Study the efficiency of Silver nanoparticles (AgNPs) and Uniform fungicide against *Alternaria solani* and *Fusarium oxysporum* and their effects on vegetative growth, chlorophyll and yield of tomato plants.

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ABSTRACT: Tomato (*Solanum lycopersicum* L.) is important member of the Solanaceae family and one of the major vegetable crops grown in Egypt. There are many diseases affected on tomato plants during growing such as early blight and wilt were caused by *A. solani* and *F. oxysporum,* respectively. Uniform fungicide and AgNPs were tested for their inhibitory activities on mycelial growth inhibition with different concentrations *in vitro* against *A. solani* and *F. oxysporum*. The data showed that the AgNPs and Uniform exhibited higher antifungal activity against *A. solani* than *F. oxysporum*. Data revealed to the increase in concentrations of AgNPs and Uniform fungicide were correlated with the increase rate of the inhibitory mycelial growth and inhibition %. Treatment with AgNPs and Uniform fungicide gave the highest decrease post-emergence damping off % and increase survived seedling % when compared with control. Also, their side effects on vegetative growth and chlorophyll of tomato plants were studied. The results were shown AgNPs and Uniform fungicide on tomato plants significantly increased plant height, plant fresh weight, dry weight, leaf area and total chlorophyll content when compared to non-treated plants in both seasons. The maximum significant increase in yield characters, fruits No. plant and fruits yield plant was obtained by AgNPs followed by Uniform when comparing with control.

Keywords: Tomato, AgNPs, Uniform, *A. solani*, *F. oxysporum*, vegetative growth, chlorophyll, yield characters.

1.INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of main vegetable crops in Egypt. Tomato is cultivated on about 199712 faddan with total production as 7943285 tones **(FAOSTAT, 2016)**. Tomato was used as a fresh vegetable besides it has a particular importance a raw material of agricultural industry. Tomato is a rich source of vitamins, minerals and lycopene and shown to be highly effective against some cancer cells **(Helyes *et al*., 2008)**. Also, it is benefiting to human health which contains high phytonutrients such as lycopene, ascorbic acid, phenolic compounds, β-carotene, ascorbic acid, and essential nutrients **(Garcia-Closas *et al*., 2014).**

The fungi are complex organisms with vegetative growth (mycelia) and produced various spores *Alternaria solani* (*A. solani*) is known also as Alternaria leaf spot or target spot which caused early blight in tomato plants. On lower leaves are appearing brown to black spots, with dark edges. When a few spots are present the leaves turn yellow and dry up. *A. solani* can infect plants at any stage during the growing season but rapidly infection after plants have set fruit. The factors of rapid spread of early blight are warm and wet weather **(Gleason and Edmunds 2006)**. *Fusarium oxysporum* (*F. oxysporum*) cause wilt in tomato cultivars, plants were infected by fungi show leaf yellowing and wilting from the base of the stem. Initially, plant will effect on one side of a leaf, one branch, or one side of a plant. The symptoms will be spread to all plant **(Gleason and Edmunds 2006)**.

Fungal diseases are controlled by foliar sprayed with fungicides **(Anonymous, 1990)** using a standard interval between sprays or based on environmental conditions to spray infected plants **(Pitblado, 1992)**. Many cases were increased tomato yields with used of fungicides reported by **(Zitter and Kodis 1987; MacNab and Gardner 1991; Shoemaker, 1992)**. The common method of seeds treatment is using fungicides before planting to protect tomato seeds and other plants seeds from *F. oxysporum* infection **(Elamawi and Al-Harbi 2014)**. The fungicides applications were shown effects on other living organisms including harmful and useful soil microorganisms **(Khalifa *et al*., 1995; Lewis *et al*., 1996)**.

Synthetic fungicides are used to control diseases of agricultural plants. It including; Ridomil gold plus, Carbendazim, Copper oxychloride, Mancozeb, Benomyl and Captafol today used to control early blight (A. solani) in tomato **(Chohan et al., 2015; Saharan et al., 2015)**. Environmental pollution is caused by used excessive fungicides, which lead to health problems **(Kim et al., 2009)**. Also, pathogens are acquiring fungicides resistance **(Namanda et al., 2004; Kirk et al., 2005)**. The pathogen populations are developing resistance to one fungicide may also become resistant to relate fungicides. Therefore, researches in the field are searching for alternative eco-friendly compounds and less hazer for controlling plant diseases and crop protection.

Nanoparticles have negative or Positive effects on plants in several reports .Nanoparticles may be act in the same a way for synthetic pesticides. Nanoparticles can use as capsules; carriers of active ingredients of pesticides **(Khan and Rizvi 2014)**. AgNPs can be use as different formulations, shapes and sizes for controlling various plant fungi pathogens when compared to synthetic fungicides **(Abdel-Hafez *et al*., 2016)**. Silver as nanoparticles was used to control microorganisms and the prevented the infections. Many studies reveal to antimicrobial activities of the silver nanoparticles is due to the positive charge reacting with the negatively charged proteins on the cell membranes and contributing to their antimicrobial activities **(Hamouda *et al*., 2000 and Dragieva *et al*., 1999)**.The antifungal activities of silver nanoparticles were tested against eighteen plant pathogenic fungi including Cladosporium, Alternaria, Corynespora, Botrytis, Cylindrocarpon, Stemphylium, Pythium and Fusarium **(Kim *et al*., 2012)**.

The objective of this research was carried out to evaluate the antimicrobial activities of silver nanoparticles and Uniform fungicide for controlling *Alternaria solani* and *Fusarium oxysporum*) and their effects on vegetative growth such as (plant height, plant fresh weight, dry weight, leaf area), chlorophyll and yield (fruits No. plant^{-1}and fruits yield plant^{-1}) of tomato plants.

2.MATERIALS AND METHODS

Fusarium oxysporum (*F. oxysporum*) and *Alternaria solani* (*A. solani*) were brought from Department of Plant Pathology, Agricultural Research Station, Itay El Baroud. These fungi were transferred into Petri-dishes containing 15 ml Potato Dextrose Agar (PDA) containing streptomycin to prevent bacteria growth in media and incubated at 28±1°C for 8 days and observed regularly for fungal growth **(Ganie *et al*., 2013)** until using *in vitro* assay.

2.1.Chemical used:

Fungicide: Uniform (Metalaxyl 28.2% + Mancozeb10.8% SE) - Syngenta.

Silver nanoparticles (AgNPs) – was obtained from Naqaa Foundation for Scientific Research, Technology and Development.

2.2.*In vitro* assay:

Different concentrations i.e., 10, 20, 40, 80, 100, 120, 140, and 160 ppm of AgNPs and Uniform as fungicide at concentrations (50, 100,200, 300, 400, 500, 600, 700, and 800 ppm) were tested against *A. solani* and *F. oxysporium*. The tested compounds were mixed in PDA medium before solidification and pouring into the petri dishes (9cm). All plates were inoculated with 0.5 cm fungal disc were taken from active margin of 7 days old culture of tested fungi. Other unamend plates were inoculated only with tested fungi as control. Three replicates were used for each concentration. All plates were incubated at 25±2ºC. When plates in control were totally colonized by fungus, radial growth of fungal mycelium was recorded. The percentages of mycelia growth inhibition (MGI) were calculated according to **Taisan *et al.*, (2014)** using following formula:

$$\text{MGI \%} = \frac{R-r}{R} \times 100$$

Where:

R is the radial growth of fungal mycelia on the control plate.
r is the radial growth of fungal mycelia on the plate treated.

2.3.Field experiment:

The field experiment was conducted during the two summer growing seasons of 2016 and 2017 in El-Bostan, El-Behaira Governorate; to determine the efficiency of AgNPs and Uniform fungicide on the vegetative growth characters, chlorophyll and yield of tomato plants (*Solanum lycopersicum* L., cv. super strain-B)

The field trial was designed in complete randomized blocks (RCBD) with three replicate plots for each treatment. Each plot was divided into 5 rows of 7 meter length. Four-week-old tomato seedlings were planted with distance 30 cm between seedlings. Each plot contains 120 seedlings. Spray the seedlings with AgNPs at 160 ppm and Uniform at recommended dose (0.5 L/faddan) three times, first time after three days, second time after 14 days and third times after 21 days from planting. Control was planting without any treatment.

2.4.Disease assessments:

The percentages of post-emergence damping off and Survived seedlings were recorded after 30 days of planting and calculated as used by **Ragab *et al.*, (2009)** as the following formulas:

$$\text{Post-emergence \%} = \frac{\text{No.of dead seedlings}}{\text{Total No.of seedlings}} \times 100$$

$$\text{Survived seedlings \%} = \frac{\text{No.of survived seedlings}}{\text{Total No.of seedlings}} \times 100$$

2.5.Vegetative growth characters:

After 50 days from transplanting, three randomly plants were chosen and gently washed with tap water and the vegetative growth traits were taken as follow:

2.5.1. Plant height (cm): The main stem height of the plant was measured in cm, from the starting point of the roots up to the top of the plant.

2.5.2.Plant fresh weight (g): The whole plant (shoot and root) samples were weighed in grams and the average weight per plant was calculated.

2.5.3.Plant dry weight (g): The collected plant samples were washed with tap water to remove the adhered soil particles then washed several times with distilled water. The plant samples were oven dried at 70 Cº in a forced air oven till the weight become constant and weighed.

2.5.4.Leaf area per plant (cm^2): Leaf area per plant was calculated using the weight method as used by **Fayed (1997)**. The leaves from the plant samples (three plants) were cleaned from dust and weighed. Then, twenty random disks were taken from the leaves, using a circular puncher and weighed. The leaf area was calculated using the following formula:

$$\text{Leaf area per plant} = \frac{\text{Leaves fresh weight} \times 20 \times \text{area of disk}}{\text{Fresh weight of 20 disk} \times 3}$$

Where: 20 = number of random disk
3 = number of plant sample

2.5.5.Total chlorophyll content (SPAD value): Leaf chlorophyll content (mg g^{-1} of plant fresh weight) was measured after three days from last spray using nondestructive dual-wavelength chlorophyll meter (SPAD-502, Minolta Corp., Ramsey, NJ, USA). Three measurements were taken per treatment.

2.5.6.Yield of tomato per plant: At the harvest time after 75 days from transplanting, tomato fruits were hand-harvested.

2.5.7.Number of fruits per plant: Fruits number of each plant per treatment was counted to calculate the average number per plant.

2.5.8.Fruits yield per plant (Kg): Total fruits for each plant were weighed in grams.

2.6.Statical Analysis:

All recorded data were statistically analyzed according to the used design by CoStat program **(Version 6.4, CoHort, USA, 1998–2008)**. Least significant difference test (LSD) was applied at 0.05 confidence level to compare means of different treatments using the same program.

3.RERSULTS AND DISCUSSION

3.1.Effect of AgNPs against *A. solani* and *F. oxysoporum:*

The inhibitory effect of AgNPs at different concentrations (10, 20, 40, 80, 100, 120, 140, 160 ppm) compared with control analyzed in vitro was shown in Table (1). The data showed that the AgNPs exhibited higher antifungal activity against *A. solani*. Also data revealed to the increase in concentrations of AgNPs was correlated with the increase rate of the inhibitory effect. *A. solani* was more sensitive to AgNPs than *F. oxysoporum* at all concentrations. The highest inhibition % of AgNPs against *A. solani* and *F. oxysoporum* were found with average 93.33 and 65.56 %, respectively, at 160 pm. Statistical analysis indicated that, there is a significant effect between AgNPs concentrations compared with the control. The efficiency of nanoparticles is due to the penetration of nanoparticles into cell of microbial with lower concentrations of AgNPs which sufficient for microbial control. The silver nanoparticles are disrupting transport systems) **Morones *et al.*, 2005)**. The AgNPs are producing active oxygen species (ROS) via reaction with oxygen, causing damage to lipids, nucleic acids and proteins **(Storz and Imlay, 1999 and Hwang *et al.*, 2008)**. The silver nanoparticles not only interact with the membrane surface, but can also penetrate inside the organism **(Srivastava *et al.*, 2011)**. In other study the colony number and growth of *Aspergillus niger were* decreased significantly at 12.5 ppm of nano-silver **(Naghsh *et al.*, 2012)**. The uses of nanoparticles as fungicides in recent years have been increasing because of many advantages over synthetic fungicides; AgNPs are highly toxic against microorganisms and preventing the growth of fungal **(Ismail *et al.*, 2016)**.

3.2.Effect of Uniform Fungicide against *A. solani* and *F. oxysoporum:* The control of plant diseases has been possible by the use of chemicals or fungicides. The effects of different concentrations of Uniform on the mycelial growth of *A. solani* and *F. oxysoporum* are describing in Table (2). The results showed that Uniform fungicide reduced the mycelial growth of the pathogens. All concentrations of Uniform were found to inhibit the growth of *A. solani* and *F. oxysoporum*. Results indicated that the inhibition percentage was increased by increasing the concentrations. Among all concentrations tested, 800 ppm causedthe greatest inhibition of mycelium growth of *A. solani* and *F. oxysoporum*. *F. oxysoporum* was more resistance than *A. solani* at all concentrations of Uniform. The highest inhibition % of Uniform to *A. solani* and *F. oxysoporum* are 91.11 and 74.44%, respectively, at 800 pm. Many researchers were reported the inhibitory effect of fungicides on fungus **(Abada *et al.*, 2008; Patil *et al.*, 2001)**. Similar data were found mancozeb was the most effective fungicide to control of *A. solani* **(Prasad and Naik. 2003)**. The data agree with used of Carbendazim and Prochloraz effective in inhibiting mycelial growth of *F. oxysporum* **(Song *et al.*, 2004)**. The

Table (1) Effect of AgNPs on mycelium growth and inhibition % of *A. solani* and *F. oxysoporum.*

Concentration (ppm)	Mycelium growth (cm)		Inhibition %	
	A. solani	*F. oxysoporum*	*A. solani*	*F. oxysoporum*
Control	*9.0 a	9.0 a	0.00 n	0.00 n
10	7.5 c	8.3 b	16.67 l	7.78 m
20	7.1 d	7.7 c	21.11 k	12.22 l
40	4.8 g	6.4 e	46.67 h	28.89 j
80	2.2 k	5.9 f	75.56 d	34.44 i
100	1.5 l	4.6 g	83.33 c	48.89 h
120	1.1 m	4.0 h	87.78 b	55.56 g
140	0.9 m	3.5 i	90.00 b	61.11 f
160	0.6 n	3.1 j	93.33 a	65.56 e

* Values with the same alphabetical letters did not differ significantly at 5% confidence level.

highest reduction 86.4% inhibition of mycelial growth of *A. solani* was obtaind when apply mancozeb at 1500 ppm **(Sadana and Didwania 2015)**. The activity of fungicides, Chlorothalonil, Propineb, Mancozeb, Thiophanate-methyl and Copper oxychloride was evaluated against *A. solani* and all fungicides inhibited the mycelial growth of *A. solani* when compared with control **(Ghazanfar *et al.*, 2016)**.The treatment with Nativo was inhibited the growth of *F. oxysporum* and caused reduction in growth 98% as compared to control **(Akhtar *et al.*, 2017)**.

3.3.Effects on post-emergence damping off % and Survived seedling %: The data of effects AgNPs and Uniform on post-emergence and survived seedling recorded in Table (3). Treatment with AgNPs gave the highest decrease post-emergence and increase survived seedling. Treatment seedling with AgNPs and Uniform decreased each of post -emergence (5 and 6.7%) and increased survived seedling (95 and 93.3 %) in the first season, while (3.3 and 4.2%) and (96.7 and 95.8 %) in second season, respectively, compared with control. In the same trend, treatment tomato with fungicide reduced post-emergence damping off 30.4% in the natural field **(Sharma *et al.*, 2007)**.While applications of Copper hydroxide, mixed Copper hydroxide with Mancozeb, Streptomycin, and mixed Streptomycin with Copper hydroxide to seedlings were increased the survival seedling compared to control in the field **(Hausbeck *et al.*, 2000)**. In greenhouse, used of Carbendazim and Prochloraz to control their effects on tomato fusarium wilt, and data indicate that the preventive effect were 69.6% and 87.0% for Prochloraz and Carbendazim, respectively **(Song *et al.*, 2004)**.

Table (2) Effect of Uniform fungicide on mycelium growth and inhibition % of *A. solani* and *F. oxysoporum.*

Concentration (ppm)	Mycelium growth (cm)		Inhibition %	
	A. solani	*F. oxysoporum*	*A. solani*	*F. oxysoporum*
Control	*9.0 a	9.0 a	0.00 q	0.00 q
50	8.1 c	8.5 b	10.00 o	5.56 p
100	7.5 e	7.7 d	16.67 m	14.44 n
200	6.3 g	7.0 f	30.00 j	28.89 k
300	4.8 i	6.4 g	46.67 h	22.22 l
400	3.1 l	5.0 h	65.56 e	28.89 k
500	2.3 m	4.5 j	74.44 d	44.44 i
600	1.9 n	4.1 k	78.89 c	50.00 g
700	1.5 o	3.6 l	83.33 b	60.00 f
800	0.8 p	2.3 m	91.11 a	74.44 d

*Values with the same alphabetical letters did not differ significantly at 5% confidence level.

3.4.Effects on vegetative growth and chlorophyll of tomato plants:

The applications of AgNPs and Uniform on tomato plants significantly increased plant height, plant fresh weight, dry weight, leaf area and total chlorophyll content when compared to non-treated plants in both seasons were shown in Tables (4). The data indicated that the AgNPs was better than Uniform in both seasons. The increments in plant height, plant fresh weight, plant dry weight, leaf area, total chlorophyll content when used AgNPs were found as 58.57 cm, 466.79 gm, 130.12 gm, 979.21 cm^2 and 54.11 mg g^{-1} in the first season and 60.3 cm, 468.45 gm, 151.47 gm, 992.71 cm^2 and 54.89 mg g^{-1} in the second season, respectively, compared with control. Results are in harmony with those of **Haghighi and Pessarakli (2013)** who reported that the application of Si or Nano-Si resulted in significant enhancement on the vegetative growth characters. Meanwhile, the effect of SA on the vegetative growth of treated plants, was confirmed by **Yildirim *et al.*, (2008)**, they reported that the application of Salicylic acid (SA) increased shoot fresh and dry weight, plant height and leaves number $plant^{-1}$ for cucumber plants. The data disagree with applied fungicides (Iprobenfos, Fentin hydroxide, Tebuconazole and Fosetly-aluminum in soil were significantly reduce up to 10% in the total chlorophyll content of all treatments compared with the control **(Shoaib *et al.*, 2014)**. Herbicides and insecticides decreased chlorophyll contents in tomato leaves after one and four days from spraying **(Salem 2016)**. In other study used Paclobutrazol (Pbz) (at 1.6 ppm) significantly reduced leaf area (LA), shoot dry weight and root dry weight, while increased chlorophyll content in both irrigated and deficit irrigated conditions as compared to control **(Pal *et al.*, 2016)**.

3.5.Effects on tomato yield per plant:

Study the efficiency of AgNPs and Uniform fungicide on fruits No. plant and fruits yield plant of tomato plants in both seasons were recorded in Table (5). The maximum significant increase in yield characters was obtained by AgNPs followed by Uniform fungicide when comparing with control. The AgNPs increasing of fruits No. $plant^{-1}$ was recorded by 20.53

Table (3) effects of AgNPs and Uniform on post-emergence damping off % and Survived seedling % of tomato plants.

Treatment	First season		Second season	
	No. of dead seedling	Post-emergence%	No. of dead seedling	Post-emergence%
AgNPs	6 c	5.0 c	4 b	3.3 c
Uniform	8 b	6.7 b	5 b	4.2 b
Control	19 a	15.8 a	16 a	13.3 a
	No. of Survived seedling	**Survived seedling %**	**No. of Survived seedling**	**Survived seedling %**
AgNPs	114 a	95 a	116.3 a	96.9 a
Uniform	112 a	93.3 b	114 a	95.0 b
Control	101 b	84.2 c	103 b	85.8 c

* Values with the same alphabetical letters did not differ significantly at 5% confidence level.

and 20.97, while fruits yield plant recorded by 2.31 and 2.36 kg as com pared with control in both seasons, respectively. The treatments with fungicides were increased in the yield and average weight of tomato per plant **(Cwalina-Ambroziak and Amarowicz, 2012)**.

The application of fungicide pretreatment after sowing followed by biweekly was increased fruit yield and quality in tomato **(Guimarães *et al.*, 2017)**. In other hand, treatments of tomato plant with unden resulted in decreasing yields though comparing with control plots **(Glover-Amengor and Tetteh, 2008)**.

Table (4) Effects of AgNPs and Uniform on vegetative growth and chlorophyll of tomato plants.

Treatment	First season	Second season
Plant height (cm)		
AgNPs	58.57a	60.3a
Uniform	56.16b	59.3a
Control	50.82c	49.24b
Plant fresh weight (g)		
AgNPs	466.79a	468.45a
Uniform	394.37b	435.42b
Control	315.62c	326.88c
Plant dry weight (g)		
AgNPs	130.12a	151.47a
Uniform	128.67b	139.53b
Control	98.45c	106.72c
Leaf area per plant (cm^2)		
AgNPs	979.12a	992.71a
Uniform	872.91b	886.46b
Control	738.94c	751.37c
Total chlorophyll content (mg g^{-1})		
AgNPs	54.11a	55.89a
Uniform	53.62b	52.72b
Control	47.23c	47.78c

*Values with the same alphabetical letters did not differ significantly at 5% confidence level.

CONCLUSION

· AgNPs had exhibited a high broad spectrum activity towards *A. solani* and *F. oxysoporum*. Fungicide application is one of a sharp tool against disease control in plants. Therefore, Uniform fungicide effective to control *A. solani* and *F. oxysoporum*. AgNPs and Uniform fungicide activities contributed to increase in vegetative growth and chlorophyll and the yield of tomato plants.

Table (5) Effects of AgNPs and Uniform on yield per tomato plant.

Treatment	First season	Second season
Number of fruits per plant		
AgNPs	20.53a	20.97a
Uniform	19.75b	20.34b
Control	15.01c	15.21c
Fruits yield per plant (kg)		
AgNPs	2.31a	2.36a
Uniform	2.09b	2.2b
Control	1.86c	1.76c

*Values with the same alphabetical letters did not differ significantly at 5% confidence level.

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دراسة فاعليه كل من الفضة فى صوره النانو والمبيد الفطرى ينيفورم على فطرى اليترناريا سولانى وفيوزريم اكيسبوريم، وتاثيرهما على النمو الخضرى، الكلورفيل ، الانتاجية للنباتات الطماطم

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مركز البحوث الزراعية-محطة بحوث ايتاى البارود - محافظة البحيرة - مصر

الملخص العربى:

تم دراسة كلا من تاثير فاعليه الفضة فى صوره النانو والمبيد الفطرى ينيفورم على فطرى اليترناريا سولانى وفيوزريم اكيسبوريم فى المعمل، فوجد ان كلا المركابين لهما تاثير على تثبيط النمو الفطر باستخدام تركيزات مختلفة منهما، وكلما زاد التركيز المستخدم زاد تثبيط نمو الفطريات المختبره.

عند معاملة الشتلات نباتات الطماطم بكلا من الفضة فى صوره النانو والمبيد الفطرى ينيفورم، وجد انهما تذيد من بقاء عدد الشتلات الحية مقارنة بالكنترول.

ووجد ايضا ان كلا من الفضة فى صوره النانو والمبيد الفطرى ينيفورم لهما تاثير واضح على النمو الحضرى، حيث اديا الى زيادة كلا من طول النبات، الوزن الطازج، الوزن الجاف، مساحة الورقة مقارنة بالنباتات الغير معاملة.

وعند دراسه ايضا تاثير كلا من الفضة فى صوره النانو والمبيد الفطرى ينيفورم على المحتوى الكلورفيل ، وجد ان لهما تاثير ايجابى بالزيادة فى نسبة الكلورفيل فى اوراق النباتات المعاملة مقارنة بالاوراق الغير معاملة.

اما عند دراسة تاثير الفضة فى صوره النانو والمبيد الفطرى ينيفورم على انتاجية نباتات الطماطم، فادى الى زيادة كلا من عدد الثمار لكل نبات و والمحصول لكل نبات مقارنة بانتاجية النباتات الغير معاملة.