Journal of Crop Nutrition Science
ISSN: 2423-7353 (Print) 2538-2470 (Online)
Vol. 3, No. 2, 2017
http://JCNS.iauahvaz.ac.ir
OPEN ACCESS

Assessment the Effect of Concentration and Application Time of Titanium Dioxide Nanoparticles on Biochemical Traits and Seed Yield of Wheat (*Triticum aestivum* L.)

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RESEARCH ARTICLE

ARTICLE INFO.
Received Date: *2 Mar. 2017*
Received in revised form: *27 Apr. 2017*
Accepted Date: *18 May. 2017*
Available online: *30 Jun. 2017*

To Cite This Article: Ali Jasemnejad, Payam Moaveni. Assessment the Effect of Concentration and Application Time of Titanium Dioxide Nanoparticles on Biochemical Traits and Seed Yield of Wheat (*Triticum aestivum* L.). ***J. Crop. Nut. Sci., 3(2):*** *25-37, 2017.*

ABSTRACT

Nanoparticles of titanium increase cell growth by improvement of photosynthetic and nitrogen metabolism and therefore, caused an increasing in weight of the plant. This re-search was conducted to evaluate seed yield and biochemical traits of bread wheat affected by different levels of titanium dioxide Nanoparticles in Ahvaz region, Khuzestan province, located at southwestern Iran by factorial experiment based on randomized complete blocks design with four replications during 2011-2012 growing season. The factors consisted of foliar application of titanium dioxide Nanoparticles at three growth stages (S_1: Tillering, S_2: Stem elongation, S_3: Ripening) and different concentrations of titanium dioxide at five levels (C_1: 0.01; C_2: 0.03; C_3: 0.05; C_4: 0.07 % titanium dioxide Nanoparticles and C_5: 0.06 % non-Nano particles of titanium dioxide or Bulk type). The results of analysis of variance indicated that the effect of spraying at different growth stage (except on harvest index and proline) and different concentration of titanium dioxide (except for proline) was significant on all measured traits. Mean comparison result showed the highest seed yield, its components and biochemical traits (expect malon di aldehyde) was achieved in 0.05% concentration at stem elongation stage, but the 0.07% concentration had a lower effect than other concentrations. It should be expected that higher concentrations of nanoparticles have inhibitory or neutral effects on growth trend.

Keywords*:* *Catalase, Nanotechnology, Phenology, Proline.*

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INTRODUCTION

Nanotechnology is an interdisciplinary research field. In recent past efforts have been made to improve agricultural yield through exhaustive research in nanotechnology (Aliabadi *et al*., 2016). Nanoparticle mediated material delivery to plants and advanced biosensors for precision farming are possible only by nano particles or Nano chips (Singh Duhan *et al*., 2017). Nanoparticles are atomic or molecular aggregates with at least one dimension between 1 to 100 nm that can drastically modify their physicochemical properties compared with the bulk material (Kim *et al.,* 2011). Nanotechnology allows wide advances in agricultural research, such as reproductive science, change of agricultural and food wastes to energy and other useful by-products through enzymatic nano bio processing, disease prevention, and treatment in plants with using various nanocides. (Carmen *et al*., 2003). Nano TiO_2 has been widely used in industry at present and has a rapidly increasing exposure to environment, especially under the driving force of nanotechnology (Su *et al*., 2007). 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 Mingyu *et al.,* 2007). Titanium compounds enhance the yield of various crops, by improving some essential elements content in the plant tissues and increasing the peroxidase, catalase, and nitrate reductase activities in plant tissues, also TiO_2 nanoparticles encourage spinach seed germination and plant growth (Zheng *et al*., 2005). Results of some studies showed that negative and positive effects of nano particles on growth and development on crop plants related to the physical and chemical properties, concentration, size and composition of engineered nanoparticles and plant species (Ma *et al.,* 2010; Shaw and Hossain, 2013). Feizi *et al.* (2012) reported that among the wheat germination indices only mean germination time was affected by treatment (10 ppm Nano TiO_2). So the lowest and the highest mean germination time (0.89 vs. 1.35 days) were obtained in 10 ppm concentration of Nano sized TiO_2 and control treatments, respectively. In addition, shoot length, seedling length, and root dry matters were affected by bulk and nano sized TiO_2 concentrations, significantly. Shoot and seedling lengths at 2 and 10 ppm concentrations of Nano sized TiO_2 were higher than those of the untreated control and bulk TiO_2 at 2 and 10 ppm concentrations. Employing nano sized TiO_2 in suitable concentration could promote the seed germination of wheat in comparison to bulk TiO_2 but in high concentrations had inhibitory or no effect on wheat. 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 with two application at 125 $ml.ha^{-1}$, increased traits of grain yield, 1000 seed weight, number of seeds per pod, pod length, number of pods per plant and leaf area. Haghighi *et al.* (2012) showed that spraying of tomato plants by Nano particle of TiO_2, increased root and shoot dry weight, flower number and shoot diameter of this plant. It was found that Nano-anatase TiO_2 promotes antioxidant stress by decreasing the accumulation of superoxide radicals, hydrogen peroxide, and malon di aldehyde content and enhances the activities of superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase and thereby increases the evolution oxygen rate in spinach chloroplasts under UV-B radiation (Lei *et al*., 2008). Aliabadi *et al.* (2016) evaluated nano TiO_2 and nano aluminum on the growth and some physiological parameters of the wheat reported that nano aluminum decreased the length of root and shoot and the content of chlorophyll; however, it increased the content of proline and malon di aldehyde (MDA) in shoot and root. Generally the application of nano titanium dioxide in low concentration can moderate the damage effects of nano aluminum in the range of 100 $mg.l^{-1}$. Taieb Baiazidi Aghdam *et al.* (2016) reported use of nano scale TiO_2 at 10 $mg.l^{-1}$ concentration improved the morphological and physiological traits of *Linum usitatissimum* compared to other doses. Mahmoodzadeh *et al.* (2013a) reported treatment of nano scale TiO_2 at 2000 $mg.l^{-1}$ (20 nm mean particle size) concentration promoted both seed germination and seedling vigor. The lowest and the highest germination rate were obtained in 1500 and 2000 $mg.l^{-1}$ treatments, respectively. Higher concentrations of nano scale TiO_2 (1200 and 1500 $mg.l^{-1}$) showed large radicle and plumule growth of seedling compared to other concentrations and control. The inhibitory effect with lower nanoparticle concentration reveals the need for judicious usage of these particles in such applications. Nano particles of titanium dioxide is one of the most important photo catalyst and considered as a unique light-activated catalyst due to strong photo catalyst properties such as high oxidative and stability of the optical properties with other benefits, such as nontoxicity, low cost and availability (Reddy *et al.,* 2004; Fujishima and Xang, 2006; Kasem and Dahn, 2010). Therefore, based on the importance of TiO_2 nano particle in increasing of growth and yield of crops, the objective of this study was to determine the effects of time of spraying at different growth stage and different concentration of nano TiO_2 on physiological parameters and biochemical constituents of wheat.

MATERIALS AND METHODS

Field and treatments information

This research was carried out to evaluate seed yield and biochemical traits of bread wheat affected different level of titanium dioxide nanoparticles in Ahvaz region, Khuzestan province located at southwestern of Iran via factorial experiment based on randomized complete blocks design with four replications during 2011-2012. The factors consisted of foliar application of titanium dioxide Nano-particles at three growth stages (S_1: Tillering, S_2: Stem elongation, S_3: Ripening) and different concentration of titanium dioxide at five level (C_1: 0.01; C_2: 0.03; C_3: 0.05; C_4: 0.07 % titanium dioxide Nano-particles and C_5: 0.06 % non-Nano particles of titanium dioxide or Bulk). Geographical information of research location (Experimental field of Islamic Azad University, Ahvaz Branch) was 48^0 32' E as longitude, 32^0 22' N as latitude and altitude was 82 m. The average annual rainfall, temperature, and evaporation in studied region were 240 mm, 22 ^{0}C and 3200 mm, respectively. The soil properties of studied field were mentioned in table 1.

Table 1. Soil properties of studied field

Soil depth (cm)	**0-30**
Acidity (pH)	7.60
Electrical conductivity ($ds.m^{-1}$)	2.45
Organic carbon (%)	0.85
Phosphorus (ppm)	7.30
potassium (ppm)	118
Nitrogen (ppm)	375
Zinc (ppm)	0.5
Clay (%)	39
Silt (%)	44
Sand (%)	17

Farm management

Before planting the land was irrigated and then the land preparing operation was done in October including the plow to a depth of 30 cm and 2 perpendicular discs. The base fertilizers were added to the soil before the second disc and were mixed with soil by disc and then the field was leveled by the trowel and finally the crop was planted. The base fertilizer used in the field consisted of 95 $kg.ha^{-1}$ of triple super phosphate and 95 $kg.ha^{-1}$ of potassium sulfate fertilizer and 200 $kg.ha^{-1}$ of nitrogen fertilizer from the urea source (46% Nitrogen) as the split in two stages (50% at planting stage and 50% at stem elongation stage). Each experimental plot included 7 planting lines along 5m, and the distance between the planting lines was 20 cm and the distance between the experimental plots was 1 m. The rate of required seeds for planting was considered based on 400 plants per square meter. Irrigation was done immediately after planting the seeds and the first irrigation was considered as the planting date and the rest of irrigations were done as required and based on the rainfall in plots. The necessary crop care was taken during the growth period as needed. The white powder of titanium dioxide Nano-particle with a purity of 99.5%, an average size of 21 nm and a specific surface area of more than 50 $m^2.gr^{-1}$, was the product of Germany. Weeds were controlled via topic (0.8 $l.ha^{-1}$) and Granestar (15 $gr.ha^{-1}$) herbicides at the beginning of tillering stage.

Traits measure

To measure seed yield and biological yield, all plants in 1 m^2 of each plot were harvested, oven dried at 75°C for 48 hour, and calculated as $Kg.ha^{-1}$. Harvest index (HI) was calculated according to formula of Gardener *et al*. (1985) as follows:

Equ.1. HI= (Seed yield/Biologic yield) ×100.

To measure protein, Malon di aldehyde, proline and leaf enzymes, one week after each foliar application, 20 leaves were separate from each treatment, and immediately after placing in pre-prepared aluminum sheets, they were frozen by liquid nitrogen and stored at -30° C. To extract the total solution protein, one gram of leaf tissue was mixed in the presence of 100 mg of poly vinyl pyrrolidone (PVP) and 3 ml of buffer (containing 50 mM potassium phosphate buffer and 1 mM Sodium metabisulfite; pH=7). For extraction, the sample was transferred to centrifuge tubes and centrifuged at + 4°C for 25 minutes and 15000 rpm (Beckman; Model Allegra-64R). The total soluble protein content was measured by using spectrophotometer according to the Bradford method (1976). Different concentrations of bovine serum albumin (BSA) were used to draw the standard curve. In order to measure proline, 5 grams of leaf wet samples were homogenized in 10 ml of sulfosalicylic acid (3%) and the extract was filtered off, in continue Bates *et al*. (1973) method was used then. The Malon di aldehyde concentration (MDA) was calculated by Heath and Packer (1968) method and used extinction coefficient equal to 1.56×10^5. The results of the measurement were calculated and presented in terms of the tissue fresh weight. To evaluate the activity of superoxide dismutase, according to Giannopolitis and Ries (1997) method, 0.2 gram of frozen leaf samples were used. Measurement of catalase activity was performed according Cakmak and Horst (1991) method. 0.2 grams of frozen sample were extracted in 3 ml of sodium phosphate buffer of 25 mM at pH 6.8. The homogeneous product was centrifuged at 15000 rpm for 15 minutes at 4^0C and a solution was used to measure the activity of the catalase enzyme. Analysis of hydrogen peroxide by decrease at wavelength of 240 nm followed and expressed for one milligram protein in enzyme extract.

Statistical analysis

The analysis of variance was done by MSTAT-C software and the means were compared via Duncan's multiple range test (DMRT) at 5% probability level.

RESULT AND DISCUSSION

Seed yield

According result of analysis of variance effect of time of spraying at different growth stage and different concentration of Nano TiO_2 on seed yield was significant at 5% probability level, but interaction effect of treatments was not significant (Table 2). Mean comparison result showed in different time of spraying the maximum seed yield (3748 $kg.ha^{-1}$) was observed in stem elongation stage and the lowest one (3454 $kg.ha^{-1}$) was found in tillering treatment, also among different concentration of Nano TiO_2 maximum seed yield (3816 $kg.ha^{-1}$) was obtained for 0.05 concentration and minimum of that (3376 $kg.ha^{-1}$) was for 0.06 (Bulk) treatment (Table 3). This concentration value was the best concentration for stimulating plant growth under test conditions, while the concentration of 0.07% had a low effect on studied traits it should be expected that higher concentrations of nanoparticles can have a deterrent or neutral effect on plant growth. The increase in the values of these traits with the use of Nano TiO_2 can be due to the interference of this nanoparticle in increasing the absorption of light, enhancing the photosynthetic system of the plant through the enhancement of the rubisco enzyme, the activity of nitrate reductase enzyme, glutamate dehydrogenase, glutamine, hill reaction, reduction iron cytochromes in chloroplasts and thus increasing chlorophyll content and photosynthesis production compare to bulk type (Pokhrel and Dubey, 2013). In fact, titanium dioxide nanoparticles have a greater effect on plant growth compared Balk type, due to the much smaller size than to bulk type, so nanoparticles is readily introduced into cells, especially chloroplasts, in comparison to its bulk (Aslani *et al*., 2014). The finding of Sheykhbaglou *et al*. (2010); Arora *et al*. (2012) and Mahmoodzadeh and Aghili (2014) were consistent with results of this research.

Table 2. Result of analysis of variance effect of different concentration of Nano TiO_2 and time of spraying at different growth stage on seed yield, its components and harvest index

S.O.V	df	Seed yield	Biologic yield	Number of seed per spike	Number of spike per m^2	Seed weight	Harvest index
Block	2	7834.33^{*}	1954.77^{*}	1184.54^{*}	2519.47^{*}	7289.09^{*}	1400.01^{*}
Spraying at different growth stage (G)	2	4677.66^{*}	4560.00^{*}	1745.12^{*}	1136.11^{*}	6232.14^{*}	1310.02^{ns}
Different concentration of Nano TiO_2 (D)	4	3504.66^{*}	3660.00^{*}	3107.45^{*}	9754.48^{*}	4736.02^{**}	2810.05^{*}
G*D	8	1106.66^{ns}	1235.00^{ns}	2451.15^{ns}	1165.70^{ns}	1925.07^{ns}	1101.03^{ns}
Error	28	1295.52	1150.87	1778.29	3675.11	6432.01	0.11072
CV (%)	-	5.06	3.78	4.45	6.22	3.69	6.67

^{ns}, * and ** are non-significant and significant at 5% and 1% probability levels, respectively.

Biological yield

Result of analysis of variance revealed effect of time of spraying at different growth stage and different concentration of Nano TiO_2 on biological yield was significant at 5% probability level, but interaction effect of treatments was not significant (Table 2). Mean comparison of result revealed that the maximum and the minimum amount of biological yield in different time of spraying belonged to stem elongation stage (9120 $kg.ha^{-1}$) and tillering (8820 $kg.ha^{-1}$). Maximum biologic yield between different concentration of Nano TiO_2 (9183 $kg.ha^{-1}$) was obtained for 0.05 concentration and minimum of that (8750 $kg.ha^{-1}$) was for 0.06 treatment (Table 3). The concentration of nanoparticles used in plant growth is important, so stimulating plant growth by nanoparticles will greatly correlate with the concentration of nanoparticles (Lin and Xing, 2007). Studies by Amuamuha *et al*. (2012) indicated use of nanoparticles at different growth stage (stem elongation, flowering and after the first, second and third cutting) on yield of *Glycyrrhiza glabra* showed that the highest amount of yield was obtained from the treatment application at stem elongation. The nanoparticles had higher discharge of elements (Such as nitrogen, phosphorus and calcium) by the plant from the soil, more permeability into plant tissues, quantum effect and photo catalytic activity (Cause to catalyst for oxidation and reduction reactions and releasing high-energy electrons) than to bulk type (Feizi *et al*., 2012; Zheng *et al*., 2005). In fact mentioned reasons led to increase photosynthetic efficiency and achieve higher yield by nanoparticles (Yang *et al*., 2006). Mahmoodzadeh *et al*. (2013b) reported that nanoparticles of titanium di oxide increased wheat stalk weight by use concentration of 100 $mg.l^{-1}$. Bradley *et al*. (2012) find similar result about increasing effect of apply nanoparticles of titanium di oxide on biomass of green algae and spinach weight, respectively.

Table 3. Mean comparison effect of different concentration of Nano TiO_2 and time of spraying at different growth stage on seed yield, its components and harvest index

Treatment	Seed yield ($kg.ha^{-1}$)	Biologic yield ($kg.ha^{-1}$)	Number of seed per spike	Number of spike per m^2	Seed weight (gr)	Harvest index (%)
Spraying at different growth stage						
Tillering	3454^{b}	8820^{b}	35.95^{b}	469^{c}	36.11^{c}	-
Stem elongation	3748^{a}	9120^{a}	39.85^{a}	481^{a}	40.29^{a}	-
Ripening	3528^{ab}	8940^{ab}	37.17^{ab}	474^{b}	38.45^{b}	-
Different concentration of Nano TiO_2 (%)						
0.01	3563^{ab}	8933^{abc}	38.78^{ab}	472^{b}	37.26^{ab}	39.81^{ab}
0.03	3660^{ab}	9083^{ab}	39.06^{ab}	475^{ab}	38.12^{ab}	40.20^{ab}
0.05	3816^{a}	9183^{a}	40.68^{a}	479^{a}	39.95^{a}	41.52^{a}
0.07	3466^{b}	8850^{bc}	36.37^{b}	469^{bc}	36.33^{b}	39.11^{b}
Bulk (0.06)	3376^{c}	8750^{c}	33.44^{c}	467^{c}	34.85^{c}	38.53^{b}

*Similar letters in each column show non-significant difference at 5% level in Duncan's multiple rang test.

Zheng *et al*. (2005) by tested effect of different concentrations of nanoparticles of titanium di oxide (0, 0.25, 0.5, 1, 1.5, 2, 2.5, 4, 6 and 8 percent) and bulk type of nanoparticles of titanium di oxide (0, 0.25, 0.5, 1, 1.5, 2, 2.5, 4, 6, and 8 percent) on spinach plant found that seedling dry weight at concentrations of 2.5% nanoparticles titanium oxide was higher than other concentrations, especially bulk type.

Number of seed per spike

Result of analysis of variance showed effect of time of spraying at different growth stage and different concentration of Nano TiO_2 on number of seed per spike was significant at 5% probability level, but interaction effect of treatments was not significant (Table 2). Mean comparison result of different time of spraying indicated that maximum number of seed per spike (39.85) was observed for stem elongation stage and minimum of that (33.95) was for tillering treatment. Also compare different concentration of Nano TiO_2 revealed maximum number of seed per spike (40.68) was obtained for 0.05 concentration and minimum of that (33.44) was for 0.06 treatment (Table 3). This result, were accordance with results of Jaberzadeh *et al*. (2013).

Number of spike per m^2

Result of analysis of variance showed effect of spraying at different growth stage and different concentration of Nano TiO_2 on number of spike per m^2 was significant at 5% probability level, but interaction effect of treatments was not significant (Table 2). Mean comparison result of different time of spraying showed that the maximum and the minimum amount of number of spike per m^2 belonged to stem elongation stage (481) and tillering (469) treatment, respectively. Also between different concentrations of Nano TiO_2 the highest value of number of spike per m^2 was belonged to the 0.05 concentration (479) and the lowest one was found in the bulk type treatment as 467 (Table 3). Janmohammadi *et al*. (2016) and Naderi *et al*. (2013) have pointed similar results in this regard.

Seed weight

According result of analysis of variance effect of spraying at different growth stage and different concentration of Nano TiO_2 on seed weight was significant at 5% probability level, but interaction effect of treatments was not significant (Table 2). Evaluation mean comparison result of different time of spraying revealed the maximum seed weight (40.29 gr) was noted for stem elongation stage and minimum of that (36.11 gr) belonged to tillering treatments. Also among concentrations of Nano TiO_2 maximum amount of seed weight (39.95 gr) was obtained for 0.05 concentrations and minimum of that (34.85 gr) was for bulk type treatment (Table 3). Some researchers such as Morteza *et al*. (2013) confirmed mentioned result.

Harvest index

Harvest index is one of the important physiological indicators that express the transfer of photosynthetic material from the vegetative organs to the seeds. Assessment result of analysis of variance indicated that effect of different concentration of Nano TiO_2 on harvest index was significant at 5% probability level, but effect of spraying at different growth stage and interaction effect of treatments was not significant (Table 2). Mean comparison result showed among different concentration of Nano TiO_2 maximum harvest index (41.52%) was obtained for 0.05 concentrations and minimum of that (38.53%) was for Bulk type treatment (Table 3). The results of this experiment showed that the application of titanium nanoparticles has a more effective role in the transfer of photosynthetic materials to the economic organs of wheat (seeds). Another researchers such as Janmohammadi *et al.* (2016) reported same result.

Soluble protein

According result of analysis of variance effect of spraying at different growth stage and different concentration of Nano TiO_2 on soluble protein was significant at 5% and 1% probability level, respectively but interaction effect of treatments was not significant (Table 4). Mean comparison result of different time of spraying indicated that the maximum and the minimum amount of soluble protein belonged to stem elongation stage (1.32 $mg.g^{-1}$ wet weights) and tillering treatments (1.20 $mg.g^{-1}$ wet weights), respectively. Also between different concentrations of Nano TiO_2 maximum soluble protein (1.39 $mg.g^{-1}$ wet weights) was obtained for 0.05 concentrations and minimum of that (1.07 $mg.g^{-1}$ wet weights) was for Bulk type treatment (Table 5). Regarding the increase in the amount of soluble protein, due to the Nano TiO_2, compared with bulk type, it can be stated that nitrogen is one of the important compounds of chlorophyll, amino acids, proteins and ETC, so Nano TiO_2, in contrast to bulk type, was more effective on nitrogen metabolism, stabilizes it, increases nitrogen content, chlorophyll and protein content in plant. Gao *et al.* (2008) have pointed similar results in this regard.

Table 4. Result of analysis of variance effect of different concentration of Nano TiO_2 and time of spraying at different growth stage on bio-chemical traits

S.O.V	df	Soluble protein	Super oxide dismutase	Catalase	Malon di aldehyde	Proline
Block	2	0.0003^{*}	11486.95^{*}	74.86^{*}	230.91^{*}	0.091^{*}
Spraying at different growth stage (G)	2	0.0322^{*}	6075.51^{**}	3465.01^{*}	4349.39^{**}	0.006^{ns}
Different concentration of Nano TiO_2 (D)	4	0.1483^{**}	49589.47^{**}	2745.05^{**}	2423.92^{**}	0.017^{ns}
D*G	8	0.005^{ns}	9443.16^{ns}	512.18^{ns}	90.33^{ns}	0.011^{ns}
Error	28	0.0011	23761.80	405.77	12.76	0.014^{ns}
CV (%)	-	2.95	4.33	5.29	3.66	4.53

^{ns}, * and ** are non-significant and significant at 5% and 1% probability levels, respectively.

Super oxide dismutase enzyme

Result of analysis of variance showed effect of spraying at different growth stage and different concentration of Nano TiO_2 on super oxide dismutase enzyme was significant at 1% probability level, but interaction effect of treatments was not significant (Table 4). Mean comparison result showed in different time of spraying the maximum super oxide dismutase (1269.25 Variation absorb H_2O_2 $mg.min^{-1}$) was observed in stem elongation stage and the lowest one (932 Variation absorb H_2O_2 $mg.min^{-1}$) was found in tillering treatment, also among different concentration of Nano TiO_2 maximum super oxide dismutase (1345.83 Variation absorb H_2O_2 $mg.min^{-1}$) was obtained for 0.05 concentration and minimum of that (872.08 Variation absorb H_2O_2 $mg.min^{-1}$) was for (Bulk) treatment (Table 5). In this experiment, the superoxide dismutase enzyme was more increase due to use of Nano TiO_2 compare to bulk type. In fact plants have a high-performance defense system to control oxidative stress and eliminate or neutralize free radicals. This defense system includes superoxide dismutase, catalase, ascorbate peroxidase and glutathione redactase (Blokhin *et al*., 2003). Some researchers such as Pokhrel and Dubey (2013) confirmed mentioned result. Hong *et al*. (2005) also reported that titanium dioxide nanoparticles, by increasing the amount of superoxide dismutase enzymes, prevent the early aging of spinach chloroplasts against high light intensity.

Table 5. Mean comparison effect of different concentration of Nano TiO_2 and time of spraying at different growth stage on of bio-chemical traits

Treatment	Soluble protein ($mg.g^{-1}$ wet weight)	Super oxide dismutase (Variation absorb H_2O_2 $mg.min^{-1}$)	Catalase (Variation absorb H_2O_2 $mg.min^{-1}$)	Malon di aldehyde (Variation absorb H_2O_2 $mg.min^{-1}$)
Spraying at different growth stage				
Tillering	1.20^{b}	932.00^{c}	334.17^{c}	112.37^{a}
Stem elongation	1.32^{a}	1269.25^{a}	414.80^{a}	97.12^{c}
Ripening	1.25^{ab}	1024.30^{b}	391.75^{b}	82.88^{b}
Different concentration of Nano TiO_2 (%)				
0.01	1.28^{ab}	1064.58^{c}	360.83^{d}	96.03^{c}
0.03	1.33^{ab}	1203.33^{b}	400.25^{b}	90.08^{d}
0.05	1.39^{a}	1345.83^{a}	444.91^{a}	81.97^{e}
0.07	1.25^{b}	905.00^{cd}	379.41^{c}	99.23^{b}
Bulk (0.06)	1.07^{c}	872.08^{d}	315.50^{e}	120.05^{a}

*Similar letters in each column show non-significant difference at 5% level in Duncan's multiple rang test.

Catalase enzyme

According result of analysis of variance effect of spraying at different growth stage and different concentration of Nano TiO_2 on catalase enzyme was significant at 5% and 1% probability level, respectively but interaction effect of treatments was not significant (Table 4). Mean comparison result of different time of spraying indicated that the maximum and the minimum amount of catalase enzyme belonged to stem elongation stage (414.80 Variation absorb H_2O_2 $mg.min^{-1}$) and tillering treatments (334.17 Variation absorb H_2O_2 $mg.min^{-1}$), respectively. Also between different concentrations of Nano TiO_2 maximum catalase enzyme (444.91 Variation absorb H_2O_2 $mg.min^{-1}$) was obtained for 0.05 concentrations and minimum of that (315.50 Variation absorb H_2O_2 $mg.min^{-1}$) was for Bulk type treatment (Table 5). These findings were in accordance with Aliabadi *et al*. (2016), where he reported that the amount of this enzyme in the stem elongation stage was greater than the flowering stage. The results of Lu *et al*. (2002) also found that soybean seed treatment with Nano-Titanium dioxide increased the catalase enzyme in germinated soybean, thus exacerbating the use of water and fertilizer. In the experiment of Feizi *et al*. (2012) on wheat, it was found that suitable concentrations of Nano-Titanium dioxide increased the catalase enzyme in wheat seedlings, but higher concentrations had less effect. It has been suggested that nanoparticles could hasten the germination and growth of crop, 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).

Malon di aldehyde (MDA)

Result of analysis of variance showed effect of spraying at different growth stage and different concentration of Nano TiO_2 on malon di aldehyde was significant at 1% probability level, but interaction effect of treatments was not significant (Table 4). Evaluation mean comparison result of different time of spraying revealed the maximum malon di aldehyde (112.37 Variation absorb H_2O_2 mg.min^{-1}) was noted for tillering and minimum of that (97.12 Variation absorb H_2O_2 mg.min^{-1}) belonged to stem elongation stage treatments. Also among concentrations of Nano TiO_2 maximum amount of malon di aldehyde (120.05 Variation absorb H_2O_2 mg.min^{-1}) was obtained for bulk type and minimum of that (81.97 Variation absorb H_2O_2 mg.min^{-1}) was for 0.05 concentration treatment (Table 5). When oxidative stress occurs, the peroxidation of unsaturated fatty acids and lipids increases. Due to free radicals attacking lipids, various aldehydes, including malon di aldehyde, are produced. Free radicals of oxygen attack proteins and cause minor changes in the amino acid location with the peptide chain. In this experiment, with the use of titanium nanoparticle, malon di aldehyde is less produced and the defense system antioxidants are also enhanced by this nanoparticle and are less susceptible to attack, decomposition and destruction of free radicals and the plant has yielded more performance due to its improved defense system. Aliabadi *et al*. (2016) have pointed similar results in this regard. Hong *et al*. (2005) showed that titanium dioxide nanoparticles reduced malon di aldehyde and by preventing peroxidation of chloroplast membrane lipids at high light intensity caused the membrane to be strengthened and treatment with balk type was less effective. Many researches have shown that the MDA content in treated plants with metal ions increased (Liu and Lal, 2015; Ren *et al*., 2011). Metal ions released from nanoparticles can cause oxidative stress and producing high ROS and consequently activate enzymatic and non-enzymatic antioxidants (Mohammadi *et al*., 2013). Plants grown in stressed environment accumulate a lot of free radicles in their cells. Peroxidation of cell membrane lipids by free radicals is an indication of the presence of toxic substances in the environment and an end product is MDA (Shaw and Hossain, 2013).

Proline

According result of analysis of variance effect of time of spraying at different growth stage, different concentration of nano TiO_2 and interaction effect of treatments on proline trait was not significant (Table 4).

CONCLUSION

The obtaining maximum biological yield, seed yield and harvest index in concentrations of 0.05%, compared with other concentrations is because of, this concentration is the best to stimulate plant growth in these conditions, but the 0.07% concentration had a lower effect than other concentrations. It should be expected that higher concentrations of nanoparticles can have inhibitory or neutral effects on growth trend. Regarding the increase in the soluble protein content, due to use the

Nano TiO_2, compared to its bulk type, it can be justified that nitrogen is one of the important compounds of chlorophyll, amino acids, proteins, etc. Catalase and superoxide dismutase enzymes have increased with the use of Nano TiO_2, while the level of malon di aldehyde has decreased, because of plants have a high-performance defense system to cope oxidative stress and eliminate or neutralize free radicals. In this experiment, the highest activity of enzymes was in the concentration of 0.05% at the stem elongation stage, which indicated effect of titanium nanoparticles for reduction oxidative damage. In other hand produce minimum amount of malon di aldehyde in mention treatments, due to less free radicals and increase defense system antioxidants and the plant has yielded more performance due to its improved defense system.

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