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Control of Tomato Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* by Grafting and Silver nanoparticles under greenhouse conditions

Naeema.A.Gomaa[1], A.M.Mahdy[1], R.N.Fawzy[1], A.S.Mohamed[2] and G.A.Ahmed[1]
[1]Plant Pathology Department, Faculty of Agric., Univ., Benha, Egypt
[2]Horticultural Crops Technology Dept., Agric. and biology research institute, National Research Centre, Dokki, Giza, Egypt
E-mail: naeema.hassan@fagr.bu.edu.eg

ABSTRACT

Grafting plays an important role in the management of pathogens infecting root and stem as this technology can be quickly deployed without causing significant changes in farming operations. Grafting vegetable crops is common to control fusarium wilt (caused by *Fusarium oxysporum* f.sp. *lycopersici*). Biological and chemical methods were used for the synthesis of silver nanoparticles (AgNPs). AgNPs synthesized by two different methods i.e., *Trichoderma harzianum*, and tri-sodium citrate. The synthesized silver nanoparticles were characterized with transmission electron microscopy (TEM), Dynamic Light Scattering nano sizer (DLS) and Zeta potential. *In vitro*, *Trichoderma harzianum* AgNPs at concentration 800 µL/L inhibited completely the mycelium growth and sporulation of *Fusarium oxysporum* f.sp. *lycopersici* (FOL). Tri-sodium citrate came in the second place for reducing the mycelium growth and sporulation of *Fusarium oxysporum* f.sp. *lycopersici* (FOL)**.** Results revealed the biological method was better than chemical method for controlling fusarium wilt caused by (FOL)**.** Grafted plants treated with silver nanoparticles exhibited an increase in activities of defense enzymes such as peroxidase, polyphenol-oxidase, phenylalanine ammonia layse, chitinase and phenol contents over the control plants.

Keywords: Silver nanoparticles, grafting, *Fusarium oxysporum*, tomato ,enzymes , phenole.

1. Introduction

Tomato *(Lycopersicon esculentum* Mill.) belongs to the family Solanaceae and it is considered one of the world's most popular vegetables [30]. It is the most important tropical vegetable crop widely used throughout the world [21] **.** It is a high-value horticultural crop for the local market and an important dietary component, contributing to improved nutrition and livelihood for both rural and urban population [38]. Fusarium wilt of tomato is considered as one of the most important diseases of tomato both in the field and greenhouse-grown tomatoes worldwide[4-21-2]. Which is characterized by wilted plants, yellow leaves and minimal/reduced or even total loss/absent crop yield.Vegetable grafting began in 1920 using resistant rootstock to control soilborne diseases**.** [10]. Diseases like fusarium wilt (caused by *Fusarium oxysporum* f.sp. *lycopersici*) can be managed relatively easily with this method as major resistance genes are available in tomato, and these resistance genes have been intro-gressed into commercial cultivars as well as inter-specific rootstocks [32]**.** Nanostructures were significantly effective to inhibit mycelial growth by up to more than 70% and, in addition, the treatment was effective to reduce the severity of the disease in *Fusarium oxysporum* inoculated seedlings after 14 days post-inoculation. [19]. Biosynthesis of AgNPs using some fungi such *Trichoderma harzianum,* has been used for AgNPs synthesis. The antifungal activity of silver nanoparticles has been studied by [1]. Treated tomato plants with silver nanoparticles exhibited an increase in activities of defense enzymes such as peroxidase, polyphenol-oxidase, chitinase, phenylalanine ammonia and phenol contents over the control plants [18].

Materials and Methods

1- Isolation and identification of the causal organism:

Diseased samples of tomato plants showing Fusarium wilt symptoms were collected from Kafr El-sheikh governorate and subjected to isolation trails. The causal of Fusarium wilt was isolated from stem vascular discoloration diseased plants. The infected tissues were cut into small pieces, surface sterilized with sodium hypochlorite (0.5%) for 2-3 minutes, washed several times with sterilized distilled water, dried between sterilized filter papers and transferred directly to the PDA medium in plate 9cm. The PDA plates were incubated at 28±2°C for 7 days [24]. The fungus was purified by the hyphal tip technique [9]**.** The emerged fungal hyphal tips were taken randomly from the peripheral ends of the growing colonies and transferred onto poured PDA plates according to [28]**.** PDA slants from the fungus were kept in the refrigerator at 4 o C for further experiments.

2. Laboratory Experiments:

2.1. Evaluation of AgNPs synthesized by two different methods *i.e. Trichoderma harzianum*, and tri-sodium citrate**.**

In this experiment,*T. harzianum* AgNPs and tri-sodium citrate AgNPs were evaluated for their effects on growth of the most virulent FOL *in vitro* and Characterization by DLS, Zeta potential and TEM.

2.2. Effect of Silver Nanoparticles (AgNPs) synthesized different methods on the linear growth and spores' production of *Fusarium oxysporum in vitro*:

Synthesis of Silver Nanoparticles by biological method according to the method described by [7].

Synthesis of Silver Nanoparticles by chemical method according to the method described by [36].

This study was designed to investigate the inhibitory effect of different methods, on the linear growth of FOL in *vitro*. The AgNPs used were tested at 6 concentrations as follows:

1) *T. harzianum* AgNPs concentrations 25, 50, 100, 200,400 and 800 µL /L
2) *T. harzianum* without Ag NPs at concentrations 25, 50, 100,200,400 and 800 µL /L
3) Tri-sodium citrate AgNPs at concentrations 25, 50, 100,200,400 and 800 µL /L

The effect of AgNPs against the tested virulent *Fusarium oxysporum* f.sp. *lycopersici* (FOL) isolate was done *in vitro*. The Ag NPs added immediately to the warmed 100 mL PDA medium. PDA medium in each conical flask was gently shacked then poured with constant volume (15 mL) into sterilized Petri dishes (9cm Ø) and left to solidify. The medium without Ag NPs served as control. Mycelium discs (5mm Ø) from the edge of 7 days old cultures of fungus were placed in the center of Petri dishes. Three plates were used for each concentration. Plates were incubated in an incubator at 27 ±2°C. The experiment was terminated when mycelium covered the medium surface in the control treatment. Fungal growth was measured by averaging the two diameters taken at right angles for each colony.

The inhibition percentages of fungal mycelial growth were calculated using the given formula by [5]. IP= ((C-T) /C) *100, where, IP = Inhibition Percentage over control, C = Growth of tested pathogen with absence of antagonist (mm) and T = Growth of tested pathogen with antagonist (mm)

3. Greenhouse Experiments

3.1 Grafting of tomato on resistant rootstocks for controlling tomato wilt disease

Plant materials used in this experiment are shown in **Table (1).** Tomato cultivar *i.e*. cv. Super Strain B was used as scion and the (1G-48-6031), (1G-48-6032) were used as rootstocks. The cleft grafting method was used in this experiment. Seeds of rootstocks were sown in 84 trays and seeds of scions were sown in 209 trays. At the fourth true-leaf stage "after 25-27 days from sowing, scions tomato seedlings (cv. Super Strain B) were grafted onto the two rootstocks. In addition, non-grafted plants were used as control treatments. The grafted plants were kept under a tunnel with more than 95% relative humidity and 5-10% of normal light density "shading". After 48hour, light density was gradually increased and 48hours more air humidity was gradually decreased for adaptation and preparation of the grafted seedlings before transplanting them in the greenhouse. After 3 weeks from the grafting process, grafted seedlings were transplanted under greenhouse condition.

Table (1) List of rootstocks and scion used in this experiment.

Cultivars		Scientific name	Source of seeds
Rootstocks	1- (1G-48-6031)	*Lycopersicon hirsutum*	Golden seeds Co Greece
	2-*(1G-48-6032)*	*Lycopersicon hirsutum*	Golden seeds Co Greece
Scion	1- *Super Strain B*	*Lycopersicon esculentum* Mill. cv. ' Super Strain B	Unigen seeds Co. Italy

3.1.2. Effect of silver nanoparticles for controlling Fusarium wilt on grafted tomato under greenhouse conditions:

Grafted plants on rootstocks namely (1G-48-6031),(1G-48-6032) were planted in pots (20 cm Ø) under greenhouse conditions at the Experimental Station, Moshtohor, Faculty of Agriculture, Benha University, Egypt during the growing season 2019.

Loamy sand soil [3 clay:1 sand w/w] was sterilized by thoroughly mixing with 5% commercial formalin solution (one L of 5% formalin solution/cubic feet of soil mixture) and covered with polythene for 2 weeks. Later, polythene cover was removed and soil was raked for 10 days for ventilation and formalin evaporation. Similarly, plastic pots (ϕ 20 cm) were sterilized by dipping in 5.0% commercial formalin solution for 15 minutes, left to dry for 24 hrs. Then filled with the previously sterilized soil. The inoculum of *F. oxysporum lycopersici* (FOL) isolate was grown on PDA plates for 10 days at 27±2°C Then transferred to a sterilized sand barley medium for two weeks. Inoculum of FOL was added to the potting soil at rate of 3.0% w/w, mixed thoroughly with the soil surface of each pot then watered and left for one week to ensure the distribution of inoculum**.** In this study, the AgNPs synthesized by two different methods (fungi and chemical method) were evaluated on the grafted tomato seedling compared with fungicide and biocide as dipping treatments for controlling fusarium wilt of tomato under greenhouse conditions. The tested treatments were:

1) *T. harzianum* Ag NPs 125 µL /L
2) *T. harzianum* Ag NPs 250 µL /L
3) *T. harzianum* Ag NPs 500 µL /L
4) *T. harzianum* without AgNPs 125 µL /L
5) *T. harzianum* without AgNPs 250 µL /L
6) *T. harzianum* without AgNPs 500 µL /L
7) Tri-sodium citrate AgNPs 125 µL /L
8) Tri-sodium citrate AgNPs 250 µL /L
9) Tri-sodium sodium citrate AgNPs 500 µL /L
10) Maxim-XL
11) Bio-Zeid

Healthy tomato grafted plants were dipped in each particular treatment for 2 h. Then raised and left to dry in the air before planting. Transplants dipped in water as control. Three transplants per pot and three pots were used as replicates for each treatment. Wilt disease incidence % (WDI %) and severity % (WDS %) were determined and calculated 60 days post transplanting based on a 0-4 scale as described by [34], where: 0=No infection, 1=Slight infection which is about 25% of full scale: one or two yellowed leaves, 2=Moderate infection: two or three yellowed leaves, 50% of wilted leaves, 3=Extensive infection: all plant leaves became yellow, 75% of wilted leaves and growth was inhibited and 4 =Complete infection: the whole plant leaves became yellow, 100% of wilted leaves, and the plant died.

Disease severity was calculated as follows:

% Disease severity = Σ (a x b) / N x K x 100,

Where: a = number of infected plants in each category, b = numerical value of his category, N = Total number of examined plants and K = the highest degree of infection category

Also, disease incidence was recorded for each individual treatment by the equation:

$$\% \text{ Disease incidence} = \frac{\text{number of infected plants}}{\text{total number of plants}} \times 100$$

Data collection:

The plants were gently removed from pots, washed with tap water, left to air dry at room conditions for about 30-60 minutes then the following parameters *i.e.*, fresh and dry weight of root and shoot as g/ pot were determined then dry biomasses of roots and shoots were determined after oven drying of samples at 65-70°C for 2–3 days until constant weight gained.

4. Determining of enzyme activities:

Leaves samples of grafted plants tomato plants treated with different treatments were taken 40 days after transplanting. Leaf samples were ground with 0.2 M Tris HCl buffer (pH 7.8) containing 14 mM β-mercaptoethanol at the rate 1/3 w/v. The extracts were centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant layer was used to determine enzyme activities [37].

4.1. Determination of **Peroxidase (PO):**

Peroxidase activity was determined according to the method described by [3].

4. 2. Determination of Polyphenoloxidase (PPO):

The polyphenoloxidase activity was determined according to the method described by [27]

4.3. Determination of phenylalanine ammonia lyase (PAL):

The activity of PAL was determined according to the method described by [11].

4.4. Determination of chitinase.

Determination of the activity of chitinase was carried out according to the method of [8].

5. Determination of phenolic compounds:

Phenol contents were determined using Spectrophotometer (SPECTRONIC 20-D) at 520 nm according to the method of [6].

Results and Discussion

1. Characterization of AgNPs

The average size distribution of the AgNPs (Dynamic Light Scattering- nano sizer (DLS)) was 17.5 nm for *Trichoderma harzanium* and 69.5 nm for Tri-sodium citrate (Fig. 1).

The negative zeta potential of about -1.73mv and -16.69 mv was observed for AgNPs synthesized from *Trichoderma harzanium* and tri-sodium citrate, respectively (Fig. 1). The values strongly supported long time stability of AgNPs (AgNPs are stable up to three months without agglomeration) [17]**.** The estimation of zeta potential is based on the direction of the velocity of particles under the influence of a known electric field [15]**.** The results indicate an ideal surface charge of the formed AgNPs. Moreover, the high absolute and negative value of zeta potential revealed a high electrical charge on the AgNPs surface, which can cause a strong repulsive force among the particles to prevent agglomeration and hence might be responsible for their high stability.

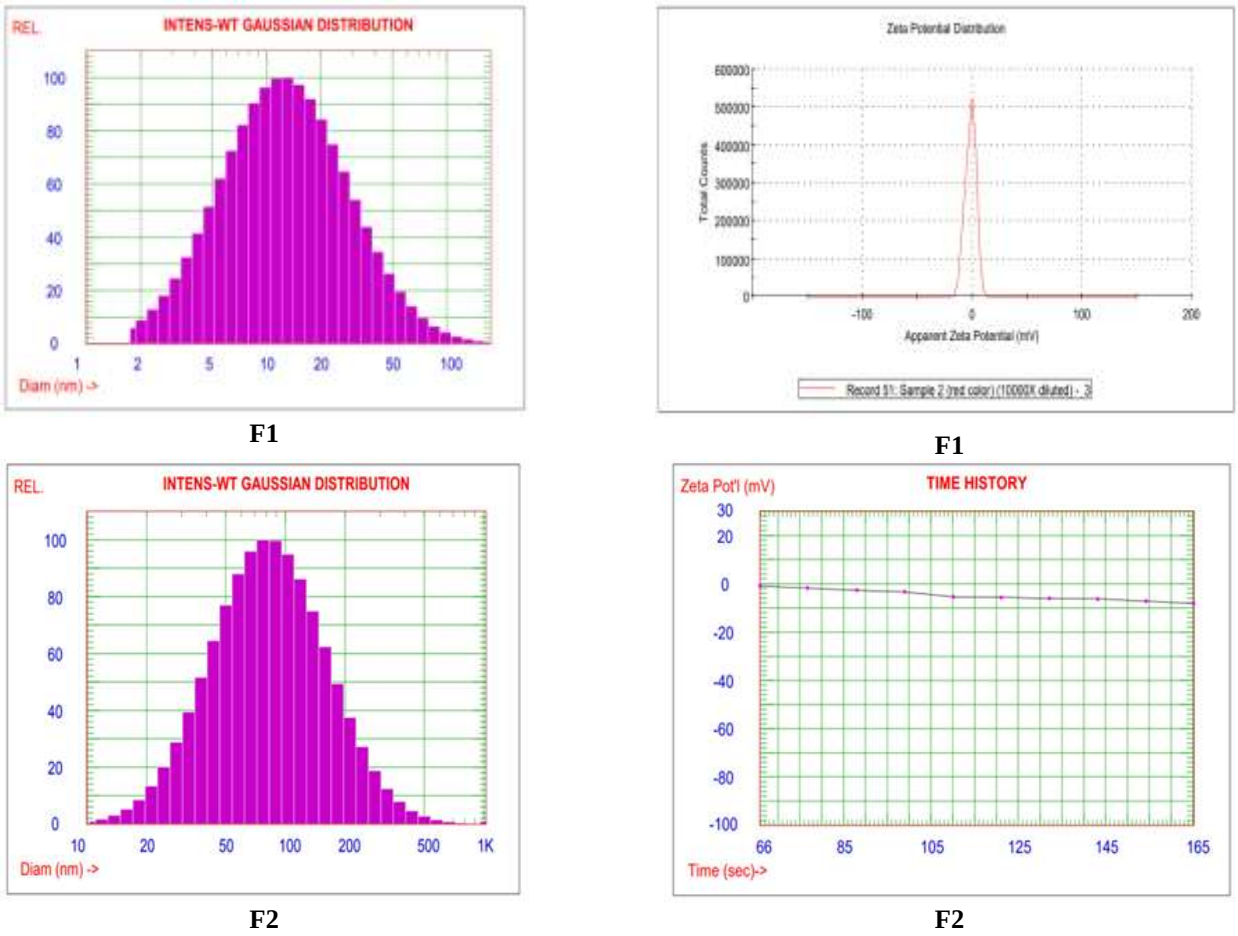

Fig.(1) F1-F2: Characterization of the Synthesized AgNPs, respectively: (Left F1-F2) Particle size distribution analysis, (Right F1-F2) Zeta potential measurements of the Synthesized AgNPs

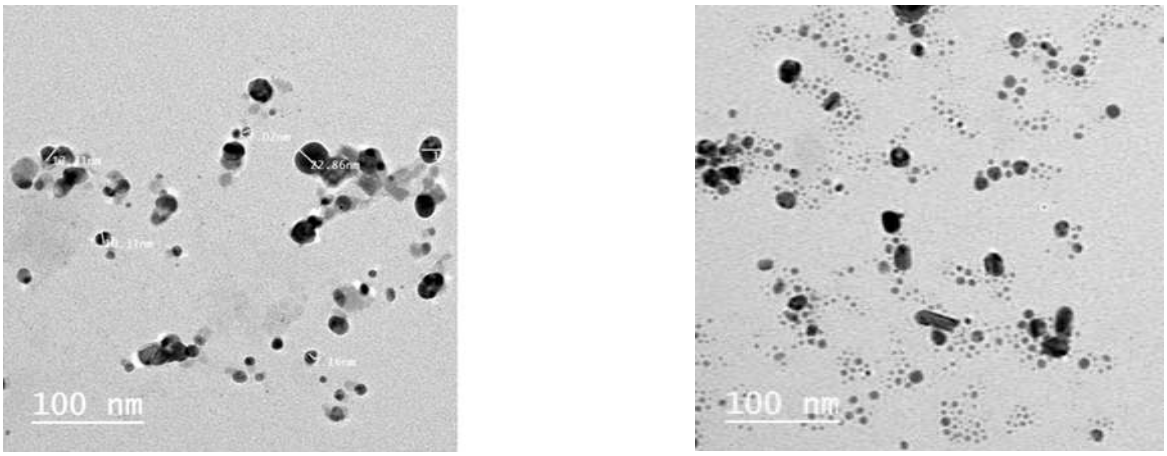

Fig. (2) F1-F2: HR-TEM images of the synthesized AgNPs by (F1) *T. harzianum*, (F2) Tri-sodium citrate AgNPs.

3. Effect of silver nanoparticles synthesized by different methods on the growth and spores' production of *F. oxysporum in vitro*:

Data in **Table 2 and Fig.3,** show that all tested Ag NPs synthesized by different methods reduced the growth and spores' production of *F. oxysporum* f. sp. *lycopersici* **(**FOL) compared with control. Reduction in linear growth and spores' production was increased by increasing the concentration of most tested treatments. *Trichoderma harzianum* Ag NPs at concentration 800 µL/L inhibited completely the mycelium growth and sporulation of FOL followed by tri sodium citrate AgNPs at concentration 800 µL/L at 78.33% and spores' production of (FOL) by 20 %. These results agree with those of [12]**.** They found that, silver nanoparticles with size 1-20 nm at a concentration of 0.5 mM was effective against *Fusarium oxysporum* and displayed 68.2% inhibition of colony formation compared with the control. [26] found that the bioactivity against *F. culmorum* of the different treatments, without and with AgNPs, was studied *in vitro* by monitoring the radial growth of the mycelium at a concentration of 62.5 µg·mL−1, 125 µg·mL−1, 250 µg·mL−1, 500 µg·mL−1. fungal mycelium growth was inhibited with 79– 98% as compared to the control. These results agree with [39 - 23] they found biosynthesized AgNPs from *Trichoderma* spp. inhibit the growth of pathogenic *Fusarium* spp *in vitro*. These results agree with this of [14] who found that AgNPs synthesized by *Trichoderma* spp. was used as an antifungal against four soil-borne *Fusarium* spp., *F. solani*, *F. semitectum, F. oxysporum* and *F. roseum*. These results agree with those of [35] who found that silver nanoparticles synthesized by chemical and biological means were tested for antimicrobial activity.

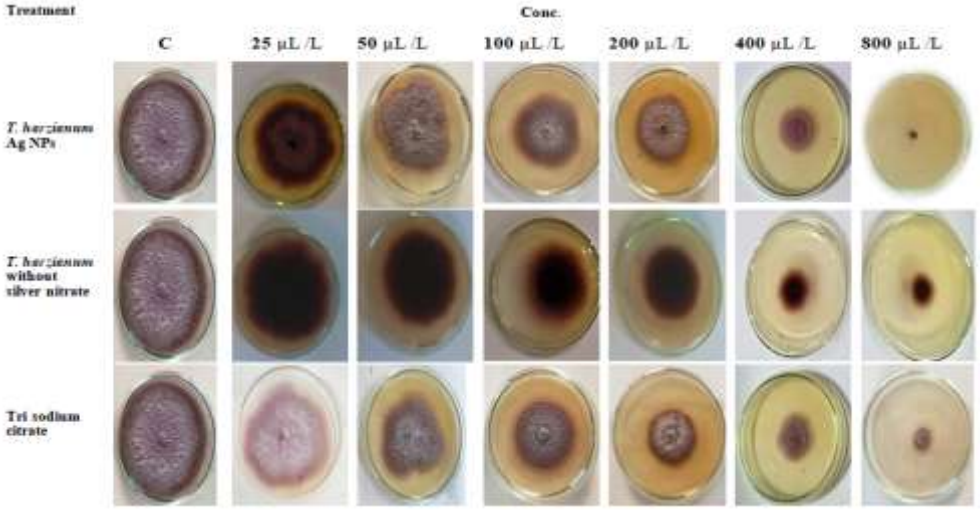

Fig. (3) Effect of silver nanoparticles synthesized by two different methods on the growth of *F. oxysporum in vitro*.

Table (2) Effect of silver nanoparticles synthesized by three different methods on the growth and spores' production of *F. oxysporum in vitro*

Treatment	Conc. µL/L	Mycelium growth	No. of spores (*10^{t})/mL	Efficacy	
				Mycelium growth	No. of spores (*10^{t})/mL
Trichoderma harzianum	25	6.50	192	27.7ᵛ	47.82
Ag NPs	50	6.33	172	29.66	53.26
	100	5.27	144	41.44	60.86
	200	3.57	120	60.33	67.39
	400	2.83	88	68.55	76.08
	800	0.00	0.00	100	100.00
T. harzianum **filtrate**	25	7.17	216	20.33	41.30
without Silver nitrate	50	6.57	184	27	50
	100	6.43	168	28.55	54.34
	200	5.67	154	37	58.15
	400	4.47	140	50.33	61.95
	800	3.17	102	64.77	72.28
Tri-sodium citrate AgNPs	25	5.96	160	33.77	160
	50	5.63	152	37.44	152
	100	5.38	120	40.22	120
	200	3.16	96	64.88	96
	400	2.98	80	66.89	80
	800	1.95	20	78.33	20
Control		9.00	368	0.00	0.00
LSD at 0.05		1.02	23.53		

2. Effect of AgNPs as dipping treatment on controlling Fusarium wilt disease on grafted tomato seedling (rootstock 1G-48-6031) under greenhouse.

Data in **Table (3)** indicate that all applied treatments significantly reduced wilt disease incidence and disease severity, as well as increased fresh and dry weight of shoots and roots, of tomato plants compared to the control. In this respect, some of the treatments are equal in the reduction percentages of disease incidence and disease severity as *T. harzianum* AgNPs 500 µL /L, tri-sodium citrate 500 µL /L, and Maxim –XL which recorded 0.00% of disease incidence and 0.00 % of disease severity. Moreover, the highest increase in fresh and dry weight of shoots and roots was recorded with *T. harzianum* AgNPs 500 µL /L followed by Maxim –XL. On the other hand, *T. harzianum* without AgNPs 125 µL /L was the least effective.These results are in harmony with the results of [25] who found that, the AgNPs caused a reduction in the severity of tomato wilt disease in the greenhouse by 90%. This was detected through reducing the number of wilted seedlings especially after placing their roots in a suspension of 500 mg/L of AgNPs for 4 h before infestation of soil with pathogenic *F. oxysporum*, compared with soil treated with the pathogen only. These results are in agreement with this of [21] Who found that Fusarium wilt can be controlled on tomato with resistant rootstocks and the grafting treatment was effective in significantly reducing disease severity and enhancing root and stem fresh weights and yield by 30% compared to non-grafted control.

Table (3) Effect of AgNPs as dipping treatment on controlling Fusarium wilt disease on grafted seedling (rootstock 1G-48-6031) under greenhouse.

Treatment	DI %	DS %	FW (g)		DW (g)	
			Shoot	Root	Shoot	Root
***Trichoderma harzianum* Ag NPs 125 µL /L**	33.33	11.08	28.54	6.29	8.34	1.06
***T. harzianum* Ag NPs 250 µL /L**	33.33	8.33	29.15	6.69	9.22	1.92
***T. harzianum* Ag NPs 500 µL /L**	0.00	0.00	32.50	9.39	10.14	2.57
***T. harzianum* filtrate without Silver nitrate 125 µL /L**	33.33	16.67	16.19	4.41	5.43	0.91
***T. harzianum* filtrate without Silver nitrate 250 µL /L**	33.33	16.67	17.80	5.25	7.28	0.93
***T. harzianum* filtrate without Silver nitrate 500 µL /L**	33.33	8.33	24.51	5.46	7.68	0.98
Tri-sodium citrate AgNPs 125 µL/L	33.33	16.67	27.44	5.65	7.91	1.08
Tri-sodium citrate AgNPs 250 µL /L	33.33	11.08	29.46	6.76	٨.59	1.24
Tri-sodium citrate AgNPs 500 µL/L	0.00	0.00	31.11	7.54	8.36	1.11
Maxim-XL	0.00	0.00	31.57	7.95	8.99	1.39
Bio-Zeid	33.33	8.33	27.97	6.49	8.07	1.04
Control 1	0.00	0.00	12.47	4.26	5.19	0.80
Control 2	100	80.55	10.88	4.19	3.43	0.63
(R1*)	66.67	41.67	9.67	3.91	2.92	0.55
(R1)**	0.00	0.00	15.63	5.10	5.80	0.83
LSD at 0.05	9.08	4.99	1.57	1.55	1.46	0.33

DI= disease incidence**, DS=** disease severity**, FW=** Fresh weight**, DW=** Dry weight, **Control 1**=Un-inoculated soil, (cv. super strain B) **Control 2**=inoculated soil with FOL (cv. super strain B), **(R1*) =** rootstock 1G-48-6031 (inoculated soil with FOL), **(R1**) =** rootstock 1G-48-6031 (Un-inoculated soil with FOL),

3. Effect of AgNPs as dipping treatment on controlling Fusarium wilt disease on grafted seedling (rootstock 1G-48-6032) under greenhouse.

Data in **Table (4)** indicate that all applied treatments significantly reduced wilt disease incidence and disease severity, as well as increased fresh and dry weight of shoots and roots, of tomato plants compared to the control. In this respect, some of the treatments are equal in the reduction percentages of disease incidence and disease severity as *T. harzianum* AgNPs 500 µL /L and Maxim –XL which recorded 0.00% of disease incidence and 0.00 % of disease severity Moreover, the highest increase in fresh and dry weight of shoots and roots was recorded *T. harzianum* AgNPs 500 µL /L followed with Maxim–XL. These results are in harmony with the results of [22] who found that AgNPs biosynthesized by the fungus *Trichoderma longibrachiatum* reduced the incidence of *F. oxysporum* in tomato. Silver ions produce active oxygen species (ROS) via their reaction with oxygen, causing damage to cells proteins, lipids, and nucleic acids. According to [4] found that, prochloraz and bromuconazole were the most effective fungicides for controlling *Fusarium oxysporum* f.sp. *lycopersici* among the following fungicides; benomyl, carbendazim, prochloraz, fludioxonil, bromuconazole and azoxystrobin. [33] found that grafting tomato onto vigorous rootstocks effectively controlled Fusarium wilt and also improved plant vigor and yield.

Table (4) Effect of AgNPs as dipping treatment on controlling Fusarium wilt disease on grafted seedling (rootstock 1G-48-6032) under greenhouse.

Treatment	DI%	DS%	FW(g)		DW(g)	
			Shoot	Root	Shoot	Root
***Trichoderma harzianum* Ag NPs 125 µL /L**	33.33	16.67	30.81	7.97	8.36	1.13
***T .harzianum* Ag NPs 250 µL /L**	33.33	8.33	31.68	7.81	9.26	2.04
***T. harzianum* Ag NPs 500 µL /L**	0.00	0.00	35.52	9.64	11.08	2.65
***T. harzianum* filtrate without Silver nitrate125 µL /L**	33.33	16.67	19.69	5.47	5.69	0.94
***T. harzianum* filtrate without Silver nitrate 250 µL /L**	33.33	16.67	21.65	6.4	7.83	1.21
***T. harzianum* filtrate without Silver nitrate500µL /L**	33.33	16.67	23.31	6.49	8.12	1.05
Tri-sodium citrate AgNPs 125 µL /L	33.33	16.67	27.10	7.36	8.26	1.10
Tri-sodium citrate AgNPs 250 µL /L	33.33	8.33	32.49	7.75	8.68	1.29
Tri-sodium citrate AgNPs 500 µL /L	33.33	8.33	34.17	9.15	8.45	1.15
Maxim-XL	0.00	0.00	35.12	9.70	9.24	1.60
Bio-Zeid	33.33	8.33	32.49	7.99	8.22	1.05
Control 1	0.00	0.00	12.47	4.26	5.19	0.80
Control 2	100	80.55	10.88	4.19	3.43	0.63
(R2*)	77.78	41.67	10.88	4.19	3.59	0.75
(R2)**	0.00	0.00	14.83	5.40	5.30	0.87
LSD at 0.05	10.84	4.64	2.38	2.31	1.85	0.58

DI= disease incidence**, DS=** disease severity**, FW=** Fresh weight**, DW=** Dry weight, **Control 1**=Un-inoculated soil, (cv. super strain B) **Control 2**=inoculated soil with FOL (cv. super strain B), **(R2*) =** rootstock 1G-48-6032 (inoculated soil with FOL), **(R2**) =** rootstock 1G-48-6032 (Un-inoculated soil with FOL).

4. Effect of AgNPs as dipping treatment on activities of defense-related enzymes in tomato plants grafted on seedling (rootstock 1G-48-6031) under greenhouse conditions.

Data in **Table (5)** reveal that all tested treatments increased the activities of peroxidase(PO), polyphenol oxidase (PPO), chitinase and phenylalanine ammonia lyase(PAL) enzymes compared with control treatment (FOL). Maxim-XL was the best treatment where it increased the activities of peroxidase, chitinase and Phenylalanine ammonia lyase enzymes and recorded efficacy % as 2143.96, 2390.90, and 1345.40%, respectively While tri-sodium citrate 500 µL /L the best treatment increased the activities the activities of polyphenol oxidase enzymes and recorded efficacy as (1141.90%). *T. harzianum* filtrate without Silver nitrate125 µL /L was the least effective compound in increasing activities of PO, and PPO and PAL enzymes while *T. harzianum* filtrate without silver nitrate 250 µL /L of chitinase in this respect. These results are in agreement with those of [31] who found that tomato seedling AgNPs synthesis by *T. harzianum* increases hydrolytic enzymes β-1,3-glucanase, N-acetylglucos aminidase (NAGase), chitinase, and acid protease.

Table (5) Effect of AgNPs as dipping treatment on activities of defense-related enzymes in tomato plants grafted on seedling (rootstock 1G-48-6031) under greenhouse conditions.

Treatment	PO	PPO	PAL	Chitinase	Efficacy % PO	PPO	PAL	Chitinase
***Trichoderma harzianum* Ag NPs 125 µL /L**	37.26	28.01	20.02	9.45	1700	789.20	810.00	472.72
***T. harzianum* Ag NPs 250 µL /L**	38.34	29.30	23.52	14.28	1752.17	830.15	969.09	765.45
***T. harzianum* Ag NPs 500 µL /L**	46.44	38.96	27.14	30.84	2143.47	1136.82	1133.63	1769.09
***T. harzianum* filtrate without Silver nitrate125 µL /L**	13.59	21.56	7.64	14.79	556.52	584.44	247.27	796.36
***T. harzianum* filtrate without Silver nitrate 250 µL /L**	**13**.78	23.93	9.14	6.30	565.70	659.68	315.45	281.81
***T. harzianum* filtrate without Silver nitrate500 µL /L**	16.02	25.83	10.98	15.72	673.91	720.00	399.09	852.72
Tri-sodium citrate 125 µL /L	36.72	31.94	15.24	19.86	1673.91	913.96	592.72	1103.63
Tri-sodium citrate 250 µL /L	46.08	38.25	12.04	16.62	2126.08	1114.28	447.27	907.27
Tri-sodium citrate 500 µL /L	44.82	39.12	14.52	29.85	2065.21	1141.90	560.00	1709.09
Maxim-XL	46.45	34.57	31.8	41.1	2143.96	997.46	1345.40	2390.90
Bio-Zeid	41.40	28.78	24.60	20.4	1900.00	813.65	1018.18	1136.36
Control 1	10.35	5.34	2.80	3.66	400.00	69.52	27.272	121.81
Control 2	2.07	3.15	2.20	1.65	0.00	0.00	0.00	0.00
R1*	7.68	4.26	2.40	1.80	271.01	35.23	9.09	9.09
R1**	12.34	6.43	5.73	4.56	496.13	104.12	160.45	176.36

* **Control 1**: Un-inoculated soil, **Control 2**: inoculated soil with FOL), **(R1*) =** root stock 1G-48-6031 (inoculated soil with FOL), **(R1**) =** root stock 1G-48-6031 (Un-inoculated soil with FOL).
*Peroxidase activity was expressed as the increase in absorbance at 425 nm/gram fresh weight/15 minutes.
* Polyphenoloxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/ minutes.
* PAL activity was expressed as µmol trans-cinnamic acid min^{-1} g^{-1} protein.
* Chitinase activity was expressed as mM N-acetylglucose amine released/g fresh weight tissue/60 minutes.

5. Effect of AgNPs as dipping treatment on activities of defense-related enzymes in tomato plants grafted on seedling (rootstock 1G-48-6032) under greenhouse conditions.

Data in Table (6) reveal that, all tested treatments increased the activities of peroxidase, polyphenol oxidase, chitinase and phenylalanine ammonia lyase enzymes compared with control treatment (FOL). *T. harzianum* Ag NPs 500 µL /L was the best treatment where it increased the activities of peroxidase and chitinase and recorded efficacy % as 4356.22 and 2282.42 %, respectively. Tri-sodium citrate 500 µL /L the was best treatment where it increased the activities of PPO and PAL and recorded efficacy % as 1281.58 and 1767.27 %, respectively. While *T. harzianum* filtrate without silver 125 µL /L was the least effective compound in increasing activities of PO, PPO , and chitinase enzymes and *T. harzianum* filtrate without silver nitrate 500 µL /L of phenylalanine ammonia lyase in this respect. These positive results agree with [29] exposure tomato AgNPs
at 10, 20, or 30 µL /L silver increased peroxidase and phenylalanine ammonia lyase enzymes.

Table (6) Effect of AgNPs as dipping treatment on activities of defense-related enzymes in tomato plants grafted on seedling (rootstock 1G-48-6032) under greenhouse conditions.

Treatment	PO	PPO	PAL	Chitinase	Efficacy %			
					PO	PPO	PAL	Chitinase
***Trichoderma harzianum* Ag NPs 125 µL /L**	70.48	33.69	18.54	19.44	3304.83	969.52	742.72	1078.18
***T. harzianum* AgNPs 250µL /L**	86.35	41.06	28.24	25.80	4071.48	1203.49	1183.63	1463.63
***T. harzianum* AgNPs 500 µL /L**	92.25	30.18	32.04	39.31	4356.22	858.09	1356.36	2282.42
***T harzianum* filtrate without Silver nitrate125 µL /L**	15.03	18.60	12.04	4.26	626.08	490.47	284.66	158.18
***T. harzianum* filtrate without Silver nitrate 250 µL /L**	22.45	22.56	11.80	9.54	984.54	616.19	436.36	478.18
***T. harzianum* filtrate without Silver nitrate500 µL /L**	24.43	23.79	10.82	13.02	1080.19	655.23	391.81	689.09
Tri-sodium citrate AgNPs 125 µL /L	39.06	29.13	32.04	13.80	1786.95	824.76	1356.36	736.36
Tri-sodium citrate 250 µL /L	43.20	33.16	33.67	20.76	1986.95	952.69	1430.45	1158.18
Tri-sodium citrate AgNPs 500 µL /L	70.02	43.52	41.08	31.20	3282.61	1281.58	1767.27	1790.90
Maxim-XL	68.40	30.36	41.00	33.60	3204.34	863.80	1763.63	1936.36
Bio-Zeid	61.20	28.95	30.00	26.25	2856.52	819.04	1263.63	1490.90
Control 1	13.78	4.28	3.82	2.34	565.70	35.87	73.63	41.81
Control 2	2.07	3.15	2.20	1.65	0.00	0.00	0.00	0.00
R2*	12.15	5.16	3.58	1.85	486.95	63.81	62.72	12.12
R2**	14.88	7.36	6.44	3.77	628.84	133.56	192.72	128.48

* **Control 1**: Un-inoculated soil, **Control 2**: inoculated soil with FOL), **(R1*) =** root stock 1G-48-6031

6. Effect of AgNPs as dipping treatment on activities of phenol content in tomato plants grafted on seedling (1G-48-6031) under greenhouse conditions.

The results in **Table (7)** indicate that phenol content was affected by the treatment with AgNPs compounds. Compared with control, all tested AgNPS synthesized increased the total phenols. The highest increase in the total phenols was *T. harzianum* Ag NPs 500 µL /L (1262.92%) followed by Bio-Zeid (874.15%). While *T. harzianum* filtrate without silver nitrate125 µL /L was the least effective and increased the total phenols by (203.37 %). As for the free phenol, all tested AgNPs compounds increased the free phenols. The highest increase in the free phenols was *T. harzianum* Ag NPs 500 µL /L (3344.08%). While treatments differed in their effect on the conjugated phenols. In this respect reduction by tri-sodium citrate 125 µL /L increased the conjugated

phenols by 150.63%. These results are in agreement with those of [16] who found that, a high amount of secreted proteins, carbohydrates and phenolic compounds in the plant system was exposed to silver ions and chemically synthesized silver nanoparticles.

Table (7) Effect of AgNPs as dipping treatment on activities of phenol content in tomato plants grafted on seedling (rootstock 1G-48-6031) under greenhouse conditions.

Treatment	Total Phenol	Conj. Phenol	Free Phenol	Efficacy % Total Phenol	Efficacy % Conj. Phenol	Efficacy % Free Phenol
***Trichoderma harzianum* Ag NPs 125 µL /L**	39.59	5.88	33.712	353.93	5.18	977.06
***T. harzianum* Ag NPs 250 µL /L**	52.33	10.982	41.35	500.00	96.46	1221.08
***T. harzianum* Ag NPs 500 µL /L**	118.87	11.07	107.80	1262.92	98.3	3344.08
***T. harzianum* filtrate without Silver nitrate125 µL /L**	26.46	12.64	18.81	203.37	126.11	500.95
***T . harzianum* filtrate without Silver nitrate 250 µL /L**	33.61	7.154	26.46	285.39	27.97	745.36
***T. harzianum* filtrate without Silver nitrate500 µL /L**	40.18	13.82	26.36	360.67	147.23	742.17
Tri-sodium citrate AgNPs 125 µL /L	53.21	14.01	39.2	510.11	150.63	1152.39
Tri-sodium citrate AgNPs 250 µL /L	58.80	2.25	61.05	574.15	-59.74	1850.47
Tri-sodium citrate AgNPs 500 µL /L	70.75	2.84	67.91	711.23	-49.19	2069.77
Maxim-XL	74.08	11.179	62.91	749.44	99.98	1909.90
Bio-Zeid	84.96	12.25	72.71	874.15	119.14	2223.19
Control 1	13.72	3.83	9.89	57.30	-31.48	215.97
Control 2	8.72	5.59	3.13	0.00	0.00	0.00
R1*	23.61	5.09	18.52	170.78	-8.94	491.69
R1**	25.45	6	19.45	191.85	7.33	521.40

* **Control 1**:Un-inoculated soil, **Control 2**:inoculated soil with FOL-5, **(R1*) =** root stock 1G-48-6031 (inoculated soil with FOL), **(R1**) =** root stock 1G-48-6031 (Un-inoculated soil with FOL).
*Phenol contents were calculated as milligrams of catechol per one -gram fresh weight

7. Effect of AgNPs as dipping treatment on activities of phenol content in tomato plants grafted on seedling (rootstock 1G-48-6032) under greenhouse conditions.

The results in **Table (8)** indicate that, phenol contents were affected by the treatment with AgNPs compounds. compared with control, all tested AgNPs synthesized increased the total phenols. The highest increase in the total phenols was resulted in Maxim-XL treatment (1035.09%) followed by *T .harzianum* AgNPs 500 µL /L(1011.46%).While *T. harzianum* filtrate without silver nitrate125 µL/L was the least effective and increased the total phenols by (673.27%). As for the free phenol, all tested AgNPs compounds increased the free phenols. The highest increase in the free phenols was Bio-Zeid (2476.68%).While treatments differed in their effect on the conjugated phenols, in this respect. Reduction by tri-sodium citrate 250 µL/L decreased the conjugated phenols by (-77.28%).Whereas *Trichoderma harzianum* AgNPs 125 µL/L increased the conjugated phenols by (622.18%). These results are in agreement with those of [13] found that the synthesis of silver nanoparticles by plant extract increased total polyphenols, fractionation of phenols, flavonoids in treatments plants.

Table (8) Effect of AgNPs as dipping treatment on activities of phenol content in tomato plants grafted on seedling (rootstock 1G-48-6032) under greenhouse conditions.

Treatment	Total Phenol	Conj. Phenol	Free Phenol	Efficacy % Total Phenol	Efficacy % Conj. Phenol	Efficacy % Free Phenol
***Trichoderma harzianum* Ag NPs 125µL /L**	75.66	40.37	35.28	767.66	622.18	1027.15
***T. harzianum* Ag NPs 250 µL /L**	76.048	35.28	40.76	772.13	513.12	1202.23
***T. harzianum* Ag NPs 500 µL /L**	96.92	31.07	65.85	1011.46	445.81	2003.83
***T. harzianum* filtrate without Silver nitrate125 µL /L**	37.43	22.83	14.60	673.27	308.40	366.45
***T. harzianum* filtrate without Silver nitrate 250 µL /L**	42.53	18.816	23.716	387.72	236.49	657.50
***T. harzianum* filtrate without Silver nitrate500 µL /L**	61.93	28.23	33.71	610.21	405.01	976.99
Tri-sodium citrate AgNPs 125 µL /L	51.25	9.11	42.14	487.72	62.96	1246.64
Tri-sodium citrate AgNPs 250 µL /L	63.30	1.27	62.03	625.91	-77.28	1881.79
Tri-sodium citrate AgNPs 500 µL /L	78.40	7.45	70.95	799.08	33.27	2166.77
Maxim-XL	98.98	38.03	60.95	1035.09	580.32	1847.28
Bio-Zeid	92.61	11.96	80.65	962.04	113.95	2476.68
Control 1	11.27	6.52	9.60	29.24	16.63	206.71
Control 2	8.72	5.59	3.13	0.00	0.00	0.00
R2*	22.93	11.67	11.17	162.95	108.76	256.86
R2**	25.31	13.08	12.23	190.25	133.98	290.73

* **Control 1**:Un-inoculated soil, **Control 2**:inoculated soil with FOL-5, **(R1*)** = root stock 1G-48-6031 (inoculated soil with FOL), **(R1**)** = root stock 1G-48-6032 (Un-inoculated soil with FOL).
*Phenol contents were calculated as milligrams of catechol per one -gram fresh weight

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