Potential effects of silver nanoparticles, synthesized from *Streptomyces clavuligerus*, for controlling of wilt disease caused by *Fusarium oxysporum*

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Background
Fusarium oxysporum causes wilt disease, which is considered a destructive disease, leading to decreased growth and death of most infected plants.
Materials and methods
After 7 days of incubation of *Streptomyces clavuligerus* on starch nitrate medium, the synthesis of silver nanoparticles (AgNPs) was done by using the supernatant from the microorganism. The color changed to dark brown, proving the formation of AgNPs. The size of AgNPs was analyzed using transmission electron microscope. Various concentrations of AgNPs (20, 40, 60, 80, and 100 µl) were investigated against *F.?oxysporum* by using agar well diffusion method. Disease symptoms, disease index percent, phytochemicals, and metabolic indicators of resistance in plant, such as the reaction to induction of systemic resistance, were recorded in tomato plants.
Results and conclusion
The resultant AgNPs had size from 4 to 38 nm and were oval to spherical in shape. The observed inhibition zones were 12, 18, 19, 23, and 27 mm in diameter correspondingly. The growth of Fusarium has been reduced by 60, 40 ppm, and followed by 20 ppm. Treatment with different concentration of nanoparticles resulted in different responses regarding the total phenol content, proline content, and total protein of Fusarium-infected plants. Applications of 60 ppm by foliar shoot+root immersion and root immersion methods were the best treatments and reduced percent disease indexes by 8 and 11%, respectively. Therefore, it could be suggested that the application of tested treatments could be commercially used for controlling Fusarium wilt disease of vegetable plants, as they are effective against this disease, are less expensive, and are safe.

Keywords:
Fusarium oxysporum, silver nanoparticles, *Streptomyces clavuligerus*, transmission electron microscope, tomato plant, wilt disease

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Introduction

Plants are affected with diseases by an assortment of pathogenic microorganisms that present in their surroundings and in soil rhizosphere. Plant disease can be caused by various pathogens, including viruses, fungi and bacteria, which lead to considerable loss in crop around the world [1]. Among the soil pathogens, *Fusarium* spp. are soil-borne fungi that synthesize various mycotoxins (secondary metabolites), which belong to the class of fumonisins, zearalenone, trichothecenes, and nivalenol [2]. The secondary metabolites are synthesized owing to various stressful conditions (fungus-plant and/or environmental interaction), and these mycotoxins have specific type of biological activities, such as toxicity, phytotoxicity, and antibiosis [3]. For example, *Fusarium oxysporum* causes the Fusarium wilt disease, which affects the crop yield negatively and significantly decreases the quality and the quantity of the crop [4,5]. Disease suppression using the biocontrol agents is the sustained manifestation of the interaction among the biocontrol agent, the pathogen, the plant, the physical environment, and the microbial community around the plant [6]. To control plant disease, one of the most important strategies for defense is the utilization of nanotechnology by synthesis of silver nanoparticles (AgNPs) [7,8]. However, nanoparticles may enhance the growth of plants by improving nitrogen-fixation capability and photosynthesis in roots and leaves, respectively, where nanoparticles could encourage conversion efficiency and the energy utilization [9,10]. AgNPs were applied for the inhibiting the harmful infections and the control of microorganisms. Moreover, many scientists have

proved that the antimicrobial activities of AgNPs are owing to the positive charge that reserve AgNPs in responsive with proteins of negatively charged on the cell membranes, and consequently contributing to the antimicrobial activities of AgNPs [11,12]. This research expected to synthesize AgNPs utilizing the actinomycetes strain *Streptomyces clavuligerus*, to characterize the resultant AgNPs using transmission electron microscope (TEM), and to investigate the antagonistic effect of the resultant AgNPs on the isolated *F. oxysporum*, which causes wilt disease in plants.

Materials and methods

Silver nanoparticles

Biological synthesis of Silver nanoparticles

S. clavuligerus was incubated for 7 days on a starch nitrate medium (g/l): starch 10.0, potassium nitrate 2.0, K_2HPO_4 1.0, $MgSO_4.7H_2O$ 0.5, NaCl 0.5, $FeSO_4$ 0.01 and $CaCO_3$ 3.0, and 1000 ml H_2O, with the pH adjusted to 7.0. Thereafter, the supernatant of *S. clavuligerus* was used for the synthesis of AgNPs. A solution of silver nitrate (1 mmol/l) was prepared by dissolving 0.017 g of the compound in 100 ml of distilled water. Then, 95 ml of silver nitrate solution was added to 5 ml of *S. clavuligerus* supernatant and incubated again for 7 days at room temperature under dark conditions, and color change was observed [13].

Silver nanoparticles shape and size characterization using transmission electron microscope

This study was undertaken to know the size and shape of AgNPs. The TEM image was carried out using electron probe micro-analyser JEOL – JXA 840 A, Model (Japan). Place. Thin films of the sample were prepared on a coated copper grid by just placing a very small amount of the sample on the grid. Then the film on the TEM grid was kept for drying, and the images of AgNPs were taken.

Isolation and maintenance of pathogen (Fusarium oxysporum)

F. oxysporum was isolated from infected wilted tomato plants according to Katan *et al.* [14] and identified macroscopically according to morphological features [15]. The isolated fungus was maintained on PDA at 24°C. To induce sporulation, cultures were transferred on PDA at 24°C for 6 days. Conidial suspensions were prepared as described in Boedo *et al.* [16]. Spore density was counted by a hemocytometer and adjusted to 107 spores per ml, and then pathogen was confirmed by pathogenicity test according to Hibar *et al.* [17].

Antagonistic effects of isolated bacteria against Fusarium oxysporum

The antimicrobial activity was investigated by using agar well diffusion method as follows: 20.0 ml of the media (SDA for pathogenic fungi at 28–30°C) was inoculated with 20.0 µl of the prepared *F. oxysporum* suspensions and poured in 9.0-cm diameter plates, mixed well, and allowed to solidify. After solidification, holes of 9.0-mm diameter were made in the agar plate by the aid of a sterile Cork borer. For each sample, duplicate holes were made and then different concentrations of the AgNPs were poured in the prepared holes using an automatic micropipette. The petri dishes were kept in a refrigerator for 1 h to permit homogenous diffusion of the antimicrobial agent before growth of the test microorganisms, and then the plates were incubated at 28°C for 72 h for *F. oxysporum*. The antimicrobial activities were determined by measuring the diameter of the inhibition zone [18].

Plant material and growing conditions

For the present investigation, 4-week-old tomato seedlings were obtained kindly from Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Greenhouse experiment

This study was conducted in a pot experiment at an experimental farm station of Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University. The minimum inhibitory concentrations of AgNPs synthesized from *S. clavuligerus* (20, 40, and 60 µl) were added by using different applications methods, which are as follows: foliar shoot (FS) until dropping, root immersion (RI) for 10 min, and FS+RI before 1 week of infection with *F. oxysporum*. Complete block design was used with nine treatments and two controls (each has five replicates). Disease development was recorded for 15 days after inoculation. Disease index was recorded. The plant samples were collected for physiological and biochemical indicators for resistance analysis when the plants were 40 days old.

Disease symptoms and disease index

Disease symptoms were assessed 60 days after inoculation, and the disease index was evaluated according to Demir *et al.* [19]. Percent disease index (PDI) was calculated using the five-grade scale according to the following formula:

$$\mathrm{PDI} = (1n_1+2n_2+3n_3+4n_4)100/4n_t,$$

where $n1$–$n4$ is the number of plants in the indicated classes and n_t is the total number of plants tested. The

percent protection was calculated using the following formula:

$$\text{Protection}\,(\%) = \frac{AB}{A} \times 100\%,$$

where A=PDI in infected control plants and B=PDI in infected-treated plants.

Determination of phytochemicals

Determination of phenolic compounds (mg/100 g of dry weight) was carried out according to the method described by Daniel and George [20].

Contents of free proline (mg/100 g of dry weight) were determined according to the method described by Bates *et al.* [21].

Total soluble proteins (mg/100 g of dry weight) were determined according to Lowery *et al.* [22].

Peroxidase activity was assayed according to that method described by Srivastava [23].

The activity of polyphenol oxidase enzyme was determined according to the method adopted by Matta and Dimond [24].

Statistical analyses

The experiment data were subjected to one-way analysis of variance, and the differences between means were separated using Duncan's multiple rang test and the LSD at 5% level of probability using Co-state software [25].

Results

Biological synthesis of silver nanoparticles

After the end of the incubation period, color changed into dark brown owing to the synthesis of Ag^+ ions, and this confirms the synthesis of AgNPs. The occurrence of a dark brown color in solution of the *S. clavuligerus* filtrate is the sign for the formation of AgNPs.

Silver nanoparticles characterization is produced by *Streptomyces clavuligerus* using transmission electron microscope

The results in Fig. 1 observed that the AgNPs showed varying sizes according to the stimulation of surface plasmon vibrations that appear in these AgNPs ranging from oval to spherical in shape. Moreover, under magnification of 100 nm, the size of AgNPs ranged from 13 to 38 nm (Fig. 1a), whereas under magnification of 200 nm, the sizes ranged from 4 to 16 nm (Fig. 1b).

Antifungal activity in-vitro of silver nanoparticles against *Fusarium oxysporum*

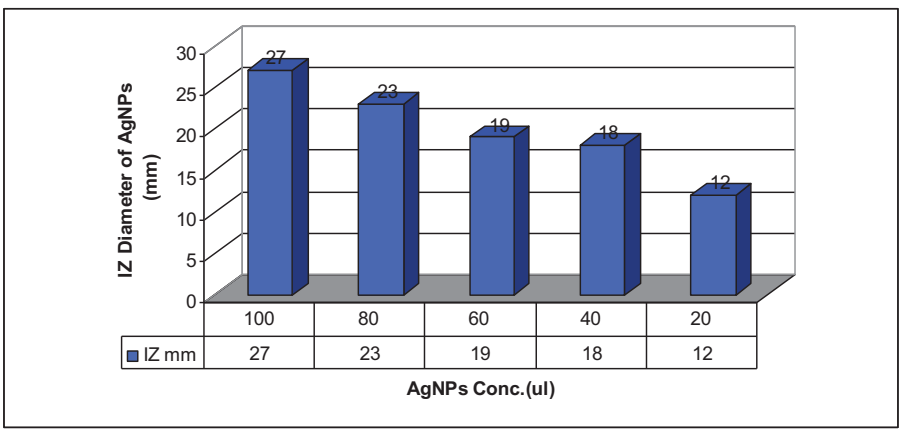
Various concentrations of AgNPs (20, 40, 60, 80, and 100 µl) were investigated against *F. oxysporum,* which causes wilt disease in tomato, by agar well diffusion method. The results observed in Fig. 2 and Photo 1 show that the concentrations of AgNPs of 20, 40, 60, 80, and 100 µl gave inhibition zones of 27, 23, 19, 18, and 12 mm in diameter, respectively.

The effect of silver nanoparticles on percent disease incidence and protection % of tomato plants infected with *Fusarium oxysporum*

Results in Table 1 showed that all applied concentrations of AgNPs synthesized from *S. clavuligerus* using different application methods (FS, FS+RI, and RI) reduced significantly wilt PDI caused by *F. oxysporum* compared with untreated infected control plants, which showed infection percentage reaching up to 83.33%.

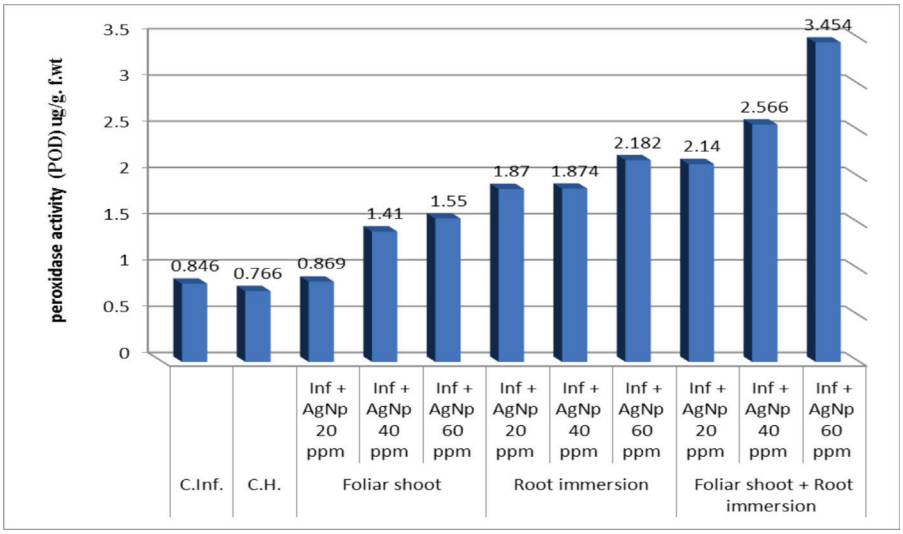
Moreover, the results proved that the application of AgNP concentrations at 20, 40, and 60 µl by FS+RI

Figure 1



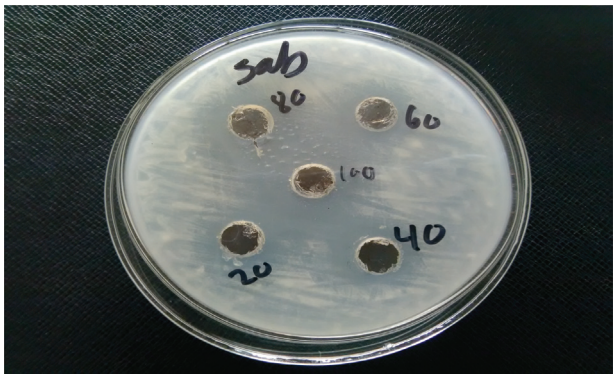
Transmission electron microscope of silver nanoparticles produced by *Streptomyces clavuligerus* (a) scale 200 nm and (b) scale 100 nm.

Figure 2



Antifungal activity of the silver nanoparticles obtained from *Streptomyces clavuligerus* against *Fusarium oxysporum.*

Photo 1



The antifungal activity of the silver nanoparticles obtained from *Streptomyces clavuligerus* against *Fusarium oxysporum.*

method was the best treatment, which gave reduced PDI (13.25, 11.25, and 8%) and caused higher protection by 84.10, 86.50, and 90.40%, respectively. This was followed by AgNP concentrations at 20, 40 and 60 µl with RI method, which reduced PDI by 26.5, 24.25, and 22.75% and caused protection by 68.20, 70.90, and 72.80%, respectively. Finally, the AgNPs at concentrations of 20, 40, and 60 µl, with FS method reduced the PDI by 63.25, 56.00, and 55.50% and cause protection by 24.10, 32.80, and 33.39%, respectively.

Physiological and metabolic changes in plant

Results shown in Table 2 revealed that total phenol and proline content as well as total soluble proteins of tomato plants significantly increased in response to the infection with *F. oxysporum*. However, treatment with AgNPs synthesized from *S. clavuligerus*, resulted in different responses of fusarium-infected plants. These responses were different owing to the concentrations of AgNPs and to the methods of application. Concerning the effect of different concentrations from AgNPs with different application methods on total phenols of the infected plants with *F. oxysporum*, it was found that treatments with concentrations of AgNPs of 20, 40, and 60% using FS+RI and RI methods were the best treatments, followed by concentrations of AgNPs at 20, 40, and 60% using RI, FS, and FS+RI methods, respectively. Then came next, AgNPs at 20, 40, and 60% using RI, FS, and FS+RI methods, respectively. This observed increased was found to be statically significant.

In addition, it is clear from Table 2 that proline contents of infected tomato plants significantly increased owing to all applied inducers. Application of AgNPs at 60, 40, and 20% using FS+RI method was the best treatments, followed by AgNPs at 60, 40, and 20% using RI methods, and next came AgNPs at 60, 40, and 20% using FS method, correspondingly. This observed increased was found to be statically significant.

In this context, the data of Table 2 showed that all tested inducers, with three exceptions, increased total soluble protein contents in infected plants compared with the untreated infected control. These exceptions were treatment by AgNPs at 20, 40, and 60 using IR and FS methods. Applications of AgNPs at 60, 40, and 20% using FS+RI method were the best treatments, followed by AgNPs at 60, 40, and 20% using RI and FS methods, respectively. These changes were found to be statistically significant.

Table 1 The effect of silver nanoparticles synthesized from *Streptomyces clavuligerus* on percent of disease incidence and protection % of tomato plants infected with *Fusarium oxysporum*

Treatments		Percent disease incidence (%)	Protection (%)
Methods of application	Concentration (µl)		
Foliar shoot	Inf+AgNP 20 ppm	63.25	24.10
	Inf+AgNP 40 ppm	56.00	32.80
	Inf+AgNP 60 ppm	55.50	33.39
Root immersion	Inf+AgNP 20 ppm	26.50	68.20
	Inf+AgNP 40 ppm	24.25	70.90
	Inf+AgNP 60 ppm	22.75	72.80
Foliar shoot+root immersion	Inf+AgNP 20 ppm	13.25	84.10
	Inf+AgNP 40 ppm	11.25	86.50
	Inf+AgNP 60 ppm	8.000	90.40
Control infected		83.33	0000

AgNPs, silver nanoparticles.

Table 2 Effect of silver nanoparticles synthesized from *Streptomyces clavuligerus* on total phenol content, proline content, and protein contents in tomato plants infected with *Fusarium oxysporum*

Treatments		Total phenol	Efficacy (%)	Proline content	Efficacy (%)	Total protein	Efficacy (%)
Methods of application	Concentration (µl)						
Foliar shoot	Inf+AgNP 20 ppm	1.37**	30	2.06**	3.5	24.61**	–6.8
	Inf+AgNP 40 ppm	1.41**	34	2.64**	32	25.92**	–1.8
	Inf+AgNP 60 ppm	1.71**	62	2.75**	38	34.18**	29.4
Root immersion	Inf+AgNP 20 ppm	1.39**	32	2.80*	40	26.41**	00
	Inf+AgNP 40 ppm	1.78**	69	3.42**	71.8	34.18**	29.4
	Inf+AgNP 60 ppm	2.23**	112	3.45**	73.3	41.6**	55
Foliar shoot+root immersion	Inf+AgNP 20 ppm	1.58**	50	3.76**	88.9	41.64**	57.6
	Inf+AgNP 40 ppm	2.36**	124	4.02**	102	43**	62
	Inf+AgNP 60▒ppm	2.58**	145	4.85**	143	45.1**	70
Control infected		1.05**	00	1.99**	00	26.41**	00
Control healthy		0.92**	–	1.35**	–	23.34**	–
LSD at 0.05*		0.40		0.4		6.6	

AgNPs, silver nanoparticles. *LSD, least significant difference. Variants possessing the different latter are statistically significant $P<0.05$. **statistically significant $P<0.05$.

Oxidative enzymes activity

Results in Figs 3 and 4 show that the alterations in the action of oxidative enzymes [peroxidase and polyphenol oxidase (PPO) enzymes] in fusarium-infected tomato plants were significantly increased than that of noninfected ones (control). Furthermore, treatment with AgNPs synthesized from *S. clavuligerus* (AgNPs) resulted in different responses of infected plants by fusarium. These reactions were different owing to the method of application and the concentration of AgNPs.

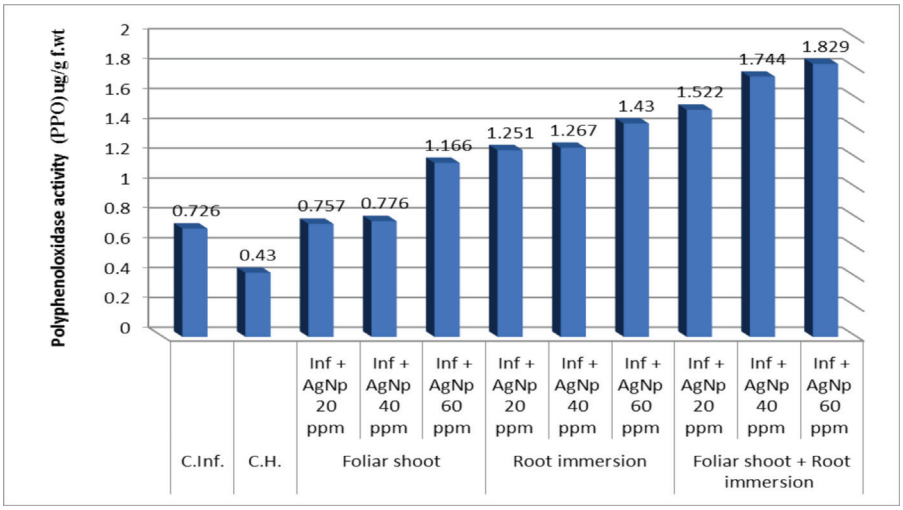
All applied inducers significantly increased polyphenol oxidase and peroxidase activities in contrast with infected control. Applications of AgNPs at 60, 40, and 20% using FS+RI and IR methods were the best treatments, followed by AgNPs at 20, 40, and 60% using FS+RI and IR methods, correspondingly. Then came the AgNPs at 60, 40 and 20% using FS method, correspondingly, which resulted in increase of peroxidase activity.

Regarding PPO activity, it was found that applications of AgNPs at 60, 40 and 20% using FS+RI method were the best treatments, followed by AgNPs at 60, 40, and 20% using RI methods, correspondingly. Then next came AgNPs at 60, 40, and 20% using FS method, correspondingly. This increase was found to be statically significant.

Discussion

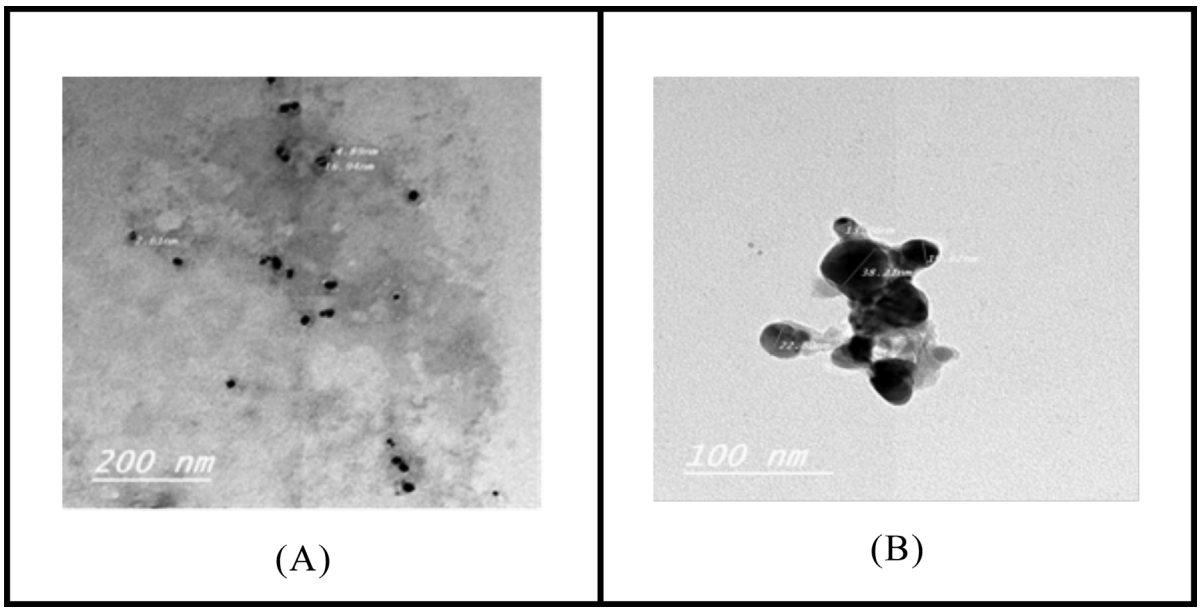
The aim of this study was the updating the results of systemic resistance (SR) in tomato plants versus wilt disease caused by *F. oxysporum*. Anyways, numerous trials have been successively done to conclude if induction of resistance was achieved and if AgNPs protect tomato plants against *F. oxysporum* by direct

Figure 3



Effect of silver nanoparticles synthesized from *Streptomyces clavuligerus* on the activity of peroxidase enzyme (µg/g fresh weight) at the shoots of infected tomato plants with *Fusarium oxysporum.*

Figure 4



Effect of silver nanoparticles synthesized from *Streptomyces clavuligerus* on the activity of polyphenol oxidase enzyme (µg/g fresh weight) at the shoots of infected tomato plants with *Fusarium oxysporum.*

inoculation or not. First, biological synthesis of AgNPs was determined by a dark brown color appearance in filtrate of the *S. clavuligerus*, and this is a sign for the synthesis of AgNPs in the filtrate [26]. Several reports stated that the biosynthesis of Ag^+ is owing to the electron shuttle quinines and reducing agents such as enzymes [27]. This was proved by TEM for the resulted AgNPs which was found to be ranging from spherical to oval in shape and ranging from 4 to 38 nm in size under different magnifications. On the contrary, the antifungal activity of using various concentrations of AgNPs (20, 40, 60, 80 and 100 µl) were investigated, and the results obtained proved that AgNPs have significant potential as an antifungal agent in treatment of *F. oxysporum*, which causes wilt disease in tomato plants. Alt *et al.* [28] demonstrated that AgNPs link to cell film and penetrate into the fungi, and then they create a site with a low molecular weight in the fungi center, and then AgNPs connect to respiratory sequence, which leads to stopping of cell division and finally cell death, as AgNPs liberate silver ions in fungal cell, leading to an increase in its function as an antifungal agent. Moreover, it was demonstrated that AgNPs link to

bacterial cell wall of negatively charged and leading to the denaturation of cell protein and cell death [29,30]. On the contrary, the results also showed different abilities of AgNPs synthesized from *S. clavuligerus* (AgNPs) according to the concentration used and the methods of applications in controlling fusarium wilt disease. The first indicator to govern the occurrence of SR in plants through treatment with AgNPs extracts is reduced percentage of disease index and highly increased protection against infection by *F. oxysporum*. The inhibition of the pathogenic infection may be owing to bioactive metabolites or siderophore production by *S. clavuligerus* through physicochemical and biological characteristics of AgNPs. The obtained results showed that, all applied concentrations of AgNPs synthesized from *S. clavuligerus* (AgNPs) using different methods of applications such as FS, FS+RI, and RI can reduce significantly disease incidence (PDI) caused by *F. oxysporum* compared with untreated infected control plants. However, several bioactive metabolites synthesized by the genus *Streptomyces* are widely recognized that can work to control phytopathogen and to give an advantage to endophytic or rhizosphere colonization [31]. On the contrary, two siderophores and two terpenes biosynthesis gene clusters were revealed. Moreover, plant defense can be stimulated by using priming phenomenon, leading to promotion of SR [32]. Furthermore, the promotion of SR in fusarium-infected tomato plants by AgNPs synthesized from *S. clavuligerus* (AgNPs) and the involved mechanisms were investigated. The obtained results showed that AgNPs worked as a potential inducer in induction of SR. All treatments with AgNPs especially using FS+RI method significantly increased phenolic level, proline amount, and total soluble protein content. Phenols work as substrates for several of antioxidant enzymes as well as free radical scavengers [33]. Many phenols are respected as inhibitors of pre-infection, supplying the plant with specific level of essential resistance against pathogenic microorganism. Therefore, cell wall lignification and phenol metabolism are engaged in and have conclusion for several cellular of ecological process and whole plant, that may supply plants the immunity against negative agents [34]. When microbial pathogens infect plants, they synthesize reactive oxygen species that promote cell death in the plant cells around the infected site to effect on the wall of pathogen and eliminate the disease [35,36]. The proline (amino acid) may work as potent scavenger of reactive oxygen species, and this prevents the promotion of cell death [37]. Our results also, proved that, the content of proline was increased in treated plant by AgNPs. These results agree with Shahnaz *et al.* [38] who showed that, the levels of proline were increased in the infected tomato plants compared with the noninfected control plant. During SR, induction all treatments AgNPs using different methods triggered peroxidase and PPO activities. This results demonstrated that in plants infected with fusarium the antioxidant enzymes activity increased significantly. PPO and PO action were greater in the treated plants with AgNPs, especially using FS +RI method, and challenged with fusarium, compared with infected plants. In this regard, improved PPO action against insect pests and disease has been proved in many beneficial microbe-plants associations [39]. These results proved also a difference in disease resistance mechanisms was engaged in promoting resistance by AgNPs synthesized from *S. clavuligerus* (AgNPs) with different application methods.

Conclusion

F. oxysporum causes wilt disease, which is considered a destructive disease, leading to decreased growth and death of most infected plants. AgNPs Bio-syntheses using *S. clavuligerus* with size from 4 to 38 nm oval to spherical shape reduce *F. oxysporum* by 60, 40 ppm and followed by 20 ppm. Applications of 60 ppm by FS+RI and RI were proved to be the best treatments that can reduce percent disease indexes by 8 and 11%, respectively. Therefore, it could be suggested that application of tested treatments could be commercially used for controlling fusarium wilt disease of vegetable plants, as they are effective against these disease, are less expensive, and are safe.

Acknowledgements

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References

1 Shabana YM, Abdel-Fattah GM, Ismail AE, Rashad YM. Control of brown spot pathogen of rice (*Bipolaris oryzae*) using some phenolic antioxidants. Braz J Microbiol 2008; 39:438–444.

2 Bhatnagar D, Ehrlich KC. Toxins of filamentous fungi. Chem Immunol 2002; 81:167–206.

3 Biro KK. Thesis: Adverse Effects of Deoxynivalenol and Ochratoxin A in Farm Animals. Comparative in vivo and in vitro studies. Dissertation University of Utrecht, Faculty of Veterinary Medicine, Department of Veterinary Pharmacology, 2003; 72–77.

4 Zaher A, Effat A, Abada KA, Zyton Marwa A. Effect of combination between bioagents and solarization on management of crown-and stem-rot of Egyptian clover. J Plant Sci 2013; 1:43–50.

5 Farrag AA, Attia MS, Younis A, Abd Elaziz AMA. Potential impacts of elicitors to improve tomato plant disease resistance. Al Azhar Bull Sci 2017; 9:311–321.

6 Barea JM, Azcon R, Azcon-Aguilar C. Mycorrhizal fungi and plant growth promoting rhizobacteria. In: Varma A, Abbott L, Werner D, Hampp R, editors. Plant surface microbiology. Heidelberg, Germany: Springer-Verlag 2004. pp. 351–371.

7 Bruchez M, Moronne J, Gin MP. Semiconductor nanocrystals as fluorescent biological labels. Science 1998; 281:2013–2016.

8 Sastry M, Ahmad A, Khan MI, Rajiv K. Biosynthesis of metal nanoparticles using fungi and actinomycetes. Curr Sci 2003; 85:162–170.

9 El-Batal AI, Sidkey NM, Ismail A, Rawhia A, Arafa A, Fathy RM. Impact of silver and selenium nanoparticles synthesized by gamma irradiation and their physiological response on early blight disease of potato. J Chem Pharm Res 2016; 8:934–951.

10 Sharaf AM, Kailla AM, Attia MS, Nofal MM. Evaluation of biotic and abiotic elicitors to control Meloidogyne incognita infecting tomato plants. Nat Sci 2016; 14:125–137.

11 Hamouda TA, Donovan MB, Shih A, Reuter JD, Baker A. Novel surfactant nano emulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi. Microbiol Res 2000; 156:1–7.

12 Dragieva I, Stoeva P, Stoimenov E, Pavlikianov XX, Klabunde K. Complex formation in solutions for chemical synthesis of nanoscaled particles prepared by borohydride reduction process. Nanostruct Mater 1999; 12:267–270.

13 El-Ghwas DE, El-Waseif AA. The synthesis of silver nanoparticals from *Streptomyces sp*. with antimicrobial activity. Int J Pharm Tech Res 2016; 9:179–186.

14 Katan T, Zamir D, Sarfati M, Katan J. Vegetative compatibility groups and subgroups in *Fusarium oxysporum f. sp. radicislycopersici*. Phytopathology 1991; 81:255–262.

15 Nelson PE, Toussoun TA, Marasas WO. Fusarium species. An illustrated manual for identification. USA, University Park and London, UK: The Pennsylvania State University Press 1983. p. 193.

16 Boedo C, Benichou S, Berruyer R, Bersihand S, Dongo A, Simoneau P, *et al.* Evaluating aggressiveness and host range of Alternaria dauci in a controlled environment. Plant Pathol 2012; 61:63–75.

17 Hibar K, Edel-Herman V, Steinberg C, Gautheron N, Daami-Remadi M, Alabouvette C, El Mahjoub M. Genetic diversity of *Fusarium oxysporum* populations isolated from tomato plants in Tunisia. J Phytopathol 2007; 155:136–142.

18 Oluwafemi F, Debiri F. Antimicrobial effect of *Phyllanthus amarus* and *Parquetina nigrescens* on *Salmonella typhi*. Afr J Biom Res 2008; 11:215–219.

19 Demir S, Türkmen Ö, Sensoy S, Akköprü A, Erdinc MC, Yıldız M, Kabay T. Reactions of melon landraces grown in the LakeVan Basin to the physiological races of *Fusarium oxysporumf. sp. melonis*. Eur J Hortic Sci 2006; 71:91–95.

20 Daniel HD, George CM. Peach seed dormancy in relation to indogenous inhibitors and applied growth substances. J Am Soc Hortic Sci 1972; 97:651–654.

21 Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies plant and soil. Plant Soil 1973; 39:205–207.

22 Lowery OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin reagent. J Biol Chem 1951; 193:265–275.

23 Srivastava SK. Peroxidase and polyphenol oxidase in Brassica juncea plants infected with *Macrophomina phaseolina* (Tassi) Goid and their implications in disease resistance. Phytopathology 1987; 77:249–254.

24 Matta A, Dimond AE. Symptoms of Fusarium within relation to quantity of fungus and enzyme activity in tomato stems. Phytopathology 1963; 53:544–578.

25 Snedecor GW, Chochran WG. Statistical methods. 7th ed. Iowa, USA: Iowa State University. Press 1982.

26 Chan PM, Yuen T, Ruf F. Method for multiplex cellular detection of mRNAs using quantum dot fluorescent in situ hybridization. Nucleic Acids Res 2005; 33:161–168.

27 Navin J, Arpit B, Sonali M, Tarfdar JC, Jitendra P. Extracellular biosynthesis and characterization of silver nanoparticles using Aspergillus flavus NJP08: a mechanism perspective. Nanoscale 2011; 3:635–641.

28 Alt V, Bechert T, Steinrucke P, Wagener M, Seidel P, Dingeldein E, *et al.* An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement. Biomaterials 2004; 25:4383–4391.

29 Lin Y, Vidic RD, Stout JE, McCartney CA, Yu VL. Inactivation of Mycobacterium avium by copper and silver ions. Water Res 1998; 32:1997–2000.

30 Zawrah MF, Abd El-Moez SI. Antimicrobial activities of gold nanoparticles against major food-borne pathogens. Life Sci J 2011; 8:37–44.

31 Chandra G, Chater KF. Developmental biology of *Streptomyces* from the perspective of 100 actinobacterial genome sequences. FEMS Microbiol Rev 2014; 38:345–379.

32 Berg G, Smalla K. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 2009; 68:1–13.

33 Martin- Tanguy J. Metabolism and function of polyamines in plants: recent development (new approaches). Plant Growth Regul 2001; 34:135–148.

34 Sudhakar N, Nagendra-Prasad D, Mohan N, Murugesan K. Induction of systemic resistance in *Lycopersicon esculentum* cv.PKM1 (tomato) against Cucumber mosaic virus by using ozone. J Virol Methods 2007; 139:71–77.

35 Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 2004; 55:373–399.

36 Adi M, Jens P, Brotman Y, Mikhail K, Iris S, Henryk C, Renal G. Stress responses to tomato yellow leaf curl virus (TYLCV) infection of resistant and susceptible tomato plants are different. Metabolomics 2012; 6:1–13.

37 Chen C, Dickman MB. Proline suppresses apoptosis in the fungal pathogen *Colletotrichum trifolii*. Plant Pathol 2005; 102:3459–3464.

38 Shahnaz AMA, Moubasher H, Mahmoud MG, Madany MY. Induced systemic resistance: an innovative control method to manage branched broomrape (Orobanche ramosa L.) in tomato. IUFS J Biol 2013; 72:9–21.

39 Harish S, Kavino M, Kumar N, Balasubramanian P, Samiyappan R. Induction of defense-related proteins by mixtures of plant growth promoting endophytic bacteria against Banana bunchy top virus. Bio Control 2009; 51:16–25.