Biological Forum – An International Journal **7**(1): 957-964(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of Different Concentrations and Time of Nano TiO_2 Spraying on Quantitative and Qualitative yield of Soybean (*Glycine max* L.) at Shahr-e-Qods, Iran

Farnaz Rezaei*, Payam Moaveni* and Hamid Mozafari*
**Islamic Azad University Shahr-e-Qods Branch, Fath Highway, Shahid Kalhor Boulevard, PO. Box: 37541-374, Shahr-e-Qods, Tehran, IRAN.*

(Corresponding author: Payam Moaveni)
(Received 12 February, 2015, Accepted 08 April, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The aim of the present investigation was to determine the effect of different concentrations and time of nano TiO_2 spraying on quantitative and qualitative yield of soybean (*Glycine max* L.). Therefore, this study was conducted as split factorial based on complete randomized block design with four replications, in the year of 2013, at Shar-Qods, Iran. Treatments consisted of two factors. First factor was, different concentrations of nano titanium dioxide (0 or control, 0.01%, 0.03% and 0.05%) and the second factor was, time of spraying of this nano particle (foliar application at vegetative and reproductive stages). Traits such as height, grain weight, number of pods per plant, pod dry weight, oil percentage and seed and oil yield were evaluated in this experiment. Results of analysis variance showed that the concentration of titanium dioxide nanoparticles was significant on all traits, with the exception of oil percentage. In this experiment, it was observed that spraying of soybean plants by nano TiO_2, had a significant and positive effect on the seed and oil yield and component of it in compared to the control treatment.

Keywords: NanoTiO_2, Quantitative and qualitative yield, Vegetative stage, Reproductive stage

INTRODUCTION

Soybean (*Glycine max* L) is one of the oldest crops that it contains essential amino acids (20 to 22%), protein (40%) and 18-22% oil of which 85% is cholesterol-free. Also this crop is an important legume with multifarious uses. Its cost effectiveness is ensured through biological nitrogen fixation and rotation with exhaustive crops. It replenishes and maintains soil fertility (Ngalamu *et al.,* 2013). It is essential, implementation of ways to increasing of production. Therefore, identification of the factors that caused an increasing or decreasing in the yield, are necessary (FAO, 2008). Nanotechnology is the general term for all advanced technologies in the field of working with nano scale and means of the nano scale is particles with dimensions of 1-100 nm (Oberdorster *et al.,* 1996).

Nanoparticles of titanium dioxide is one of the most important photocatalyst and considered as a unique light-activated catalyst due to strong photocatalyst properties such as high oxidative and stability of the optical properties with other benefits, such as non-toxicity, low cost andavailability (Reddy *et al.,* 2004, Fujishima *et al.,* 2006, Kasem *et al.,* 2010).

Furthermore, nanoparticles have photocatalytic property and can be easily placed into the cells due to the very small dimension (Xuming *et al.,* 2008). The obtained results indicated that treatments of nano TiO_2 induced an increase of the Hill reaction and of the activity of chloroplasts, which accelerated FeCy reduction and oxygen evolution. Moreover non cyclic photophosphorylation activity was higher than cyclic photophosphorylation activity. The explanation of these effects, on the opinion of the authors, could be that the nanoTiO_2 might enter the chloroplast, and its oxidation-reduction reactions might accelerate electron transport and oxygen evolution (Monica and Cremonini, 2009). Moreover, nanoparticles of titanium increase cell growth by improvement of photosynthetic and nitrogen metabolism and therefore, caused an increasing in weight of the plant (Hong *et al.,* 2005 and Mingyuand *et al.,* 2007). In one study, Klancnik *et al.,* (2011) found that nano TiO_2, increased mitotic division index of onion root tips. But its effect was not significant on the root length and root number of onion. Previous studies have shown that some NMs could enhance the plants' ability to absorb of water and nitrogenated fertilizers, promote the vigor of root system and the activities of the nitrate reductase, and accelerate the breakdown of organic substances, formation of essential amino acids (Harrison *et al.,* 1996).

Moaveni *et al.,* (2011a) declared that the foliar application of marigold by nano TiO_2 at concentration of 0.03%, reduced dihydrozingoanozin and dinitrozin content. Moaveni *et al* (2011b) investigated the effect of nano titanium on traits of wheat plant and showed that the highest amount of 1000 grain weight, number of grain per spike, was achieved by use of this nanoparticle compared with the control treatment. In one study, it was found that the growth of treated spinach seeds with nano TiO_2, increased compared with the bulk treatment, because of the easier passage of this nanoparticle into the seeds of plant (Zheng *et al.,* 2005). Navaro *et al.,* (2008) in a study showed that nanoparticles can gather nutrients in their surface and act as a nutritional source for the plant. Hghighi *et al.,* (2012) showed that the use of one mg per liter of titanium, increased fresh and dry weight of shoot compared to the control treatment. Furthermore, Moaveni and Kheiri, (2011c) studied the effect of titanium nanoparticle in two stages of appearance of the flower and stem and concluded that the highest amount of corn yield was obtained by application of this nanoparticle in the appearance of the flower. Increasing of shoot length and root dry weight by use of titanium in comparison with the control treatment, was observed in studies of Feizi *et al.,* (2012). In addition, they stated that application of nano TiO_2 at appropriate concentrations improved seed germination and seedling growth of wheat compared to the bulk and control treatments so that high concentrations of nano TiO_2, have inhibitory effects on the growth of wheat plants. Owolade *et al.,* (2008) tested foliar application of titanium at concentrations of 62 and 125 $ml.ha^{-1}$ and number of spraying (once and two) treatments on plants of cowpea, and they concluded that spraying of this plant with two application at 125 $ml.ha^{-1}$, increased traits of grain yield, 1000 grain weight, number of seeds per pod, pod length, number of pods per plant and leaf area.

It has been suggested that nanoparticles could hasten the germination and growth of soybean, could destroy mold and could increase the strength of roots and the activity of nitrate reductase, enhancing the root's ability to absorb water and fertilizers and to increase the activities of the antioxidant enzymes like superoxide dismutase and catalase, resulting in an improvement of the soybean resistance to stress (Harrison, 1996).

Mandeh *et al.,* (2005) reported that the use of nanoparticles of TiO_2 in vitro, increased the size of callus, number of callus and production of callus in comparison with the control, but it had no effect on the qualitative characteristics such as color and shape of callus. In accordance with the role of nano TiO_2 application on the increasing of growth of plants, therefore, the objectives of this study were evaluation of height, 1000 grain weight, grain weight, number of pods per plant, pod dry weight, oil percentage and seed and oil yield of soybean.

MATERIAL AND METHODS

This experiment was conducted as a factorial experiment based on the complete randomized block design with four replications, at Islamic Azad University of Shahr-e-Qods Branch, Iran in the year of 2013. Treatments consisted of two factors. First factor was different concentrations of nano titanium dioxide (0 or control, 0.01%, 0.03% and 0.05%) and the second factor was time of foliar application of this nano particle (vegetative and reproductive stages).

Soybean seeds were taken from the plant and seed Institute of Karaj, Iran. Seeds of soybean, planted within pots with height of 90 cm in July. Fertilization and feeding of plants recommendations were made based on the soil test. Spraying of treatments was based on growth stages and concentrations of nanoTiO_2. The characters were measured consist of plant height, pod dry weight, number of seeds per pod, grain weight, grain yield, oil percentage and grain and oil yield. At the end of the growth and in the maturity stage, pods of soybean plants for each treatment were harvested. Then the number of grain was determined by the average of five pods from of the each treatment and after pod drying, the average weight of treated seed, pod dry weight and grain yield was determined with the carriage scale.

The oil extraction with conventional solvents were performed in a Soxhlet-type apparatus using petroleum benzene as a solvent. For extraction, 10 g of soybean seeds and 300 mL of petroleum benzene were used. Time of extraction was 4-4.5 hours. After 4.5h, oil of soybean was accumulated in the erlen of Soxhlet extractor. Finally, oil yield was determined by the following formula.

Oil yield = Oil percentage × grain yield.

After normalization test, data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS Institute) and followed by Duncan's multiple range tests. Terms were considered significant at $P \leq 0.05$.

RESULTS

A. Pod dry weight

Results of analysis of variance (Table 1), indicated a significant effect of nano titanium dioxide and interaction effect between concentrations and time of spraying of this nanoparticle on this attribute ($P \leq 0.01$) but time of spraying on this trait was not significant.

According to the results of means comparison (Fig. 2), the highest amount of pod's dry weight, was obtained by interaction effect of spraying of soybean plant by 0.05% of nano TiO_2 at reproductive stage with the amount of 1.66g and the minimum amount of this trait was obtained by application of nano TiO_2 at concentration of 0.01% at vegetative stage (0.87 g) and interaction effect of control treatment in the vegetative and reproductive stage (0.86 and 0.83 g).

Table 1: Results of analysis variance of the soybean traits under different concentrations of nanoTiO_2 foliar application in two stages of plant growth.

		Means squares						
Sources of variation	**df**	**Height**	**Pod dry weight**	**Number of seed in pod**	**Grain weight**	**Grain yield**	**Oil**	**Oil yield**
Replication	3	919.57 ^{ns}	0.09 ^{ns}	0.193 ^{ns}	0.00003 *	61039.40 ^{ns}	0.469 ^{ns}	3965.96 ^{ns}
Concentrations of nanoTiO_2	1	1507.94 *	0.28 *	0.089 ^{ns}	0.0002 **	548822.04 **	1.681 ^{ns}	30869.13 **
Time of spraying (stages of plant growth)	3	79.38 ^{ns}	0.15 ^{ns}	0.180 ^{ns}	0.00006 *	564307.04 **	3.215 ^{ns}	42610.10 *
Interaction of Time and Concentrations	3	61.29 ^{ns}	0.49 **	0.069 ^{ns}	0.00001 ^{ns}	214118.71 *	9.428 ^{ns}	14615.35 ^{ns}
Error	21	320.32	0.06	0.109	0.00001	62625.92	4.63	6052.12

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

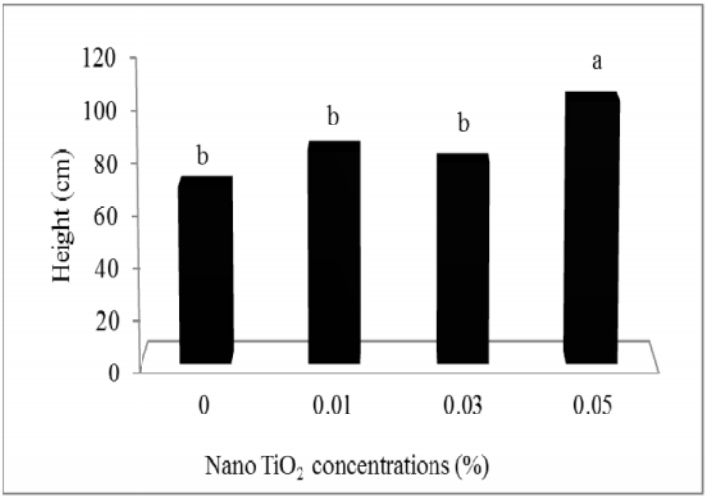

Fig. 1. Effect of nano TiO_2 concentrations on height of soybean.

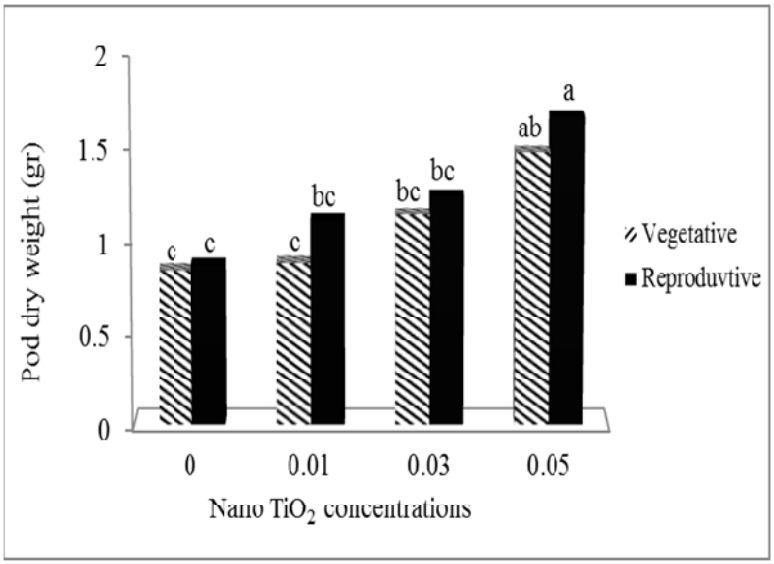

Fig. 2. Interaction effect of nano TiO_2 concentrations and time of foliar application of it on pod dry weight of soybean.

B. Number of seeds per pod

Results of analysis of variance showed that the simple effect of nano titanium dioxide concentrations and time of spraying and as well as the interaction effects between the nanoparticles concentrations and time of spraying on this trait was not significant (Table 1).

C. Grain weight

In accordance to the analysis variance (Table 1), the effect of nano titanium dioxide concentrations treatment on the grain weight of soybean was significant at the probability level P≤0.01 and time of spraying effect of nano TiO_2 on this trait was significant at the probability level P≤0.05. Nevertheless, the interaction effect of concentration of nano TiO_2 and time of spraying on this trait, was not significant (Table 1). The results of the means comparison showed that the treatment of nano TiO_2 at concentrations of 0.05% and 0.03% (0.106 and 0.104 g respectively) had the maximum amount of grain weight and control treatment (0.094 g), had the lowest seed weight (Fig. 3).

In addition, means comparison of data revealed that the use of nanoparticles in the reproductive stage of the plant, had a highest value of grain weight so that the highest grain weight (0.102g), was obtained by treatment of nano TiO_2 spraying at reproductive stage and the lowest amount of this trait (0.099 g) was achieved by foliar application of this nanoparticle at vegetative stage.

D. Gain yield

According to the results of analysis of variance (Table 1), the main effect of each treatment of titanium concentrations and time of spraying on the trait of grain yield, was significant at the probability level P≤0.01 and interaction effects of these treatments on this trait, was significant at the probability level P≤0.05. The results of means comparison showed that the application of nano TiO_2 at concentration of 0.05% in reproductive stage has the highest values of grain yield (2416.16 kg ha^{-1}) that it had not significant difference with the interaction effect of 0.05% concentration of nano TiO_2 in the vegetative stage and interaction effect of 0.03% concentration of nano TiO_2 in the reproductive stage, so that these treatments were in the same statistical group. While treatment of no application of nano TiO_2 at vegetative stage (1494.8 $kg.ha^{-1}$) and reproductive stage (1532.1 $kg.ha^{-1}$) had the lowest grain yield values (Fig. 5) that these treatments had not significant difference with the interaction effect of 0.03% concentration of nano TiO_2 in the vegetative stage.

E. Oil percentage

Results of analysis of variance (Table 1), shows that the simple effects of each treatment of nano TiO_2 concentrations and time of its spraying on this plant, and interaction effects of these treatments was not significant on the oil percentage treatment.

F. Oil yield

According to the results of analysis of variance (Table 1), the effect of nano TiO_2 concentrations on oil yield of soybean plant, was significant at the probability level P≤0.01 and the spraying time effect on this trait, was significant at the probability level P≤0.05 but the interaction effects of different concentrations of this nanoparticle and time of spraying on this trait was not significant.

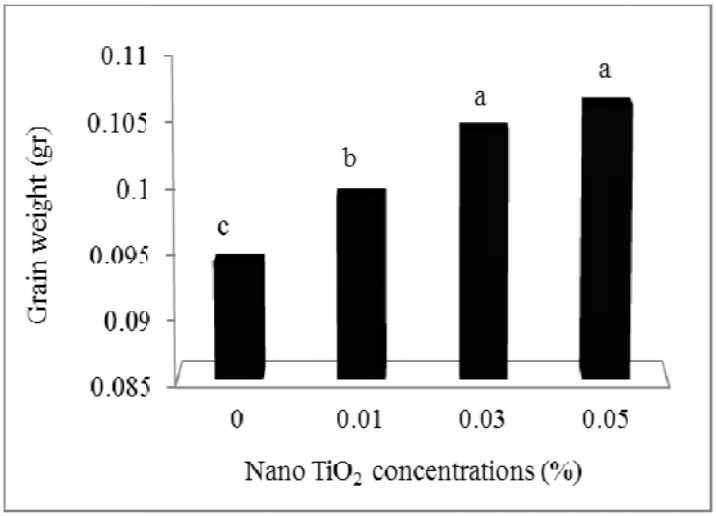

Fig. 3. Effect of nano TiO_2 concentrations on grain weight of soybean.

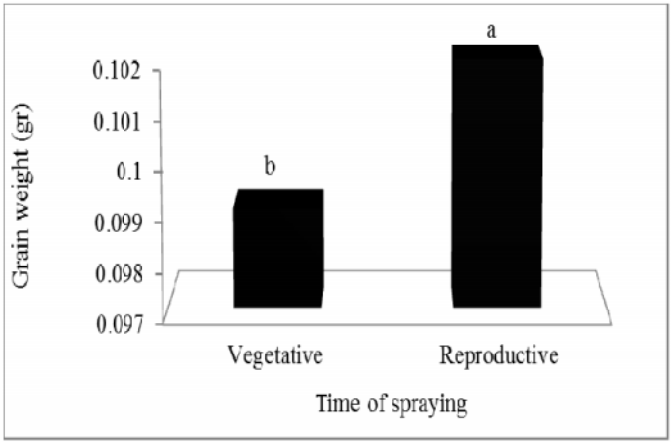

Fig. 4. Effect of time of nano TiO_2 spraying on grain weight of soybean.

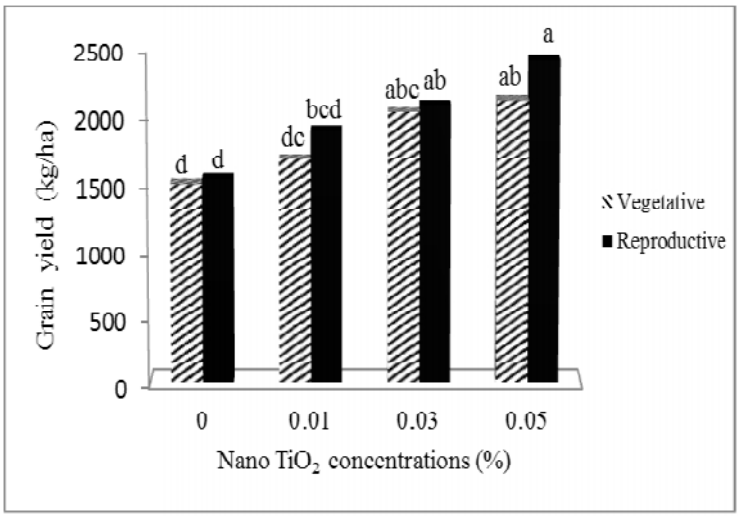

Fig. 5. Interaction effect of nano TiO_2 concentrations and time of spraying of it on the grain yield of soybean.

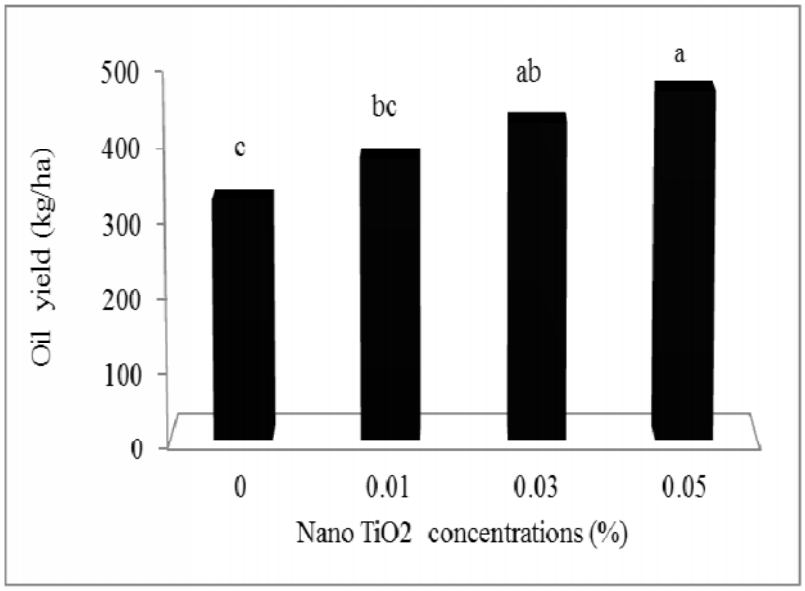

Fig. 6. Effect of nano TiO_2 concentrations on oil yield of soybean.

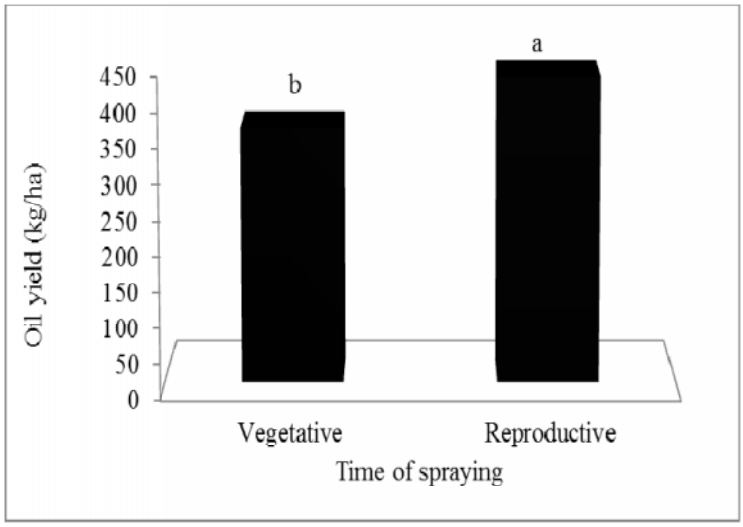

Fig. 7. Effect of time of nano TiO_2 spraying on oil yield of soybean.

Foliar application of soybean by TiO_2 nanoparticle at concentration of 0.05% with the yield of 466.12 kg.ha^{-1} had the maximum yield of soybean that it had not significant difference with the treatment of 0.03% concentration of nano TiO_2 so that control treatment with the yield of 321.75 kg.ha^{-1} had the minimum yield of soybean that this treatment had not significant difference with the treatment of 0.03% concentration of nano TiO_2. Therefore, application of titanium nanoparticles, caused an increasing in soybean oil yield (Fig. 6).

According to the Fig. 7, the highest amount of oil yield (433.40kg.ha^{-1}), was obtained by nano TiO_2 spraying at reproductive stage and the lowest amount of oil yield (360.43 kg.ha^{-1}), was achieved by foliar application of this nano particle at vegetative stage.

DISCUSSION

Nano particles of TiO_2 with photocatalytic property, enhance photosynthetic apparatus, Therefore photosynthetic activity increases, and it should be expected that increase of all traits are related to the plant production, including biochemical, morphological and yield, In fact, with increasing of plant photosynthesis, it should be expected that, in plots that treated with this nanoparticle, increased plant height and oil and seed yield and yield components of soybean compared with the no application of it.

In this experiment, since the oil percentage was not affected by any treatment, however, because of oil yield was obtained by multiplication of the oil percentage and seed yield traits therefore, seed yield in treatment of 0.05% of nano TiO_2 application at reproductive stage was more than the other treatments. It was observed that, oil yield in the treatment of 0.05 percent application of nano TiO_2 at reproductive stage, was higher than the other treatments, especially control treatment and it should be emphasized that the concentrations of nanoparticles was used in plant growth is extremely important so that stimulation of plant growth by nanoparticles, will be correlated with the concentration of nanoparticles (Lin and Xing, 2007).

In fact, nano-anatase TiO_2 treatments could obviously increase the activities of nitrate reductase, glutamate dehydrogenase, glutamine synthase, and glutamic-pyruvic transaminase during the growing stage. Nano-anatase TiO_2 treatments could also promote plant to absorb nitrate, accelerate inorganic nitrogen (such as NO_3--N and NH_4 +-N) to be transformed into organic nitrogen (such as protein and chlorophyll), and enhance height of plants and the fresh weight and dry weight (Yang *et al.*, 2006). So that results of Jabrazadh *et al.*, (2013) research and Owolade *et al.*, (2008) showed that the use of titanium nano dioxide increased wheat and soybean grain weight. In fact, this nanoparticle, has the major role in the remobilization of the photosynthetic materials at reproductive stage as well as, titanium nanoparticle increase seed and oil yield with increasing of plant resistance against stress and disease and preventing of yield loss. Increasing of yield by titanium, was reported by Chao *et al.* (2005) and in accordance with these results, Moaveni and Kheiri, (2011) and Jaberzadh *et al.*, (2013) reported that effects of time of nano TiO_2 spraying were significant on yield of plant. Therefore, plants treated with this nanoparticle in the reproductive stage, transfer higher photoasmylat and photosynthetic materials into the grain compared with the control. Thus foliar application of this nanoparticle in the reproductive stage caused an increasing in yield and component of it.

CONCLUSION

It can be inferred that application of nano TiO_2 0.05% as foliar application during flowering stage in soybean can improve yield component, seed yield that results in increasing of oil yield of this plant in comparison with the other treatments.

REFERENCES

Chao SHL, Choi H.S. (2005). Method for providing enhanced photosynthesis. Korea Research Institute of Chemical Technology, Jeonju, South Korea. *Bulletin*, **10**.

FAO (Food and Agriculture Organization of the United Nations) FAOSTAT. (2008). Available: http://faostat.fao.org/

Feizi H., Rezvani Moghaddam P., Shahtahmassebi N, Fotovat A. (2012). Impact of bulk and nanosized titanium dioxide (TiO_2) on wheat seed germination and seedling growth. *Biological Trace Element Research*, **146**,101-106.

Fujishima A, Zhang X. (2006). Titanium dioxide photocatalysis: present situation and future approaches. *C.R. Chemie*, **9**: 750-60.

Haghighi M., Heidarian, S., Teixeira Silva J.A. (2012). The effect of titanium amendment in N-withholding nutrient solution on physiological and photosynthesis attributes and micronutrient uptake of tomato. *Biological Trace Element Research*, **150**: 381-90.

Harrison, C.C. (1996). Evidence for intramineral macromolecules containing protein from plant silicas, *Phytochemistry*, **41**, 37-42.

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.

Jaberzadeh A, Moaveni P., Tohidi Moghadam H.R., 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.

Kasem KK, Dahn M. (2010). Photodissociation of water using colloidal nanoparticles of dopedtitanium (IV) oxide semiconductors for hydrogen production. *Current Science*, **99**, 1068-73.

Klancnik K, Drobne D, Valant J, Dolenc Koce J. (2011). Use of a modified allium test with nano TiO_2. *Ecotoxicology and Environmental Safety*, **74**, 85-92.

Lin, D. and Xing, B. (2007). Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. *Environmental Pollution*, **150**, 243-250.

Monica, R.C. and Cremoniniimme, R. (2009). Nanoparticles and higher plants. *Caryologia*, **62**, 161-165.

Mandeh M, Omidi M, Rhaie M. (2011). In vitro influences of TiO_2 nanoparticles on barely (*Hordeum vulgar* L.) tissue culture. *Biological Trace Element Research*, **150**, 376-80.

Mingyu S, Fashui H, Chao L, Xiao W, Xiaoqing L, Liang C, Fengqing G, Fan Y, Zhongrui L. (2007). Effects of Nano-anatase TiO_2 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). Investigation of nanoparticles tio2 affected on some of enzymes in calendula (*Calendula officinalis* L.). *Advances in Environmental Biology*, **5**, 2234-2237.

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

Moaveni, P. and Kheiri T. (2011). TiO_2 nano particles affected on maize (*Zea mays* L.). *2nd International Conference on Agricultural and Animal Science IPCBEE 22, IACSIT Press, Singapore.*

Navarro, E., Baun A., Behra R., Hartmann N.B., Filser J., Miao A., Quigg A, Santschi P.H., Sigg L. 2008. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. *Ecotoxicology*, **17**, 372-386.

Ngalamu, T., Ashraf, M., Meseka, S. (2013). Soybean (*Glycine max* L.) genotype and environment interaction effect on yield and other related traits. *American Journal of Experimental Agriculture*, **3**, 977-987.

Oberdorster, G. (1996). Significance of particle parameters in the evaluation of exposure-dose-response relationships of inhaled particles. *Inhalation Toxicology*, **8**, 73-89.

Owolade O.F., Ogunleti D.O., Adenekan M.O. (2008). Titanum dioxide affects diseases, development and yield of edible cowpea. *Electronic Journal of Environmental Agricultural and Food Chemistry* **7**, 2942- 2947.

Reddy B.M., Ganesh I., Khan A. (2004). Stabilization of nanosizedtitania-anatase for hightemperature catalytic applications. *Journal of Molecular Catalysis A: Chemical* **223**, 295-304.

Xuming W., Gao F., 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.

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.