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USE SILICA NANOPARTICLES IN CONTROLLING LATE WILT DISEASE IN MAIZE CAUSED BY *HARPOPHORA MAYDIS*

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ABSTRACT

Green synthesized silica nanoparticles (SiNPs) and their optimization under different pH values *i.e.*, (5, 7, 9, 11) giving sizes 20, 40.2, 70.2 and 95.5 nm, were evaluated for controlling *Harpophora maydis* the causal agent of late wilt disease in *Zea maize in vitro* and *in vivo*. Under laboratory conditions, PDA medium revealed that all tested SiNPs sizes, 20, 40.2, 70.2 and 95.5nm at different concentrations (0.5, 2.5, 5 and 10 ppm) significantly inhibited the mycelia radial growth of *Harpophora maydis*. Reduction increased by increasing concentration compared to control. The most efficient treatment was SiNP- 20 nm followed by SiNP-40.2 nm. Greenhouse experiment indicated that seed coated by SiNPs significantly reduced the infection percentage of late wilt and enhanced the germination percentage compared with check treatment (70.8%). The SiNP-20 nm followed by SiNP-40.2 nm introduced superior reduction in disease incidence (88.2 and 87.7% reduction, respectively) at concentration 10 ppm. The lowest effect was SiNP-95.5 nm which gave 52.9% reduction. Results of field trails during 2019-2020 growing season at Giza and Gemmeza disease nurseries indicated that treated seeds with SiNPs showed significant reduction in maize infected with late wilt compared with check plants (78.3% and 81.7% at Giza 2019, Gemmeza 2020, respectively). Also, there were significant differences between treatments in yield average of the two seasons. The disease reduction and yield increased with increasing concentrations. The SiNPs-20 nm and SiNPs-40.2 nm treatments were the most efficient treatments in decreasing disease incidence and enhancing yield when recorded 6.7% at 10 ppm at first season (Giza-2019) for both treatments while gave 6.7% and 8.3% in the second season at Gemmeza-2020, respectively. On the other hand, average yield were 29 ard/fd in cases of SiNP-20 nm and 27.3 ard/fd in case of SiNP-40.2 nm. In contrary, the lowest treatments were the concentration 0.5 ppm of treatments SiNPs 95.5, 70.2 nm. Where, the infection was 34% and average yield of 17.6 ard/fd for SiNP-95.5 nm and it was 26.3% infection % which yielded 18 (ard/fd) in treatment SiNP-70.2 nm at Gemmeza location. It could be concluded that using the green synthesized SiNPs ecologically welcomed at sizes 20 and

40.2 nm, were more efficient than that with higher sizes in controlling maize late wilt disease and enhancing maize yield productivity.

INTRODUCTION

Maize (*Zea mays* L.) is considered as one of the most important cereal crops in Egypt. The late wilt disease of maize caused by *Harpophora maydis* **(Games, 2000)** synonymous: *Cephalosporium maydis* **(Samra, *et al.*, 1963)** is one of the most important diseases on maize in Egypt **(Sabet *et al.*, 1966b; Ali, 2000 and Saleh & Leslie, 2004).** Moreover, Egyptian isolates of *C. maydis* vary in their morphological characters and capability to cause infection as well as their genetic structures **(Saleh *et al.*, 2003)**. Furthermore, the yield losses may reach up to 40% in naturally infested fields with infection up to 80% **(El-Shafey and Clafline, 1999).** Late wilt appears during tasseling as a rapid wilting of the lower leaves and develops to hollow and shrunken stalks with a dark yellow-to-brown or black-stained pith **(El-Shafey and Claflin 1999)**. The pathogen is mainly a soil-borne fungus whichable to invade root tissue and colonizes the xylem **(Sabet *et al.* 1970).** Breeding of resistant varieties of maize is the most effective method for controlling this disease **(El-Shafey *et al.* 1988).** In recent years, using pesticide for controlling plant disease resulted in many environmental hazards. Many attempts were made to control the pathogen using chemical and biological methods **(El-Mehalowy *et al.* 2004; Ashour *et al.* 2013; El-Moghazy *et al.*, 2017 and Elshahawy and El-Sayed, 2018).** Some tested fungicides worked well in pots but failed in field experiments. Therefore, many researchers are trying to find an alternative method for pesticides as inorganic nanoparticles. Nanotechnology is characterized by the formation of particles with variable sizes, shapes, chemical compositions, depending on their applications. Although chemical and physical methods may produce pure and well-defined nanoparticles, these methods are just costly and critical to the habitat **(Reddy *et al.*, 2012).**

Green synthesis of nanoparticles with the help of plants as reducing agents is considered an efficient, cost effective, fast and eco-friendly in manner **(Yugandhar and Savithramma, 2015a).** In recent past, most of the scientists adopted green synthesis methods for the production of narrow-ranged particles, like calcium **(Yugandhar and Savithramma, 2013),** copper **(Shende *et al.* 2015),** gold **(Gopinath *et al.* 2014),** iron **(Naseem and Farrukh, 2015),** silica **(Athinaranan *et al.* 2015)**, silver **(Yugandhar and Savithramma, 2016)**, and zinc **(Bala *et al.* 2015)** from different medicinal plants including *Nerium oleander* among them, silica nanoparticles (SiNPs) were recognized as important in the fields of chemistry, physics and plant disease control due to their distinctive properties.

Present study aims to investigate the antifungal activity of green synthesized silica nanoparticles with different sizes against *Harpophora maydis*, the causal agent of maize late wilt disease under *in vitro* and *invivo* conditions.

MATERIAL AND METHODS

Synthesis of silica nanoparticles

Synthesis of silica nanoparticles (SiNPs) was achieved with slight modifications of **Adam *et al*. (2011)** protocol. The well-ground (20 g) of *Nerium oleander* plant leaf powder was subjected to acid treatment by mixing it with 500 ml of 1 M HNO_3 in a 1000-ml Erlenmeyer conical flask and stirred for 24 h. this step was done before SiNPs synthesis to purify the reaction mixture from plant impurities **(Yugandhar *et al*. 2015a)**.Mixture was centrifuged at 14.000 rpm for 20 minutes, yielded raw silicon dioxide (SiO_2)in the form of a pellet at the bottom of centrifuge tubes. Filtrate part was discarded and SiO_2 pellet was collected and washed several times with distilled water the pH up to (4.0–5.0). The solution was dried in an oven between 100 and 110 $^{\circ}$C for 12 h. The evaporated turbid solution was stirred with 500 ml of 1 M (NaOH) solution up to 24 h with a magnetic stirrer to reach the pH up to 12 to form sodium silicate. The obtained reaction mixture was separated with a suction pump and titrated with 3 M (HNO_3) until the pH was attained up to 8.5–9.0 to get the pure form of SiO_2, sodium nitrate and water molecules **(Yugandhar and Savithramma, 2015 b; Yugandhar *et al*., 2015).** When 3M (HNO_3) solutions was added drop wise to the purified plant mixture, color pattern of the mixture was gradually changed from brown to whitish precipitate. The contents were centrifuged at 5000 RPM for 10 min to separate biological admixtures. The contents were washed 3 to 4 times with distilled water and dried in a hot air oven for 12 h at 80°C. The preliminary indication of (SiNPs) formation can be confirmed by its color change from brown to whitish precipitate. The obtained powder was well-ground with a mortar and pestle, and was utilized for characterization and antimicrobial studies.

Characterization and optimization of SiNPs

Ultraviolet–visible (UV–Vis) spectroscopic characterization of the obtained nanoparticles before dryingwas analyzed by using UV–Vis Spectrophotometer (Shimadzu UV-1800) at wavelengths ranged in the 200–800 nm compared with negative control (plant filtrate). Dynamic light scattering (DLS) was carried out by using a Malvern Zetasizer Nano ZS 90 (Worcestershire, UK) to determine SiNPs sizes. Microstructures were recorded on a MSAL-XD2 X-ray di-ractometer (XRD, Bruker, Karlsruhe, Germany) employing Cu target in the 2q range from 0^{o} to 80

°(40 kV, 30 mA,ג= 1.540513A). Transmission electron microscopic (TEM) analysis of nanoparticles was performed by using JEOL JEM-2010 (USA) with high-resolution transmission electron microscope operated between 80 and 200kV accelerating voltages.

Optimization of silica nanoparticles sizes

Inan attempt to produce better size controlled silica nanoparticles, the effect of the pH reaction was studied by varying it at a time, keeping the other experimental conditions the same. In all, the reactions, the concentrations of silica nanoparticles and the plant extract were set at 1 mM and 10 g (wet weight) of plant extract/100 ml. The mixtures were incubated at different pH values (5, 7, 9 and 11) for various time periods for one month with respect to SiNPs stability. Simply by varying the pH value of the reaction system, size of the nanoparticles could be turned. When the reaction is completed, products were collected and thoroughly washed for several times with ethanol to obtain pure SiNPs without any by-products and finally subjected to dry vacuum at 80 °C for 3 h. optimum reaction parameters were then selected by measuring the absorbance of resulting solutions spectrophotometric ally using a UV–visible spectrophotometer (Shimadzu UV- 1800) at wavelengths ranged in the 200–800 nm. For each condition, respective controls were maintained. The hydrodynamic diameter of the formed SiNPs were measured by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS 90 (Worcestershire, UK).

***In vitro* effect of nanoparticles on *Harpophora maydis* radial growth**

A laboratory experiment was carried out to screening the inhibitory effect of SiNPs against a highly virulent *Harpophora maydis* (No.9) liner growth. In vitro assay was performed on Potato dextrose agar (**Bilgrami &Verma, 1981).** The tested SiNPs sizes 20, 40.2, 70.2 and 95.5 nm at different concentrations (0.5, 2.5, 5 and 10 ppm) were poured into growth media prior to pouring in a Petri dish (9cm in diameter). Three replicates were used for each concentration. Five mm indiameter agar plugs were obtained from the actively growing *Harpophora maydis* (7 old day cultures) inoculated in the center of plates supplemented with different treatments. The plates were incubated at 28°C for 9 days. Colony diameters were measured every 72h until full growth in control. Control plates inoculated by *Harpophora maydis* in growth medium without SiNPs. The percentage of inhibition zones were measured compared with control using the following formula: Inhibition rate (%)= $(R - r)/R \times 100$

Where R is radial growth of fungi in control and r is the radial growth of fungi in treated plates.

In vivo effect* of nanoparticles on *Harpophora maydis

-Seed coating technique

Seed coating was done following the method described by **Bardin *et al.* (2004).** Seeds were soaked for 15min. in 1% methyl cellulose (MC) solution at the rate of 3ml per 100 seeds. Thereafter, seeds were removed and placed in plastic bags containing silica nanoparticles with four different sizes (20, 40.2, 70.2, and 95.5nm) at the rate of 5 ml per 100 seeds from different four concentrations 0.5, 2.5, 5, and 10 ppm of each SiNPs. Bags were inflated with air and shaken vigorously. Thereafter, seeds were directly planted in the infested-potted soil. Seed coated with sterile distilled water only acted as the control.

-Inoculum preparation

Substrate for fungal growth was prepared in 500 ml glass bottles each contained 100 g of sorghum grains, 50 g of washed and 90 ml of tap water. Bottles were autoclaved at 121ºC for 30 min then cooled down. The fungus propagules of aggressive *Harpophora maydis* isolate No. 9 obtained from culture type collection of Maize and Sugar Crops Dis. Dept., Plant Pathol. Res. Inst., ARC, taken from 7 day-old culture grown on PDAY medium, were aseptically transferred into each bottle and allowed to colonize on sorghum medium for 2-3 weeks at 27 ± 2ºC until sufficient growth of the fungus was obtained **(El-Shafey *et al.*, 1979).** The incubated bottles were shack every three days to ensure uniform of fungal growth. The content of the bottles of was poured out and mixed to get homogenized, and then inoculum was used for soil infestation.

-Soil infestation

Soil infestation was carried out according to **(Samra *et al.*, 1966)** as follows: Batches of autoclaved clay loam soil were infested with inoculum of *Harpophora maydis* with soil at the rate of 30 g/kg soiland mixed thoroughly. Infested soil was dispensed into pottery pots (25-cm-diameter) sterilized by 0.4 % formaldehyde. Maize grains (cv. Boushy) were surface sterilized in 5% sodium hypochlorite solution for 3 min. then washed in sterilized distilled water for 5 min. and air dried before sowing. Ten grains were sowed in each pot. Four pots were used for each treatment as replicates. On the other hand, autoclaved sterilized sorghum grains, mixed thoroughly with soil at the rate of 30 g/kg soil and kept as control (check) treatment. Pots were regularly watered every other day for a week before sowing.

Field experiment

Silica nanoparticles with their four varied sizes at concentration (0.5, 2.5, 5 and 10 ppm)were studied under field conditions in disease nursery at Giza and Gemmeza Research Stations, ARC, during summer of 2019-2020 growing seasons. Seed coating treatment, were used. Maize seeds cv. Boushy, susceptible to *Harpophora maydis*, was used in this

study. Untreated seeds were used as check treatment. Four replicate plots, 20 maize plants were used in each plot.

Disease assessment

Disease incidence as percentage of infection was recorded 90 day after sowing according to **Sabet *et al*. (1966a)**.

Statistical analysis

Data were subjected to statistical analysis ofvariance (ANOVA) test. A complete randomize design was applied and Duncan's multiple rangetests were used for comparing means **(Gomez and Gomez, 1984).**

RESULTS

Synthesis and optimization of silica nanoparticles

In this regard and before silica nanoparticles (SiNPs) synthesis, plant material was subjected to acid treatment by using 1M (HNO_3) in a way to purify the reaction mixture from plant impurities. Finally, the preliminary indication for the formation of (SiNPs) can be confirmed by its color change from brown to whitish precipitate as mention before in material and methods. Solutions after titration with 3 M (HNO_3) were analyzed with the help of UV–Vis spectrophotometer, which displayed a broad peak at 350nm (Fig. 1) and (Fig.2A) due to the surface plasmon resonance nature of SiNPs in the reaction solution. The obtained nanoparticles absorb light at different wave lengths and are excited to give a broad peak.

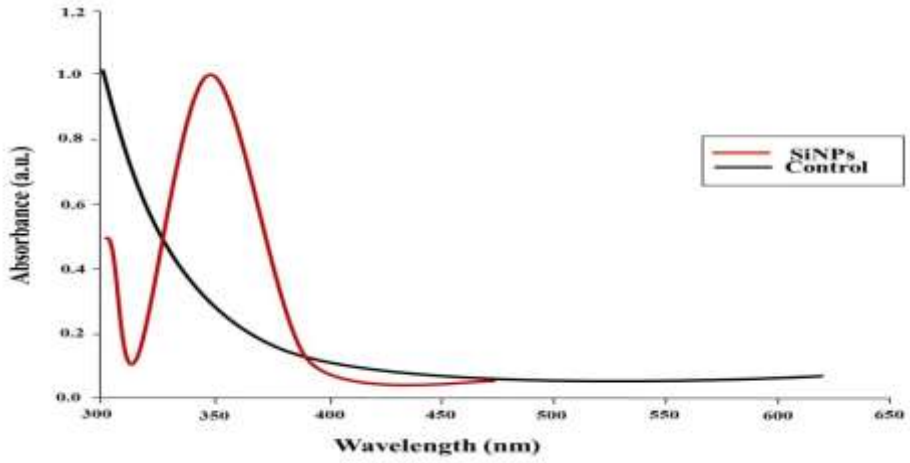

Fig. (1): Plant synthesis of the formed silica nanoparticles: a UV– Vis spectra of the formed SiNPs in comparison with the plant filtrate (negative control).

The Morphology of the biosynthesized SiNPs were examined using HR-TEM (Fig.2B). Measurements indicated the formation of poly-dispersed spherical shaped SiNPs with 50 nm ± 5.5 average sizes and -

21.5 mV value of zeta potential as indicated by DLS analysis (Fig.2C), which indicated ideal surface charge of the formed SiNPs, which prevent the agglomeration and generate a strong repulsive force among the particles that increase their stability. Also, crystallographic studies of the synthesized SiNPs with XRD instrument displayed a broad and high intensity peak at 22◦ of 2θ values of x-axis corresponding to the amorphous nature of SiNPs which is correlated with JCPDS file No. 01-0787 of the Joint Committee on Powder Diffraction Standards (Fig.3).

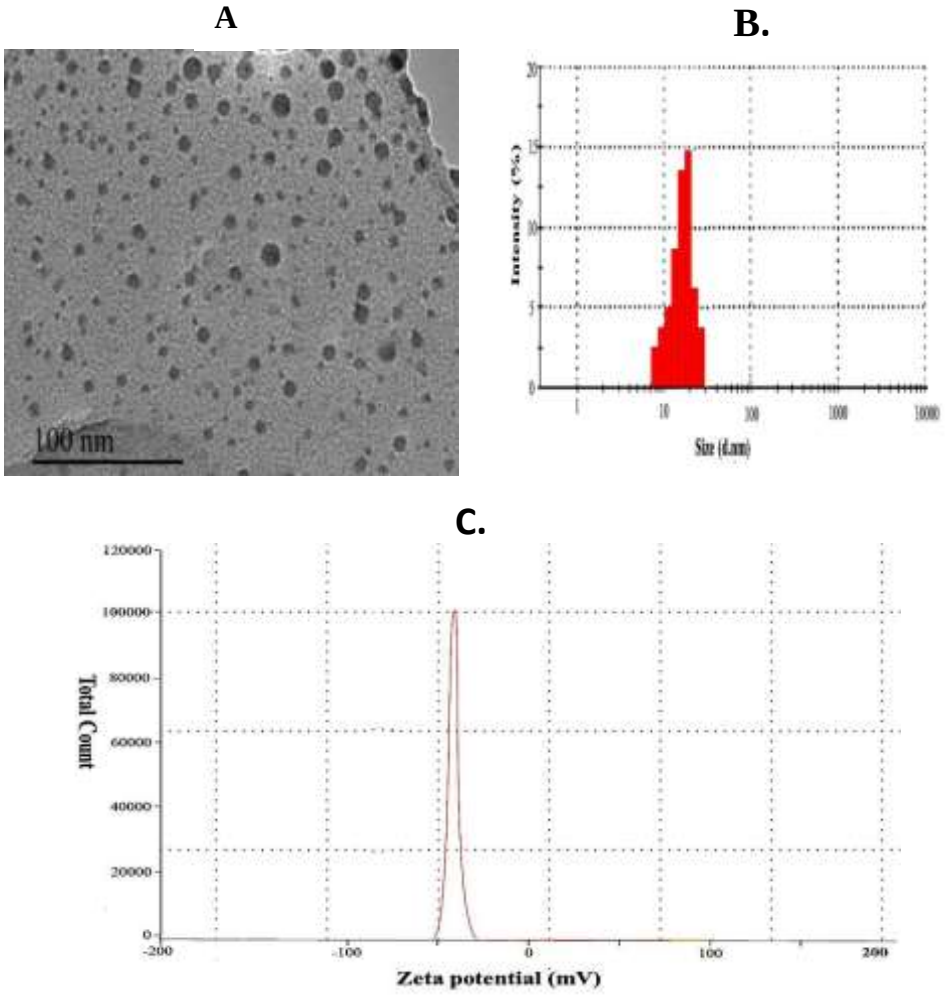

Fig.(2): Characterization of the formed silica nanoparticles:(A.) TEM image of the formed SiNPs, (B) & (C) the particle and the zeta potential of the formed SiNPs respectively.

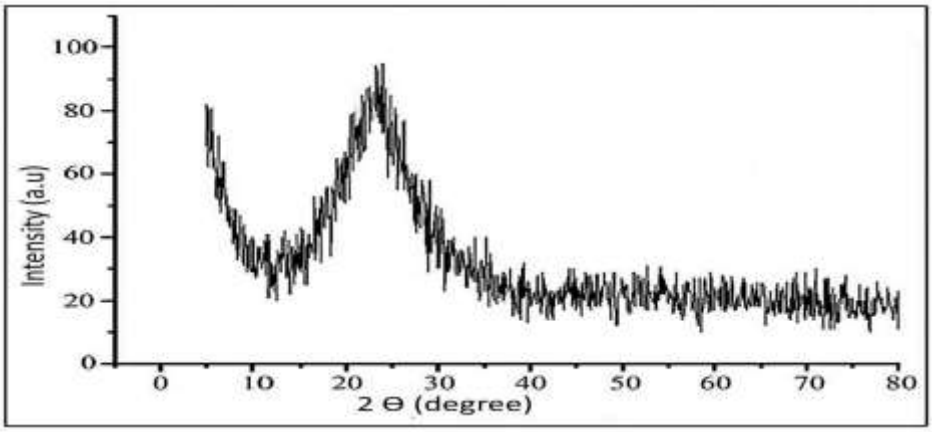

Figure (3): X-ray Diffraction patterns of the formed SiNPs.

The absence of any other XRD peak indicating that the synthesized nanoparticles were pure crystalline in nature. On the other hand, in a way to obtain a smaller SiNPs size, the reaction medium of SiNPs was changed over varied degrees of pH (5, 7, 9, 11). The color of the reaction mixture and the intensity of the absorbance peaks were pH dependent. Where, the results showed that SiNPs synthesized at pH of 5, 7, 9 and 11 presented absorption peaks at 300, 350, 360, and 380 nm respectively.

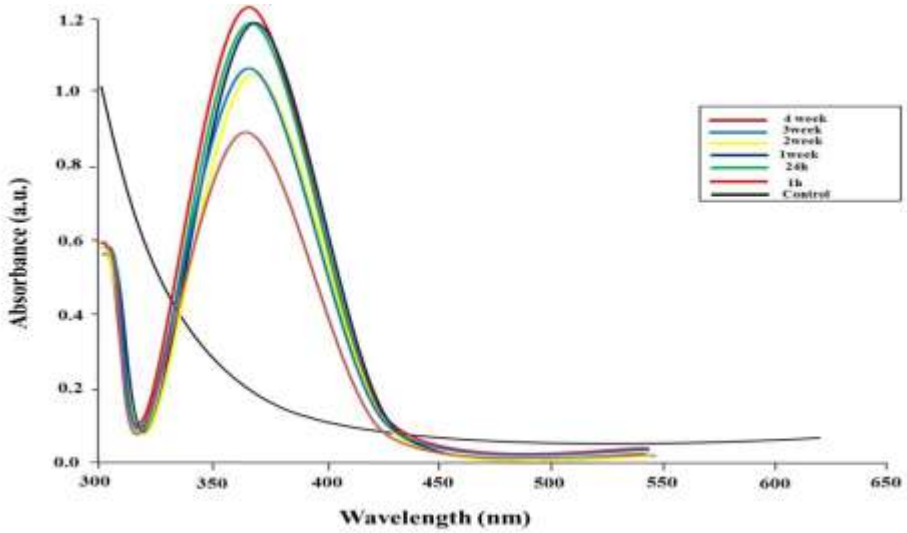

Figure (4): Plant mediated silica nanoparticles: a UV– vis spectra of the formed SiNPs (20 nm) at different times as indicated during the synthesis and after 4weeks of storage.

The absorption peaks shifted to shorter wavelength and became narrower at alkaline pH values, possibly due to the decreased size or anisotropy degree of SiNPs. DLS measurements were carried out to observe the size of the produced SiNPs under pH values. The results indicated that the smallest SiNPs produced were at pH=11 with 20 nm in size. Particles obtained at pH= 9, 7 were larger size, (40.2, 70.5 nm) respectively. At acidic condition (pH=5), the particle size was 95.5 nm and cannot observe any characteristic absorbance band for SiNPs formation at better size. Those results indicated that SiNPs were produced in more small size at alkaline medium. Most importantly, the UV spectra results showed that the produced silica nanoparticles (20 nm) have a high stability over four weeks this was indicated by the absence of red shifting in UV absorbance analysis over a course of time as indicated in Fig. 4.

***In vitro* effect of nanoparticles on *Harpophoramaydis* radial growth**

The inhibitory effect of SiNPs were evaluated against the linear growth of the highly virulent *Harpophora maydis* isolate (No. 9) using the culture technique on PDAY medium.

Table 1. Effect of silica nanparticals on *Harpophora maydis* radial growth

Treatments		Radial growth (cm) 8 days	Reduction
SiNPs sizes	**Concentration ppm**		
SiNP- 95.5 nm	**0.5**	**3.8**	**55.2**
	2.5	**1.5**	**82.3**
	5	**1.1**	**87.0**
	10	**0.5**	**94.1**
	mean	**1.73**	**79.5**
SiNP-70.2 nm	**0.5**	**2.5**	**70.5**
	2.5	**0.6**	**92.9**
	5	**0.5**	**94.1**
	10	**0.5**	**94.1**
	mean	**1.03**	**88**
SiNP-40.2 nm	**0.5**	**0.7**	**91.7**
	2.5	**0.7**	**91.7**
	5	**0.6**	**92.9**
	10	**0.6**	**92.9**
	mean	**0.65**	**92.5**
SiNP- 20 nm	**0.5**	**0.6**	**92.9**
	2.5	**0.5**	**94.1**
	5	**0.5**	**94.1**
	10	**0.5**	**94.1**
	mean	**0.53**	**93.8**
Control		**8.5**	
L.S.D. 0.05		**1.519**	

Results in Table (1) reveal that all tested nanoparticles, SiNP-20, SiNP-40.2, SiNP-70.2 and SiNP-95.5 nm at the different tested concentration (0.5, 2.5, 5 and 10 ppm) significantly inhibited radial growth of *Harpophora maydis* on PDA compared to control. Generally, growth inhibition (%) was increased with the increasing of the

concentrations of all substances. However, the highest effect was recorded beginning from 2.5 ppm concentration being 94.1, 92.9, 91.7 and 82.3% reduction of SiNP-20 nm, SiNP-70.2 nm, SiNP-40.2 nm and SiNP-95.5 nm, respectively. It was observed that on average, SiNP-20 nm (93.8%) and SiNP-40.2 nm (92.5%) where, the most efficient treatment at any concentration followed by treatment SiNP-70.2 nm (88%) and SiNP-95.5 nm achieved 79.5% reduction.

In vivo effect of SiNPs nanoparticles on maize late wilt:

Pot experiment was carried out to study the effect of seed coated SiNPs treatments at the tested four concentrations (0.5, 2.5, 5 and 10 ppm) against *Harpophora maydis* the casual organism of maize late wilt. Results in Table (2) show that, all seed treatments with SiNPs significantly reduced the infection %of late wilt compared with check treatment (70.8%) under artificial infestation.

Table. 2 Effect of SiNPs treatments on maize late wilt under greenhouse conditions, Giza, 2019.

Treatment		Ger%	Infection (%)	Reduction (%)
SiNPs sizes	Concentration			
SiNP- 95.5 nm	0.5	85	33.3	52.9
	2.5	85	27.8	60.7
	5	85	25.0	64.6
	10	81	20.1	71.6
SiNP- 70.2 nm	0.5	87.5	27.8	60.7
	2.5	87.5	19.4	72.5
	5	85	17.0	75.9
	10	85	14.2	79.9
SiNP- 40.2 nm	0.5	90	19.4	72.5
	2.5	90	16.7	76.4
	5	89	11.8	83.3
	10	86	8.7	87.7
SiNP- 20 nm	0.5	90	16.7	76.4
	2.5	90	11.1	84.3
	5	90	8.7	87.7
	10	88	8.3	88.2
Control		85	70.8	-
L.S.D.			10.952	

The most efficient treatments in reducing disease incidence were SiNP-20 and SiNP- 40.2 nm at 10 and 5ppm concentrations gives (76.4%, 72.5% reduction at 5 ppm, respectively) and at 10 ppm (88.2, 87.7% reduction, respectively) followed by SiNP-70.2 nm (79.9%). The lowest treatment was SiNP-95.5 nm ranged between 52.9-71.6% reductions. Germination percentages were not negatively affected and sometimes slightly enhanced.

Field experiments:

The influence of seed coating with four SiNPson maize late wilt and growth parameters was studied in disease nursery of *Harpophora maydis* at Giza and Gemmeza Research Stations under field conditions. Untreated plants were used as check. Data presented in Table (3) reveal

that seed treatment with SiNPs significantly reduced maize infection with late wilt compared with check plants (78.3% infection at Giza 2019 and 81.7% at Gemmeza 2020). It was observed that disease incidence was higher in Gemmeza location than Giza. There were significant differences between treatments in yield average of the two seasons. The disease reduction and yield were increase with increasing concentrations. The SiNP-20, 40.2 nm treatments were the most efficient in decreasing disease incidence and enhancing yield where infection percentage recorded 6.7% at 10 ppm in first season resulted from both treatments and 6.7% and 8.3% in second season in SiNP-20nm and SiNP-40.2nm, respectively. On the other hand, average yield were 29 (ard/fd) in SiNP-20 nm and 27.3 (ard/fd) in case of SiNP-40.2 nm. In contrary, the lowest treatments were those at concentration 0.5 ppm for treatments SiNPs 95.5nm, 70.2 nm where infection was 34% and average yield 17.6 (ard/fd) for SiNP-95.5 nm and it was 26.3% infection which yielded 18 (ard/fd) in SiNP-70.2nm.

Table. 3 Effect of SiNPs on incidence of maize late wilt and yield under field conditions at disease nursery, Giza and Gemmeza, 2019-2020 growing seasons.

Treatment		Giza Seasons 2019		GemmezaSeasons 2020		Yield* (ard/fd)
SiNPs sizes	Conc.	Infection%	Redaction %	Infection%	Redaction %	
SiNP- 95.5 nm	0.5	26.7	65.9	34	58.3	17.6
	2.5	18.3	76.6	24	70.6	20
	5	15.0	80.8	20	75.5	20.2
	10	13.3	83.0	18	77.9	21.7
SiNP- 70.2 nm	0.5	23.3	70.2	26.3	67.8	18
	2.5	16.7	78.6	21.7	73.4	18
	5	15.0	80.8	18.3	77.6	23.0
	10	11.7	85.0	13.3	83.7	26.0
SiNP- 40.2 nm	0.5	20.0	74.4	23.3	71.4	19
	2.5	13.3	83.0	11.9	85.4	23
	5	10.0	87.2	11.7	85.6	25.6
	10	6.7	91.4	8.3	89.8	27.3
SiNP- 20 nm	0.5	18.3	76.6	18.2	77.7	19.7
	2.5	10.0	87.2	11.7	85.6	23.7
	5	8.3	89.3	10.0	87.7	26
	10	6.7	91.4	6.7	91.7	29
Control		78.3		81.7		14
L.S.D.		9.588		7.321		1.571

***: Grain yield per feddan (GYPF), in ardab by adjusting grain yield / plot to grain yield per feddan(adjusted at 15.5% grain moisture).**

DISCUSSION

Late wilt disease of maize caused by *Harpophora maydis* is one of the most important diseases on maize in Egypt. Breeding of resistant varieties of maize is the most effective method for controlling this disease **(El-Shafey *et al.*, 1988)**. Many attempts were made to control the disease using chemical and biological methods **(Abdel-Hamid *et al.*,**

1981; Singh and Siradhana, 1989; El-Mehalowy *et al.*, 2004 and Ashour *et al.*, 2013). In recent years, using pesticide for controlling plant disease resulted in many environmental hazards. Therefore, this work demonstrates the use of safe management method that pose less danger to humans and animals also controlling the disease. In this study, maize seeds coated with silica nanoparticales (20, 40.2, 70.2 and 95.5 nm) were used *in vivo* and *in vitro*to control late wilt disease. All tested SiNPs *in vitro* inhibited the fungal radial growth at different tested concentrations resulted in significant reduction of *Harpophora maydis* on PDA compared to check treatment. Reduction (%) increased with increasing of SiNPs concentrations. The reduction in SiNPs sizes 20, 40.2 nm were higher at any concentration ranged between 94- 92% followed by SiNP-70.2 nm then SiNP-95.5 nm. The results were in harmony with those obtained by **Suriyaprabha *et al.*(2014)** who stated that maize nanosilica-treated plants showed a higher expression of phenolic compounds and a lower expression of stress-responsive enzymes against fungal infection. Maize expressed more resistance to *Aspergillus* spp., than *Fusarium* spp. These results showed significant higher resistance in maize treated with silica nanoparticales than with bulk silicon. Hence, silica nanoparticles can be used as an alternative potent antifungal agent against phytopathogens. **El-Gazzar and Rabie (2018)** reported that using AgNPs against *Harpophora maydis* alone was efficient more when combined with chemical fungicides MaximXI and Vitavax reduced fungal growth to 51%. The mechanisms responsible for inhibiting of fungal growth by silicon are not well understood. In spite of that, some hypotheses were made by some investigators to illustrate the mode of action of Si in this respect. **Bi *et al,* (2006)** stated that Si resulted in a collapse and shrinkage of fungal hyphae and spores, which consequently causing the loose of fungal sporulation. **Li *et al,* (2009)** observed that ultrastrucural alterations were happened by using transmission electron microscopy, including thickening of the hyphal cell walls. Meanwhile, the inhibitory effect of nanoparticles may be due to release of extracellular enzymes and metabolities **(Perez-de-Luque, and Rubiales, 2009)** also some studies proposed that nanoparticles may cause structural changes of microbial cell membrane, causing cytoplasm leakage and eventually the cells **(Sawai and Yoshikawa, 2004; Brayner *et al.*,2006).**

Nanoscience and technology are enabling the development of a wide range of materials for plant growth enhancement **(Nair *et al.*, 2010).** Nano-materials such as titanium and alumina penetrate the plants, thereby improving or decreasing their growth characteristics **(Carmen *et al.*, 2003; Yang and Watts, 2005)**. Silica (SiO2) is an essential element for monocotyledon plants and it is known to confer biotic and abiotic

stress tolerance **(Rains *et al.*, 2006 and Epstein, 2001).** The present study raveled that, *in vivo* results supported the *in vitro* ones that all SiNPs treatments, whether in the greenhouse or in field significantly reduced the disease infection when compared to the control. Efficiency in decreasing the disease incidence was increased by increasing the SiNPs concentrations. Also the SiNPs enhanced the germination (%) than check treatment. Similar results were found by **Suriyaprabha *et al.*(2012)** they reported that maize treated with nanosilica showed the highest germination (98.5%) while sodium silicate treatment gave 92.5% germination (bulk silicon). They suggested that immediate uptake of SiNPs through seeds and its role in biochemical induction. Current results also showed that, under greenhouse conditions the SiNPs-20, 40.2 nm were the most significant treatments in reducing disease incidence followed by SiNPs70.2, 95.5nm. The same trend was obtained in the field. In previous study on bulk silicon, El-Shabrawy*et al.*(2014) reported that using sodium silicate as an eco-friendly compound, in managing stalk-rot disease complex of maize thatreduced significantly the linear growth of *Cephalosporium maydis, Rhizoctonia solani, Fusarium verticillioides* and *Sclerotium rolfsii* in Si amended-PDA medium. Sodium silicate completely suppressed the tested fungi at concentration 3.0 %. Using sodium silicate as seed coating, seed soaking or soil treatment, managed significantly late-wilt infection in greenhouse and stalk-rot disease complex of maize in field trials of sick plots with efficiency reached 76.9% under field conditions. In contrary in the present investigation, reduction in *H.maydis* by SiNPs achieved 88.2% under greenhouse and 91.7 % under field conditions. Moreover, average yield of the two seasons were 29 (ard/fd) in SiNP-20nm the highest efficient treatment and while it was 17.6 (ard/fd) in case of the lowest ones SiNP-95.5 nm when compared to control treatment yielded 14 (ard/fd). These findings are in agreement with **(Yuvakkumar *et al.*,2011)** who reported that SiNPs were important for maize growth enhancement with respect to morphological parameters. However, it was essential to soil microbial biomass and silica during maize cultivation. Their results suggested the use of nanofertilizers in maize crop to enhance yield due to its cost-effectiveness. In addition, the effect of silica sources on the changes in the microbial biomass (C and N) components in soil as well as the rhizosphere should also be considered. Soil beneficial microorganisms such as nitrogen fixers and phosphate-solubilizing bacteria (PSB) are potent plant growth promoters. On the other hand, silica nanoparticlescould be easily synthesized with a controlled size, shape, and structure, making them highly advantageous delivery vehicles **(Mody *et al.*, 2014).** They are commonly produced in a spherical shape with pore-like holes; for example, porous hollow silica

nanoparticles (PHSNs) or mesoporous silica nanoparticles (MSNs). PHSN and MSN commonly load the pesticide into the inner core to protect the active molecules and, therefore, provide a sustained release. The shell structure of PHSNs protects the active molecules inside the nanoparticles against degradation by UV light. The available literatures suggest that silicon has already been used to enhance plant tolerance against various abiotic and biotic stresses and, therefore, silica nanoparticles seem to be the natural choice for the development of agri-products against pests **(Barik *et al.*, 2008).** Numbers of studies have shown that SiNPs may directly interact with plants and help in improving plant growth and yield **(Strout *et al.*, 2013 and Suriyaprabha, 2014).** In this regard, silica nanoparticles were observed to form a binary film at the epidermal cell wall after absorption, which may add structural color to plants **(Strout *et al.*, 2013).** The impact was not limited to coloring; SiNPs were also speculated to act as a strengthening material that may act as an agent to prevent fungal, bacterial, and nematodes infections and thus, may increase disease resistance.

In conclusion, the efficiency of green synthesis SiNPs in reducing late wilt disease incidence in maize was higher. All tested treatments showed similar trends in both *vitro* and *vivo* studies. These results suggested the possibility of using SiNPs to control the disease and enhancing maize yield productivity. Moreover, SiNPs may be considered as a green method of controlling plant diseases as an environmentally safe substitute of the synthetic fungicide.

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استخدام النانوسيليكا فى مكافحة مرض الذبول المتأخر فى الذرة الشامية المتسبب عن *Harpophora maydis*

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تم استخدام جزيئات النانوسيليكا المخلقة نباتيا والمحسنة بالتحضين تحت ارقام مختلفه من الحموضة pH 5،7،9 و11 لانتاج الاحجام 20،40.2،70.2 و95.5 النانومترية لتقييمها فى مكافحة الفطر *Harpophora maydis* المسبب لمرض الذبول المتأخر فى الذرة الشامية تحت ظروف المعمل، الصوبة والحقل. باستخدام بيئة الدكستروز اجار، اظهرت النتائج ان جميع احجام النانوسيليكا تحت الاربع تركيزات المستخدمه خفضت معنويا النمو الخطى لفطر

H. maydis وانخفض النمو اكثر بزيادة التركيزبالمقارنه بالكنترول. وكانت نانوسيليكا-20 نانوميتر افضل المعاملات تبعها نانوسيليكا-42.2 نانوميتر. اظهرت نتائج تجربة الصوبة ان التقاوى المعاملة بالنانوسيليكا خفضت معنويا نسبة الاصابة بالذبول المتاخر وحسنت من درجة الانبات بالمقارنة بنسبة الاصابة فى الكنترول 70.8%. وكانت المعاملتين نانوسيليكا-20 نانوميتروالنانوسيليكا-40.2 نانوميتر اكثر المعاملات خفضا لدرجة الاصابة (88.2%،87.7% على الترتيب) تحت تركيز ppm10. بينما كانت المعامله نانوسيليكا-95.5نانوميتر اقل المعاملات تاثيرا حيث كانت نسبة خفض الاصابة 52.9%. اظهرت تجارب الحقل المنفذه فى حقول العدوى بالذبول بمحطتى الجيزه والجميزة مواسم 2019-2020 ان التقاوى المعاملة بجزيئات النانوسيليكا اظهرت خفضا معنويا فى درجة الاصابة بالذبول المتأخر بالمقارنة بالكنترول 78.3% اصابة بحقل الجيزة 2019 و81.7% بحقل الجميزة 2020. ايضا كما كانت هناك اختلافات فى متوسط المحصول لموسمى الزراعة باختلاف المعاملة حيث زاد المحصول بارتفاع التركيز. وكانت المعاملات نانوسيليكا- 20 نانوميتر ونانوسيليكا-40.2 نانوميتر من افضل المعاملات فى خفض الاصابة وزيادة المحصول، حيث كانت نسبة الاصابة فى المعاملتيين 6.7 % فى الموسم الاول بالجيزة عن تركيز ppm10 وكانت فى الموسم الثانى بالجميزة 6.7% نانوسيليكا- 20 نانوميتر و8.3% فى نانوسيليكا-40.2 نانوميتر فى الموسم الثانى بالجميزه. من ناحية اخرى كان متوسط المحصول 29 اردب/فدان فى معاملة نانوسيليكا-20 نانوميتر و27.3 اردب/فدان فى نانوسيليكا -40.2 نانوميتر. فى المقابل كانت اقل المعاملات هى نانوسيليكا-95.5 نانوميتر ونانوسيليك ا- 70.2 نانوميتر حيث كانت الاصابة 34% ومحصول 17.6 اردب/فدان بالمعاملة حجم (95.5 نانوميتر) و بينما كانت نسبة الاصابة بالمعاملة حجم (70.2 نانوميتر) 26.3% ومحصول 18 اردب /فدان فى حقل الجميزة. من النتائج المتحصل عليها يمكن استخلاص ان استخدام جزيئات النانوسيليكا المخلقه نباتيا والامنه للبيئة ومنخفضة التكلفةفى الاحجام الصغيرة 20 و 40.2 نانوميتر عن الاحجام الكبيرة، كانت فعالة فى مكافحة مرض الذبول المتأخر ورفع انتاجية محصول الذرة.