Influence of TiO_2 Nanoparticles on Growth, Chemical Constituents and Toxicity of Fennel Plant

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ABSTRACT

The present work is carried out to evaluate the effect of TiO_2 nanoparticles treatments on fennel (*Feoniculum Vulgare* Mill) plants. The plants were sprayed with different concentrations of TiO_2 nanoparticles 2, 4 and 6 ppm. In most cases, the tallest plants, the highest number of branches, the highest fruit yield per plant and the highest values of Pigments, Carbohydrates, Sugars nitrogen, phosphorus, potassium were obtained from the treatment of 6 ppm TiO_2 nanoparticles of fennel. Results showed that sprayed fennel plant with concentrations of TiO_2 nanoparticles 0, 2, 4 and 6 ppm is safe and enhanced chlorophyll synthesis and consequently enhanced photosynthesis.

Key word: Laser, Titanium nanoparticles, Toxicity, fennel Plant .

INTRODACTION

Fennel (*Foeniculum Vulgare Mill*) is a member of the Apiaceae family. The Florence fennel (*Foeniculum vulgare* var. *azoricum*) is a variety group with inflated leaf bases which form a bulb-like structure [(1)]. It is used in many of the culinary traditions of the world and widely used in the preparation of various dishes[(2)]. Fennel is used as a spice because some terpenic compounds can be isolated from its fruits volatile oil [(3-5)]. The essential oil [(6)] of fennel is used to flavor different food preparations and in perfumery industries[(7,8)]. Fennel contains anethole, which can explain some of its medical effects such as mitigating flatulence in infant, treating babies with colic or painful teething, relaxing the intestines and reducing bloating caused by digestive disorders, and many other medicinal uses [(1,9,10)] .It is still widely used in traditional Arabian medicine as diuretic appetizer and digestive [(11)].

Nanoparticles (NP) are particulate with size between 1-1000nm. Nanotechnology has many applications in different research areas such as agriculture, medicine, etc. Today, NP-TiO_2 is widely used in industry [(12)] . NP-TiO_2 can stimulate antioxidant system, enhance abilities of absorbing and utilizing water, and hasten germination and growth in Glycine max [(13)]. NP-TiO_2 influenced the industry and research. This nanoparticle usually exists in crystal forms with three titles: rutile, anatase and brukite, and there are also noncrystallines. NP-TiO_2 is applied in coated surfaces, dipole electron tubes, optical module, disinfectant sprays, sporting goods, etc. It affects biological systems and physiologic parameter of plants. NP-TiO_2 has various effects on redox systems oxygen (ROS) in the presence of UV radiation [(14)]. NP-TiO_2 has an increasing effect on seed germination of fennel [(15)].

The ninth most abundant element and the second most abundant transition metal in the earth's crust is titanium element. The most important effects of Ti compounds on plants are enhancement of the yield of various crops. It improves some essential element contents in plant tissues and increases the peroxidase, catalase, and nitrate reductase activities in plant tissues. TiO_2 nanoparticles encourage seed germination and plant growth of spinach (*Spinacia oleracea*) [(16)]. TiO_2 NPs can promote plant photosynthesis and nitrogen metabolism and then greatly improve growth of spinach at a suitable concentration [(16-18)].

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Application of nano-TiO_2 improved light absorbance and promoted the activity of Rubisco activase thus accelerated spinach growth [16,19]. Nano-TiO_2 [anatase] improved plant growth by enhancing nitrogen metabolism [18] by promoting the absorption of nitrate in spinach and accelerating conversion of inorganic nitrogen into organic nitrogen, thereby increasing the fresh weights and dry weights. Studies also showed the effects of nitrogen photoreduction on the improved growth of treated spinach plant [18,20]. It increased the content of light harvesting complex II (LHC II) on thylakoid membranes of spinach [20,17] .Also, it promoted energy transfer and oxygen evolution in photosystem II (PS II) of spinach [20] .Nano-anatase TiO_2 promotes antioxidant stress by decreasing the accumulation of superoxide radicals, hydrogen peroxide, malonyldialdehyde 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 [19] .The ability of nano-anatase TiO_2 to improve the light harvesting complex content of plants is highly comparable with the use of TiO_2-quantum dot (QD) assembly for the conversion of solar energy [21] .Uptake and distribution of QD through plant cells can be exploited for efficient and increased solar energy trapping that might improve the photosynthetic efficiency of plants and the photoluminescence property of quantum dots can be used for cell imaging too.

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

MATERIAL AND METHOD

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

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

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

Chemical properties of the soil in this study were determined in (Table 1) according to [22,23] .

Table (1): The soil chemical analysis of the field planted with Coriander plants.

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				0.6			
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 plate (99.9% in purity) in 10 ml deionized water . Q-switched Nd:YAG (Quantel) pulse laser generating 8 ns pulses at the wavelength of 1064 nm 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 metal plate immersed in water according to [24].

Characterization of TiO_2 Nanoparticles

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

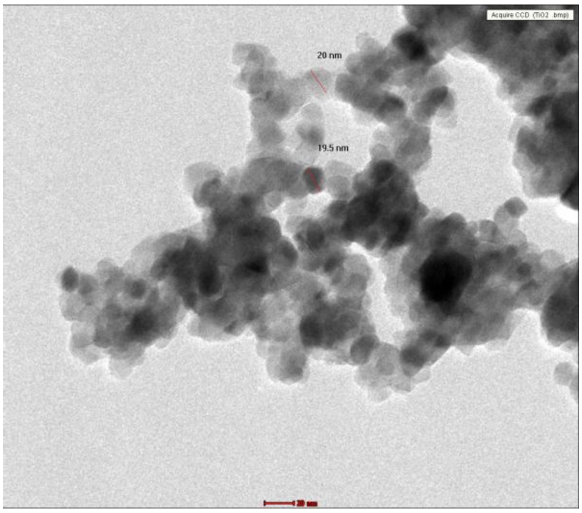

Fig. (1): TEM imaging of the prepared TiO_2 nanoparticles revealed a spherical shape of the particles, with an average size of 20 ±2.0 nm (inset shows electron diffraction pattern).

Chemical Analysis

The following determinations had been carried out:

- Sugars content according to [25] .
- Carbohydrates content was determined according to [26].
- Nitrogen content was determined according to [27] .
- Potassium content was determined according to [28] .
- Phosphorus content was determined according to [29] .
- Chlorophyll a (Chla), Chlorophyll b (Chlb) and Carotenoids content were determined according to the method described by [30].

Determination of Heavy Elements

Fe, Mn, B, Ni, Pb, Ti, Zn and Cu determined by atomic absorption spectroscopy (Thermo Jarrell Ash Model AA SCAN1) according to the method described by [31] .

Statistical Analyses

All statistical analyses were carried out by SAS version 9 software for all data . R- Squared values (R^2) are considered significant (p-values <0.05) for the analysis of variance test (ANOVA).

RESULTS AND DISCUSSION

Plant Growth

There was a significant interaction between foliar spray with TiO_2 Nano particles and plant height. (Table 2), the tallest plants were recorded for that treated with 6 ppm of TiO_2 nanoparticles giving (115.2 and 117.6 cm) as compared with the untreated plants (82.5 and 85cm) for the first and the second seasons respectively. These results agreed with that reported by Zheng et al and Yang et al [16-18].

Number of Branches / Plants

Data in (Table 2) revealed that spraying fennel plants with TiO_2 at 2,4 and 6 ppm significantly increased the number of branches/plant . The greatest number of branches /plant (7.1 and 7.8) had been recorded for the plants treated with 6 ppm TiO_2 Nano particles as compared with (4.6 and 5.3) branches for the untreated plants in the first and the second seasons respectively. These results are in harmony with that found by Zheng et al, Yang et al and Lei et al [16,18,19] .

Fruit Yield

Foliar treatment of fennel with Tio_2 nanoparticles at 2, 4 and 6 ppm had significantly increased the fruit yield/plant. (Table 2) treating the plants with the highest concentration (6 ppm) resulted in the highest fruit yield (50.4 and 51.2 g/plant) as compared with the untreated plants (32.3 and 33.5 g/plant) for the first and the second seasons respectively. The present findings are in agreement with that found by Yang et al [18].

Table (2): Effect of different concentrations of TiO_2 NPs foliar spray on growth and yield (g/pl.) of coriander 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	82.5	85	4.6	5.3	32.3	33.5
2 ppm	95.4	94.7	5.3	6.1	42.5	43.1
4 ppm	104	105.1	6.3	6.7	46.6	48.4
6 ppm	115.2	117.6	7.1	7.8	50.4	51.2
R^2	0.99	0.99	0.99	0.98	0.93	0.93

Photosynthetic Pigments Analysis

As shown in (Table 3) the foliar treatment of fennel with TiO_2 nanoparticles at 2, 4 and 6 ppm increased significantly the chlorophyll content in the trated plants as compared to the control samples .These results are in agreement with those of Zheng et al and Lei et al [16,19].

Table (3): Effect of different concentrations of TiO_2 NPs on chl.a, chl.b and carotenoids of coriander plants during 2012 and 2013.

Treatment	Chlorophyll a		Chlorophyll b		Carotenoids	
	2011/ 2012	2012/2013	2011/ 2012	2011/ 2012	2012/2013	2011/ 2012
Control	2.039	2.247	1.281	1.352	0.378	0.476
2 ppm	2.29	2.364	1.43	1.51	0.449	0.528
4 ppm	2.698	2.846	1.765	1.937	0.548	0.657
6 ppm	2.703	2.934	2.130	2.247	0.686	0.748
R^2	0.90	0.91	0.96	0.97	0.97	0.97

Soluble Sugars Content

Data in (Table 4) showed that soluble sugars content had been significantly affected with spraying the plants with TiO_2 nanoparticles giving the highest content 1.06 and 1.17 mg/g for plants treated with 6 ppm TiO_2 nanoparticles as compared with 0.577 and 0.636 mg/g for control plants in the two successive seasons , respectively. These results agree with those of Rutskaya et al [32] who found that the development of sugar-beet was favorably influenced by the addition of ammonium titanyl sulfate to soil according to their results the chlorophyll content of the leaves increased and the sugar content of the beet became higher.

Total Carbohydrates

Total carbohydrates content increased with TiO_2 nanoparticles (Table 4) however, the highest total carbohydrates content 39.64 and 40.75 mg/g resulted from 6 ppm of TiO_2 nanoparticles compared with control 28.04 and 29.36 mg/g.

Table (4): Effect of different concentrations of TiO_2 NPs on sugars contents and total carbohydrates contents of coriander plants during 2012 and 2013.

Treatment	Sugars		Carbohydrates	
	Season 2012	Season 2013	Season 2012	Season 2013
Control	**0.577**	**0.636**	**28.04**	**29.36**
2 ppm	**0.854**	**0.953**	**32.02**	**33.65**
4 ppm	**0.973**	**0.978**	**33.2**	**35.3**
6 ppm	**1.062**	**1.165**	**39.64**	**40.75**
R^2	**0.92**	**0.89**	**0.93**	**0.96**

Nitrogen Content

It is clear that spraying 6ppm of TiO_2 nanoparticles on fennel plant exhibited the highest content of nitrogen 2.1 and 2.24 % compared with 1.4 and 1.6 % for controal plants, in the first and the second seasons, respectively (Table 5). These results were in agreement with the findings of [18], who reported that Nano-TiO_2 (anatase) improved plant growth by an enhanced nitrogen metabolism that promotes the absorption of nitrate in spinach and, accelerates the conversion of inorganic into organic nitrogen, thereby increasing the fresh and dry weights. They added that nitrogen photoreduction improved growth of treated spinach plant.

Phosphorus Content

Data on phosphorus (%) in fennel plants presented in (Table 5) showed that, the highest content of phosphorus 0.867 and 0.947 % resulted from spraying the plants with 6 ppm of. TiO_2 nanoparticles as compared with control plants giving 0.346 and 0.434% in the two seasons, respectively. These results were in agreement with the findings of Bieleski et al [33], who reported that samples of the treated plants with titanium contained higher concentrations of P, Fe, Cu, Mn, and Zn in leaves as compared to the control. This is in addition to the enhancement of growth, biomass, productivity and fruit quality obtained from many plant species treated with titanium, and increasing the concentrations of some essential elements such as nitrogen (N), phosphorus (P) and magnesium (Mg) [34,35].

Potassium Content

Results presented in (Table 5) revealed that, among different exposure treatments with TiO_2 nanoparticles on fennel, 6ppm of TiO_2 nanoparticles gave the highest content of potassium 1.64 and

1.73 %, as compared to control plants, containing the lowest potassium content 1.31 and 1.33 % treatments for the first and the 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	2011/ 2012	2012/2013	2011/ 2012
Control	1.4	1.6	0.346	0.434	1.31	1.33
2 ppm	1.82	1.85	0.504	0.624	1.47	1.53
4 ppm	1.96	2.1	0.560	0.657	1.52	1.57
6 ppm	2.1	2.24	0.867	0.947	1.64	1.73
R^2	0.91	0.98	0.91	0.91	0.96	0.94

Heavy Metals Content

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

These results confirmed that TiO_2 nanoparticles at 2, 4 and 6 ppm are safe when sprayed on fennel plants. The present results are in agreement with Larry et al, Haider et al [36-39].

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	Allowable concentration (ppm)	Cu	Allowable concentration (ppm)	Fe	Allowable concentration (ppm)	Mn	Allowable concentration (ppm)
Control	0.215	250	0.021	20	2.948	50	0.088	300
2	0.253		0.024		4.284		0.211	
4	0.358		0.032		4.383		0.226	
6	0.453		0.098		4.433		0.290	
main	0.319		0.043		4		0.203	

Table (6) B

Concentration (ppm)	Ni	Allowable concentration (ppm)	Pb	Allowable concentration (ppm)	Ti	Allowable concentration (ppm)	Zn	Allowable concentration (ppm)
Control	0.014	5	0.001	1	0.084	200	0.020	15
2	0.019		0.003		0.257		0.046	
4	0.020		0.004		0.279		0.085	
6	0.022		0.011		0.283		0.095	
main	0.018		0.004		0.225		0.615	

Anatomical Structure of Fennel Leaf

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

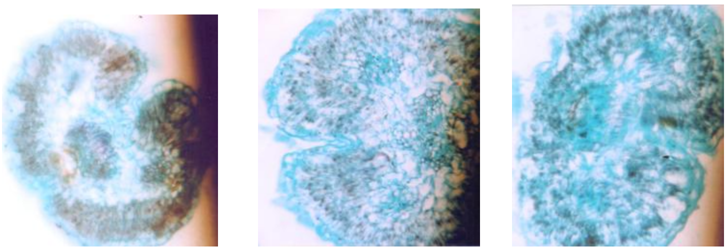

Fig. (2) A **Fig. (2) B** **Fig. (2) C**

Fig. (2): Fennel plant leaf anatomy.

CONCLUSION

TiO_2 nanoparticles foliar spray significantly enhanced on the chlorophyll a, b, carotenoids, sugars content, total carbohydrates, nitrogen, phosphorus and potassium elements, as well plant growth characteristics which will increase the total yield of fennel. The results of this work show strong evidence for the high efficiency of this new nanofertilizer on plant growth enhancement. These powerful and 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 consumers, producers and farmers. This work confirmed that TiO_2 nanofertilizers use is eco-friendly, safe and produces healthier products.

REFERENCES

(1) R. Chiej, The Macdonald Encyclopedia of Medicinal Plants. Macdonald & Co., London, pp: 446.(1984).

(2) Germplasm Resources Information Network: *Foeniculum vulgare*. http://www.ars-grin .gov/cgibin /npgs /html/taxon.pl?300219.

(3) D.S. Bhati, M.S. Shaktawat, L.L. Somani and H.R. Agarwal, Response of fennel (*Foeniculum vulgare* Mill.) to nitrogen and phosphorus. Transactions of Indian Society of Desert Technology, 2: 79-83. (1988).

(4) L. Bremness, Herbs, DK Pocket Encyclopedia. Dorling Kindersley. London, pp:240. (1997).

(5) R. Philips and M. Rix, Herbs for Cooking. Macmillan Publishers Limited. London, pp: 95. (1998).

(6) Y. Masada, 1976. Analysis of Essential oils by Gas Chromatography and Mass Spectrometry.Copyright by the Hirokawa Publishing Company, INC printed in Japan.

(7) N. Abdallah, S. El-Gengaihi and E. Sedrak, The effect of fertilizer treatments on yield of seed and volatile oil of fennel (*Foeniculum vulgare*Mille). Pharmazie, 33(9): 607-608. (1978).

(8) J.E. Simon, A.F. Chadwick and L.E. Craker,1984. Herbs. The scientific literature on selected herbs, and aromatic and medicinal plants of the temperate zone. Archon Books, pp: 770, Hamdan,CT, U.S.A. Fennel and anise as estrogenic agents, J.Ethnopharmacology PMID 6999244.

(9) A.Y. Leung and S. Foster, 1996. Encyclopedia of common natural ingredients used in food, drugs and cosmetics. Second ed. New York : John Wiley and Sons, Inc.

(10) D. Bown, Encyclopaedia of herbs and their uses. New York: DK Publishing, Inc., 283-284. (1995).

(11) C.R. Karnick, Pharmacopoeial standards of herbal plants, 1-2. Delhi: Sri. Atguru Publications,1: 139-141, 2: 71. (1994).

(12) VJ. Mohanraj, Chen Y.Nanoparticles– A Review. Tropical Journal of Pharmaceutical Research.,5(1):561-573. (2006).

(13) D . Lin, B. Xing Phytotoxicity of nanoparticles: Inhibition of seed germination and growth. Environ Pollut.,150(2):243-250. (2007).PMID. 17374428

(14) E. Kim, SH. Kim, HC. Kim, SG. Lee, SW. Jeong Growth inhibition of aquatic plant caused by silver and titanium oxide nanoparticles, Division of Nano & Bio Technology. Daegu Gyeongbuk Institute of Science and Technology (DGIST).,3(1):1-6. (2010).

(15) H. Feizi, M. Kamali, L. Jafari, P.R. Moghaddam, Phytotoxicity and stimulatory impacts of Nano sized and bulk titanium dioxide on fennel (Foeniculum vulgare Mill). Chemosphere. 2013;91:506. DOI: 10.1016/j. chemosphere. 2012.12.012.

(16) L. Zheng, F. Hong, S. Lu and C. Liu Effect of nano-TiO_2 on strength of naturally aged seeds and growth of Spinach. Biological Trace Element Research.;105:83-91. (2005).

(17) F. Hong, J. Zhou, C. Liu, F. Yang C. Wu and L. Zheng Effects of nano- TiO_2 on photochemical reaction of chloroplasts of spinach. Biol Trace Elem Res. ;105:269-279. (2005).

(18) F. Yang, F. Hong, W. You, C. Liu, F. Gao, C. Wu, and Yang P . Influence of nano-anatase TiO_2 on the nitrogen metabolism of growing spinach. Biol Trace Elem Res 110(2):179‑190. (2006). doi:10.1385/ bter:110:2:179

(19) Z. Lei, S. Mingyu, W. Xiao, L. Chao, Q. Chunxiang, C. Liang, H. Hao, L. Xiao- qing and Fashui H. Antioxidant stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation. Biol Trace Elem Res; 121:69–79. (2008).

(20) Z. Lei, S. Mingyu, L. Chao, C. Liang, H. Hao, W. Xiao, L. Xiaoqing, Y. Fan, G. Fengqing and H. Fashui. Effects of nanonatase Tio_2 on photosynthesis of spinach chloroplasts under different light illumination. Biol Trace Elem Res J 119:68–76 (2007).

(21) A. Kongkanand, K. Tvrdy, K. Takechi, M. Kuno, and P.V. Kamat . Quantum Dot Solar Cells. Tuning Photoresponse through Size and Shape Control of CdSe−TiO_2 Architecture .J. Am. Chem. Soc., 130 (12), 4007-4015(2008).

(22) M.L. Jackson, Soil chemical analysis. Published by prentice Hall Indian Private Limited. M.97, Connght Citrus, New Delhi-1. PP. 200 – 250. (1973).

(23) A. Cottenie, M. Verloo, L. Kiekens, G. Velghe, R. Camerlynck, Chemical analysis of plant and soil. Laboratory of Analytical and Agrochemistry, State Univ., Ghent, Belgium, PP. 100 – 129. (1982).

(24) K. Siuzdak, M. Sawczak, M. Klein, G. Nowaczyk,S. Jurga and A. Cenian . Preparation of platinum modified titanium dioxide nanoparticles with the use of laser ablation in water. Royal society of chemistry.6 (2014) .

(25) W. Thomas, and R.A. Ductcher, The Colourimetric determination of carbohydrates . In plants by picric acid reduction methods,1- The estimation of reducing sugars and sucrose . J. Amer. Chem. Soc., 46: 1662- 1669. (1924).

(26) A.O.A.C., 1985. Association of Official Agriculture Chemistry. Official methods of analysis. 14th Ed. Pub. Benjamin Franklin station,Washington D.C., U.S.A.

(27) F. Pregl, Quantita tive Organic Microanalysis. 4 ed. J.A. Churchill Ltd., London, th pp: 126-129. (1945).

(28) J.D. Brown and O. Lilleland, 1946. Determination of Potassium and Sodium in plant material and soil extract by flame photometry. Proc. Amer. Soc. [C.F. Hort. Sci., 73: 813].

(29) N.L. Jackson, Soil chemical Analysis constable. Ltd. Co., London, pp: 498. (1958).

(30) D. Wettstein, , Chlorophyll-letale und der Submikroskopische Form wechsel der Plastiden. Experimental Cell Research 12, 427–506(1957).

(31) D.J. Reuter, 1980. Distribution of cooper and zinc subterranean clover in relation to deficiency diagnosis .Ph.D. Thesis, Murdoch Univ. West Aust.

(32) S.I. Rutskaya, S.V. Ruzhabskaya, - Рутская С.И.-Рыжанская С.В. Содержане микрозлемнтов в различных частях свекловичного растения в завис-мости от метеоролоических условий и налчия титана. Физиолю и биохим. Культ.раст., 6, 469-473. (1974).C.f., Pais, I., Feher, M.

(33) R.L. Bieleski and I.B. Ferguson, Physiology and Metabolism of Phosphate and its Compounds. In Encyclopedia of Plant Physiology, New Series; A. Lauchli, R.L. Bieleski, , Eds.; Springer-Verlag: Berlin, Germany, Vol. 15A, 422±449. (1983).

(34) I. Pais, The biological importance of titanium. J. Plant Nutr. 6 (1), 131. (1983).

(35) M. Carvajal, J.L. Gime´nez, F. Riquelme and C.F. Alcaraz, Antioxidant content and colour level in different varieties of red pepper (Capsicum annuum L.) affected by plant-leaf Ti4þ spray and processing. Acta Alim. Hung., 27 (4), 365–375.(1998).

(36) L.P. Gough, H.T. Shacklette, and A. Arthur Case., Element Concentrations Toxic to Plants, Animals, and Man. Superintendent of Documents, U.S. Government Printing Office Washington, D.C. 20402 Stock Number 024-001-03201-5. (1979).

(37) U. Gupta,1975 . Copper in the environment , Ed.J.O. Nariago, J. Wiley and Sons, New York, 255.

(38) M. Radojevic and M. Vladimir. 1999. Practical environmental analysis, Royal Society of Chemistry, Cambridge, UK. 366.

(39) S. Haider, V. Naithani, J. Berthwal and P. Kakkas , Heavy metal content in some therapeutically important medical plants, Bull. of Environ. Contamination and Technology, 1: 119-127. (2004).