Effects of TiO$_2$ (nano and bulk) foliar application on physiological traits and grain yield of Safflower (Carthamus tinctorius L.)

Elham Morteza*, Payam Moaveni**, Tayebeh Morteza***, Hadi Saemi**** and Ali Joorabloo*****

*Ph.D. Student, Agronomy and Plant Breeding Department, Aburaihan Campus, University of Tehran, Tehran, IRAN
**Assistant Professor, Agronomy Department, Islamic Azad University, Shahr-e-Qods Branch, Shahr-e-Qods IRAN
***Graduated, Plant Protection Department, Islamic Azad University, Damghan Branch, Damghan, IRAN
****Graduated, Horticulture Department, Rasht University, Rasht, IRAN
*****Graduated, Agronomy and Plant Breeding Department, Islamic Azad University, Varamin Branch, Varamin, IRAN and M.Sc., Agronomy Department, Agriculture Jahad Office of Garmsar, Garmsar, IRAN

Corresponding author: Elham Morteza

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 nano-TiO$_2$ had a significant effect on the traits of total chlorophyll, carotenoids, soluble proteins, grain yield of safflower and the treatment of the application of nano-TiO$_2$ improved physiological traits of this plant so that the highest amount of these traits was obtained by the treatment of nano-TiO$_2$ at concentration of 0.04%. Therefore these results showed that application of nano-TiO$_2$ 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-TiO₂ promoted the germination of spinach seeds, whereas 0.25% rutile nano-TiO₂ enhances the spinach photosynthesis by promoting cyclic and linear photophosphorylation. This promotion is closely related to Mg²⁺-ATPase activity (Hong et al., 2005). An experiment was carried out to evaluate the effect of TiO₂ nano particles spraying some of the enzymes in calendula (Calendula officinalis L.), it is revealed that, TiO₂ 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 TiO₂(bulk) (Moaveni et al., 2011a). Moaveni et al., (2011b) studied effect of nanoparticles of TiO₂ and TiO₂ (bulk) spraying on some yield components in barley (Hordeum vulgare L.). Their results showed that effect of TiO₂ nano particles spraying was significant on grain yield and number of spikelets but the effect of TiO₂ nano particles spraying was not significant on harvest index, weight of spikes and the highest grain yield, weight of spikelets and number of spikelets were achieved by TiO₂ 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 TiO₂ 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 TiO₂ (bulk) at concentration of 0.04%, T3: foliar application with nano-TiO₂ at concentration of 0.02% and T4: concentration of 0.04%.

The size of the TiO₂ nanoparticles and TiO₂ (bulk) were determined by scanning electron microscopy (SEM) at the Rezaei laboratory at Tehran, Iran. Nano sized of TiO₂ was with average particle size of 44.17 nm (Fig. 1) and TiO₂ (bulk) was with average particle size of 132.16 nm (Fig. 2).

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 P₂O₅ 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 75-80°C. Harvest index was calculated by following formula (Bar-Tal et al., 2008):

\[
\text{Harvest index} = \frac{\text{Seed yield}}{\text{Biological yield}} \times 100
\]

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):

\[
\begin{align*}
\text{Chl a} & = 12.25 \times \text{AAbs}_663.2 - 2.79 \times \text{AAbs}_646.8 \\
\text{Chl b} & = 21.5 \times \text{AAbs}_646.8 - 5.1 \times \text{AAbs}_663 \\
\text{C (x + c)} & = (1000 \times \text{AAbs}_470 - 1.82 \times \text{Chl a} - 85.02 \times \text{Chl b}) \\
\text{Chl T} & = \text{Chl a} + \text{Chl b}
\end{align*}
\]
RESULTS

Table 1: Analysis of variance results of the safflower (Carthamus tinctorius L.) traits under different concentrations of TiO$_2$ (nano and bulk) spraying.

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>df</th>
<th>Total chlorophyll</th>
<th>Carotenoids</th>
<th>Soluble proteins</th>
<th>Grain yield</th>
<th>Biological yield</th>
<th>Harvest index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>3</td>
<td>0.0003</td>
<td>0.001</td>
<td>0.01</td>
<td>13943.05**</td>
<td>97083.33</td>
<td>3.83</td>
</tr>
<tr>
<td>NanoTiO$_2$</td>
<td>3</td>
<td>0.0185**</td>
<td>0.0107**</td>
<td>0.08</td>
<td>38270.26**</td>
<td>73830.66**</td>
<td>2.22**</td>
</tr>
<tr>
<td>and TiO$_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* 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 $P \leq 0.01$. 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 total carotenoids content was significant at the probability level $P \leq 0.01$ (Table 1). Means comparison with Duncan's 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.

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 $P \leq 0.05$. 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).
**D. Grain yield**
The results of analysis of variance (Table 1), indicated that the effect of foliar safflower by nano TiO₂ and TiO₂ was significant (P ≤ 0.01). According to the Fig. 6, the results of means comparison showed that treatment of 0.04% nano TiO₂ with amount of 2430.08 kg.ha⁻¹, had the highest grain yield and treatments of 0.02% nano TiO₂ with yield of 2315.54 kg.ha⁻¹, treatment of TiO₂ (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 TiO₂ nanoparticles and TiO₂ 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 TiO₂ and TiO₂ with the amount of 6345 kg.ha⁻¹, respectively, had the highest and the lowest values of this trait (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).

**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 chloroplasts 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.

REFERENCES


