The effects of nano TiO2 and Nano aluminium on the growth and some physiological parameters of the wheat (*Triticum aestivum*)

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Abstract

Nanoparticles are atomic or molecular particles with at least 1–100 nanometer diameters. They have entered vastly into the world of agriculture and biology; this is because of their special effect and their unique features. Their unknown impact on living things and the ecosystems has caused a lot of concern. This research was done in order to study the effects of nano-TiO_2 and nano aluminium on wheat (*Triticum aestivum*). Both Nano titanium dioxide and nano aluminium were used in four concentrations {(0, 100, 1000, 2000 mgL^{-1}) and (0, 500, 100, 1000, 2000 mgL^{-1}) respectively}. The experiment was done as factorial in a completely randomized design in 3 replications. The seeds were sterilized and transferred into pots containing sand. When the plants reached four leaves stages, the nanoparticles were sprayed on plants two times in a week internal. The results revealed that nano aluminium decreased the length of root and shoot and the content of chlorophyll; however, it increased the content of proline and malondialdehyde in shoot and root. The treatment of plants in different levels of nano aluminium with nano titanium dioxide especially in 100 mgL^{-1} concentration increased the length of both the root and shoot. Nano titanium dioxide in 1000 and 2000 mgL^{-1} increased the MDA and proline content. Generally the application of nano titanium dioxide in low concentration can moderate the damage effects of nano aluminium in the range of 100 mgL^{-1}.

Keywords: Nanotechnology, Nanoparticle, Plant, Proline, Titanium dioxide

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Introduction

Nanotechnology as an interdisciplinary science can have a wide range of applications in the main sections of agriculture, including the increasing agricultural production (Aslani *et al*., 2014), decreasing the amount of toxins and fertilizers (Sekhon, 2014), increasing the length of time spent to preserve farming productions (Ma *et al*., 2010). In addition, nanotechnology experts have produced industrial compounds which are generally used as commercial and consuming products (Yashveer *et al*., 2014). The dispersing of nanoparticles into the air, water and soil leads to an environmental problem (Lopez-Moreno *et al*., 2010). People also are sometimes exposed to this substance. Thus, it is obvious that harmless nanoparticles should be produced more and more (Kumar et al., 2014).

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Titanium dioxide, which is known by the name Titania and chemical formula as TiO_2, when it is used as pigment it is called white titanium or white pigment (Ze *et al*., 2011). Titanium dioxide (TiO_2) is used for removing organic pollutions such as polychlorobiphenyl, production of surfactants, insecticides and organic colors. It is also used for producing self-cleaning hydrophobic products and Sunscreen. They are also used as light reactions (Photocatalist) in purifying wastages and cleaning the air (Karunakaran *et al*., 2013).

Alumina is one of the engineering materials that have a special importance in different industries. Alpha alumina is the most sustainable and compressed phase of alumina and due to having special properties such as high hardness, resistance to corrosion and scrubbing and chemicals (Doshi *et al.,* 2008). It has extensive applications in different industries including its usage in different material with high energy, including explosive material and propulsions, because of having considerable electrical properties, resistance to heat shock and high mechanical resistance in high temperature (Burklew *et al.,* 2012).

In studies done on the effects of nano titanium dioxide on plants has been found that it can increase yield to %30 by increasing photosynthesis through influencing the light absorption. TiO2 NPs are encouraging as valuable nutrient source for plants to increase biomass production due to enhanced metabolic activities, and application of local nutrients by improveing microbial activities (Palmqvist *et al*., 2015). Inhibition of root elongation of wheat was reported by Riahi–Madvar *et al*. (2012) as an effect of nano alumina. They revealed that nano aluminium induced oxidative stress in wheat roots cells. It is mentioned that the toxic effect is probably not nano-specified but is due to the dissolution of Al_2O_3 nanoparticles. Moreover the effects of nanoparticles on plants depending on the type of plant and concentration of nanoparticles used (Stewart *et al*., 2015).

The aim of the present study was to investigate the effect of both nano aluminium and nano titanium dioxide on growth and some physiological parameters of wheat.

Material and Methods

Experimental design

In this experiment FALAT cultivar of wheat was used. The seeds of the plant were supplied by the JIHAD KESHAVARZI office in Neyshabur. At first, seeds were sterilized and transferred into pots which contained sand. They were kept under conditions with 15 - 30 °C min/max temperature and 16-8 light/dark period in greenhouse.

Nano TiO2 (< 50 nm) and nano Al_2O_3 (< 100 nm) were purchased from Sigma-Aldrich. The experiment was done as factorial in a completely randomized design in 3 replications. The samples were irrigated by Hoagland nutrient solution. After reaching to four leaves stage (14 days), the treatments were performed on the plants. The procedure of treatment was implemented by spraying solutions on the leaves (both side) with specific concentrations of nano titanium dioxide (0, 100, 1000, 2000 mgL^{-1}) and nano Al_2O_3 (0, 100, 500, 1000 mgL^{-1}). Solutions were sprayed on the leaves two times with on an interval of 7 days, simultaneously. 21 days plants were collected and used for the next measurements.

Chlorophyll determination

To measure chlorophyll content Lichtenthaler (1987) method was used. 1 gram fresh leaf with 4 ml acetone %80 was grounded in a mortar and pestle. Then the resulted solution was centrifuged for 5 min at 3000 rpm. Finally, the absorbance of the supernatant was read to determine the content of chlorophylls a, b in the wavelength of 644, 647 and 664 nm. The concentration of photosynthetic pigments was calculated using following equations:

$$\text{Chl. a} = [12/25\,(A_{644}) - 2/79\,(A_{647})] \times \frac{V}{1000 \times w}$$

$$\text{Chl. b} = [21/51\,(A_{647}) - 5/1\,(A_{664})] \times \frac{V}{1000 \times w}$$

Determination of proline content

To measure the content of proline Bates et al. (1973) method was used. 0.2 gram of fresh leaf was grounded in 4 ml of watery acid sulphosalycilic (%3). 2 ml of filtered solution mixed with 2 ml of nainhidrin and 2 ml of acetic acid. Then the resulted solution was placed in water bath (100 °C). Then 4 ml of toluene was added into the tube and was strongly vortex for 30 seconds.

Finally, the absorbance of the solution at 520 nm was read. Finally the content of proline was calculated by a standard curve as µM per gram at fresh weight in plant samples according the following equation:

µmoles per gram fresh tissue = [(µg proline/ml) x ml toluene)/115.5 µg/µmole] / [(g sample)/5]

Determination of MDA content

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content (Dhindsa et al., 1981). A 1 ml aliquot of supernatant was mixed with 4 ml of 20% trichloroacetic acid containing 0.5% thiobarbituric acid. The mixture was heated at 100 °C for 30 min, quickly cooled, and then centrifuged at 10000×g for 10 min. The absorbance of the supernatant was read at 532 nm. The unspecific turbidity was corrected by A600 subtracting from A530. The concentration of MDA was calculated using an extinction coefficient of $155 mM^{-1} cm^{-1}$.

Statistical analysis

Experimental data were statistically analyzed by ANOVA with Statistical Analysis System (SAS) software. Probabilities of significant difference were used to test the significance among treatments and interactions, and Fisher's Protected least significant difference (LSD) (P = 0.05) was used to compare the means.

Results

The results showed that the individual effect of nano aluminium, nano TiO2 and the combined effects had a significant impact on all parameters, except combined effects on root length and MDA content (Table 1).

Growth parameters and Chlorophyll a and b

Comparison of means demonstrated that nano aluminium and nano TiO_2 considerably decreased shoot and root length and chlorophylls content and only at the 100 mgL^{-1} concentration showed a slight positive effect. The highest shoot and root length and chlorophylls content were found in plants which were treated with 100 mgL^{-1} of nanoparticles (Figs. 1-5).

Malondialdehyde (MDA) and proline content

The application of nano titanium dioxide and nano aluminium in wheat significantly increased the contents of MDA and proline comparing with the control condition (not treated with nano particles), so that the highest contents of MDA and proline were observed in maximum concentration of nano particles. In combined effects at low concentration of nano aluminium the application of 100 mgL^{-1} of the nano titanium dioxide decreased the content of proline (Figs. 6-8).

Table 1
Analysis of variance of the effects of nano titanium dioxide and nano aluminium on some growth and physiological parameters of wheat.

S.O.V	df	MS					
		Shoot length	Root length	Chlorophyll a	Chlorophyll b	MDA	Proline
Nano titanium dioxide (T)	3	271.91**	475.17**	113.52**	102.42**	0.0134**	6.92**
Nano aluminium (A)	3	55.13**	32.95**	32.02**	30.47**	0.0010**	5.16**
T×A	9	3.82 *	1.17ns	2.65**	3.50**	0.00001ns	0.82*
error	32	0.95	6.51	0.69	1.11	0.00001	0.33
CV		7.10	9.86	9.83	13.78	2.33	17.26

* and **: non-significant, significant at the 5% and 1% levels of probability, respectively

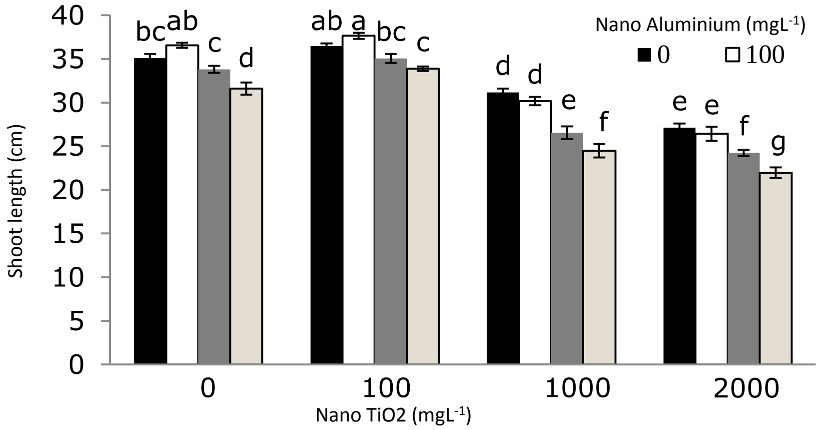

Fig. I. The effects of simultaneous application of nano titanium dioxide and nano aluminium on the shoot length in wheat. (Means with common letters in each column based on the LSD test are not statistically significant at the 5% probability level and Bars indicate standard errors).

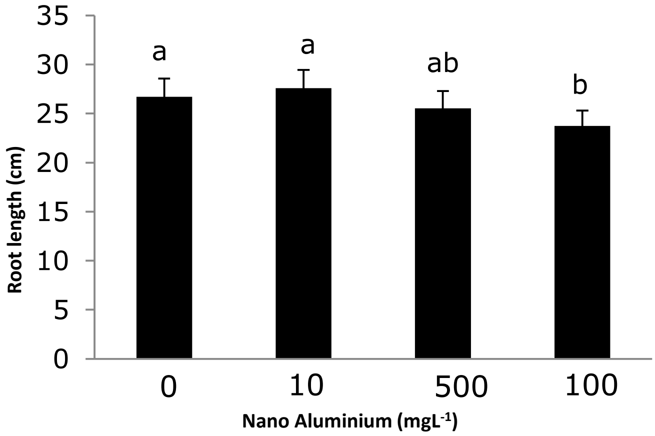

Fig. II. The effect of nano aluminium on the root length of wheat (Means with common letters in each column based on the LSD test are not statistically significant at the 5% probability level and Bars indicate standard errors).

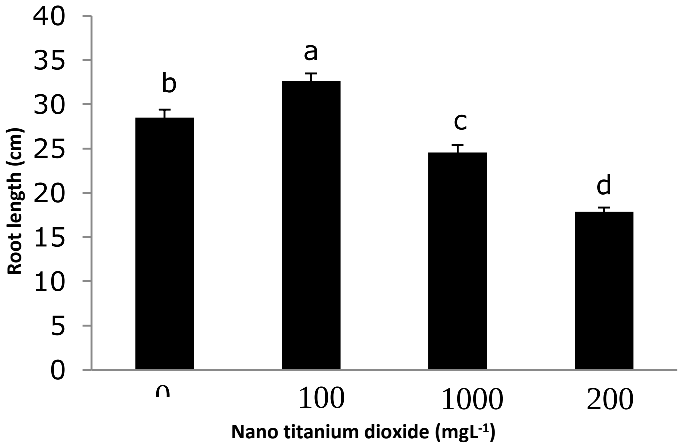

Fig. III. The effect of nano titanium dioxide on the root length of wheat. (Means with common letters in each column based on the LSD test are not statistically significant at the 5% probability level and Bars indicate standard errors).

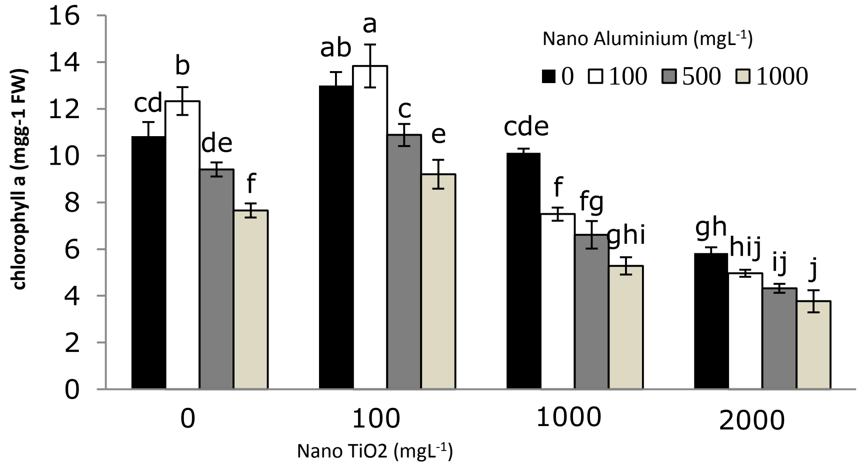

Fig. IV. Simultaneous application of nano titanium dioxide and nano aluminium on the content of chlorophyll of wheat (Means with common letters in each column based on the LSD test are not statistically significant at the 5% probability level and Bars indicate standard errors).

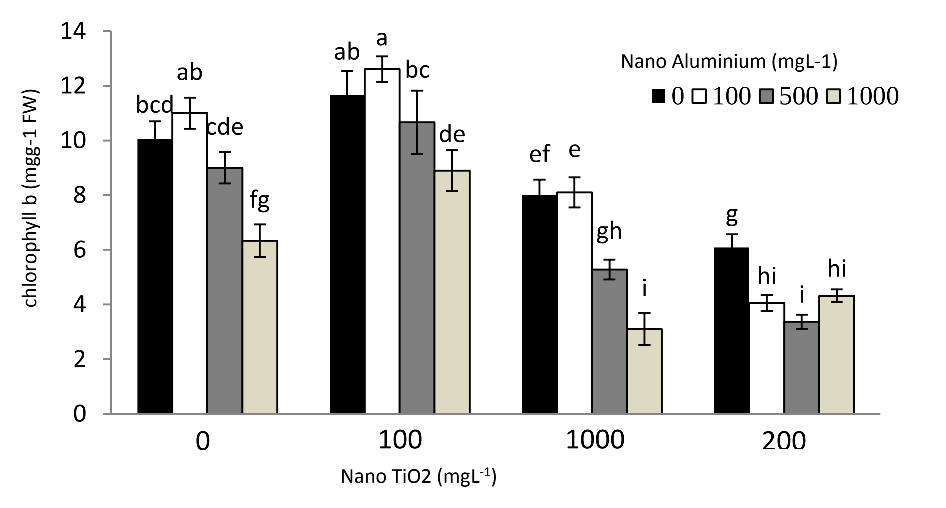

Fig. V. Simultaneous application of nano titanium dioxide and nano aluminium on the content of chlorophyll b of wheat (Means with common letters in each column based on the LSD test are not statistically significant at the 5% probability level and Bars indicate standard errors).

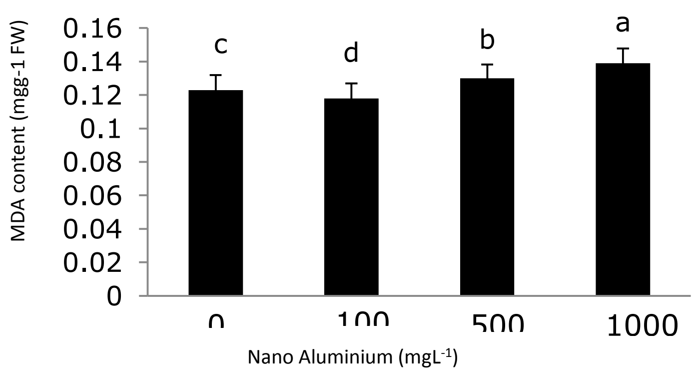

Fig. VI.The effect of nano aluminium on the content of MDA in wheat (Means with common letters in each column based on the LSD test are not statistically significant at the 5% probability level and Bars indicate standard errors).

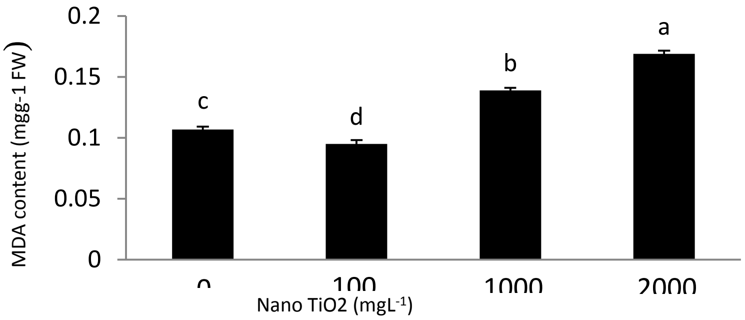

Fig. VII. The effect of nano titanium dioxide on the content of MDA in wheat (Means with common letters in each column based on the LSD test are not statistically significant at the 5% probability level and Bars indicate standard errors).

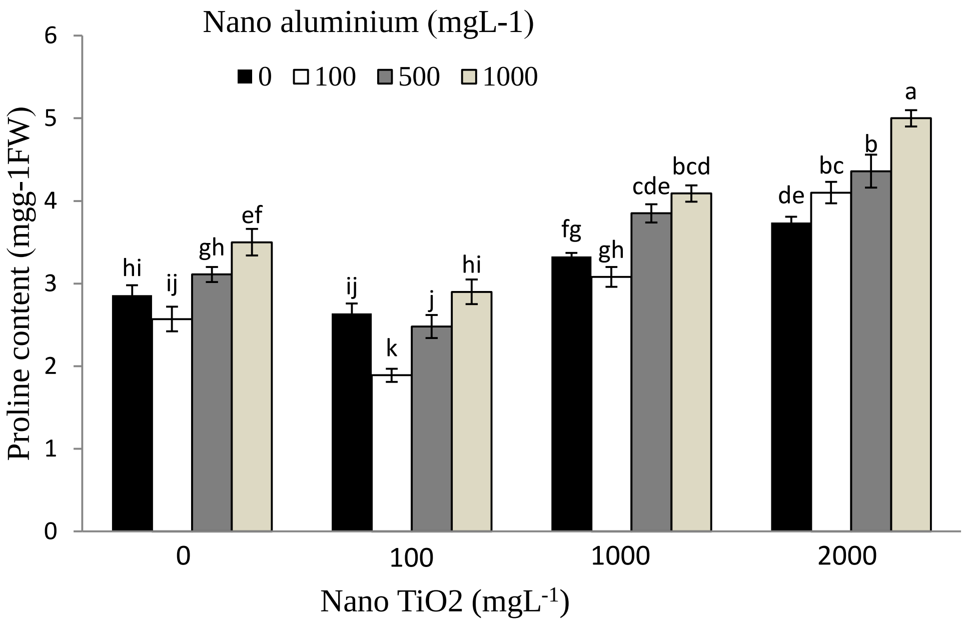

Fig. VIII. Effects of simultaneous application of nano titanium dioxide and nano aluminium on the content of proline in wheat. (Means with common letters in each column based on the LSD test are not statistically significant at the 5% probability level and Bars indicate standard errors).

Discussion

Growth parameters

The results of the present study suggest a negative and toxic effect of nano aluminium and nano TiO_2 on wheat. Generally with increasing the concentration of nanoparticles the length of shoot and root decreased. Only at the 100 mgL^{-1} concentration it showed a slight positive effect. The application of nano titanium dioxide at 100 mgL^{-1} concentration showed positive effect on growth parameters and it could alleviate the negative effects of nano aluminium in combined effects. The growth of plant is the main index for Evaluating the survival and adoption of plant with environmental conditions. The rate of growth in plant is determined by indices like the surface and number of the leaves, the dry/fresh weight and height of shoot and root (Zhao et al., 2013).

Different studies have shown the effects of nanoparticles on growth parameters of plants. For example, treatment of spinach seeds with nano titanium dioxide (5 nm) in low concentrations increased the fresh/dry weight of shoot. The content of chlorophyll increased %17 and rate of photosynthesis % 29 in comparison to control group (Zheng et al., 2005). In another study Peyvandi et al (2011) showed that consumption of one kilogram per hectare of nano-Fe chelate in comparison to Fe chelate, increased dry weight of shoot and root, leave number and the lengths of sweet basil. Titanium and nano titanium were more effective on root than the shoot and were improved the fresh/dry weight of the root (Song et al., 2012). The result of the research on the soybean showed that the movement of titanium is very slow and is more effective in treated organs. Therefore, application of the nano titanium dioxide solution in the root environment stimulated the root more than other shoot (Calza et al., 2014). Treatment of wheat seedlings with nano alumina decreased the root length and as result of oxidative stress the activity of superoxide dismutase and catalase enzymes increased (Riahi-Madvar et al., 2012). Mahmoodzade and Aghili (2014) showed that nano titanium dioxide at its optimal concentration has a stimulating effect and high concentrations have an inhibiting effect on the growth of root and shoot of the wheat. They also showed that the fresh and dry weights of the root remarkably are affected by nano titanium dioxide.

Physiological parameters

In present study the consumption of nano aluminium and nano titanium dioxide in wheat caused significant reduction in the content of chlorophyll a and b in compared to control plants (not consuming nano aluminium) specially at high concentrations. The nano TiO_2 increases the content of chlorophylls a and b in concentration 100 mgL^{-1} (Fig. 5 and 4). In oxidative stress conditions caused by nanoparticles, oxygen radicles damage and break the photosynthetic pigments and structural protein of photosynthetic systems (Dimkpa et al., 2013). Also, proteins that participate in the metabolism of chlorophyll are targeted by types of active oxygen and are damaged (Foltete et al., 2011). Nano Tio2 improves the structure of chlorophyll, increases the sun light absorption, facilitates the pigments formation, increases the transfer of energy to active electrons and finally increases photosynthesis (Jiang et al., 2014). It has also been reported that these physiological effects depending on the size of nanoparticles (Lee et al., 2013).

Our results showed that in response to nanoparticle treatments the content of proline increased (Fig. 8). Among the Amino acids, proline is more sensitive to environmental stresses (X. Li et al., 2015). Induction of proline biosynthesis is the first response of plant to the environmental stress (Lin & Xing, 2007). An increase in proline makes the cell to be more adapted to the stress and protect cytosol enzymes and cellular structures. Proline plays different roles such as regulating pH of the cell, stabilizing the proteins, protecting against the cold and regulating the potential redox (Liu & Lal, 2015). Proline is accumulated mainly in cytoplasm to balance the osmotic potential of vacuole (Mukherjee et al., 2014). Proline acts as an antioxidant to reduce the free radicals produced by nanoparticles (Pokhrel & Dubey, 2013).

The result shows the content of MDA increases by nano aluminium and nano TiO_2 (Fig. 7). Many researches have shown that the MDA content in treated plants with metal ions increased (J. Li et al., 2013; 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 & Hossain, 2013).

Conclusion

The study described here demonstrates that nano TiO_2 has positive effects on the wheat plant at low concentration. Nano alumina by oxidative stress induction and creating oxygen radicles has a negative effect on in wheat plant. Therefore, we can conclude that the application of nano titanium dioxide at low concentration could alleviate the toxic effects of nano aluminium.

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