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# EFFECT OF $TIO_2$ NANOPARTICLES SPRAYING ON FENNEL PLANT

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## ABSTRACT

The present investigation was carried out to investigate effects of $TiO_2$ nanoparticles on growth and chemical composition of fennel plants .

Previous work showed that $TiO_2$ nanoparticles could significantly raise photosynthesis and greatly improve growth of fennel (*Feoniculum vulgare* Mill) plants . The plants were sprayed with different concentrations of $TiO_2$ nanoparticles 2, 4 and 6 ppm . The results showed that, $TiO_2$ nanoparticles at treatment 6 ppm could obviously get the highest number of branches , the tallest plants , the highest fruit yield per plant and the highest values of Pigments,Carbohydrates, phosphorus ,Sugars nitrogen , potassium . sprayed fennel plant with concentrations of $TiO_2$ nanoparticles 0, 2, 4 and 6 ppm is safe for human .

**Keywords:** *Titanium nanoparticles, yield, biochemical constituents,growth, Foeniculum vulgare.*

## INTRODACTION

Fennel (*Foeniculum vulgare Mill*) belongs to family Apiaceae. The Florence fennel is a variety group with inflated leaf bases which form a bulb-like structure (Chiej,1984). Fennel is used as a condiment due to volatile oil compounds isolated from its fruits (Bhati *et al*., 1988; Bremness and Herbs ,1997 ; Philips and Rix ,1998). The fennel essential oil is used to flavor different food preparations and in perfumery industries (Masada ,1976; Abdallah *et al* .,1978; Simon et al.,1984) .Fennel have a medical effects such as : to ease flatulence, relax the intestines and many other medicinal uses (Chiej ,1984; Leung and Foster ,1996; Bown ,1995) .It is still used in traditional medicine as digestive ,diuretic and appetizer (Karnick ,1994).

Nanoparticles (NP) are particles with size between 1-1000 nm. Nanotechnology has many applications in different areas such as industry medicine, agriculture etc. $TiO_2$ nanoparticles can stimulate antioxidant system, enhance absorbing of utilizing water abilities, hasten germination and growth in Glycine max (Lin and Xing , 2007). This nanoparticle generally be in crystal shape with three titles: anatase, rutile, and brukite, and there are also noncrystallines. $TiO_2$ nanoparticles is applied in optical module ,coated surfaces, dipole electron tubes, sporting goods ,disinfectant sprays, etc. $TiO_2$ nanoparticles has effects on redox oxygen systems (ROS) in the presence of Ultraviolet (UV) light (Kim *et al*., 2010). $TiO_2$ nanoparticles enhanced seed germination, plant growth of spinach and fennel (Feizi *et al*., 2013; Zheng *et al*., 2005) and enhance light absorbance and promoted the activity of Rubisco thus accelerated growth of spinach plant (Zheng *et al* ., 2005; Lei *et al*., 2008) .The important effects of $TiO_2$ compounds on plants are enhancement the yield of different crops , increase the activity of peroxides catalase, improve some essential element contents , enhance nitrate reductase activities in plant tissues , improve plant growth by enhancing nitrogen metabolism and promote plant photosynthesis by promoting the absorption of nitrate (Yang *et al*.,2006 ; Lei *et al*., 2007).Application of Foliar $TiO_2$ nanoparticles in spinach plant accelerate conversion of inorganic nitrogen into organic nitrogen by increasing the fresh and dry weights (Yang *et al.* , 2006) .

$TiO_2$ nanoparticles raised oxidative stress tolerance by decreasing the accumulation of superoxide radicals, hydrogen peroxide, malonyldialdehyde content and raise the activities of superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase and thereby enhanced the antioxidant activities in spinach chloroplasts under UV-B radiation (Lei *et al* ., 2008) .The ability of $TiO_2$ nanoparticles to enhance the light harvesting complex content of plants is highly comparable with the use of Titanium dioxide ( Anusorn *et al.* , 2008) .

The present work is conducted to investigate effects of $TiO_2$ nanoparticles on growth yield and chemical composition of fennel plants.

## MATERIALS AND METHODS

The field research was carried out during two successive seasons, 2011/2012 and 2012/2013 at a Farm in Sakara, Giza , Egypt,.

Seeds were obtained from the National Research Centre, Doki, Giza, Egypt. The fennel plants were sprayed by $TiO_2$ nanoparticles at the concentrations of 0,2,4 and 6 ppm . The seeds were sown on 1 November of both years, the plot area was 2m x 4m. The distance between lines was 75 cm and 30 cm between plants.

The layout of the experiment is a complete randomized block design, comprising 4 treatments and each of them is replicated three times and the replicate consisted of approximately 30 plants.

chemical properties of the soil in this study were determined and illustrated in (Table 1) according to (Jackson ,19973 ; Cottenie et al. ,1982) .

**Table (1): The soil chemical analysis of the field planted .**

| measurements | Season 2011-2012 | | | | Season 2012-2013 | | | |
|---|---|---|---|---|---|---|---|---|
| Soil depth (Cm) | 0-60 | | | | 0-60 | | | |
| pH (1:2.5) | 7.2 | | | | 7.5 | | | |
| E.C. (mmhos/Cm) | 1.37 | | | | 1.3 | | | |
| Calcium Carbonates (%) | 7.1 | | | | 7.4 | | | |
| Soluble cations (meq/L) | $K^+$ | $Na^+$ | $Mg^{+2}$ | $Ca^{+2}$ | $K^+$ | $Na^+$ | $Mg^{+2}$ | $Ca^{+2}$ |
| | 0.97 | 2. 2 | 2.8 | 2.0 | 0.42 | 2.43 | 0.8 | 4.8 |
| Soluble anions (meq/L) | $SO_4^{-2}$ | $Cl^-$ | $HCO_3^-$ | $CO_3^{-2}$ | $SO_4^{-2}$ | $Cl^-$ | $HCO_3^-$ | $CO_3^{-2}$ |
| | 4.77 | 1. 1 | 1.2 | _- | 3.21 | 1.25 | 2.8 | -_ |

**Preparation of $TiO_2$ Nanoparticles**

Titanium nanoparticles ($TiO_2$) were prepared by laser ablation of a Titanium($TiO_2$) plate (99.9% in purity) in 10 ml deionized water . Q-switched Nd:YAG (Quantel) pulse laser at the wavelength of 1064 nm and 8 ns pulses with the repetition rate of 10 Hz and the energy density was 400 mJ $cm^{-2}$, was focused using a 100 mm focal length lens on the Titanium($TiO_2$) plate immersed in water according to (Siuzdak et al., 2014).

**Characterization of $TiO_2$ Nanoparticles**

Physicochemical properties of $TiO_2$ nanoparticles were characterized via TEM imaging (Fig. 1 ) illustrating a spherical shape and an average particle size of 19.5 to 20 nm.

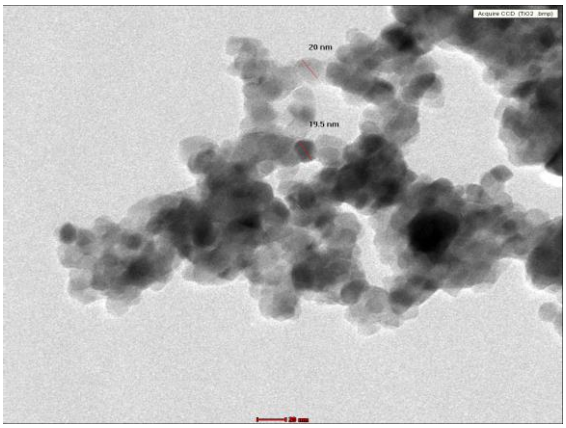


**Figure 1: TEM imaging of the prepared $TiO_2$ nanoparticles revealed a spherical shape of the particles, with an average size of 20 ±2.0 nm .**

**Chemical analysis**

**The following determinations had been measured:**

- Total soluble Sugars (TSS) content was measured according to Thomas and (Ductcher ,1924) .
- Phosphorus content was determined according to (Jackson ,1958) .
- Insoluble Carbohydrates content was measured according to (A.O.A.C ,1985).
- Potassium content was determined according to (Brown and Lilleland, ,1946) .
- Nitrogen content was determined according to (Pregl ,1945) .
- Chlorophyll a (Chla), Chlorophyll b (Chlb) and Carotenoids content were determined according to the method (Wettstein ,1957).

**Determination of heavy elements**

Fe, Mn, Ni, B ,Pb ,Zn,Ti and Cu concentrations were determined by atomic absorption spectrophotometer (Thermo Jarrell Ash Model AA SCAN1) according to the method of (Reuter ,1980).

**Statistical Analyses**

Data were statistically analyzed using SAS software version 9. R- Squared values ($R^2$) are considered significant at (p-values <0.05) , least significant difference (LSD) at 5 % was used to compare the deference between the means of treatment values by analysis of variance test (ANOVA) as described by (Snedecor and Cochran., 1990).

## RESULTS AND DISCUSSION

**Plant growth**

There was interaction between plant height and foliar spray with $TiO_2$ Nanoparticles (Table 2). The highest plants were recorded for the plants treated with 6 ppm of $Tio_2$ Nanoparticles giving (116.3 and 118.7 cm) as compared with untreated plants (83.3 and 85.8cm) for first and second season respectively. These results agreed with that reported by (Zheng et al., 2005 ; Yang et al.,2006) .

**Number of branches / plants**

Data in (Table 2 ) revealed that spraying fennel plants with $TiO_2$ at 2,4 and 6 ppm increased significantly the number of branches/plant . The highest number of branches /plant (7.1 and 7.8) had been found for the plants treated with 6 ppm $TiO_2$ Nanoparticles as compared with (4.6 and 5.3) branches for the untreated plants in first and second season respectively. These resulted are in harmony with that found by (Zheng et al ., 2005; Yang et al ., 2006; Lei et al., 2008).

**Fruit yield**

Foliar treatment of fennel with $Tio_2$ Nanoparticles at 2, 4 and 6 ppm had significantly increased Fruit yield/plant (Table 2) treating the plants with the highest concentration (6 ppm) recorded the highest fruit yield (50.9 and 51.7 g/plant) as compared with the untreated plants (32.6 and 33.8 g/plant) for first and second season respectively, Our finding agreed with that found by (Yang et al., 2006).

**Table 2: Effect of different concentrations of $TiO_2$ NPs foliar spray on Plant height, Number of branches and yield (g/pl.) of Fennel during 2012 and 2013**

| Treatment | Plant height (cm) | | Number of branches (Branch/Plant) | | Fruit yield (g/pl.) | |
|---|---|---|---|---|---|---|
| | 2011/ 2012 | 2012/2013 | 2011/ 2012 | 2012/2013 | 2011/ 2012 | 2012/2013 |
| Control | 83.325 | 85.850 | 4.646 | 5.353 | 32.623 | 33.835 |
| 2 ppm | 96.354 | 95.647 | 5.353 | 6.161 | 42.925 | 43.531 |
| 4 ppm | 105.040 | 106.151 | 6.363 | 6.767 | 47.066 | 48.884 |
| 6 ppm | 116.352 | 118.776 | 7.171 | 7.878 | 50.904 | 51.712 |
| $R^2$ | 0.99 | 0.99 | 0.99 | 0.98 | 0.93 | 0.93 |
| LSD | 1.1 | | 0.32 | | 0.6 | |

**Photosynthetic Pigments Analysis**

In (Table 3) The foliar treatment of fennel with $Tio_2$ Nanoparticles at 2, 4 and 6 ppm increased the chlorophyll content in the leaves of the treated plants as compeared with untreated plant .These results are in agreement with those of (Lei et al., 2008; Zheng et al ., 2005).

**Table 3: Effect of different concentrations of $TiO_2$ NPs on chl.a, chl.b and carotenoids of Fennel plants during 2012 and 2013**

| Treatment | Chlorophyll a (mg/g$^{-1}$ fw) | | Chlorophyll b (mg/g$^{-1}$ fw) | | Carotenoids (mg/g$^{-1}$ fw) | |
|---|---|---|---|---|---|---|
| | 2011/ 2012 | 2012/2013 | 2011/ 2012 | 2012/ 2013 | 2011/2012 | 2012/ 2013 |
| Control | 2.059 | 2.269 | 1.294 | 1.366 | 0.382 | 0.481 |
| 2 ppm | 2.313 | 2.388 | 1.444 | 1.525 | 0.453 | 0.533 |
| 4 ppm | 2.725 | 2.874 | 1.783 | 1.956 | 0.553 | 0.664 |
| 6 ppm | 2.730 | 2.963 | 2.151 | 2.269 | 0.693 | 0.755 |
| $R^2$ | 0.90 | 0.91 | 0.96 | 0.97 | 0.97 | 0.97 |
| LSD | NS | | NS | | 0.33 | |

**NS = not significant**

**Soluble sugars content (TSS)**

Data in (Table 4) showed that soluble sugars content was increased with spraying the plants with $TiO_2$ Nanoparticles giving the highest content 1.07 and 1.17 mg/g$^{-1}$ Dw for plants treated with 6 ppm $TiO_2$ Nanoparticles as compared with 0.583 and 0.642 mg/g$^{-1}$ Dw for control plants in the seasons 2011/2012 and 2012/2013, respectively . These results agreed with (Rutskaya et al.,1974) who found that the development of sugar-beet was enhanced by inserting ammonium titanium sulfate to the soil .According to their results the chlorophyll content of the leaves became higher and the sugar content of the beet root increased.

**Total carbohydrates**

Total carbohydrates content increased with $TiO_2$ Nanoparticles (Table 4). The highest total carbohydrates content 40.03 and 41.15 mg/g$^{-1}$ Dw resulted from 6 ppm of $TiO_2$ Nanoparticles compared with untreated plant 28.3 and 29.6 mg/g$^{-1}$ Dw.

**Table 4 : Effect of different concentrations of $TiO_2$ NPs on sugars contents and total carbohydrates contents of Fennel plants during 2012 and 2013**

| Treatment | Sugars (mg/g$^{-1}$ Dw) | | Carbohydrates (mg/g$^{-1}$ Dw) | |
|---|---|---|---|---|
| | 2011/ 2012 | 2012/2013 | 2011/ 2012 | 2012/ 2013 |
| Control | 0.583 | 0.642 | 28.320 | 29.654 |
| 2 ppm | 0.863 | 0.963 | 32.340 | 33.987 |
| 4 ppm | 0.983 | 0.988 | 33.532 | 35.653 |
| 6 ppm | 1.073 | 1.177 | 40.036 | 41.158 |
| $R^2$ | 0.92 | 0.89 | 0.93 | 0.96 |
| LSD | 0.25 | | 1.6 | |

**Nitrogen Content**

It is clear that spraying 6ppm of $TiO_2$ Nanoparticles on fennel plant exhibited the highest content of nitrogen 2.1 and 2.2 % compared with 1.4 and 1.6 % for untreated plants, in the first and second season, respectively (Table 5). These findings are agreement with (Yang et al ., 2006) who reported that Nano-$TiO_2$ (anatase) improved plant growth by an increase the nitrogen metabolism that enhance the absorption of nitrate in spinach and accelerating the transforming of inorganic into organic nitrogen. They added that nitrogen photoreduction was reflected on the improved growth of treated spinach plants.

**Phosphorus Content**

Data on phosphorus (%) in fennel plants found in (Table 5) showed that, the highest content of phosphorus resulted from spraying the plants with 6 ppm $TiO_2$ Nanoparticles (0.87 and 0.95 %. as compared with untreated plants 0.34 and 0.43 % in the two seasons, respectively). These results were in agreement

with (Bieleski et al., 1983), who reported that treated plants with titanium contained higher concentrations of P in leaves as compared to the control. As well as, the enhancement of biomass, growth and fruit quality obtained from many plant species treated with titanium ($TiO_2$), increased the concentrations of some essential elements such as phosphorus (P), magnesium (Mg) and nitrogen (N) (Pais ,1983 ; Carvajal et al ., 1998).

**Potassium Content**

Results presented in (Table 5) found that, among different exposure treatments with $TiO_2$ Nanoparticles on fennel, 6ppm of $TiO_2$ Nanoparticles gave the highest content of potassium 1.65 and 1.74 %, as compared to control plants, in which potassium content was 1.32 and 1.34 % for the first and second seasons, respectively.

**Table 5. The effect of different concentrations of $TiO_2$ NPs foliar spray on N, P, K (%),of Fennel during 2012 and 2013**

| Treatment | N(%) | | P(%) | | K(%) | |
|---|---|---|---|---|---|---|
| | 2011/ 2012 | 2012/2013 | 2011/ 2012 | 2012/ 2013 | 2011/2012 | 2012/ 2013 |
| Control | 1.414 | 1.616 | 0.349 | 0.438 | 1.323 | 1.343 |
| 2 ppm | 1.838 | 1.869 | 0.509 | 0.630 | 1.485 | 1.545 |
| 4 ppm | 1.980 | 2.121 | 0.566 | 0.664 | 1.535 | 1.586 |
| 6 ppm | 2.121 | 2.262 | 0.876 | 0.956 | 1.656 | 1.747 |
| $R^2$ | 0.91 | 0.98 | 0.91 | 0.91 | 0.96 | 0.94 |
| LSD | 0.14 | | 0.98 | | 0.62 | |

**Heavy metals content**

The data in (Table 6A and 6B) showed that the application of $TiO_2$ Nanoparticles resulted in lower concentrations of Zn,Cu , Fe , Mn , Ni , Pb , Ti and B but higher than the control treatment as compared with the allowable concentrations for each of them.

These results confirmed that concentration 2, 4 and 6 ppm of $TiO_2$ Nanoparticles are safe when sprayed on fennel plants. Our results agreed with (Larry et al., 1979 ; Gupta ,1975; Radojevic and Vladimir ,1999 ; Haider et al ., 2004).

**Table 6 (A,B) : Effect of $Tio_2$ nanoparticles on heavy metals (B,Cu,Fe,Mn,Ni, Pb,Ti and Zn) content in Fennel plant**
**Table 6 A :**

| Concentration (ppm) | B (ppm) | Allowable concentration (ppm) | Cu (ppm) | Allowable concentration (ppm) | Fe (ppm) | Allowable concentration (ppm) | Mn (ppm) | Allowable concentration (ppm) |
|---|---|---|---|---|---|---|---|---|
| Control | 0.217 | 250 | 0.021 | 20 | 2.977 | 50 | 0.089 | 300 |
| 2 | 0.256 | | 0.024 | | 4.327 | | 0.213 | |
| 4 | 0.362 | | 0.032 | | 4.427 | | 0.228 | |
| 6 | 0.458 | | 0.099 | | 4.477 | | 0.293 | |
| Mean | 0.322 | | 0.043 | | 4.040 | | 0.205 | |

**Table 6 B :**

| Concentration (ppm) | Ni (ppm) | Allowable concentration (ppm) | Pb (ppm) | Allowable concentration (ppm) | Ti (ppm) | Allowable concentration (ppm) | Zn (ppm) | Allowable concentration (ppm) |
|---|---|---|---|---|---|---|---|---|
| Control | 0.014 | 5 | 0.001 | 1 | 0.085 | 200 | 0.020 | 15 |
| 2 | 0.019 | | 0.003 | | 0.260 | | 0.046 | |
| 4 | 0.020 | | 0.004 | | 0.282 | | 0.086 | |
| 6 | 0.022 | | 0.011 | | 0.286 | | 0.096 | |
| Mean | 0.018 | | 0.004 | | 0.227 | | 0.062 | |

**Anatomical structure of fennel leaf**

As shown in (Figure 2a) the epidermis cells of the control were similar in size and shape, while the epidermal cells of the NP-treated leaves became larger in size and reached a maximum size at treatment 6 ppm of $TiO_2$ Nanoparticles foliar spray (Figure 2b) . In addition, the thickness of mesophyll tissue, which is specialized photosynthetic tissue that contains chloroplasts in palisade and spongy parenchyma tissue, was a great compared to control leaves. This finding was based on the chlorophyll concentration, which was higher in 6 ppm of TiO2 Nanoparticles foliar spray treatment compared to control (Figure 2c) . This confirms that $TiO_2$ Nanoparticles enhanced chlorophyll synthesis,photosynthesis and plant growth.

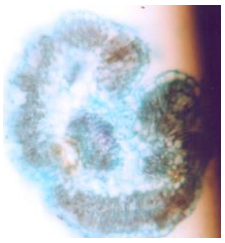
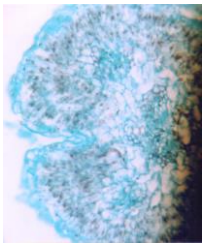
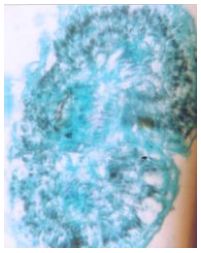

**Figure 2 a** **Figure 2 b** **Figure 2 c**

**Figure 2: Fennel plant leaf anatomy**

## CONCLUSION

$TiO_2$ NPs foliar spray had a significant enhancement on the chlorophyll a, b, carotenoids, , phosphorus ,nitrogen, potassium, total carbohydrates, and sugars content as well plant growth characteristics and which will increase the total yield of fennel plant. The results show strong evidence for the high efficiency of this new nanofertilizer on enhanced plant growth. These inexpensive nanofertilizers could replace traditional methods of plant growth enhancement. Furthermore $TiO_2$ nanofertilizers development could have large scale, economic implications and multiple benefits for farmers, consumers, producers. This work confirmed that $TiO_2$ nanofertilizers using is eco-friendly, safe and produce healthier products.

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## تاثيرالرش بالنانوالتيتانيوم على نبات الشمر

**محمد سليمان خاطر**

**المعهد القومى لعلوم الليزر- جامعة القاهرة**

اظهرت الدراسة ان الرش بالنانو تيتانيوم يحسن بشكل كبير التمثيل الضوئى والنمو لنبات الشمرحيث تم رش نباتات الشمر بتركيزات مختلفة من النانو تيتانيوم ٢و٤و٦ جزئ فى المليون - واظهرت النتائج ان الرش بتركيز ٦ جزئ فى المليون تم الحصول على اعلى محصول واعلى نمو للنباتات وكذلك اعلى عدد فى افرع النباتات و الصبغات والسكريات والكربوهيدرات والنيتروجين والفسفور والبوتاسيوم .

كما ان رش نبات الشمر بتركيزات ٢و٤و٦ جزئ فى المليون من النانو تيتانيوم يكون امن على الانسان.