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Effects of TiO_2 (nano and bulk) foliar application on physiological traits and grain yield of Safflower (*Carthamus tinctorius* L.)

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ABSTRACT: This study was done to investigate the effects of TiO_2 (nano and bulk) foliar application on the physiological traits and grain yield of safflower (*Carthamus tinctorius* L.). Therefore, this study was conducted during the growing seasons of 2013 and 2014. This research was done as a RCBD experiment with four replications at the one personal farm, at Garmsar, Iran. This experiment consisted of four treatments, T1: no-foliar application, T2: foliar application of TiO_2 (bulk) at concentration of 0.04%, T3: foliar application with nano-TiO_2 at concentration of 0.02% and T4: concentration of 0.04%. Final results showed that application of nanoTiO_2 had a significant effect on the traits of total chlorophyll, carotenoids, soluble proteins, grain yield of safflower and the treatment of the application of nanoTiO_2 improved physiological traits of this plant so that the highest amount of these traits was obtained by the treatment of nanoTiO_2 at concentration of 0.04%. Therefore these results showed that application of nanoTiO_2 could be used to enhance the yield of safflower.

Keywords: Nano TiO_2, Soluble proteins, Biological yield, Carotenoids

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an annual plant originally grown for its flowers. Today, safflower it is primarily cultivated for its oil, which is used for food and industrial purposes. Safflower can be used as animal feeds, birds feed and in small quantity for ruminants feed as whole seeds or meals (Oelke *et al.,* 1992). The seed contains nearly 35-50% oil, 15-20% protein, and 35-45% hull fraction (Betschart, 1975; Rahamatalla *et al.,* 2001). It contains 72 percent linolenic acid, the factor which reduces blood cholesterol. Moreover, due to high content of unsaturated fatty acids and it is being used as an excellent drying oil for use in paints and varnishes (Ravi *et al.,* 2008). In recent years, a lot of attention is being paid to nanotechnology with its research and applications for its beneficial effects. Nanoparticles with sizes typically below 100 nm, have been applied in several fields of bioscience and biomedicine with an increasing number of commercial applications. There are many types of man-made nanoparticles produced by nanomanufacturing technologies, which are being used in nanomedicine and classified elsewhere in detail (Thul *et al.,* 2013).

Nanoscale titanium dioxide, one of the most popular manufactured nanomaterials, is used in a variety of consumer products, such as sunscreens, cosmetics, paints and surface coatings (Fisher and Egerton, 2001; Kaida *et al.,* 2004), and in the environmental decontamination of air, soil and water (Esterkin *et al.,* 2005; Choi *et al.,* 2006). The effect of nanomaterials on plant growth is complex; even the same kind of nanomaterials may have different biological effects on different plant types. Nano-TiO_2 can improve light absorbance and promote the activity of Rubisco activase. Therefore, accelerate plant growth (Yang *et al.* 2006). Nano-TiO_2 can significantly promote the genetic expression of *Arabidopsis thaliana* light-harvesting complex II b (Ze *et al.,* 2011). Lu *et al.* (2002) indicated that a combination of nanosized SiO_2 and TiO_2 increased ability of soybean for absorbing and utilizing water and fertilizer, the nitrate reductase enzyme activity and therefore accelerate its germination and growth of this plant. Moreover, nano-TiO_2 improved plant growth by enhanced nitrogen metabolism (Zheng *et al.,* 2005).

Zheng *et al.* (2005) reported that 2.5% rutile nano-TiO_2 promoted the germination of spinach seeds, whereas 0.25% rutile nano-TiO_2 enhances the spinach photosynthesis by promoting cyclic and linear photophosphorylation. This promotion is closely related to Mg^{2+}-ATPase activity (Hong *et al.,* 2005). An experiment was carried out to evaluate the effect of TiO_2 nano particles spraying some of the enzymes in calendula (*Calendula officinalis* L.), it is revealed that, TiO_2 nano particles could increased antioxidants enzymes of superoxide dismutase (SOD) and CAT and decreased malondialdehyde (MDA) content in compare with un-spraying situation and spraying with TiO_2(bulk) (Moaveni *et al.*, 2011a). Moaveni *et al.*, (2011b) studied effect of nanoparticles of TiO_2 and TiO_2 (bulk) spraying on some yield components in barley (*Hordem vulgare* L.). Their results showed that effect of TiO_2 nano particles spraying was significant on grain yield and number of spikelets but the effect of TiO_2 nano particles spraying was not significant on harvest index, weight of spikelets and the highest grain yield, weight of spikelets and number of spikelets were achieved by TiO_2 nano particles of 0.03 percentage. Whereas, in the literature there is little evidence on the effect of titanium on crop plants, the objective of this study is investigation the effect of nano TiO_2 concentrations on the physiological traits, seed yield of safflower (*Carthamus tinctorius* L.).

MATERIAL AND METHODS

This study was conducted during the growing seasons of 2013 and 2014. This research was done as a RCBD experiment with four replications at the one personal farm, at Garmsar, Iran. This experiment consisted of four treatments, T1: no-foliar application, T2: foliar application of TiO_2 (bulk) at concentration of 0.04%, T3: foliar application with nano-TiO_2 at concentration of 0.02% and T4: concentration of 0.04%.

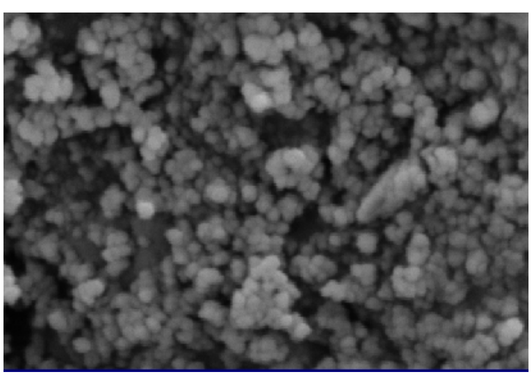

Fig. 1. Image of TiO_2 by scanning electron microscopy (SEM).

The size of the TiO_2 nanoparticles and TiO_2 (bulk) were determined by scanning electron microscopy (SEM) at the Rezaei laboratory at Tehran, Iran. Nano sized of TiO_2 was with average particle size of 44.17 nm (Fig. 1) and TiO_2 (bulk) was with average particle size of 132.16 nm (Fig. 2).

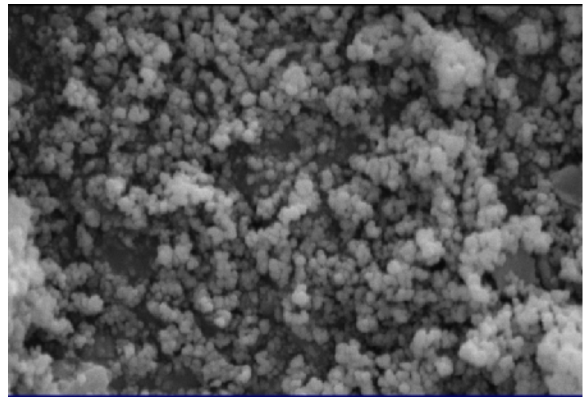

Fig. 2. Image of nano TiO_2 by scanning electron microscopy (SEM).

Each experimental plot consisted of 4 rows, 3 m long with 50 cm spaced between rows and 10 cm distance between plants on the rows. Seeds were sown on October 17th, 2013. 1/3 of nitrogen fertilizer was applied before sowing, and the remaining of N fertilizer was applied during the vegetative growth and p fertilizer was applied at a rate of 150 kg P_2O_5 in the form of the superphosphate triple in the soil before sowing. Phosphorus fertilizer according to the results of soil analysis pointed out for the sufficient level of this element in the soil. Titanium dioxide nanoparticles and Titanium dioxide (bulk) were sprayed by a portable spray machine on the safflower plants at vegetative and reproductive stages of plant.

In this test characteristics of soluble proteins, total chlorophyll content, carotenoid's content, grain yield, biological yield and harvest index was evaluated. Seed yield in each plot measured with 14% humidity. After harvesting, branches were dried in the shade and grain yield was measured using a carriage scale using standard moisture at 14%. Biological part of plant were dried to uniform moisture content for 24-48 h at 75-80ºC. Harvest index was calculated by following formula (Bar-Tal *et al.*, 2008):

Harvest index = Seed yield/ Biological yield ×100

Total chlorophyll and carotenoid's content was measured against a blank of 80% (V/V) acetone at wavelengths of 646.8 and 663.2 nm for chlorophyll assays and 470 nm for carotenoid assays. Finally, evaluations for traits were determined by the following formula (Lichtenthaler, 1987) ;

$\text{Chl a} = 12.25\ _{AAbs}663.2 - 2.79_{AAbs}646.8$

$\text{Chl b} = 21.5\ _{AAbs}646.8 - 5.1_{AAbs}663$

$\text{C (x + c)} = (1000\ _{AAbs}470 - 1.82\text{Chl a} - 85.02\text{Chl b})/189$ Chl T = Chl a + Chl b

Protein content was estimated following the method of Bradford (1976) at 595 nm wavelength by spectrophotometer using serum albumin as standard protein and amount of it was recorded mg.fw^{-1}. The analysis of variance of data was done by as software, and duncan' multiple range test was used to compare treatment means.

RESULTS

Table 1: Analysis of variance results of the safflower (*Carthamus tinctorius* L.) traits under different concentrations of TiO_2 (nano and bulk) spraying.

Mean squares							
Sources of variation	**df**	**Total chlorophyll**	**Cartenoids**	**Soluble proteins**	**Grain yield**	**Biological yield**	**Harvest index**
Replication	3	0.0005^{ns}	0.002^{ns}	0.01^{ns}	13943.05^{ns}	93750.00^{ns}	0.13^{ns}
NanoTiO_2 and TiO_2	3	0.0185^{**}	0.0107^{**}	0.08^{*}	38270.26^{**}	73830.66^{ns}	2.22^{ns}
Error	9	0.0003	0.001	0.01	7280.04	97083.33	3.83

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

A. Total chlorophyll content

According to the results of Table 1, effect of TiO_2 (nano and bulk) application on the total chlorophyll content, was significant at the probability level P ≤0.01. Maximum amount of this trait (1.01 mg. fw^{-1}) was obtained by 0.04% of nano TiO_2 and minimum amount (0.87 mg. fw^{-1}) of it was obtained by no application of this nanoparticle or control and TiO_2 (bulk) treatments (Fig. 3).

B. Carotenoid's content

The results of analysis of variance showed that effect of TiO_2 (nano and bulk) application on the on the carotenoids content was significant at the probability level P≤0.01 (Table 1). Means comparison with Duncans' test (Fig. 4) showed that amount of carotenoids increased with the increasing of nano TiO_2 concentration. So that treatments of 0.04% and 0.02% of nano TiO_2 application with amounts of 1.20 mg.g fw^{-1} and 1.18 mg.g fw^{-1}, respectively caused a significant increase in carotenoids content in comparison with the other treatments. Treatments of control or no application of this nano TiO_2 with amount of 1.10 mg.g fw^{-1} and foliar application with TiO_2 (bulk) with the amount of 1.11 mg.g fw^{-1}, had the least amount of this trait and were in the same group.

C. Soluble proteins

According to the results in Table 1, the effect of different application of nanoparticles of titanium and titanium dioxide on soluble protein's content had significant effect at the probability level P≤0.05. The use of TiO_2 nanoparticles at concentration of 0.04% had the highest amount of soluble proteins (1.57 g) in comparison with the other treatments, while the no-application of this nanoparticles and TiO_2 (with 1.20 g soluble proteins) and the use of bulk or titanium dioxide (with 1.36 g soluble proteins), had the lowest soluble protein's content and treatment of 0.02% of nano TiO_2 application with the amount of 1.40 g soluble proteins was placed between the maximum and minimum values of this trait (Fig. 5).

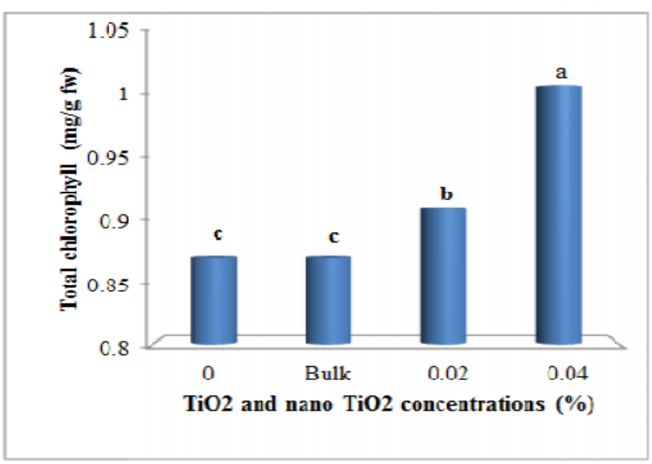

Fig. 3. Effect of TiO_2 (nano and bulk) concentrations on total chlorophyll content of safflower.

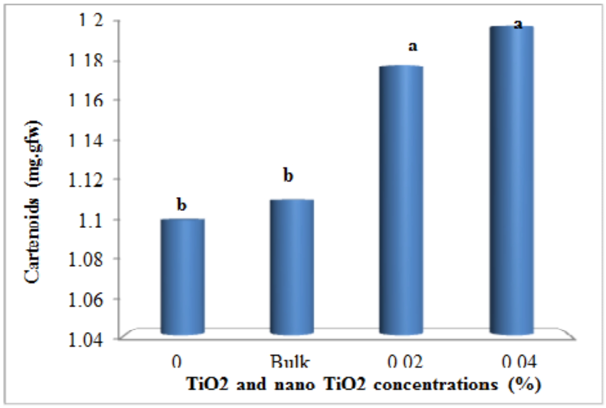

Fig. 4. Effect of TiO_2 (nano and bulk) concentrations on carotenoids content of safflower.

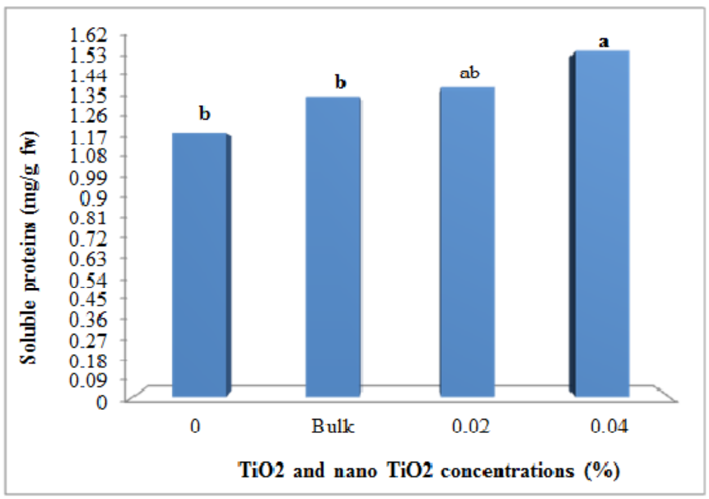

Fig. 5. Effect of TiO_2 (nano and bulk) concentrations on soluble proteins content of safflower

D. Grain yield

The results of analysis of variance (Table 1), indicated that the effect of foliar safflower by nano TiO_2 and TiO_2 was significant ($P \leq 0.01$). According to the Fig. 6, the results of means comparison showed that treatment of 0.04% nano TiO_2 with amount of 2430.08 $kg.ha^{-1}$, had the highest grain yield and treatments of 0.02% nano TiO_2 with yield of 2315.54 $kg.ha^{-1}$, treatment of TiO_2 (bulk) with yield of 2260.50 $kg.ha^{-1}$ and control treatment with yield of 2200.06 $kg.ha^{-1}$respectively showed the lowest yield.

E. Biological yield

The results of analysis of variance (Table 1), showed that the effect of application of TiO_2 nanoparticles and TiO_2 sprayed on the biological yield of this plant was not significant but foliar application with 0.04% of this nanoparticle with the amount of 6650 $kg.ha^{-1}$ and treatment of no-use of nano TiO_2 and TiO_2 with the amount of 6345 $kg.ha^{-1}$, respectively, had the highest and the lowest values of this trait (Fig. 7).

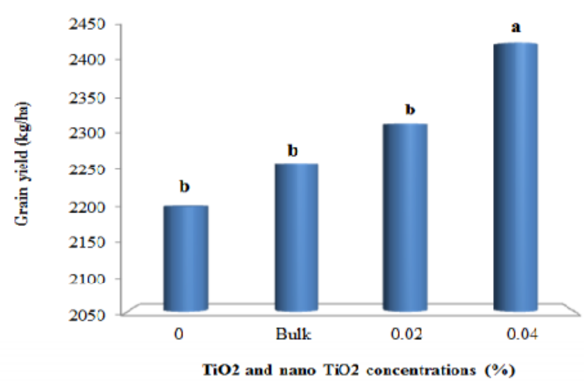

Fig. 6. Effect of TiO_2 (nano and bulk) concentrations on grain yield.

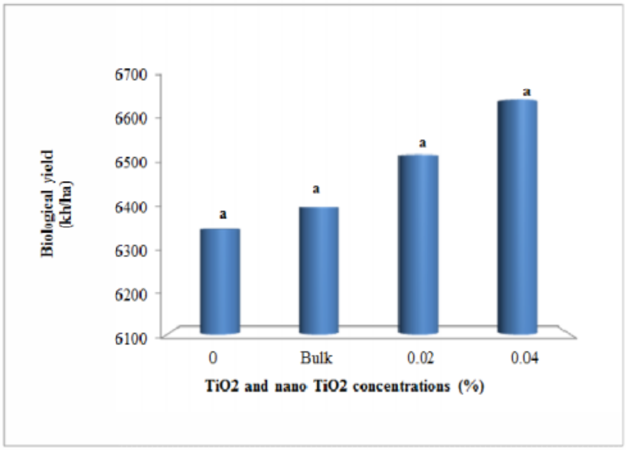

Fig. 7. Effect of TiO_2 (nano and bulk) concentrations on biological yield of safflower.

F. Harvest index

The results showed that nano TiO_2 and TiO_2 (bulk) on the harvest index was not significant (Table 1). However, the results showed that treatments of nano TiO_2 application, particularly concentrations of 0.04% in comparison with the control and titanium dioxide (bulk) treatments, had more harvest index value (Fig. 8).

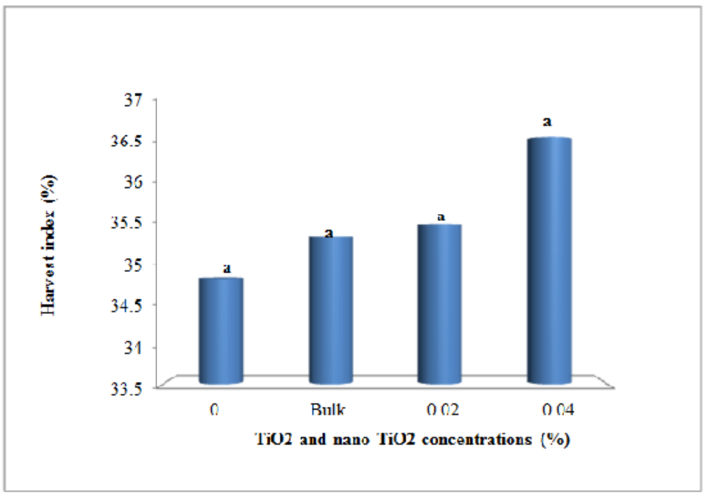

Fig. 8. Effect of TiO_2 (nano and bulk) concentrations on the harvest index of safflower.

DISCUSSION

The results for this study showed that the treatment of safflower plant with nano TiO_2 had the more positive and significant effect on many of the measured traits in comparison with the bulk (TiO_2) and control treatments. The concentration of nanoparticles used in plant growth, is important so that stimulate of the plant growth by nanoparticles, will be correlated with the concentration of nanoparticles (Lin and Xing, 2007). In this experiment concentration of 0.04 nano TiO_2 in comparison with the other concentrations and other treatments (control and bulk) was the best treatment for increasing of total chlorophyll, carotenoids, proteins solution, harvest index, biological yield, grain yield traits.

In fact, nanoparticle of titanium dioxide in comparison with the TiO_2 (bulk) has a greater effect on plant growth and simply enters the cells, especially chloroplats because of the much smaller size compared with the bulk and acts as a photocatalyst (Xuming *et al.*, 2008) and this leads to more efficient of photosynthetic system and thus increase the yield of the plant. The photocatalytic activity and quantum effects of titanium nanoparticle is more than the titanium dioxide (bulk) because nanoparticles of TiO_2 could catalyze the oxidation and reduction reactions and thereby release high-energy electrons (Yang *et al.*, 2006).

Treatment of this plant with nano TiO_2 compared with the bulk type affects on the nitrogen metabolism and stabilizing the nitrogen in the plant and increase nitrogen content and for this reason, treatment of plant with nano TiO_2, caused a significant increasing in chlorophyll and protein content in the plant (Hong *et al.*, 2005a; Yang *et al.*, 2006; Zheng *et al.*, 2005) and increasing the amount of soluble protein and chlorophyll can increase production of dry matter and morphological characteristics of the plant and ultimately increase the plant's yield. In fact nanoparticles during grain filling, moved photoassimilate into the grains with increasing of photosynthetic rate and photoassimilate and prevention of leaves senescence or in other words, by increasing the power of source. Also with the use of this nanoparticle, accumulation of photoassimilate due to increasing of absorption of solar radiation in the shoot, CGR, LAI, plant height, dry weight of leaves and stems, thereby caused an increasing in the biological yield of the plant.

HI, is one of the important physiological parameters that indicate the transmission of the photoassimilate from vegetative organs to the grains. Results of this experiment showed that foliar application of nano TiO_2 in this experiment had a more efficient role in the remobilization of photoassimilate into economic organs of safflower (grain).

Therefore, in this experiment, the use of nanoparticles of titanium dioxide compared with the titanium dioxide (bulk) and no-application of this nanoparticles and titanium dioxide, had an effective role in enhancing the physiological characteristics and grain yield of safflower.

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