Investigation of different concentrations and times of nano-TiO$_2$ foliar application on traits of soybean (*Glycine max* L.) at Shahr-e-Qods, Iran

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**Abstract**

In order to investigation of different concentrations and times of nano-TiO$_2$ foliar application on traits of soybean (*Glycine max* L.) cultivar of M9, this experiment was done as split factorial based on complete randomized block design with four replications, in 2013 year, at Shar-Qods, Iran. Treatments included 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, times of foliar application of this nano particle (foliar application at vegetative and reproductive stages). Final results indicated that, effect of nano titanium dioxide concentrations with the exception of anthocyanins, was significant on the other traits (chlorophyll a, b and total and carotenoids content) but times effect of nano-TiO$_2$ foliar application on whole traits was not significant. However, interaction’s effects of concentrations and times of nano-TiO$_2$ foliar application, was significant on the chlorophyll b and carotenoids. Maximum content of chlorophyll a and total chlorophyll, were achieved by foliar application of nano-TiO$_2$ at 0.05% concentration and the highest amount of chlorophyll b, was obtained by interaction effect of concentration of 0.05% and foliar application at vegetative stage and the highest content of carotenoids, was achieved by spraying of 0.05% concentration of nano-TiO$_2$ at reproductive stage.

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Introduction

Soybean (Glycine max L.) is a legume that grows in tropical, subtropical and temperate climate and this plant is a crop which can provide complete protein and containing eight amino acids essential for human (Asadi and Faraji, 2009). Soybean is a valuable resource for both oil and protein, and it used for a wide variety of consumer uses today. So that, soybean oil can be separated into industrial and edible products. Edible uses include margarine, shortenings, salad oils, deserts, and drug manufacturing, while industrial uses include soaps, inks, putty, insecticides, adhesives, linoleum, and lectin. Soybean meal is used for livestock feed, fish and pet feed, fertilizers, and protein concentrate. Soybean meal can also be used as soy flour for baked goods, cereals, and baby food (Hauck et al., 1972). Also soybean oil is a rich source of vitamin E. Vitamin E is essential to protect the body fat from oxidation and to scavenge the free radicals and therefore, helps to prevent their potential effect upon chronic diseases such as coronary heart diseases and cancer (Lu and Liu, 2002). As a result, it can play a major role in elevating nutritional standards of foods in developing countries, where human beings are facing protein deficiencies (Asadi and Faraji, 2009).

Changes in agricultural technology have been a major factor shaping modern agriculture. Among the latest line of technological innovations, nanotechnology occupies a prominent position in transforming agriculture and food production. The development of nano-devices and nano-materials could open up novel applications in plant biotechnology and agriculture. Currently, the main thrust of research in nanotechnology focuses on applications in the field of electronics, energy, medicine and life sciences (Scrinis and Lyons, 2007). Nano-TiO$_2$ has shown to be potential for agricultural application because of its photocatalytic disinfection and photobiological effects (Ichinose et al., 2008). Therefore, because of having a photocatalyzed property, nano-TiO$_2$ could enhance the photosynthetic rate of chloroplasts; here, we hypothesized that nano-TiO$_2$ might protect chloroplasts from aging under light. It is relative to reactive oxygen scavenging (Hong et al., 2005) so that, Gao et al., (2006) showed that treated Solanum oleracea by nano-anatase TiO$_2$, Rubisco carboxylase activity was 2.67 times that of control rubisco. In one study, Zheng et al., (2005) declared that nano-TiO$_2$ accelerated water absorption by spinach seeds. Lu et al., (2002) reported that mixture of nano-TiO$_2$ and nano-SiO$_2$, increased nitrate reductase enzyme in soybean (Glycine max) and accelerated absorption and utilization of water and fertilizer, promoting the antioxidant system, and finally accelerate its germination and growth. Moreover, increasing of chlorophyll content (a, b and total) and carotenoids of corn by foliar application of nano-TiO$_2$ has been reported by Morteza et al., (2013). In this case, Mohammadi et al., (2014) concluded that, nano-TiO$_2$ could increase content of chlorophyll a, b and total and carotenoids of chickpea, so that they emphasized, TiO$_2$ nanoparticles (NPs) have been found to change the dry weight, chlorophyll (Chl) synthesis, and some characteristics of metabolisms in photosynthetic organisms due to their unique properties.

Thus, in accordance with the importance of the TiO$_2$ nanoparticles in increasing of plant yield and this point that it has not been done any study about the effects of nanoparticles of titanium on physiological and biochemical characteristics of the soybean plant, therefore, the aim of this study, was an investigation of different concentrations and times of nano-TiO$_2$ foliar application, on chlorophyll, carotenoids and anthocyanin’s content of soybean (Glycine max L.) at Shar-e-Qods, Iran.

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 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, times 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 growing stages and concentrations of nano-TiO$_2$. The characters were measured consist of: chlorophyll a, b and total, carotenoids and anthocyanins. Samples for traits measurement, were obtained from the youngest fully expanded leaves of different individuals, 7 days after the latest of treatments spraying and were frozen in liquid nitrogen and then stored at -80°C.

**Chlorophylls and carotenoids assay**
Total chlorophyll (Chl a+b), chlorophyll a (Chl a) and chlorophyll b (Chlb) and carotenoids (Car), were determined spectrophotometrically, using 80% acetone as a solvent (Lichtenthaler, 1987). The pigment extract was measured against a blank of 80% (v/v) acetone at wavelengths of 647 and 663 nm for chlorophyll assays and at wavelengths of 470 for carotenoids. Finally, amounts of traits, was determined by the following formula.

\[
\text{Chl a} = 12.25 \frac{A_{663}}{A_{647}} - 2.79
\]

\[
\text{Chl b} = 21.5 \frac{A_{647}}{A_{663}} - 5.1
\]

\[
\text{Chl T} = \text{Chl a} + \text{Chl b}
\]

\[
C (x+c) = \frac{1000 A_{470} - 1.82 \text{Chl a} - 85.02 \text{Chl b}}{189}
\]

**Anthocyanins content assay**
Anthocyanins of samples, were analysed according to Wanger (1979). For determination of anthocyanins content, frozen tissue of samples were soaked immediately in acidified methanol (methanol: HCl 99:1 (v/v)). Tissues, were 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 (model Jenway 4506). Anthocyanins of samples, were calculated by the following formula.

\[
A_{550} = \varepsilon bc
\]

\[
A = \text{Read absorbance at } 550 \text{ nm}
\]

\[
\varepsilon = \text{Extinction coefficient } = 33,000 \text{ mol}^{-1} \text{cm}^{-1}
\]

\[
b = \text{Cell width} = 1 \text{ cm}
\]

\[
c = \text{Concentration of anthocyanin (µ mol.g}^{-1} \text{.fw})
\]

Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (12) and followed by Duncan’s multiple range tests (significant at P ≤ 0.05).

**Results and discussion**
Analysis of variance showed that the effect of the concentration of nano-TiO$_2$ was significant on the content of chlorophyll b and total chlorophyll (P ≤ 0.01), and on the chlorophyll a content (P ≤ 0.05). But the effect of foliar application time of this nanoparticle on whole traits, was not significant (Table 1). According to the results of analysis of variance (Table 1), the interactions between the different concentrations of nano-TiO$_2$ and time of foliar application of this nano particle, were significant on the chlorophyll b (P ≤ 0.01) and on the carotenoids (P ≤ 0.05) (Table 1).

**Table 1.** Results of variance analysis of the soybean (*Glycine max* L.) traits under different concentrations of nano titanium dioxide and times of foliar application of it.

<table>
<thead>
<tr>
<th>Means square</th>
<th>Chlorophyll a</th>
<th>Chlorophyll b</th>
<th>Total chlorophyll</th>
<th>Carotenoids</th>
<th>Anthocyanins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>0.006**</td>
<td>0.00025**</td>
<td>0.006**</td>
<td>0.088**</td>
<td>2219.76**</td>
</tr>
<tr>
<td>Concentration of nano-TiO$_2$ (C)</td>
<td>0.038*</td>
<td>0.02267**</td>
<td>0.118**</td>
<td>0.001**</td>
<td>375.28**</td>
</tr>
<tr>
<td>Time of foliar application (T)</td>
<td>0.012**</td>
<td>0.00001**</td>
<td>0.013**</td>
<td>0.001**</td>
<td>13.04**</td>
</tr>
<tr>
<td>$T \times C$</td>
<td>0.013**</td>
<td>0.00180**</td>
<td>0.024**</td>
<td>0.029**</td>
<td>186.80**</td>
</tr>
<tr>
<td>Error</td>
<td>0.009</td>
<td>0.0001</td>
<td>0.009</td>
<td>0.006</td>
<td>376.64</td>
</tr>
<tr>
<td>Cv (%)</td>
<td>5.88</td>
<td>2.51</td>
<td>4.58</td>
<td>8.01</td>
<td>15.15</td>
</tr>
</tbody>
</table>

Note: * and **, significant at 5 and 1% levels respectively.
Chlorophyll content
Chlorophyll a content
Mean comparison using Duncan’s multiple range tests showed that with increasing of nano titanium concentration, the amount of chlorophyll a increased. Treatment of 0.05% of nano titanium with 1.75 mg/g.fw, caused increasing of chlorophyll a content, so that it hadn’t significance difference with the concentration of 0.03% of this nano particle and concentration of the 0.01% of this nano particle and control treatment had the highest amount of this trait (Fig. 1). In accordance with these results, increasing of the chlorophyll content in treated plants of spinach with the nano titanium dioxide was reported by studies of Zheng et al., (2005) also Hruby et al., (2012) showed that chlorophyll content of green algae and pepper, increased with application of nano-TiO₂ in these plants.

Fig. 1. Effect of nano-TiO₂ concentrations on chlorophyll a content in soybean.

Chlorophyll b content
In accordance with the Fig 2, amount of chlorophyll b, increased with increasing of nano-TiO₂ concentration so that, interaction of 0.05 percent of nano-TiO₂ treatment and treatment of nano particle foliar application at vegetative stage, with 0.48 mg/g.fw, had the highest chlorophyll b content in compared with the other treatments. While, the lowest chlorophyll b content, was obtained by the control treatment with the 0.36 mg/g.fw so that, study results of Morteza et al., (2013) on corn and results of Hong et al., (2005) on spinach in investigation on the effect of nano titanium particles on this trait, were in accordance to these results.

Fig. 2. Effect of interaction of nano-TiO₂ concentrations and times of application of it on chlorophyll b content in soybean.

Total chlorophyll content
Results of means comparison showed that the highest amount of total chlorophyll with the amount of 2.12 and 2.22 mg/g.fw, were achieved by spraying of soybean plants with 0.05 and 0.03 percent concentrations of nano-TiO₂ respectively, so that these treatments hadn’t significant difference together and were in the same statistical group. Nevertheless, the lowest content of total chlorophyll, was obtained by foliar application of 0.01 percent concentration of nano-TiO₂ and control treatments, so that these treatments were in the same statistical group (Fig. 3) also, increasing of total chlorophyll content by nano-TiO₂ has been reported in research of Haghighi et al., (2012).

Fig. 3. Effect of nano-TiO₂ concentrations on total chlorophyll content in soybean.

Therefore, results of means comparison showed that chlorophyll content (a, b and total) increased with spraying of nano-TiO₂, because nano-TiO₂ have a photocatalyzed property and could enhance the photosynthetic rate of chloroplasts so that nano-TiO₂
might protect chloroplasts from aging under light. It is relative to reactive oxygen scavenging (Hong et al., 2005) especially spraying in vegetative stage had the highest chlorophyll content, because of higher vegetative growth of young tissues and stop of vegetative growth with the start of reproductive stage.

Fig. 4. Effect of interaction of nano-TiO$_2$ concentrations and times of application on carotenoids content in soybean.

### Carotenoids content

Foliar application of soybean plants with the concentration of 0.05 percent of this nanoparticle at reproductive and vegetative stages, and spraying with nano TiO$_2$ at concentration of 0.03 percent at reproductive stage had maximum content of carotenoids and treatment of control at vegetative stage, had the lowest carotenoids content so that other treatments were between these two treatments (Fig. 4). It seems that plants are faced with different stresses in reproductive stage, and in this research, carotenoids as the non antioxidant system, increased for the fight with the free radicals in chloroplast (Polyakov et al., 2001) affected by stress condition in reproductive stage plant. In confirmation of these results, increasing of the carotenoid's content of chickpea and corn treated with the nanoparticles of titanium was reported by Mohammadi et al., (2014) and Morteza et al., (2013). Furthermore, in this case, Lu et al., (2002) reported 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 accelerate its germination and growth.

### Conclusion

Results of this study showed that, nano-TiO$_2$ significantly increased chlorophyll content and decreased the accumulation of reactive oxygen-free radicals in chlorophyll, by increasing of carotenoids non antioxidant. So that these results showed that nano-TiO$_2$ by increasing of chlorophyll, can increase yield of soybean as an oil seed plant.

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