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EFFECTS OF NANOANATASE TiO_2 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_2 <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_2 under light could cause an oxidation–reduction reaction. The nano-TiO_2 treatments have obvious effects on the improvement of growth and development in lettuce; however, bulk TiO_2 treatments have only little effects.

It was demonstrated that nanoanatase TiO_2 (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_2 nano particles (0.03%) recorded the highest total yield (16.434 ton fed^{-1}) and the lowest content of nitrate in lettuce leaves (182.5 µg g^{-1} DW) as an average in both seasons.

Nano-anatase TiO_2 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_3^-). 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_2^-). 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 *el 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 unhealthful concentrations of nitrate in plants (Schonbeck, 1988).

As a photocatalyst, nano-TiO_2 under light could cause an oxidation–reduction reaction and release an energetic electron (Crabtree, 1998). The research of nano-TiO_2 on improving photosynthesis of spinach suggested that nano-TiO_2 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_2 (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_2 could promote photosynthesis and greatly improve spinach growth. Also, Yang *et al*., 2006 indicated that nano-anatase TiO_2 promoted the activation of nitrate reductase (NR), accelerated the transformation from NO_3^- to NH_4^+ and improved spinach growth.

This experiment was conducted to study the effects of TiO_2 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_2) 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^2.

Table 1: Experimental design

Treatments	Description
T1	TiO_2 Nano particles (0.01%)
T2	TiO_2 Nano particles (0.02%)
T3	TiO_2 Nano particles (0.03%)
T4	Bulk TiO_2 (0.01%)
T5	Bulk TiO_2 (0.02%)
T6	Bulk TiO_2 (0.03%)
T7	Distilled water (Control)

Nano-titanium dioxide (TiO_2) (<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-1)	O.M (%)	CaCO3 (%)	Soil available macronutrients (mg kg-1)		
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.$^{-1}$ 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_2) 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_2 gave the highest plant growth as compared with all other treatments. However, bulk TiO_2 effect was not as significant as nanoanatase TiO_2. It is because grain size of nanoanatase TiO_2 (<10 nm) is much smaller than that of bulk TiO_2, which entered lettuce cell more easily. And total surface of nanoanatase TiO_2 is higher than that of bulk TiO_2, the absorbance and transfer efficiency of light on the thylakoids membrane was increased, which lead to much more evolution of electrons from nanoanatase TiO_2 and transfer. This is consistent with the research of Lei *et al*., 2007 on spinach.

Nano-anatase TiO_2 treatment could also promote lettuce to absorb nitrate, accelerate inorganic nitrogen (such as NO_3^-–N and NH_4^+–N) to be translated into organic nitrogen (such as chlorophyll), and enhance the fresh and dry weights.

The results also, showed that TiO_2 Nano particles (0.03%) recorded the lowest content of nitrate in lettuce leaves (182.5 µg g^{-1} DW) as an average of both seasons. It is because Nano-anatase TiO_2 increase the activities of nitrate reductase (Yang *et al*., 2006). The plants which spray with TiO_2 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_2) on the growth, yield and nitrate content of lettuce.

Treatments	Fresh weight g $plant^{-1}$	Dry weight g $plant^{-1}$	Total yield ton fed^{-1}	NO_3-N content in Leaves µg g^{-1} DW
		First season (2010/2011)		
TiO_2 Nano particles (0.01%)	531.08 c	30.25 c	14.235 b	290.6 c
TiO_2 Nano particles (0.02%)	592.44 b	33.08 b	16.044 a	213.4 b
TiO_2 Nano particles (0.03%)	621.74 a	35.87 a	16.331 a	180.7 a
Bulk TiO_2 (0.01%)	419.87 f	25.35 d	11.120 e	632.3 e
Bulk TiO_2 (0.02%)	424.50 f	26.82 d	11.326 d	632.9 e
Bulk TiO_2 (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
		Second season (2011/2012)		
TiO_2 Nano particles (0.01%)	552.40 c	30.13 b	15.005 c	273.3 c
TiO_2 Nano particles (0.02%)	593.77 b	33.50 a	16.011 b	227.8 b
TiO2 Nano particles (0.03%)	616.04 a	33.84 a	16.536 a	185.6 a
Bulk TiO_2 (0.01%)	482.18 e	24.57 d	13.015 e	650.2 e
Bulk TiO_2 (0.02%)	491.05 e	25.66 cd	13.217 e	640.4 e
Bulk TiO_2 (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_2 (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_2) on photosynthesis pigments of lettuce leaves.

Treatments	β-Carotene	Chlorophyll a	Chlorophyll b	Total chlorophyll
	mg 100 g^{-1} FW			
	First season (2010/2011)			
TiO_2 Nano particles (0.01%)	4.06 c	14.75 b	5.27 b	20.02 b
TiO_2 Nano particles (0.02%)	5.05 b	18.96 a	6.23 a	25.19 a
TiO_2 Nano particles (0.03%)	6.07 a	19.08 a	6.55 a	25.63 a
Bulk TiO_2 (0.01%)	3.31 d	12.61 d	4.11 d	16.72 d
Bulk TiO_2 (0.02%)	3.52 d	13.00 c	4.98 b	17.98 c
Bulk TiO_2 (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_2 Nano particles (0.01%)	3.94 b	15.24 c	5.26 c	20.50 c
TiO_2 Nano particles (0.02%)	4.96 a	17.57 b	6.44 a	24.01 b
TiO2 Nano particles (0.03%)	5.17 a	19.67 a	6.17 a	25.84 a
Bulk TiO_2 (0.01%)	3.51 c	12.70 e	4.44 d	17.14 e
Bulk TiO_2 (0.02%)	3.80 b	12.32 e	5.05 c	17.37 e
Bulk TiO_2 (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_2 treatments showed obvious positive effects on the improvement of growth and recorded the lowest content of nitrate in lettuce leaves; however, bulk TiO_2 treatments had only little effects. It is because improve pigment of photosynthesis which improve photosynthesis process (Scott, 2008).

CONCLUSIONS

As a photocatalyst, nano-TiO_2 under light could cause an oxidation–reduction reaction and release an energetic electron. It might reduce NO_3^- to NH_4^+ directly and accelerate the $NO_3^- \rightarrow NH_4^+$ reaction.

The nano-TiO_2 treatments have obvious effects on the improvement of growth and development in lettuce; however, bulk TiO_2 treatments have only little effects. This may be due to that grain size of nanoanatase TiO_2 (<10 nm) is much smaller than that of bulk TiO_2, which can penetrate lettuce cell more easily. Consequently, total surface of nanoanatase TiO_2 is higher than that of bulk TiO_2, the absorbance and transfer efficiency of light on the thylakoids membrane was increased, which lead to much more evolution of electrons from nanoanatase TiO_2 and transfer.

Nano-anatase TiO_2 treatment could also promote lettuce to absorb nitrate, accelerate inorganic nitrogen (such as NO_3^-–N and NH_4^+–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_2 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 فى نباتات الخس

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

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

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