages respectively.

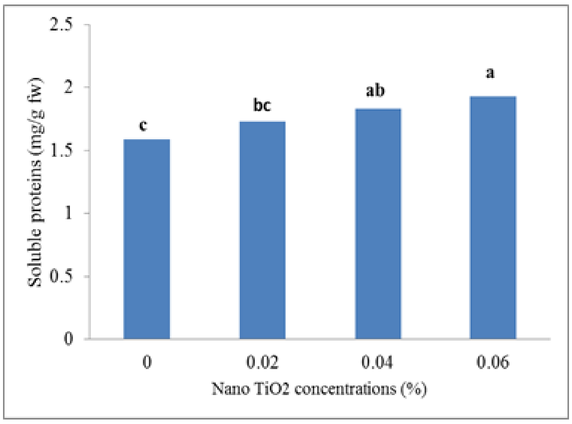

Fig. 2. Effect of nano-TiO_2 concentrations on soluble proteins content of cumi.

CAT enzyme activity

The results of analysis of variance (Table 1), showed the simple effect of nano TiO_2 concentrations treatment on the catalase enzyme activity, was significant at the level of one percent ($p \leq 0.01$), While the effects of spraying time treatment and interaction of this nanoparticle concentration and spraying times was not significant. As the results shows, it is clear that treatment of 0.06% nano titanium with 118.83 µmol.g protein.min^{-1} and concentration of 0.04% nano titanium with 117.11 µmol.g^{-1} protein.min^{-1} and concentration of 0.02% nano titanium with 112.64 µmol.g^{-1} protein.min^{-1} had not significant difference with together and had the highest amount of this enzyme activity and were placed in the superior group while no application of nano TiO_2 with the amount of 112.64 µmol.g^{-1} protein.min^{-1} had the least amount of the enzyme activity (Fig. 5).

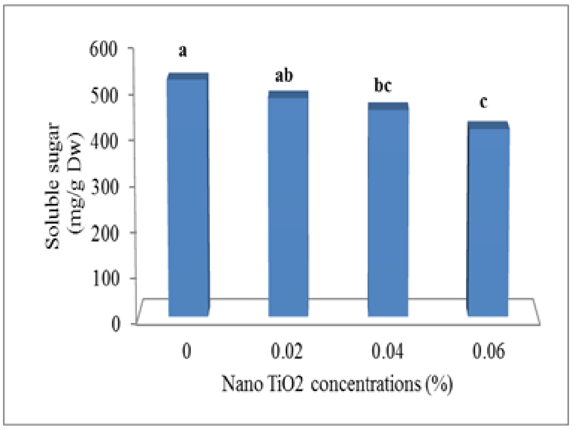

Fig. 3. Effect of nano-TiO_2 concentrations on soluble sugar of cumin.

Grain yield

According to the results of analysis of variance (Table 1), the main effect of nano TiO_2 concentrations treatment on the grain yield trait, was significant at the level of $p \leq 0.01$and the results of means comparison (Fig. 6) showed that use of 0.06% concentration of nano TiO_2 had the highest grain yield (463.01 kg.ha^{-1}), while the treatment of no-use of this nanoparticle (control) had the least (364.17 kg.ha^{-1}) grain yield.

Discussion

The results of this experiment showed that the use of different concentrations of titanium nanoparticle, increased traits of soluble proteins, catalase enzyme activity and seed yield and decreased soluble sugars content in comparison with the control treatment. It should be emphasized that nanoparticle of titanium because of a high specific surface area and very small diameter in comparison with the bulk of TiO_2 can easily pass through the tissues of the plants, and can do more and faster reactions in the target tissue.

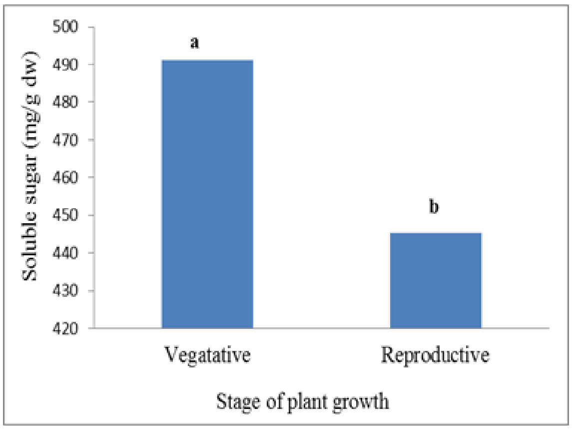

Fig. 4. Effect of times of nano-TiO_2 spraying on soluble sugar content of cumin.

Therefore, the probability of nanoparticles absorption through the foliar application, is faster and easier. In this experiment, As was observed, nano TiO_2 at concentration of 0.06%, compared with the other concentrations of this nanoparticle in this test, had a very effective role in increasing of biochemical characteristics and yield of cumin and as well as, nitrogen is one of the main components of chlorophyll and proteins and this nanoparticle with enhanced nitrogen fixation in plant systems and catalytic activity, it's expected that plants treated with this nanoparticle, had additional soluble protein content (Hong *et al.*, 2005). Consistent with these results, the results of Gao *et al.*, (2008), showed that the application of this nanoparticle increased soluble proteins of cumin so that the amount of protein in plants that were sprayed with this nanoparticle, was more than the control treatment also Li *et al.*, (1978) showed that nano TiO_2 significantly increased spinach protein content. As we know, plants use the enzymatic defense system (catalase, peroxidase, superoxide dismutases) and non-enzymatic (carotenoids and anthocyanins) to eliminate of the oxygen-free radicals produced (Chalkr-Scott, 2002). Increasing of catalase enzyme activity in plants that treated with nano titanium dioxide in comparison with the control plants, showed that nano titanium dioxide with increasing of catalase enzyme activity to combat free radicals of oxygen, prevents from degradation of proteins by oxygen-free radicals. Plants can tolerate stress by accumulating large amounts of soluble sugars and these are signals of stress (Ma *et al.*, 2004) that with increasing of stress, amounts of soluble sugars increase. Moreover, in this test, plants treated with this nanoparticle, by decreasing the amount of soluble sugars as a signal of stress, cleared free radicals caused by drought stress, thus decreased the negative effects of stress. Therefore, when the plants have a strong defense system, they can produce more yield. In support of this conclusion, in one study Hruby *et al.*, (2002), investigated the effect of nano TiO_2 particle on the antioxidant enzyme activity of pepper and green algae plants, finding of their researches showed that this nanoparticle had a positive effect on these traits, and therefore catalase enzyme activity increased in plants treated with this nanoparticle. In addition, increasing of antioxidant enzymes (eg. catalase) of Marigold (*Calendula officinalis* L.) by nanoparticle of TiO_2 in comparison with the control plants were reported by Moaveni *et al.* (2011b). During the reproductive stage, the plants are more susceptible to the stresses. Therefore, foliar application of plants by this nanoparticle in the reproductive stage, decreased soluble sugar content.

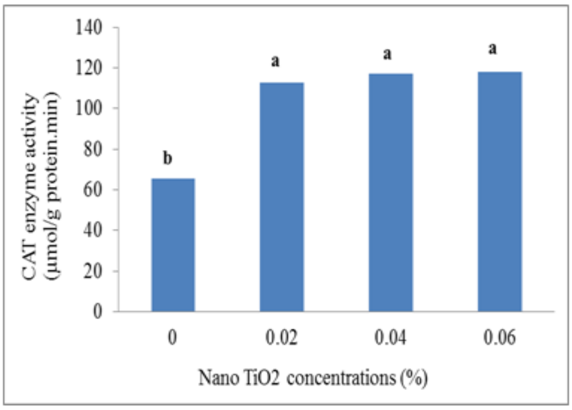

Fig. 5. Effect of nano-TiO_2 concentrations on CAT enzyme activity content in cumin.

Increasing of biochemical characteristics of cumin plant in treatment of 0.06% concentration of nano TiO_2 and decreasing of soluble sugars in the reproductive phase led to increasing of yield of cumin in conditions of the use of titanium nanoparticles. Consistent with these results, also the research of Moaveni *et al.* (2011a) showed that spraying of titanium nanoparticles on wheat plants, increased the seed yield. So that the highest value of this attribute, was obtained by application of 0.03% of this nanoparticle. Also the results of Jaberzadeh *et al.* (2013), showed that the simple effect of spraying time of this nanoparticle was significant on the seed yield trait.

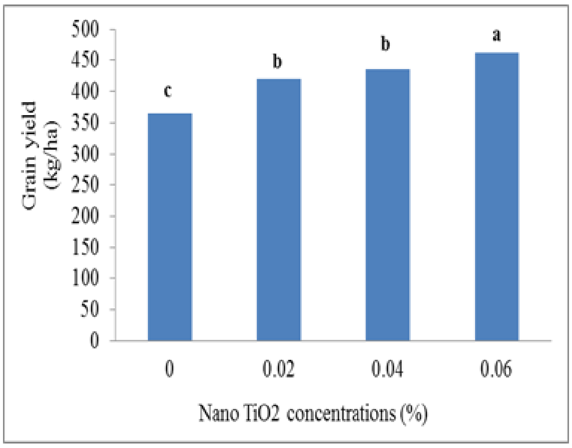

Fig. 6. Interaction effect of nano-TiO2 concentrations and times of application of it on grain yield of cumin.

Conclusion

According to these results, the use of nano TiO_2 nanoparticle at concentration of 0.06%, improved biochemical characteristics and yield of cumin because of decreasing of soluble sugar content and increasing of catalase activity and soluble protein content and decreasing of soluble sugar content at reproductive stage while the control or no application of titanium nanoparticle in comparison with the use of nanoparticles of titanium had the least amount of catalase activity and yield traits and had the highest amount of soluble sugars. Thus, due to very positive effect of nano TiO_2 on the increasing of plant yield, application of this nanoparticle can be recommended to the farmers as a strategic plan.

References

Bradford MM. 1979. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.

Analytical Biochemistry **72,** 248-254.

Buchanan BB, Gruissem W, Johones RL. 2002. Biochemistry and molecular biology of plants, Science Press, Beijing. 786- 824 P.

Carmen IU, Chithra P, Huang Q, Takhistov P, Liu S, Kokini JL. 2003. Nanotechnology: a new frontier in food science, Food Technology **57,** 24- 29.

Chalker-Scott L. 2002. Do anthocyanins function as osmoregulators in leaf tissues?. Advances in Botanical Research **37,** 103- 106.

Chance B, Maehly AC. 1955. Assay of catalase and peroxidase. Methods in Enzymology **2,** 764- 775.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry **28,** 350- 356.

Gao F, Hong F, Liu C, Zheng L, Su M, Wu X, Yang F, Wu C, Yang P. 2008. Mechanism of nano-anatase tio_2 on promoting photosynthetic carbon reaction of spinach. Biological Trace Element Research **111,** 239- 253.

Hong F, Yang F, Gao Q, Wan Z, Gu F, Wu C, Ma Z, Zhou J, Yang P. 2005. effect of nano tio_2 on spectral characterization of photosystem II particles from spinach. Chemical Research in Chinese Universities **21,** 196- 200.

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

Jaberzadeh A, Moaveni P, Tohidi Moghadam HR, Zahedi H. 2013. Influence of bulk and nanoparticles Titanium foliar application on some agronomic traits, seed gluten and starch contents of wheat subjected to water deficit stress. Notulae Botanicae Horti Agrobotanici Cluj-Napoca **41,** 201-207.

Li SJ, Cai JP, Wan GQ, Wang MQ, Zhao HY. 1978. Studies on structure and function of chloroplasts II. Isolation and interchangeability of pure coupling factors, Acta Botanica Sinica **20,** 103-107.

Ma, QF, Turner DW, Levy D, Cowling WA. 2004. Solute accumulation and osmotic adjustment in leaves of brassica oilseeds in response to soil water deficit. Australian Journal of Agricultural Research **55,** 939- 945.

Mingyu S, Fashui H, Chao L, Xiao W, Xiaoqing L, Liang C, Fengqing G, Fan Y, Zhongrui L. 2007. Effects of Nano-anatase TiO2 on Absorption, Distribution of Light, and Photoreduction Activities of Chloroplast Membrane of Spinach. Biological Trace Element Research **118,** 120- 130.

Moaveni P, Aliabadi Farahani H, Maroufi K. 2011a. Effect of TiO_2 nanoparticles spraying on quality and quantity of wheat (*Triticum Aestivum* L.). Advances in Environmental Biology **5,** 2211- 2213.

Moaveni P, Valadabadi SAR, Aliabadi Farahani H, Maroufi K. 2011b. Nanoparticles tio_2 spraying affected on calendula (*Calendula Officinalis* L.) under field condition. Advances in Environmental Biology **5,** 2242- 2244.

SAS Institute. 1988. Statistics analysis system user's guide: statistics. SAS Inst, Cary, NC.

Yang F, Hong F, You W, Liu C, Gao F, Wu C, Yang P. 2006. Influences of nano-anatase TiO_2 on the nitrogen metabolism of growing spinach. Biological Trace Element Research **110,** 179- 90.

Zheng L, Hong F, Lu S, Liu C. 2005. Effect of nano-tio_2 on strength of naturally aged seeds and growth of spinach. Biological Trace Element Research **104,** 83- 91.