

## **EFFECTS OF NANOANATASE TiO<sub>2</sub> ON GROWTH AND NITRATE ACCUMULATION OF LETTUCE**

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### **ABSTRACT**

Two field experiments were carried out at the experimental Farm of the Faculty of Agriculture, Moshtohor, Benha university, Qalubiya Governorate, Egypt during the two successive seasons of 2010/2011 and 2011/2012, to study the effect of foliar application with bulk or nano-titanium dioxide (TiO<sub>2</sub> <10 nm) with three levels (0.01, 0.02 and 0.03 %) on the growth and nitrate content of lettuce (*Lactuca sativa* var. *capitata* cv. Great lakes)

As a photocatalyst, nano-TiO<sub>2</sub> under light could cause an oxidation–reduction reaction. The nano-TiO<sub>2</sub> treatments have obvious effects on the improvement of growth and development in lettuce; however, bulk TiO<sub>2</sub> treatments have only little effects.

It was demonstrated that nanoanatase TiO<sub>2</sub> (0.03%) could greatly improve growth (fresh and dry weight) and pigments of photosynthesis (β-carotene, chlorophyll a and b) of lettuce. The results also, showed that TiO<sub>2</sub> nano particles (0.03%) recorded the highest total yield (16.434 ton fed<sup>-1</sup>) and the lowest content of nitrate in lettuce leaves (182.5 μg g<sup>-1</sup> DW) as an average in both seasons.

Nano-anatase TiO<sub>2</sub> treatment could enhance growth, photosynthesis efficiency, activities of nitrate reductase and consequently decrease nitrate content in lettuce plants.

**Keywords:** Lettuce, Nano particles, Titanium dioxide, Nitrate accumulation

### **INTRODUCTION**

Lettuce (*Lactuca sativa* L.) is the most popular salad vegetables according to the highest consumption rate and economic importance throughout the world. It is considered as an excellent nutritive source of minerals and vitamins as it consumed as fresh green salad (Deshpande and Salunkhe, 1998). An important problem facing lettuce production is nitrate accumulation (Blom-Zandstra, 1989).

Nitrogen is an essential element in protein, and thus is essential for all plants and animals. Plants absorb nitrogen from the soil mostly in the form of nitrate ion (NO<sub>3</sub><sup>-</sup>). Before the nitrogen can be combined with the carbon-based compounds produced by photosynthesis to make proteins, the nitrate ion must be reduced to the nitrite form (NO<sub>2</sub><sup>-</sup>). This chemical reaction is facilitated by nitrate reductase enzyme (Salunkhe and Zarogiannis, 1999). Nitrate accumulates not only when its concentration is excessive in soils, but also when plants are growing so slowly and consequently they failed to metabolize it into proteins. So, in addition to have too much nitrate available, slow plant growth, even in soils with only moderate amounts of nitrogen, can also result in nitrate accumulation (Santamaria *et al.*, 1997). In the winter,

when light levels are low and growth is slow, a relatively low concentration of nitrate in the soil can provide sufficient surplus to cause unhealthy concentrations of nitrate in plants (Schonbeck, 1988).

As a photocatalyst, nano-TiO<sub>2</sub> under light could cause an oxidation–reduction reaction and release an energetic electron (Crabtree, 1998). The research of nano-TiO<sub>2</sub> on improving photosynthesis of spinach suggested that nano-TiO<sub>2</sub> could increase light absorbance, accelerate the transport and transformation of the light energy, protect chloroplasts from aging, and prolong the photosynthetic time of the chloroplasts (Hong *et al.*, 2005 a,b and c).

The research by Li *et al.* (2003) showed that the sensitized nano-TiO<sub>2</sub> (anatase) by chlorophyll in vitro could strongly absorb light not only in ultraviolet region, but also in visible region from 400 to 800 nm, e.g., there was an obvious absorption in the 437- and 675-nm region. Lei *et al.*, 2007, proved that nano-TiO<sub>2</sub> could promote photosynthesis and greatly improve spinach growth. Also, Yang *et al.*, 2006 indicated that nano-anatase TiO<sub>2</sub> promoted the activation of nitrate reductase (NR), accelerated the transformation from NO<sub>3</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup> and improved spinach growth. This experiment was conducted to study the effects of TiO<sub>2</sub> nano particles on growth yield and nitrate accumulation of lettuce.

## MATERIALS AND METHODS

### 1. Experimental design

This experiment was carried out during two successive seasons of 2010/2011 and 2011/2012 at the Experimental Farm, Faculty of Agriculture, Moshtohor, Benha University to study the effect of foliar application with bulk or nano-titanium dioxide (TiO<sub>2</sub>) on the growth and nitrate content of lettuce plants, (*Lactuca sativa var. capitata cv. Great lakes*), grown under clay loam soil. Lettuce plants were transplanted at the first week of November in the two growing seasons and harvested after 10 weeks from transplanting. The experimental treatments were arranged in a randomized complete blocks design and included seven treatments with four replicates as tabulated in Table 1. Each experimental plot included 5-ridges each of 60 cm wide and 3.5 long with 25 cm between plants and plot area was 10.5 m<sup>2</sup>.

**Table 1: Experimental design**

Treatments	Description
T1	TiO <sub>2</sub> Nano particles (0.01%)
T2	TiO <sub>2</sub> Nano particles (0.02%)
T3	TiO <sub>2</sub> Nano particles (0.03%)
T4	Bulk TiO <sub>2</sub> (0.01%)
T5	Bulk TiO <sub>2</sub> (0.02%)
T6	Bulk TiO <sub>2</sub> (0.03%)
T7	Distilled water (Control)

Nano-titanium dioxide (TiO<sub>2</sub>) (<10 nm) was provided by Nanotech Egypt for Photoelectronics, Bahgat group, 6 October region, Giza Governorate.

## 2. Soil and plant analyses

**Table 2: Physical and chemical analyses of the experimental soil before transplanting**

Soil texture				pH	EC (dS m <sup>-1</sup> )	O.M (%)	CaCO <sub>3</sub> (%)	Soil available macronutrients (mg kg <sup>-1</sup> )		
Sand (%)	Silt (%)	Clay (%)	Texture					N	P	K
24.4	24.6	51	Clay loam	7.9	2.16	1.41	1.53	22.5	9.1	120

Random soil samples were taken before lettuce transplanting for chemical and physical analysis as described by Chapman and Pratt (1961) and Jackson (1965). The experimental farm soil was clay loam in texture with pH 7.9.

At harvest, four plants from each plot were randomly taken to evaluate vegetative growth characteristics, i.e., fresh and dry weight (g) per plant. Nitrate content in lettuce leaves was determined according to the method described by Cataldo *et al.* (1975). Carotene, Chlorophyll a, b and total Chlorophyll content by A.O.A.C. (1990).

The nitrogen fertilizer with ammonium nitrate (33.5% N) were applied at a total rate of 42 kg N fed.<sup>-1</sup> each and applied at three different times during plant development. The first amount (25 % N) was added 3 weeks after transplanting, the second (25 %) 5 weeks after transplanting and the third (50 %) 7 weeks after transplanting according to the suggestion of Bianco and Pimpini, 1990.

The plants were sprayed 3 times with bulk and nano - titanium dioxide (TiO<sub>2</sub>) at 3, 5 and 7 weeks after transplanting in the two seasons.

### 3. Statistical analysis

Analysis of variance (ANOVA) was performed using the SPSS program (2006). Significant differences between means were determined by Duncan's multiple range test.

## RESULTS AND DISCUSSION

Data in Table, 3 show that, Nanoanatase TiO<sub>2</sub> gave the highest plant growth as compared with all other treatments. However, bulk TiO<sub>2</sub> effect was not as significant as nanoanatase TiO<sub>2</sub>. It is because grain size of nanoanatase TiO<sub>2</sub> (<10 nm) is much smaller than that of bulk TiO<sub>2</sub>, which entered lettuce cell more easily. And total surface of nanoanatase TiO<sub>2</sub> is higher than that of bulk TiO<sub>2</sub>, the absorbance and transfer efficiency of light on the thylakoids membrane was increased, which lead to much more evolution of electrons from nanoanatase TiO<sub>2</sub> and transfer. This is consistent with the research of Lei *et al.*, 2007 on spinach.

Nano-anatase TiO<sub>2</sub> treatment could also promote lettuce to absorb nitrate, accelerate inorganic nitrogen (such as NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N) to be translated into organic nitrogen (such as chlorophyll), and enhance the fresh and dry weights.

The results also, showed that TiO<sub>2</sub> Nano particles (0.03%) recorded the lowest content of nitrate in lettuce leaves (182.5 µg g<sup>-1</sup> DW) as an average of both seasons. It is because Nano-anatase TiO<sub>2</sub> increase the activities of nitrate reductase (Yang *et al.*, 2006). The plants which spray with TiO<sub>2</sub> Nano particles (0.02 and 0.03%) gave the highest total yield as compared with all other used treatments. This increase in yield as a result of using this particular treatment reached 29.39 and 32.67 % as an average of both seasons for total yield per feddan, respectively, as compared with control.

**Table (3): Effect of foliar application with bulk or nano-titanium dioxide (TiO<sub>2</sub>) on the growth, yield and nitrate content of lettuce.**

Treatments	Fresh weight g plant <sup>-1</sup>	Dry weight g plant <sup>-1</sup>	Total yield ton fed <sup>-1</sup>	NO <sub>3</sub> -N content in Leaves µg g <sup>-1</sup> DW
<b>First season (2010/2011)</b>				
TiO <sub>2</sub> Nano particles (0.01%)	531.08 c	30.25 c	14.235 b	290.6 c
TiO <sub>2</sub> Nano particles (0.02%)	592.44 b	33.08 b	16.044 a	213.4 b
TiO <sub>2</sub> Nano particles (0.03%)	621.74 a	35.87 a	16.331 a	180.7 a
Bulk TiO <sub>2</sub> (0.01%)	419.87 f	25.35 d	11.120 e	632.3 e
Bulk TiO <sub>2</sub> (0.02%)	424.50 f	26.82 d	11.326 d	632.9 e
Bulk TiO <sub>2</sub> (0.03%)	494.56 d	28.82 c	13.142 c	616.5 d
Distilled water (control)	431.41 e	26.70 d	11.454 d	645.9 f
<b>Second season (2011/2012)</b>				
TiO <sub>2</sub> Nano particles (0.01%)	552.40 c	30.13 b	15.005 c	273.3 c
TiO <sub>2</sub> Nano particles (0.02%)	593.77 b	33.50 a	16.011 b	227.8 b
TiO <sub>2</sub> Nano particles (0.03%)	616.04 a	33.84 a	16.536 a	185.6 a
Bulk TiO <sub>2</sub> (0.01%)	482.18 e	24.57 d	13.015 e	650.2 e
Bulk TiO <sub>2</sub> (0.02%)	491.05 e	25.66 cd	13.217 e	640.4 e
Bulk TiO <sub>2</sub> (0.03%)	510.88 c	28.74 b	13.566 d	597.2 d
Distilled water (control)	494.13 d	26.65 c	13.320 e	664.6 f

Means of the same column followed by the same letter were not significantly differed due to Duncan MRT at 5%.

Data presented in Table 4, indicate that nano-anatase TiO<sub>2</sub> (0.02 and 0.03%) could greatly improve pigment of photosynthesis (β-Carotene, Chlorophyll a and b) of lettuce as compared with all other treatments in both seasons. Nano particles were prolonged the photosynthesis mechanism by transforming light energy to active electrons and chemical activity, in chloroplast. This procedure increase photosynthesis efficiency, motivate Rubisco activates complex and gain carbon photosynthesis. This photosynthesis amplification could trepan increasing in dry matter (Yang *et al.*, 2006 on spinach). This is consistent with obtained results in this study (Table, 3).

**Table (4): Effect of foliar application with bulk or nano-titanium dioxide (TiO<sub>2</sub>) on photosynthesis pigments of lettuce leaves.**

Treatments	β-Carotene	Chlorophyll a	Chlorophyll b	Total chlorophyll
		mg 100 g <sup>-1</sup> FW		
First season (2010/2011)				
TiO <sub>2</sub> Nano particles (0.01%)	4.06 c	14.75 b	5.27 b	20.02 b
TiO <sub>2</sub> Nano particles (0.02%)	5.05 b	18.96 a	6.23 a	25.19 a
TiO <sub>2</sub> Nano particles (0.03%)	6.07 a	19.08 a	6.55 a	25.63 a
Bulk TiO <sub>2</sub> (0.01%)	3.31 d	12.61 d	4.11 d	16.72 d
Bulk TiO <sub>2</sub> (0.02%)	3.52 d	13.00 c	4.98 b	17.98 c
Bulk TiO <sub>2</sub> (0.03%)	4.24 c	13.04 c	5.06 b	18.10 c
Distilled water (control)	4.12 c	12.85 c	4.88 c	17.73 c
Second season (2011/2012)				
TiO <sub>2</sub> Nano particles (0.01%)	3.94 b	15.24 c	5.26 c	20.50 c
TiO <sub>2</sub> Nano particles (0.02%)	4.96 a	17.57 b	6.44 a	24.01 b
TiO <sub>2</sub> Nano particles (0.03%)	5.17 a	19.67 a	6.17 a	25.84 a
Bulk TiO <sub>2</sub> (0.01%)	3.51 c	12.70 e	4.44 d	17.14 e
Bulk TiO <sub>2</sub> (0.02%)	3.80 b	12.32 e	5.05 c	17.37 e
Bulk TiO <sub>2</sub> (0.03%)	3.86 b	12.79 e	5.68 b	18.47 d
Distilled water (control)	3.84 b	12.80 d	4.90 c	17.70 e

Means of the same column followed by the same letter were not significantly differed due to Duncan MRT at 5%.

It can be concluded that, nano-TiO<sub>2</sub> treatments showed obvious positive effects on the improvement of growth and recorded the lowest content of nitrate in lettuce leaves; however, bulk TiO<sub>2</sub> treatments had only little effects. It is because improve pigment of photosynthesis which improve photosynthesis process (Scott, 2008).

## CONCLUSIONS

As a photocatalyst, nano-TiO<sub>2</sub> under light could cause an oxidation–reduction reaction and release an energetic electron. It might reduce NO<sub>3</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup> directly and accelerate the NO<sub>3</sub><sup>-</sup>→NH<sub>4</sub><sup>+</sup> reaction.

The nano-TiO<sub>2</sub> treatments have obvious effects on the improvement of growth and development in lettuce; however, bulk TiO<sub>2</sub> treatments have only little effects. This may be due to that grain size of nanoanatase TiO<sub>2</sub> (<10 nm) is much smaller than that of bulk TiO<sub>2</sub>, which can penetrate lettuce cell more easily. Consequently, total surface of nanoanatase TiO<sub>2</sub> is higher than that of bulk TiO<sub>2</sub>, the absorbance and transfer efficiency of light on the thylakoids membrane was increased, which lead to much more evolution of electrons from nanoanatase TiO<sub>2</sub> and transfer.

Nano-anatase TiO<sub>2</sub> treatment could also promote lettuce to absorb nitrate, accelerate inorganic nitrogen (such as NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N) to be translated into organic nitrogen (such as chlorophyll), enhance the fresh and dry weights, increase photosynthesis efficiency and activities of nitrate reductase. Finally, nanoanatase TiO<sub>2</sub> may lead to produce clean lettuce plants containing few or little nitrate content.

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## تأثير جزيئات أكسيد التيتانيوم المتناهية الصغر على النمو وتراكم النترات في نباتات الخس

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أجريت تجربتان حقليتان في مزرعة الخضر بكلية الزراعة بمشهر ، جامعة بنها ، محافظة القليوبية، مصر خلال الموسمين من ٢٠١١/٢٠١٠ ، ٢٠١٢/٢٠١١ على التوالي، لدراسة تأثير إستخدام جزيئات أكسيد التيتانيوم العادي أو المتناهي الصغر للرش الورقي بتركيزات (٠.٠١ ، ٠.٠٢ ، ٠.٠٣ %) على النمو وتراكم النترات في نباتات الخس. وقد أظهرت النتائج أن الرش بأكسيد التيتانيوم المتناهي الصغر كان له تأثير كبير على زيادة النمو إلا أن جزيئات أكسيد التيتانيوم العادية كان لها تأثيرات طفيفة في ذلك. وقد وجد أن الرش بجزيئات أكسيد التيتانيوم المتناهي الصغر بتركيز ٠.٠٣ % قد أعطى أعلى نمو خضري (وزن طازج ، وزن جاف) وكذلك أعلى تركيز من صبغات التمثيل الضوئي (كاروتين ، كلوروفيل أ ، ب) وبفارق معنوي عن باقي المعاملات كما أعطت هذه المعاملة أعلى محصول كلي للفدان (١٦.٤٣٤ طن للفدان) وأقل تراكم للنترات ١٨٢  $\mu\text{g g}^{-1}$  كمتوسط لكلا الموسمين

ويعود ذلك لقدرة هذا المركب المتناهي الصغر على الدخول بسهولة داخل النبات كما أنه يلعب دور في رفع كفاءة التمثيل الضوئي وتنشيط إنزيم إختزال النترات وبالتالي إنتاج نباتات خس منخفضة المحتوى من النترات.

**الكلمات الدالة:** الخس – الجزيئات المتناهية الصغر – أكسيد التيتانيوم – تراكم النترات