Comparative Studies Using Nanotechnology on Fungal Diseases Defense to Productivity Improvement of Squash Crop

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ABSTRACT

The objective of this paper was to study the effect of silicon dioxide (SiO_2) and titanium dioxide (TiO_2) nanoparticles NPs sprayed separate or in combination compare with traditional practices on plant ability to resist fungal diseases and improving growth and productivity of zucchini squash (*Cucurbita pepo L.*) crop in Al-Kantara Sharq experimental station located 30º 49' 41.4" N and 32º 24' 11.4" S, Eastern part of Al-Ismailia governorate within Sinai, Egypt during summer seasons of 2015 and 2016.

The treatments were: 1.Control spraying with distilled water (T_1), 2.Copper oxychloride $Cu_2(OH)_3Cl$ at concentration of 5000 ppm (T_2), 3.SiO_2 25ppm (T_3), 4.SiO_2 50ppm (T_4), 5.TiO_2 25ppm (T_5), 6.TiO_2 50ppm (T_6), 7.SiO_2 25ppm + TiO_2 25ppm (T_7), 8.SiO_2 25ppm + TiO_2 50ppm (T_8), 9.SiO_2 50ppm + TiO_2 25ppm (T_9) and 10.SiO_2 50ppm + TiO_2 50ppm (T_{10}).

The results indicated that incidence and severity of powdery and downy moilew significantly decreased with SiO_2 NPs whether sprayed at low or high concentration or if it was separate or in mixed with TiO_2 NPs when compared with control (T_1). Powdery mildew not response to individual sprayed TiO_2 NPs but in opposite, incidence and severity of downy moilew significantly decreased with increasing TiO_2 NPs concentration. up to 50 ppm compared with control.

The obtained results revealed that foliar application with the tested materials include $Cu_2(OH)_3Cl$ compared with control showed significant effect on all studied morphological growth characters, yield components and marketable yield as well as photosynthetic pigments and nitrogen, phosphorus and potassium (NPK) contents. Results refer that individual TiO_2NPs whether in low or high concentration achieved superior promotion than SiO_2 NPs on vegetative growth characters which expressed as shoot height, number of branches and leaves plant^{-1}, largest leaf area, fresh and dry weight plant^{-1} and dry matter percentage, while the maximum values were attained from T_{10} compared with T_1 and T_2. Furthermore, no significant effects were recorded between T_{10}, T_8, T_6, T_5, T_9 and T_7 but there were achieved the highest values of fresh and dry fruit weight and fruit dry matter percentage when compared with control while the highest number of fruits were attained from all tested materials except, TiO_2 NPs. Subsequently, control treatment and TiO_2 NPs in low or high concentration whether individual or in combination with low concentration of SiO_2 NPs significantly produced the highest heavy fruit weight per plant and plot while the lowest un-marketable fruit weights and number were achieved from foliar sprayed SiO_2 NPs with their concentrations in addition to T_{10}. For what it was no significant differences were found between T_{10}, T_2, T_9, T_8, T_7, T_4 and T_3 treatments, there achieved the maximum production whether per plant, plot or feddan as well as marketable yield compared with control treatment. Spraying SiO_2 NPs at concentration of 50 ppm achieved minimum un-marketable yield. Chemically, photosynthetic pigments, NO_3^-, $P_2O_5^{--}$ and K^+ contents significantly increased with all treatments compared with control while the highest accumulate in plant tissues were gotten from T_{10}. Anatomically, results and photomicrograph indicated that thin epidermis layers surrounded malformed and destroyed palisade and spongy tissues as a result to fungal diseases infection were observed in untreated plants (control). On opposite, leaf thickness and upper and lower epidermis layers as well as space area (%) were increased when plants treated with separate or mixing at low or high concentration SiO_2 and TiO_2 NPs compared with control or cupper oxychloride. Also, of observation that TiO_2 NPs increased photosynthetic cortical and exchangeable gas spaces more than SiO_2 NPs which increased cell wall thickness. The maximum leaf thickness, palisade length, sponge length, upper epidermis thickness, lower epidermis thickness and area space (%) were attained from T_{10}, T_6, T_8, T_6, T_6 and T_6.

Key words: Zucchini squash (Cucurbita pepo L.), silicon dioxide nanoparticle, titanium dioxide nanoparticle, Powdery mildew, Downy mildew.

INTRODUCTION

Zucchini squash (*Cucurbita pepo* L.) is one of the most important Cucurbitaceae family plants and is one of popularity vegetable crops in Egypt. Squash, cucumber, crab apples, grapes, lilacs, monarda , roses and phlox are all likely targets for downy and powdery mildew which causes leaf curling, yellowing, premature defoliation and in some cases death of the plant. However, using constant fungicides result in resistant populations of the pathogens and environmental pollution (McGrath *et.al*, 1996).

The increment of yield and improving quality of zucchini squash is depending on many factors such as suitable climate, fertility soil have good drainage, fresh

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water and good agricultural practices on suitable plant variety, those have been led to diseases resistance, enhancing plant growth, increase fruit set and improving crop quality and productivity.

The material properties can be change if it form or size changed. Nano is a unit equal a billionth of a meter. Nano-particle is every particle has at least one dimension 1 – 100 nm (Ball, 2002). Nanoparticles (NPs) have unique physicochemical properties *i.e.,* high surface area, high reactivity, tunable pore size and particle morphology for that, it can open a large scope of novel application in biotechnology and agricultural industries through containing herbicides, nano-pesticide, fertilizers or genes which target specific cellular organelles in plants to release their content (Siddiqui *et al.,* 2015).

The soils are content high amount of silicon with an average of 28% Si by weight. Silicon dioxide, aluminosilicates and silicate minerals are consists of the vast majority of Si compounds in the soil, none of which are available for plant uptake. The only plant bioavailable silicon compound is monosilicic acid (MSA) with synonym orthosilicic acid (OSA), the concentration of which is very low in the soil, it's meanly found in sea water and rivers water (Laane, 2018). MSA is found in dynamic equilibrium with disilicic acid, which is considered the bioavailable form of silicon. Silicates are salts of silicic acid used as fertilizers like calcium silicate, potassium silicate, sodium silicate and combinations of diatomaceous earth with minerals. The plants are classified according to contain Si, accumulators plants, intermediate types, or Si excluder types Takahashi, *et al.* 1990). The differences are back to roots capacity to absorb MSA (Ma, *et al*., 2003). Monocotyledonous plants are active to uptake and classified as MSA accumulators. The soluble uptake MSA deposited in plant mainly in epidermal cell walls, middle lamellae, and intercellular spaces within sub epidermal tissues, beneath the cuticle in epidermal cell walls, in the outer regions of epidermal cell walls as biogenic opal (Sangster, *et. al*., 2001; Kim *et al.,* 2002 and Canny, 2006). Sufficient uptake of MSA exerts many beneficial effects on plants because it's play an active role in the uptake of other plant nutrients, structural strength, leaves resistance to blast, enhancing growth and development especially when plants are exposed to abiotic stresses like drought, salinity, acidity, etc. and biotic stresses, because Si increases plant resistance by stimulating defense reaction mechanism (Kim *et al.,* 2002; Fauteux, *et al.,* 2005 and Shallan *et al.,*2016). Also, decreasing damage from insects and rodents due to the fortification of the plant, acting also as a functional deterrent to herbivore. In this respect, using silicate compounds as fungal resistance, Guével, *et al.* (2007) cleared that accumulate Si in wheat leaves resulted in a maximum reduction of powdery mildew disease severity of 80%. Also, the foliar sprays of KSi reduced the powdery mildew significantly, but less than the root-applied. Also, Wolff, *et al.* (2012) reported that one or two foliar sprays of potassium silicate at concentration of 28 and 56 mM were applied to cucumbers plants reduced the powdery mildew infection rates by up to 87%. Also, Rangaraj *et*, *al.* (2014) reported that maize treated with nanosilica recorded significantly higher resistance to *Aspergillus* spp. than with bulk silica.

Regarding to the effect of silicate application on plants, Guével, *et al.* (2007) found that soil- and foliar-applied KSi did not effect in wheat growth, but foliar stabilized silicic acid sprays showed growth-promoting effects, resulting in significantly taller plants. Suriyaprabha *et al.* (2014) found that silica nanoparticles (20–40 nm) significantly increased the concentrations of organic compounds and silica contents in maize when compared to the silicate treatment in a pot study,, while, the soil application of silicates appeared to be more effective than the foliar Si NPs. Janmohammadi *et al.* (2016) found that Si NPs foliar sprays on Safflower, significantly improved canopy spread, stem diameter, plant height, ground cover and the number of achene, while, there was no effect on the achene yield or the harvest index. Also, Shwethakumari (2017) showed that three sprays of stabilized silicic acid with concentrations of 2 mL/L (15 or 30 ppm Si) on soybean increased the plant height, number of leaves, pod and seed yield. Also, the protein and oil yield significantly increased with same treatment. Furthermore in a field study, Yassen, *et al*. (2017) found that spraying Si NPs at concentration of 60 mg/L increased cucumber growth parameters expressed as plant height, number of leaves/plant, fresh and dry weight of leaves/plant as well as number of fruits/plant, mean weight of fruit and fruit length and in turn yield of plant and total yield /ha when compared to untreated plants.

A large number of studies on the effects of titanium dioxide NPs on germination and growth of plants have been documented. In this respect, Lu *et al.* (2002) found that the nitrate reductase activity and stimulated antioxidant system improved in soybean by the mixture of TiO_2 and SiO_2 NPs. Hong *et al.* (2005) and Zheng *et al.* (2005) reported that the treatments of TiO_2 NPs on spinach increased germination, photosynthesis, chlorophyll formation and plant dry weight. Furthermore, Singh *et al*., (2010) studied the biological and physical changes of *Brassica oleracea* in presence of TiO_2 NPs (5- 8 nm) at concentration of 0.05- 2 mL in 500 mL of hoagland solution, they found that the high concentrations had negative impact on shoot length whereas positive impact on root length of broccoli. In addition, Dehkourdi and Mosavi (2013) reported that soaked parsley seeds or sprayed plants by TiO_2 NPs enhanced germination and promoted plant growth where seedling shoot and root length and chlorophyll content increased. Also, Feizi *et al.* (2013) found that the germination rate of salvia improved when the seeds were treated with TiO_2 NPs.

Laware and Raskar (2014) found that TiO_2 at lower concentration (10 and 20 µg mL^{-1}) enhanced seed germination and seedlings growth in onion and however the germination and growth showed inhibition at higher concentrations (40 and 50 µg mL^{-1}). The TiO_2 NPs also induced significant changes in activities of hydrolytic and antioxidant enzymes. Activities of amylase and protease were enhanced in lower concentration, but showed decrease at higher concentrations. Finally, Jiang *et al.* (2017) reported that jasmonic acid (JA) content in wheat seedling increased according to TiO_2 nanoparticles applied, also they found that Ti accumulation showed a dose response manner in both wheat shoots and roots as TiO_2 NPs concentrations increased. Also, Farhat *et.al.* (2018) tested nanoparticles of silicon and titanium synthesized biologically from different bacterial and fungal isolates. They found that Si NPs and Ti NPs as soaking and subsequent foliar spray against powdery mildew on wheat seedling reduced the powdery mildew severity in range 83.3 to 91.0 % according to used microbe synthesized.

The aim of this investigation was to study the effect of silicon dioxide (SiO_2) and titanium dioxide (TiO_2) nanoparticles NPs sprayed separate or in combination compare with traditional antifungals compounds and testing their ability on plant to resist fungal diseases and improving growth and crop production of zucchini squash (*Cucurbita pepo* L.) crop *cv.* Rosena under Egyptian environmental conditions.

MATERIALS AND METHODS

An experiment was conducted at Al-Kantara Sharq experimental station located 30º 49' 41.4" N and 32º 24' 11.4" S, Eastern part of Al-Ismailia governorate within Sinai for comparative study of nanoparticles and commercial fungicides in face of some fungal zucchini squash diseases to improve crop productivity.

Traditional agricultural practices, *i.e.*, fertilization, irrigation, weeds and pest control were applied on zucchini squash Rosena cultivar during two consecutive summer seasons of 2015 and 2016 until to first true two leaves then the following treatments were applied:

1. Control spraying with distilled water (T_1).
2. Copper oxychloride $Cu_2(OH)_3Cl$ at 5000 ppm (T_2).
3. SiO_2 25ppm (T_3).
4. SiO_2 50ppm (T_4).
5. TiO_2 25ppm (T_5).
6. TiO_2 50ppm (T_6).
7. SiO_2 25ppm + TiO_2 25ppm (T_7).
8. SiO_2 25ppm + TiO_2 50ppm (T_8).
9. SiO_2 50ppm + TiO_2 25ppm (T_9).
10. SiO_2 50ppm + TiO_2 50ppm (T_{10}).

The used nanoparticles (NPs) were prepared and tested in laser research institute according to the methods described by Tacchini, *et al.* (2011). SiO_2 and TiO_2 NPs powders were amorphous (50-56 and 30-36 nm) with 99.9 and 98.6 % purity respectively. NPs and the recommended dose of copper oxychloride (5000 ppm) were sprayed four times with 15 days intervals.

Complete block randomized design with three replicates was applied. Experimental plot was consisted of five ridges each of 3 meters long and 0.7 meter wide forming a plot area of 10.5 m^2. The distance between holes within the row was about 50 cm.

Physical and chemical analysis of the experimental soil used were estimated according to the methods described by Piper (1950) and Jackson (1962) respectively, while irrigation water analysis was determined by methods of Richards (1954) as shown in Tables (A,B and C).

Table A. Mechanical properties of the experimental soil

Seasons	Soil depth (cm)	Coarse sand	Fine sand	silt	Clay	Class Texture
				%		
1st	0-40	29.33	46.51	14.65	9.51	Sandy loam
2nd		26.89	49.83	13.02	10.26	Sandy loam

Table B. Chemical analysis of the experimental soil

Seasons	Soil depth (cm)	pH	EC dS/m^2	$CaCO_3$ (%)	Saturation soluble extract							
					Soluble anions (ppm)				Soluble Cations (ppm)			
					CO_3^{-2}	HCO_3	SO_4^{-2}	Cl^-	Ca^{++}	Mg^{++}	Na^+	K^+
1st	0-40	7.37	1.62	12.31	-	45.7	157.8	542.3	55.4	43.1	268.1	34.2
2nd		7.21	1.44	10.05	-	27.4	137.2	488.7	41.3	31.0	249.5	21.1

Table C. Chemical analysis of the irrigation water

Season	pH	EC dS/m	Soluble anions (ppm)				Soluble cations (ppm)			
			$CO_3^{=}$	HCO_3^-	So_4^{-2}	Cl^-	Ca^{++}	Mg^{++}	Na^+	K^+
1st	7.12	2.22	-	57.50	294.3	602	98.4	61.4	547.8	16.4
2nd	7.10	2.07	-	55.5	380.2	540.1	88.8	87.6	319.5	21.1

Data recorded:

Pathogenic measurements:

Before and after each spray date, the disease incidence and severity of powdery mildew and downy mildew were recorded. Disease incidence percentage (D.I) was calculated according to following formula:

$$\text{Disease incidence (D.I.)} = \frac{\text{No. of infected plants}}{\text{Total plants}} \text{X} 100$$

The disease severity was recorded on 0 - 5 scale. Five infected leaves were collected from each one of randomly selected five infected plants in experimental unit for scoring data of disease severity as described by Singh, 2004. Percent of disease severity (PDS) was calculated by using the following formula:

$$\text{PDS} = \frac{\text{Sum of all disease rating}}{\text{Total no. of rating x Maximum disease grade}} \times 100$$

Table D. Score chart for disease severity of powdery mildew and downy mildew on leaves

Disease grade	Disease severity
0	No infection
1	0.1- 1.0 per cent leaf area affected
2	1.1- 10.0 per cent leaf area affected
3	10.1- 25.0 per cent leaf area affected
4	25.1-50.0 per cent leaf area affected
5	< 50.1 per cent leaf area affected

Plant measurements:

1- *Morphological vegetative growth characteristics:* Five plant from each experimental plot at 75 days old from sowing zucchini squash seeds were randomly selected for plant growth measurements were expressed as: Plant height (cm) which measured from cotyledonary node up to the upper most point of the plant, number of secondary branches per plant, number of leaves per plant, fourth leaf area per plant (cm^2) using leaf area meter, fresh and dry weight of shoot per plant (g) and dry matter (%) was calculated.

2- ***Anatomical studies:*** The anatomical studies were for more light on structure changes of zucchini squash leaves tissues *i.e.* leaf thickness (µm), palisade length (µm), sponge length (µm), upper epidermis thickness (µm), lower epidermis thickness (µm) and area space (%) in response to the different treatments . Leaves samples were taken after 75 days from sowing cleaned and cut to suitable parts and fixed in fixing solution. The sections were prepared according to the method described by Johansen (1940).

3- ***Yield and its components*:** At a horticultural maturity, when the fresh fruit was about 15 cm, average of fruit number per plant, average of fruit weight and dry matter content, average yield/plant and average of total yield/fed. were measured.

4- ***Chemical assay* :** Total chlorophyll content was measured in fourth upper leaf using minolta chlorophyll meter SPAD-502. Also, nitrogen, phosphorus and potassium were determined in representative samples of fruit dry matter according to the methods of Peach and Tracey (1959), Frie *et. al*. (1964) and Brown and Lilliland (1964) for N, P and K, respectively.

Statistical analysis:

Data were subjected to statistical analysis by costat (Russel, 1991). The differences among means were performed using least significant difference range (LSR) at 5% level. The results of both investigated seasons were tested for homogeneity for applying combined analysis.

RESULTS AND DISCUSSION

Effect of nanoparticles foliar treatments against powdery mildew and downy mildew disease:

NPs are a modern uses technique in agriculture. The application of tested SiO_2 and TiO_2 NPs separate or in combination as foliar treatments are significantly effective in both diseases incidence and severity of powdery mildew and downy mildew under field conditions during two successive growing seasons. Data presented in Table 1 indicated that SiO_2 NPs whether at low or high concentration significantly reduced both diseases incidence and severity when compared with control. While tested concentrations of TiO_2 NPs resulted in no significant differences were found on powdery mildew but, downy mildew significantly decreased when they compared with control treatment. The mixture treatments were significantly reduced both powdery mildew and downy mildew diseases incidence and severity. The maximum decreasing in both diseases incidence and severity were achieved from T_{10} ($SiO_2$50ppm+$TiO_2$50ppm) followed by T_9 ($SiO_2$50ppm+$TiO_2$25ppm) and T_8 ($SiO_2$25ppm+$TiO_2$50ppm) for powdery mildew. While were attained from T_{10} followed T_2 then T_9 for downy mildew. These results agree with those found by Kim *et al.*, (2002), Rangaraj *et. al.* (2014) and Farhat *et.al.* (2018). These results may be due to silica NPs has been benefit plant in number of ways: (1) enhanced host resistance to fungi due to the accumulation and polymerization of silicic acid in cell walls and rapidly emerging infected area in plant (2) increasing canopy photosynthesis by keeping leaves erect (3) reducing the toxicity of heavy metals (4) reducing rate of transpiration (Ali *et. al.*, 2015; Elsharkawy *et. al.*, 2015 and Jiang *et al.*, 2017)

Effect of NPs foliar treatments on zucchini squash crop:

A. Morphological vegetative growth characteristics:

Data presented in Table 2 indicated that all treatments significantly increased all growth parameters when compared with control treatment. Data cleared that individual TiO_2NPs treatments whether if at low or high concentration led to superior increases in all growth parameters when compared on SiO_2 NPs. The mixture of TiO_2 and SiO_2 NPs promoted growth parameters to more enhancements which led to achieved the highest values compared with control and $Cu_2(OH)_3Cl$ as a traditional practices. The highest values attained of T_{10} for shoot height, number of branches and leaves plant^{-1}, largest leaf area, fresh and dry weight plant^{-1} and dry matter percentage were 66.3, 94.4, 78.9, 79.1, 227.1, 278.9 and 15.8 %, respectively above control treatment and were 9.6, 42.7, 23.1, 22.9, 60.3, 77.1 and 10.7%, respectively above plants treated with $Cu_2(OH)_3Cl$. These results agree with those found by Dehkourdi and Mosavi (2013), Feizi et al. (2013) and Laware and Raskar (2014). These results may be due to that TiO_2 NPs can activate the bio-metabolism process when promote forming the enzymes, hormones and some vital amino acids, in turn increase plant growth parameters (Jiang et al., 2017). Also, NPs mixtures of SiO_2+TiO_2 may be can increase plant growth parameters among to plant diseases resistance promoting and increase vital plant components which reduced disease incidence and accumulated dry matter in plant tissues (Lu et al., 2002; Ali et. al., 2015 and Elsharkawy et. al., 2015).

B. Plant anatomical characteristics:

Data presented in Table 3 revealed that leaves tissues significantly affected with investigated treatments where leaves thickness were reached to maximum increases when plants sprayed with TiO_2 NPs at low or high concentration individual or in mixture with SiO_2 NPs concentrations. Picture (A) in Figure 1 refer to malformation and destroying photosynthetic cortical (palisade tissue), parenchyma cells (spongy tissues), thinness upper and lower epidermis and decreasing intercellular space due to fungal disease effect which in turn reduce leaf thickness when plants not treated with fungicides or other material can decrease fungal diseases. On opposite, picture (B) and Table 3 cleared that leaf in good healthy case where palisade and spongy tissues were appeared, leaf thickness and upper and lower epidermis layers as well as space area (%) were increased when plants treated with $Cu_2(OH)_3Cl$ compared with control treatment.

Furthermore, palisade and spongy tissues and protection layers as well as intercellular space in turn leaf thickness increased in sequenced plants treated with separate or mixing at low or high concentration silicon and titanium nanoparticles compared with control or cupper oxychloride; but the observation, TiO_2 NPs increased palisade tissue (photosynthetic cortical) and intercellular space (exchangeable gas spaces) more than SiO_2 NPs which increased cell wall thickness (Table 3 and pictures C and D in Figure 1). These results explained why plants treated with TiO_2 NPs have been including photosynthetic pigments and their ability to increase plant growth more than SiO_2 NPs in this study. The absolute maximum values of leaf thickness, palisade length, sponge length, upper epidermis thickness, lower epidermis thickness and area space (%) were attained from T_{10}, T_6, T_8, T_6, T_6 and T_6 respectively. These results are agree with those reported by Ali *et al.* (2015); Elsharkawy *et. al.* (2015) who found that microscopic observations revealed that silica nanoparticles caused damage effects on fungal structures of rose powdery mildew.

Table 1.Effect of nanoparticles foliar treatments on diseases incidence and severity

Treatments \ Parameters	powdery mildew		downy mildew	
	D.I. %	**P.D.S**	**D.I. %**	**P.D.S**
Mean±SD	28.2±7.1	9.39±2.4	14.2±4.8	4.8±1.6
Control -**T1**	47.96 a	15.71 a	31.44 a	10.88a
$Cu_2(OH)_3Cl$-**T2**	13.89 d	4.66 c	5.92 f	2.15ef
SiO_2 25ppm **T3**	26.66 b	8.64 b	15.59 c	5.72bc
$SiO_2$50ppm **T4**	25.11 b	7.96 b	15.18 c	5.08cd
TiO_2 25ppm **T5**	46.62 a	16.33 a	20.03 b	6.84b
$TiO_2$50ppm **T6**	43.51 a	14.53 a	16.11 c	5.30bc
$SiO_2$25ppm+$TiO_2$25ppm **T7**	22.73 bc	7.52 bc	13.33 cd	4.37cd
$SiO_2$25ppm+$TiO_2$50ppm **T8**	23.11 bc	7.67 b	10.70 de	3.53de
$SiO_2$50ppm+TiO_2 25ppm **T9**	19.51 c	6.48 bc	8.33 ef	2.73defg
$SiO_2$50ppm+$TiO_2$50ppm **T10**	13.29 d	4.42 c	5.00 f	1.65fg

*Means having similar letters in the same column are not statistically differed at P≥0.05.

Table 2.Effect of nanoparticles on growth parameters of zucchini squash *cv.* Rosena

Treatments \ parameters	Shoot height (cm)	No. branches plant^{-1}	No. leaves plant^{-1}	Fourth leaf area cm^2	Weight g plant^{-1}		Dry matter %
					Fresh	**Dry**	
Mean±SD	56.0±2.6	5.8±0.5	23.6± 2.0	253.3± 30.2	1597.3± 259.7	345.9± 58.7	21.5± 0.6
Control -**T1**	38.3 g	3.6 f	15.2 c	227.5 c	691.3f	135.3e	19.6e
$Cu_2(OH)_3Cl$-**T2**	58.1 cd	4.9 d	22.1 b	331.5 b	1410.9d	289.5cd	20.5d
SiO_2 25ppm **T3**	49.0 f	4.2 e	20.7 b	310.0 b	1169.1de	249.3d	21.3c
$SiO_2$50ppm **T4**	53.6 e	5.0 d	20.5 b	307.5 a	1131.5e	248.3d	21.9bc
TiO_2 25ppm **T5**	56.4 d	6.2 c	25.3 a	380.0 a	1723.2c	368.7bc	21.4c
$TiO_2$50ppm **T6**	57.5 cd	6.8 ab	26.0 a	390.0 a	1750.0bc	378.9bc	21.6c
$SiO_2$25ppm+$TiO_2$25ppm **T7**	59.8 bc	6.2 c	25.8 a	387.5 a	1854.9bc	395.8bc	21.3c
$SiO_2$25ppm+$TiO_2$50ppm **T8**	61.7 ab	7.2 a	26.7 a	400.0 a	2015.1ab	440.8bc	21.9bc
$SiO_2$50ppm+TiO_2 25ppm **T9**	62.4 a	6.5 bc	26.1 a	391.5 a	1965.9bc	440.3ab	22.4ab
$SiO_2$50ppm+$TiO_2$50ppm **T10**	63.7 a	7.0 ab	27.2 a	407.5 a	2261.5 a	512.6a	22.7a

*Means having similar letters in the same column are not statistically differed at P≥0.05.

Table 3. Effect of nanoparticles on leaf anatomical characteristics of zucchini squash cv. Rosena

Treatments \ parameters	Leaf thickness (µm)	Palisade length (µm)	Sponge length (µm)	Upper epidermis thickness (µm)	Lower epidermis thickness (µm)	Area space (%)
Control -**T1**	227.7 d	Malformed	Malformed	10.9 c	10.8 c	2.75 e
$Cu_2(OH)_3Cl$-**T2**	257.3 c	102.1 f	129.7 c	10.9 c	14.6 ab	5.19 d
SiO_2 25ppm **T3**	284.0 b	104.2 f	148.7 b	16.4 b	14.8 ab	5.95 c
$SiO_2$50ppm **T4**	288.3 b	106.4 ef	151.5 b	16.2 b	14.3 ab	6.06 c
TiO_2 25ppm **T5**	312.0 a	124.4 b	154.9 b	16.8 b	15.8 a	7.04 b
$TiO_2$50ppm **T6**	318.3 a	136.9 a	148.3 b	17.8 a	15.9 a	8.72 a
$SiO_2$25ppm+$TiO_2$25ppm **T7**	315.0 a	110.7 de	173.0 a	16.6 b	14.6 ab	6.92 b
$SiO_2$25ppm+$TiO_2$50ppm **T8**	325.0 a	112.0 d	180.5 a	17.4 a	15.2 a	7.22 b
$SiO_2$50ppm+TiO_2 25ppm **T9**	323.7 a	117.7 cd	175.5 a	16.9 b	13.6 b	7.02 b
$SiO_2$50ppm+$TiO_2$50ppm **T10**	327.0 a	118.3 c	176.1 a	17.0 ab	15.7 a	7.04 b

*Means having similar letters in the same column are not statistically differed at P≥0.05.

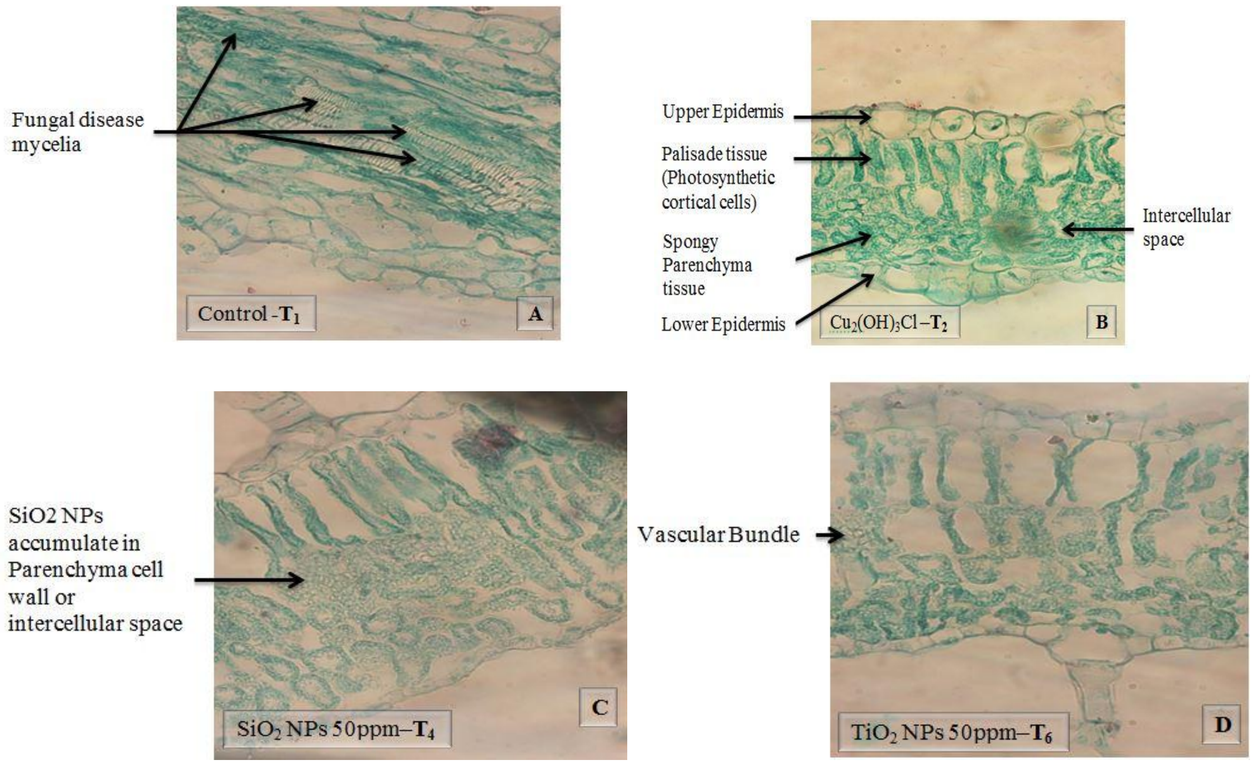

Figure 1. Photomicroscope of zucchini squash leaf cross section at 75 days old treated with: (A) control sprayed with distilled water 52x (B) $Cu_2(OH)_3Cl$ 40x (C) SiO_2 NPs at concentration of 50 ppm 40x (D) TiO_2 NPs at concentration of 50 ppm 40x

C.Yield and its components:

Regarding, data related to the influence NPs of SiO_2 and TiO_2 on zucchini squash quality which expressed as number of fruits plant^{-1}, fresh and dry fruit weight, fruit dry matter %, number and weight of un-marketing fruit plant^{-1} and for plot^{-1} were presented in Table 4. Data cleared that significant increases were recorded in all late recently mentioned parameters when NPs are tested. The treatments increased number of fruits plant^{-1} when compared with control. The highest values were associated with tested treatments except TiO_2. Also, tested materials increased fresh and dry fruit weight and fruit dry matter percentage when compared with control while the highest values were attained by T_{10}, T_8, T_6, T_5, T_9 and T_7 respectively. Control treatment and TiO_2 NPs in low or high concentration whether individual or in combination with SiO_2 NPs significantly produced the highest heavy un-marketable fruit weight for plant and plot while the lowest weights were achieved of foliar sprayed with both concentration of SiO_2 NPs. Also data cleared that high significant increases of un-marketable fruits number had been gotten from control treatment and the minimum values were attained from sprayed SiO_2 NPs. Also, data presented in Table 5 indicated that all treatments include $Cu_2(OH)_3Cl$ led to significantly increased yield whether per plant, plot or feddan as well as marketable yield when compared with control treatment. The maximum increases of total yield per feddan were attained in sequence from T_{10}, T_2, T_9, T_8, T_7, T_4 and T_3 treatments. While the maximum increase of marketable yield were achieved from T_{10}, T_2, T_4, T_9, T_8, T_7 and T_3 treatments compared with control treatment. Total and marketable yield per feddan increases when compared with control treatment were 120.4 and 143.2 % respectively when plants treated with $SiO_2$50ppm+$TiO_2$50ppm, while the $Cu_2(OH)_3Cl$ spraying increased them 119.3 and 141.3 % respectively. On other hand, the highest un-marketable yield was achieved from treatment T_8, T_9, T_1, T_5, T_6 and T_7. The minimum un-marketable yield was 780 kg/feddan achieved from SiO_2 NPs at concentration 50 ppm. These finding agree with Hong *et al*., (2005), Yang *et al.*, (2006), Jaberzadeh *et al.* (2013) and Karunakaran *et al.* (2013) who reported that TiO_2 NPs and SiO_2 NPs at low concentration (0.02 %) individual or in mixture increased dry matter (%) in spinach and some hydrolytic and antioxidant enzymes activity in germination and seedling phases of onion, also, seed number and 1000-seed weight, final yield, biomass, harvest index, gluten, and starch contents of wheat. These results are reflect that TiO_2 NPs can enhancing growth and increase plant weight through promoting plants absorb more essential nutrient elements (as shown in Table 6). In addition, there were no complex components like protein, carbohydrates, fats, hormones, enzymes, vitamins ….. etc. were formed without promote producing some plant hydrolytic enzymes such as amylase and protease (Laware and Raskar, 2014 and Shallan *et al.*,2016) which analyses starch and protein to more simple components in turn were plants more sensitive for diseases. On the other hand, SiO_2 NPs can resist plant diseases through particles accumulation within or between cells or as part of the cell wall in the case of the leaf epidermis as biogenic opal as postulated in Figure 1 and Table 3, (Sangster, *et. al*., 2001 and Canny, 2006). For the above, the combination of NPs TiO_2 and SiO_2 can produce healthy plants and improve growth and productivity.

D. Chemical composition:

Data related to assayed chemical components of zucchini squash effected by recommended practices and NPs of SiO_2 or / and TiO_2 were scheduled in Table 6. Chlorophyll content significantly increased with all treatments which were tested compared with control treatment; the highest increase (35.5 % more than control) was achieved from NPs mixture sprayed at high concentration (T_{10}), while the lowest increase (16.9 % more than control) had been gotten from T_2.

Nitrogen and potassium accumulation in squash leaves significantly increased with TiO_2 NPs at tested concentrations separate or in combination with low and high concentrations of SiO_2 NPs. The maximum of nitrogen and potassium accumulations were associated with treatments of T_{10}, T_8, T_9, T_7, T_6 and T_5. On other side, no significant differences were found between SiO_2 NPs at low concentration. and $Cu_2(OH)_3Cl$ or control treatments.

Regarding to the tested treatments impact on $P_2O_5^{--}$ accumulate in zucchini squash leaves, data cleared that NPs treatments significantly increased $P_2O_5^{--}$ compared with T_1 and T_2 (Table 6). The highest values of $P_2O_5^{--}$ were attained from T_{10}, T_8, T_9 and T_7. These results agree with Hong *et al*., (2005), Yang *et al.* (2006) and Laware and Raskar, (2014) who reported that TiO_2NPs at low concentrations (10-30 ppm) increased photosynthesis pigments, nitrogen metabolism and photosynthetic rate in Spinach, increased stimulates ribulose 1, 5-bisphosphate carboxylase activity, hydrolytic and antioxidant enzymes activity in germination and seedling phases. The present results postulated that the nano size of TiO_2 may have increased the absorption of inorganic nutrients, accelerated the breakdown of organic substances, and also caused quenching of oxygen free radicals formed during the photosynthetic process, hence increasing the photosynthetic rate (Zheng *et al*., 2005 and Shallan *et al.*,2016).

Table 4. Effect of nanoparticles on fruit quality of zucchini squash *cv.* Rosena

parameters / Treatments	No. fruits /plant	Fruit weight g		Fruit dry matter %	Un marketable fruit		
		Fresh	Dry		No. / plant	Weight g/plant	Weight kg/plot
Mean±SD	9.5±0.4	142.5±3.8	17.2±1.3	12.0±0.8	0.60±0.06	85.3±9.1	2.56±0.27
Control -**T_1**	5.3 c	128.2 d	15.6cd	12.13abc	0.74 a	94.3 a	2.83 a
$Cu_2(OH)_3Cl$-**T_2**	10.4 a	142.1bc	14.9d	10.47d	0.57 cd	81.3 bc	2.44 b
SiO_2 25ppm **T_3**	10.2 a	140.9 c	15.9cd	11.27cd	0.52de	73.3 cd	2.20 bc
$SiO_2$50ppm **T_4**	10.1 a	143.0abc	16.7bc	11.67bc	0.45 e	65.1 d	1.95 c
TiO_2 25ppm **T_5**	9.1 b	145.4 ab	17.6ab	12.10abc	0.63 bc	91.0 ab	2.73 ab
$TiO_2$50ppm **T_6**	9.5 b	146.3 ab	18.3a	12.53ab	0.61 bc	89.0 ab	2.67 ab
$SiO_2$25ppm+$TiO_2$25ppm **T_7**	10.2 a	143.2abc	17.7ab	12.33ab	0.62 bc	88.8 ab	2.66 ab
$SiO_2$25ppm+$TiO_2$50ppm **T_8**	10.0 a	146.0 ab	18.4a	12.57ab	0.67 b	97.3 a	2.92 a
$SiO_2$50ppm+TiO_2 25ppm **T_9**	10.3 a	143.3abc	17.6ab	12.27ab	0.67 b	95.9 a	2.88 a
$SiO_2$50ppm+$TiO_2$50ppm **T_{10}**	10.1 a	146.7 a	19.0 a	12.97a	0.53	77.2 c	2.32 b

*Means having similar letters in the same column are not statistically differed at P≥0.05.

Table 5. Effect of nanoparticles on yield of zucchini squash *cv.* Rosena

parameters / Treatments	Average of fruit yield kg		Total of fruit yield ton/fed.	Un marketable fruit ton/fed.	Marketable yield ton/fed.
	$Plant^{-1}$	$Plot^{-1}$			
Mean±SD	1.36±0.06	40.9±1.8	16.35±0.72	1.02±0.11	15.32±0.76
Control -**T_1**	0.67 d	20.22d	8.09d	1.13 a	6.95 d
$Cu_2(OH)_3Cl$-**T_2**	1.48 a	44.36a	17.74a	0.98 bc	16.77 a
SiO_2 25ppm **T_3**	1.44 ab	43.13ab	17.25ab	0.88 cd	16.37 a
$SiO_2$50ppm **T_4**	1.45 ab	43.38ab	17.35ab	0.78 d	16.57 a
TiO_2 25ppm **T_5**	1.32 c	39.55c	15.82c	1.09 ab	14.73 c
$TiO_2$50ppm **T_6**	1.39 b	41.73b	16.69b	1.07 ab	15.62 b
$SiO_2$25ppm+$TiO_2$25ppm **T_7**	1.46 a	43.67ab	17.47ab	1.07 ab	16.40 a
$SiO_2$25ppm+$TiO_2$50ppm **T_8**	1.46 a	43.94a	17.58a	1.17 a	16.41 a
$SiO_2$50ppm+TiO_2 25ppm **T_9**	1.47 a	44.24a	17.70a	1.15 a	16.55 a
$SiO_2$50ppm+$TiO_2$50ppm **T_{10}**	1.49 a	44.57a	17.83a	0.93 c	16.90 a

*Means having similar letters in the same column are not statistically differed at P≥0.05.

Table 6. Effect of nanoparticles on chemical composition of zucchini squash *cv.* Rosena

parameters / Treatments	Chlorophyll content	mg (g DW)$^{-1}$		
		NO_3^-	$P_2O_5^{--}$	K^+
Mean±SD	50.94±3.44	22.76±0.54	2.51±0.05	33.33±0.71
Control -**T_1**	41.57 f	21.63 e	2.39 e	31.84 e
$Cu_2(OH)_3Cl$-**T_2**	48.63 e	21.77 e	2.41 e	32.02 de
SiO_2 25ppm **T_3**	49.60 de	22.19 de	2.45 cd	32.55 de
$SiO_2$50ppm **T_4**	50.67 cde	22.85bc	2.47 c	32.90 bcd
TiO_2 25ppm **T_5**	49.77 cde	23.04abc	2.52 b	33.45 abc
$TiO_2$50ppm **T_6**	50.70 cde	23.18 ab	2.55 b	33.89 abc
$SiO_2$25ppm+$TiO_2$25ppm **T_7**	52.73 bcd	23.30 ab	2.56 ab	34.04 abc
$SiO_2$25ppm+$TiO_2$50ppm **T_8**	53.33 abc	23.40 ab	2.57 ab	34.17 ab
$SiO_2$50ppm+TiO_2 25ppm **T_9**	56.03 ab	23.30 ab	2.56 ab	34.04 ab
$SiO_2$50ppm+$TiO_2$50ppm **T_{10}**	56.33 a	23.57 a	2.59 a	34.39 a

*Means having similar letters in the same column are not statistically differed at P≥0.05.

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الملخص العربي

دراسات مقارنه باستخدام تكنولوجيا النانو على مقاومة الامراض الفطريه لتحسين إنتاجيه محصول الكوسه

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أجريت هذه التجربة لدراسة مدى تأثيرتركيزات مختلفة من أكسيد السليكون وأكسيد التيتانيوم كمواد متناهية الصغر كلاً على حدى أو مخاليطهم على مقاومة الأمراض الفطرية وتحسين نمو وإنتاجية محصول الكوسة مقارنة بالممارسات التقليدية. وقد أجريت التجربة بمحطة بحوث القنطرة شرق التى أحداثياتها "41.4 '49 30º شمالاً و "11.4 '24 32º جنوباً فى الجانب الشرقى من محافظة الأسماعيلية بسيناء – جمهورية مصر العربية خلال صيف موسمي ٢٠١٥ و ٢٠١٦م.

أستخدمت المعاملات: ١. الرش بالماء المقطر للمقارنه (م١)، ٢. الرش بأكسي كلوريد النحاس ٥٠٠٠ جزء فى المليون للمقارنه (م٢)، ٣. الرش بثانى أكسيد السليكون بمعدل 25 جزء فى المليون (م٣)، ٤. الرش بثانى أكسيد السليكون بمعدل 50 جزء فى المليون (م٤)، ٥. الرش بثانى أكسيد التيتانيوم بمعدل 25 جزء فى المليون (م٥)، ٦. الرش بثانى أكسيد التيتانيوم بمعدل 50 جزء فى المليون (م٦)، ٧. الرش بثانى أكسيد السليكون والتيتانيوم بمعدل 25 جزء فى المليون لكل منهما (م٧)، ٨. الرش بثانى أكسيد السليكون بمعدل 25 جزء فى المليون وأكسيد التيتانيوم بمعدل 50 جزء فى المليون (م٨)، ٩. الرش بثانى أكسيد السليكون بمعدل 50 جزء فى المليون وأكسيد التيتانيوم بمعدل 25 جزء فى المليون (م٩)، ١٠. الرش بثانى أكسيد السليكون بمعدل 50 جزء فى المليون وأكسيد التيتانيوم بمعدل 50 جزء فى المليون (م١٠).

أظهرت النتائج الى أن معدل حدوث وشدة الأصابة بمرضى البياض الدقيقى والزغبى إنخفضتا معنوياً عند الرش بالجزيئات متناهية الصغر من أكسيد السليكون سواء بالتركيز المنخفض أو التركيز العالى أو إن كان منفرداً أو مخلوطاً بتركيزى أكسيد التيتانيوم وذلك عند المقارنه بمعاملة المقارنة (م١). لم يستجيب البياض الدقيقى لمعاملات الرش بالتيتانوم المنفرد بينما أنخفض معدل حدوث وشدة الأصابة بالبياض الزغبى معنوياً بزيادة التركيز حتى 50 جزء فى المليون مقارنة بالمعاملة (م١).

أظهرت النتائج الى أن جميع معاملات الرش بالمواد المختبرة متضمنة أوكسي كلوريد النحاس قد أثرت معنوياً فى جميع صفات النمو الظاهريه المدروسه و المحصول ومكوناته وكذلك المحصول القابل للتسويق بالأضافة الى صبغات التمثيل الضوئي ومحتوى النبات من النيتروجين والفوسفور والبوتاسيوم مقارنة بالمعاملة التى أستخدم فيها الرش بالماء المقطر. وقد أظهرت النتائج أن الرش بالمواد متناهية الصغر من أكسيد التيتانوم منفرداً بالتركيزين المخفض والعالى أدت الى أستحداث عالى أكثر من ثانى أكسيد السليكون للنمو الخضرى المتمثل فى أرتفاع النبات وعدد الأفرع والأوراق ومساحة الورقة وكذلك الوزن الطازج والجاف بالإضافة الى النسبة المئوية للمادة الجافة وقد أدت المعاملة (م١٠) الى الحصول على أعلى قيم النمو الخضرى مقارنة المعاملة (م١) و (م٢).

أظهرت النتائج أن المعاملات (م١٠)، (م٨)، (م٦)، (م٥)، (م٩)، (م٧) لم تختلف معنوياً فيما بينها ولكنها أحرزت أعلى قيم الوزن الطازج والجاف والنسبة المئوية للمادة الجافة بالثمارمقارنة بالمعاملة (م١) فى حين أن أعلى قيم عدد الثمار قد نتج من جميع المعاملات المختبرة ماعدا معاملات أكسيد التيتانيوم المنفردة.

أدت المعاملات (م١)، (م٥)، (م٦)، (م٧)، (م٨)، (م٩) الى الحصول على أعلى وزن من الثمار الغير قابلة للتسويق سواء أنتجت من النبات أو القطعة التجريبية بينما أقل القيم بالأضافة الى عدد الثمار الغير قابلة للتسويق تم الحصول عليها من معاملات أكسيد السليكون المنفردة بالأضافة الى المعاملة (م١٠).

أظهرت النتائج أن المعاملات (م١٠)، (م٢)، (م٩)، (م٨)، (م٧) ، (م٤)، (م٣) لم يوجد بينها فروق معنوية ولكنها أدت الى الحصول على أعلى قيم للمحصول الكلى والقابل للتسويق سواء للنبات أو القطعة التجريبية أو الفدان مقارنة بمعاملة الرش بالماء المقطر. كما أدت المعاملة (م٤) الى الحصول على أقل محصول غير قابل للتسويق.

أدت جميع المعاملات المختبرة وخاصة المعاملة (م١٠) الى زيادة قيم صبغات التمثيل الضوئى والنترات والفوسفات والبوتاسيوم معنوياً مقانة بالمعاملة (م١).

أظهرت نتائج تشريح الورقة والتصوير الميكروسكوبى أن النباتات التى لم تعامل بأى من المواد المدروسة كانت طبقات خلايا البشرة العلوية والسفلية رفيعة وقد أحاطة بأنسجة مشوهه ومدمرة نتيجة لوجود الأصابات الفطرية. على النقيض فأن سمك الورقة وطبقات البشرة العلوية والسفلية وكذلك النسبة المئوية لمساحة فرغات تبادل الغازات قد زادت نتيجة لمعاملة النباتات بالجزيئات بأكاسيد السيليكون والتيتانيوم متناهية الصغرسواء منفردة أو فى مخاليط مقارنة المعاملة (م١) و (م٢). أيضاً من الجدير بالملاحظة أن معاملات أكسيد التيتانيوم أدت الى زيادة نسيج الخلايا العمادية المسئولة عن التمثيل الضوئى وفراغات تبادل الغازات أكثر من المعاملة بأكسيد السيليكون الذى أدى الى زيادة سمك الجدر الخلوية. سجلت أعلى قيم لسمك الورقة والنسيج العمادىوالنسيج الأسفنجى وطبقات البشرة العلوية والسفلية وكذلك النسبة المئوية لمساحة فراغات تبادل الغازات عند تطبيق المعاملات (م١٠)، (م٦)، (م٨)، (م٦)، (م٦) بالأضافة الى (م٤) بالترتيب.

الكلمات الدالة:

محصول الكوسة، أكسيد السليكون متناهى الصغر، أكسيد التيتانيوم متناهى الصغر، معدل حدوث وشدة الأصابة الفطرية، البياض الدقيقى، البياض الزغبى، القياسات الظاهرية والتشريحية، المحصول ومكوناتة.