OPEN ACCESS Plant Protection, 08 (04) 2024. 635-648 DOI:10.33804/pp.008.04.5343

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Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)

http://esciencepress.net/journals/PP

Research Article

EFFICACY OF SILVER NANOPARTICLES FROM *FUSARIUM SOLANI* AND MYCORRHIZAL INOCULATION FOR BIOLOGICAL CONTROL OF *FUSARIUM* WILT IN TOMATO

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ARTICLE INFO

Article history

Received: 19th August, 2024

Revised: 19th October, 2024

Accepted: 27th October, 2024

Keywords

Silver nanoparticles

Fusarium solani

Mycorrhizal inoculation

Fusarium wilt

Biological control

ABSTRACT

The present study investigates the characterization of silver nanoparticles (AgNPs) synthesized using *Fusarium solani* and their impact on tomato seed germination, plant growth, and disease resistance. A visible color change from yellow to dark smoky indicated the formation of AgNPs, while UV-visible spectrophotometry revealed an absorbance peak at 437 nm, confirming their presence. Atomic force microscopy analysis showed that the AgNPs ranged from 0 to 39.27 nm in size, with an average height of 5.772 nm, while scanning electron microscopy highlighted their diverse surface morphology. The application of AgNPs and mycorrhizal fungi significantly improved tomato seed germination rates, plant height, and dry weight compared to untreated plants infected with *Fusarium oxysporum*. The germination rate increased to 81.15% with mycorrhizal fungi and 80.02% with AgNPs treatment alone, compared to 35.63% in infected plants. Plant height also increased, reaching 17.95 cm in mycorrhiza-treated plants and 17.08 cm in those treated with AgNPs. Furthermore, the dry weight and chlorophyll content were significantly higher in treated plants, with mycorrhizal-inoculated plants showing a dry weight of 0.63 g and a chlorophyll content of 28.53 mg/g. AgNP treatment similarly enhanced these parameters infection severity of *F. oxysporum* was reduced, with the lowest rate observed in plants receiving both AgNPs and mycorrhizal treatments. These results indicate that AgNPs and mycorrhizal fungi offer effective protection against fungal pathogens while promoting overall plant health, highlighting their potential for use in sustainable agricultural practices.

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INTRODUCTION

Tomatoes are among the most important crops globally and are considered a staple food for many people. They are highly valued in various food industries due to their rich nutritional content, including essential vitamins and minerals such as iron and phosphorus, as well as their therapeutic properties, being abundant in antioxidants. As a result, tomatoes are widely cultivated in numerous countries (Alnuaimy et al., 2016).

Tomato plants are highly vulnerable to a variety of biotic stressors that can adversely affect their growth, yield, and quality (Asghar et al., 2020; Mukhtar and Kayani, 2020; Saeed and Mukhtar, 2024; Yaseen et al., 2024a, b). These stressors include pathogenic organisms such as fungi, bacteria, viruses, and nematodes, which are responsible for diseases like late blight (*Phytophthora*

infestans), bacterial wilt (*Ralstonia solanacearum*), tomato mosaic virus (ToMV), and root-knot nematode infestation (*Meloidogyne* spp.) (Aslam et al., 2017a, 2017b, 2019; Ahmed et al., 2021; Aslam and Mukhtar, 2023a, 2023b, 2024). Additionally, insect pests such as whiteflies (*Bemisia tabaci*), aphids (Aphidoidea), and tomato fruit worms (*Helicoverpa armigera*) pose significant threats by feeding on plant tissues and acting as vectors for disease transmission (Gulzar et al., 2020).

The impact of these biotic stressors can result in diminished fruit quality, reduced yields, and potentially total crop failure if not effectively managed. The severity of biotic stress on tomato crops is often intensified by suboptimal crop management practices, environmental conditions conducive to pest and pathogen proliferation, and the overuse or misuse of chemical pesticides, which can lead to the emergence of resistant pest strains.

To mitigate the effects of biotic stressors on tomato crops, it is essential to implement effective management strategies. These include the cultivation of resistant varieties, the adoption of integrated pest management (IPM) practices, and the application of sustainable agricultural techniques (Azeem et al., 2021; Mukhtar et al., 2021; Saeed et al., 2021, 2023; Aziz et al., 2024; Yaseen et al., 2023, 2024c).

To enhance agricultural productivity, many farmers rely on chemical pesticides. However, these pesticides can cause poisoning and pose significant health risks to farmers when not used properly. Over the long term, improper pesticide use also threatens the environment by contributing to environmental degradation and disrupting the activities of beneficial soil microorganisms. Moreover, the misuse of pesticides can lead to the development of pesticide-resistant pest strains, further complicating agricultural production (Al-Mousawi et al., 2017).

In response to the challenges posed by conventional chemical pesticides, researchers have increasingly turned to alternative, more sustainable pest control methods. Among these, the use of nanomaterials in agriculture has emerged as a promising approach with significant ecological benefits. Nanomaterials are known for their biodegradability, which reduces the likelihood of pests developing resistance. Additionally, they release active ingredients gradually, ensuring prolonged effectiveness. Numerous studies have demonstrated that various nanomaterials not only contribute to effective pest control but also promote overall plant growth and health (Nazir et al., 2019; Alloosh, 2020; Shahbaz et al., 2022).

Silver nanoparticles (AgNPs) stand out due to their exceptional properties, including high thermal and electrical conductivity and remarkable chemical stability. These nanoparticles are widely recognized for their potent antimicrobial activity, which has proven valuable in both industrial and medical applications. Importantly, the synthesis of AgNPs does not involve the production of toxic byproducts, making the process environmentally friendly. The synthesis can be achieved using a range of natural materials, such as plants, fungi, and bacteria, further highlighting the versatility and sustainability of this approach (Alloosh, 2020; Khan et al., 2021; Jabbar et al., 2022; Shahbaz et al., 2023).

Research has demonstrated that AgNPs provide superior control of plant diseases compared to synthetic fungicides. For instance, studies have reported that AgNPs, in combination with biocontrol agents such as *Fusarium oxysporum*, *Aspergillus niger*, and *A. flavus*, offer effective disease management and enhanced crop protection (Abd El-Aziz et al., 2015; Khan et al., 2017). Additionally, four nanomaterials viz. zinc oxide nanoparticles (ZnONPs), nickel oxide nanoparticles (NiONPs), iron oxide nanoparticles (Fe3O4NPs), and copper oxide nanoparticles (CuONPs) have been shown to control Fusarium wilt and promote the growth of common beans under greenhouse conditions (El-Sayed et al., 2023).

In addition to nanomaterials, the role of arbuscular mycorrhizal (AM) fungi in agriculture has gained significant attention. These fungi, prevalent in various agricultural ecosystems, form a mutualistic symbiotic relationship with plants. This symbiosis benefits both the plant and the fungus, as AM fungi facilitate the uptake of essential micro and macro-minerals by the plant. Moreover, AM fungi enhance the plant's resistance to soil-borne pathogens, improve tolerance to environmental stressors such as salinity and drought, and stimulate the production of growth-regulating substances like auxins and cytokinin. These benefits collectively contribute to increased agricultural productivity, surpassing the performance of non-mycorrhizal plants (Pagano et al., 2023).

The integration of nanomaterials with *Fusarium solani* and AM fungi in agricultural practices offers a powerful strategy for sustainable crop production, combining effective pest control with enhanced plant growth and resilience. Wang et al. (2022) reported that AM fungi mitigate the accumulation and potential phytotoxic effects

of nanomaterials, thereby reducing their damaging impact on plants and promoting growth. Therefore, the objective of the present study was to evaluate the effectiveness of silver nanoparticles in conjunction with *F. solani* and mycorrhizal inoculation for the biological control of Fusarium wilt disease in tomato plants (*Lycopersicon esculentum*), with a focus on enhancing disease resistance and promoting plant health.

MATERIALS AND METHODS

Sample collection

Samples were collected from the roots and rhizospheric soil of tomato plants showing symptoms of wilt. These samples were placed in clean plastic bags and transported to the laboratory for the isolation and purification of the pathogenic fungus, *Fusarium* sp.

Isolation and purification of the fungus

The fungus, *Fusarium* sp., was isolated from the infected plant roots following the method described by Abomughaid (2021). The roots were thoroughly washed with tap water to remove any soil debris, and then cut into 0.5 cm segments. These segments were surface-sterilized using 1% sodium hypochlorite solution for 5 min, followed by five rinses with sterile distilled water to eliminate any traces of the sterilizing agent. The sterilized root pieces were cultured on Potato Dextrose Agar (PDA) medium, with four pieces placed in each dish, and incubated at 25°C for five days.

For soil samples, fungal isolation was carried out according to the procedure of Fadhil et al. (2015). Serial dilutions were prepared using sterile distilled water, and 1 ml from each dilution was plated onto PDA medium. The plates were incubated at 25°C for five days.

Fusarium oxysporum and *Fusarium solani* were identified based on the morphological characteristics of the colonies, using the taxonomic key provided by Ellis (1971). Further confirmation was done through microscopic examination, and the Vitek2 compact system was employed for accurate diagnosis.

Preparation of fungal biomass

The biomass of the fungus *F. solani* was prepared by culturing it in a liquid medium containing the following components (g/L): KH_2PO_4 (7), K_2HPO_4 (2), $MgSO_4 \cdot 7H_2O$ (0.1), $(NH_4)_2SO_4$ (1.6), yeast extract (1.6), and glucose (10). The culture was incubated at 25°C for 7 days. After incubation, the fungal biomass was filtered using Whatman filter paper No. 1 and washed with distilled water to remove any residual medium components.

Next, 20 g of the fungal biomass was suspended in 200 ml of sterile distilled water and incubated at room temperature for 72 h. Following the incubation, the fungal cells were filtered again using Whatman filter paper No. 1 (Kamiar et al., 2016).

Synthesis of *F. solani* silver nanoparticles (AgNPs)

For the synthesis of silver nanoparticles, 50 ml of the fungal filtrate was mixed with 1 mM of silver nitrate ($AgNO_3$) solution and incubated at room temperature in the dark (Abeer et al., 2013). A color change from yellow to dark smoky grey indicated the successful synthesis of the nanomaterials. A negative control, consisting of 1 mM $AgNO_3$ solution alone, was incubated under the same conditions for comparison.

Examination of silver nanoparticles solution

a) UV-Visible spectrophotometry

The formation of silver nanoparticles was analyzed using UV-Visible absorption spectroscopy, measuring absorbance at wavelengths between 250 and 550 nm.

b) Atomic force microscopy (AFM)

A few drops of the AgNPs solution were placed on glass slides and left to dry at room temperature. The dried samples were then analyzed using Atomic Force Microscopy (AFM) at the Materials Research Department, Ministry of Science and Technology, following the method of Pavani et al. (2012).

c) Scanning electron microscopy (SEM)

The structural characteristics of the AgNPs solution were investigated using scanning electron microscopy (SEM) at the Materials Research Department, Ministry of Science and Technology, as described by Kamiar et al. (2016).

Isolation and identification of AM fungi from root-soil mixtures

The wet sieving and decanting method described by Gerdemann and Nicolson (1963) was used to isolate spores of arbuscular mycorrhizal (AM) fungi. The root-soil mixture was vigorously stirred with tap water using a glass rod for 30 sec. After allowing the mixture to settle for 10 sec to separate larger particles and organic matter, the remaining suspension containing soil, roots, hyphae, and spores was carefully poured through a series of three sieves with pore sizes of 85 µm, 65 µm, and 25 µm, respectively. The material retained on the sieves was then transferred to 9 cm diameter petri dishes. Spores, aggregates, and sporocarps were extracted using a pipette under a dissecting microscope. The identification of *Glomus mosseae* was based on the morphology of fresh spores, spore-bearing structures,

and, when present, the morphology of sporocarps (Powell and Bagyaraj, 2000).

Soil sterilization for the experiment

The soil mixture used in the experiment was sterilized using the formalin method described by Shanter Al-Qaysi et al. (2016). A 2% formalin solution was sprayed onto the soil, which was then tightly covered with nylon sheets for three days. After the designated period, the cover was removed, and the soil was exposed to sunlight for three additional days with regular turning to ensure the complete evaporation of formalin residues.

Seeds used in the study

Tomato (*Lycopersicon esculentum*) seeds, obtained from the College of Agriculture at the University of Baghdad, were used in this study. The seeds were divided into two groups: one group was soaked in silver nanoparticles (AgNPs) synthesized using the fungus *F. solani*, while the other group was soaked in distilled water for 24 h prior to planting.

Experimental design

The sterilized soil was evenly distributed into pots, each with a capacity of 3 kg. The experiment included the following treatments:

T1 = Tomato seeds only

T2 = Tomato seeds inoculated with *G. mosseae*

T3 = Tomato seeds inoculated with the pathogenic fungus *F. oxysporum*

T4 = Tomato seeds soaked in AgNPs solution prepared using *F. solani*

T5 = Tomato seeds soaked in AgNPs solution and inoculated with *F. oxysporum*

T6 = Tomato seeds soaked in AgNPs solution, inoculated with *G. mosseae* and the pathogenic fungus *F. oxysporum*

T7 = Tomato seeds inoculated with both *G. mosseae* and *F. oxysporum*

Measurements of plant growth rate

The growth parameters for all treatments were calculated at the end of the experiment as follows:

1. The germination Rate (%) was **c**alculated using the formula:

$$\text{Germination Rate} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

2. Plant height (cm)
3. Dry weight of the plant (g)
4. Total chlorophyll content in the plant (mg/g)

Percentage of infection with the pathogenic fungus *F. oxysporum*

The percentage of shoot and root infection was determined following the method described by Hassana and Hassan (2020).

Percentage of infection with mycorrhiza

The percentage of root infection by mycorrhiza was estimated by staining root fragments with Acid Fuchsin dye and examining them under a light microscope. The calculation was performed using the following formula:

$$\text{Percentage of mycorrhizal infection} = \frac{\text{Number of infected root fragments}}{\text{Total number of examined root fragments}} \times 100$$

Statistical analysis

Data were analyzed using the Statistical Analysis System (SAS) software (2018) to assess the effects of different treatments on the studied traits, employing a completely randomized design (CRD). Significant differences between means were determined using the Least Significant Difference (LSD) test.

RESULTS

Diagnosis of AgNPs solution by *F. solani*

Color change

The initial identification of the AgNP solution involving *F. solani* was based on a visible color change from yellow to a dark smoky color as illustrated in Figure 1.

UV-Visible spectrophotometer examination

UV-Visible spectroscopy was employed to detect AgNPs produced by F. solani. The analysis, conducted within the wavelength range of 250 to 550 nm, revealed a prominent absorbance peak at 437 nm, as shown in Figure 2.

Atomic force microscopy

AFM imaging was used to characterize the size, surface roughness, and protrusions of AgNPs synthesized by *F. solani*. The sizes of the biogenically produced silver particles ranged from 0 to 39.27 nm, with an average height of 5.772 nm and a root mean square height of 7.250 nm, as depicted in Figure 3.

Scanning electron microscopy

SEM images revealed the diverse surface morphology of AgNPs generated by F. solani, as shown in Figure 4. The nanoparticles displayed various shapes, with some appearing non-aggregated and others aggregated, likely due to the slide preparation process. The sizes of the biologically synthesized nanoparticles ranged from 26.71 to 51.05 nm, with an average size of 38.8 nm.

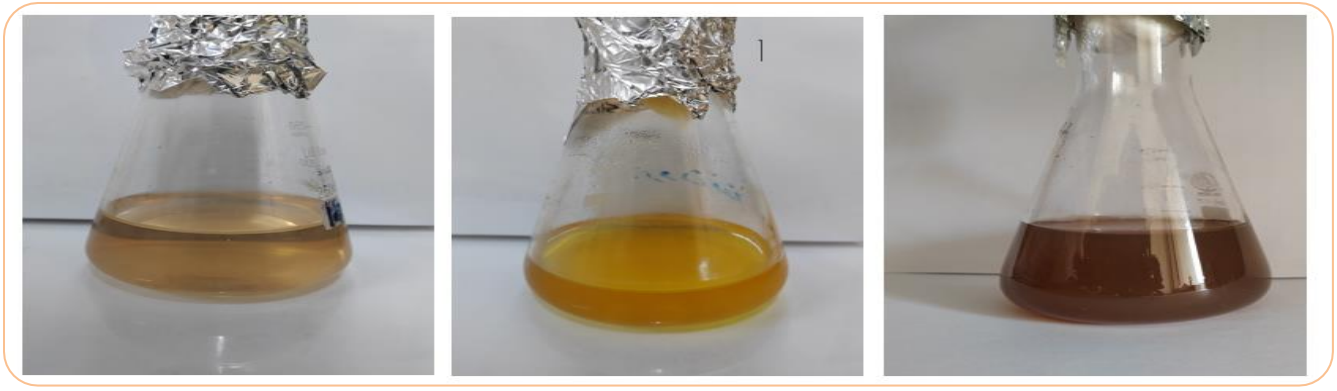

Figure 1. A (AgNO3) solution, (B) biomass of *F. solani* PDA and AgNO3) solution, (C) The initial of the silver nanoparticle solution by *F. solani*.

Measurements of plant growth rate

Germination rate (%)

The results demonstrated a significant improvement in the germination percentage of tomato seeds for all treatments compared to those treated with the *F. oxysporum* fungus, as shown in Table 1. Moreover, there were no significant differences in the germination percentage between tomato seeds inoculated with mycorrhiza and those treated with the AgNPs solution.

The intervention involving mycorrhizal fungi and AgNPs solution in the presence of the pathogenic fungus significantly enhanced the germination percentage of tomato seeds to 17.82% ($P < 0.05$), compared to the 8.23% germination rate observed in plants treated only with *F. oxysporum*. Similarly, the treatment with AgNPs solution alone, in the presence of the pathogenic fungus, also significantly improved the germination percentage of tomato seeds to 17.8%, compared to the plants treated with *F. oxysporum*.

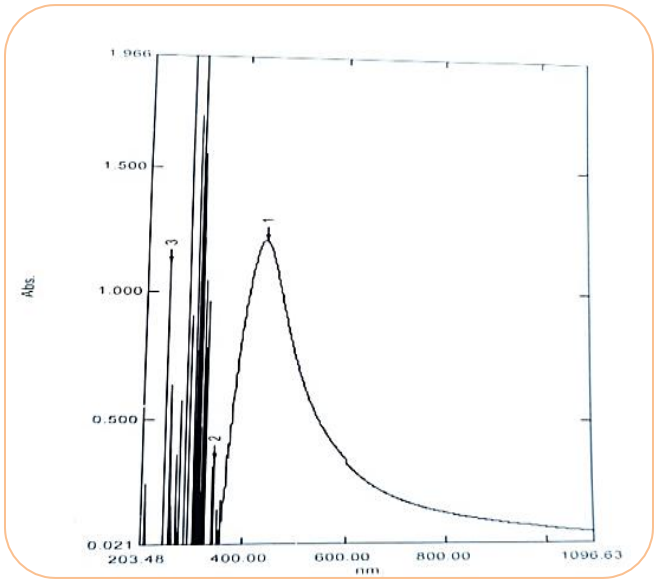

Figure 2. UV–visible spectroscopy for the AgNPs solution from *F. solani* absorbance peaks at at 437 nm.

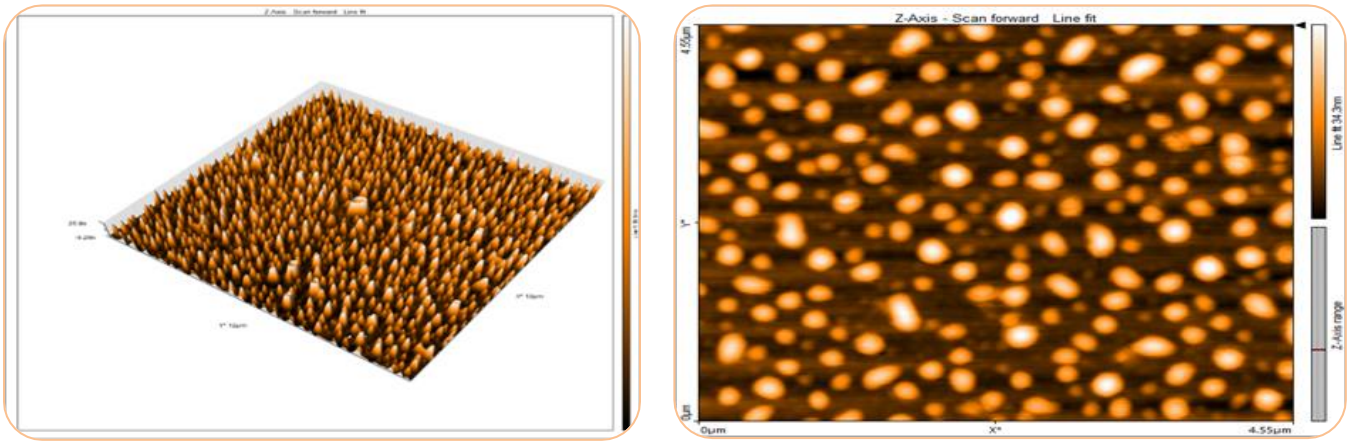

Figure 3. The size of nanoparticles of Silver (AgNPs) by *F. Solani* by AFM examination.

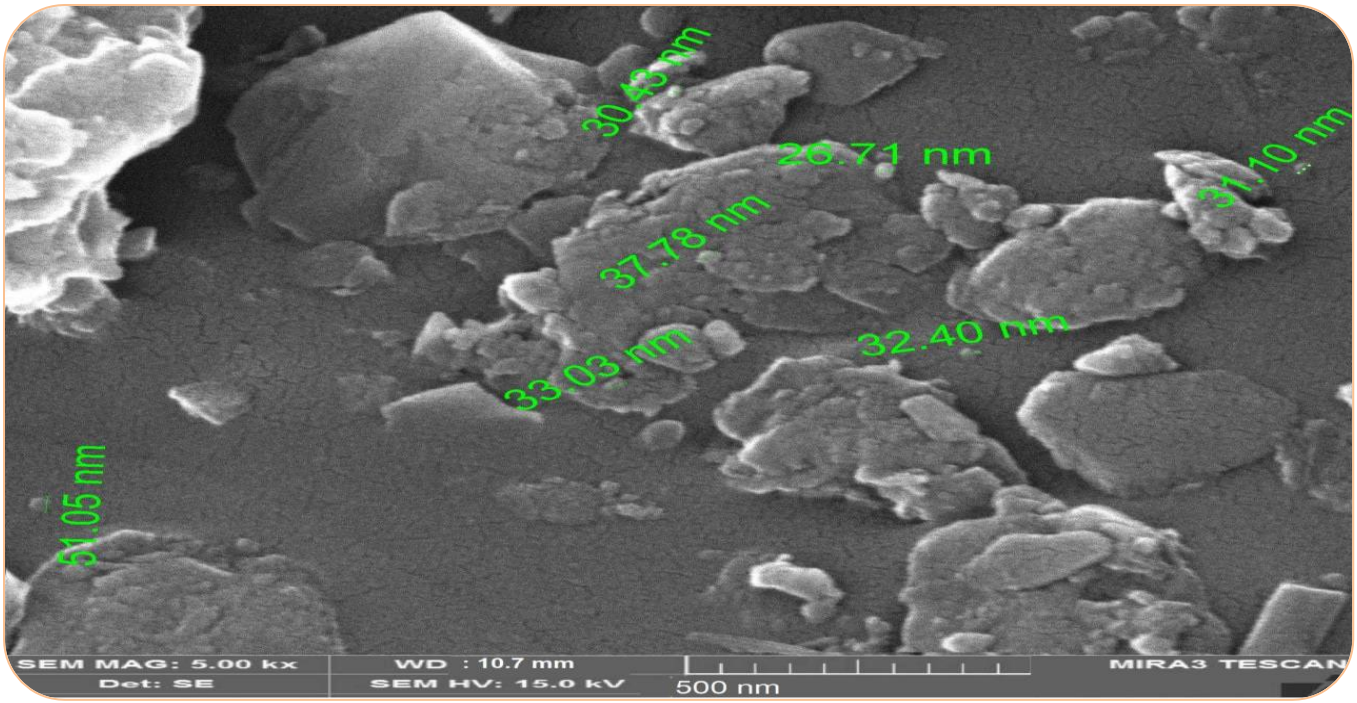

Figure 4. The size of nanoparticles of Silver (AgNPs) by *F. Solani* by SFM examination.

Table 1. Effect of AgNPs solution and mycorrhiza on germination rate (%) of tomato plants.

Treatment	Description	Germination rate (%)
T1	Tomato seeds only	0.71±16.82
T2	Tomato seeds inoculated with *G. mosseae*	0.84±17.95
T3	Tomato seeds inoculated with the pathogenic fungus *F. oxysporum*	0.57±8.23
T4	Tomato seeds soaked in AgNPs solution prepared using *F. solani*	0.82±17.8
T5	Tomato seeds soaked in AgNPs solution and inoculated with *F. oxysporum*	0.86±16.35
T6	Tomato seeds soaked in AgNPs solution, inoculated with *G. mosseae* and the pathogenic	0.69±17.82
Significant at level P≤0.05		* 9.552

Plant height (cm)

The results demonstrated a significant increase in plant height of tomato for all treatments compared to plants infected with *F. oxysporum*, as shown in Table 2. Specifically, plants inoculated with mycorrhizae reached an average height of 35.95 cm, while those treated with AgNPs solution reached 35.8 cm, both showing a notable increase compared to untreated plants.

Regarding the interaction between mycorrhizal inoculation and AgNPs solution in the presence of *F. solani* and *F. oxysporum*, the average height of plants was significantly greater at 35.82 cm compared to untreated plants. Moreover, plants treated with AgNPs solution exhibited a significant increase in height, reaching 23.35 cm, in contrast to plants infected with *F. oxysporum*.

Dry weight of the plant (g)

The results revealed a significant increase in the average dry weight of mycorrhiza-inoculated plants, averaging 0.63 g, compared to non-mycorrhiza plants. In contrast, plants treated with AgNPs solution by *F. solani* showed no significant difference in average dry weight, averaging 0.59 g, compared to untreated plants. Table 3 illustrates the effect of the treatments on the average dry weight.

The interaction between mycorrhiza inoculation and AgNPs solution by *F. solani*, in the presence of the pathogenic fungus *F. oxysporum*, resulted in a significant increase in average dry weight, reaching 0.65 g compared to untreated plants. Furthermore, plants treated with AgNPs solution exhibited a notable increase in dry weight, averaging 0.54 g, compared to plants infected with the pathogenic fungus *F. oxysporum*.

Table 2. Effect of AgNPs solution and mycorrhiza on plant height of tomato plants.

Treatment	Description	Plant height (cm)
T1	Tomato seeds only	0.71± 22.82
T2	Tomato seeds inoculated with *G. mosseae*	0.84± 35.95
T3	Tomato seeds inoculated with the pathogenic fungus *F. oxysporum*	0.57± 10.23
T4	Tomato seeds soaked in AgNPs solution prepared using *F. solani*	0.82± 35.8
T5	Tomato seeds soaked in AgNPs solution and inoculated with *F. oxysporum*	0.86±23.35
T6	Tomato seeds soaked in AgNPs solution, inoculated with *G. mosseae* and the pathogenic	0.69± 35.82
Significant at level P≤0.05		* 3.094

Table 3. Effect of AgNPs solution and mycorrhiza on dry weight of tomato plants.

Treatment	Description	Dry weight of tomato plants (g)
T1	Tomato seeds only	0.08± 0.57
T2	Tomato seeds inoculated with *G. mosseae*	0.11± 0.63
T3	Tomato seeds inoculated with the pathogenic fungus *F. oxysporum*	0.05± 0.28
T4	Tomato seeds soaked in AgNPs solution prepared using *F. solani*	0.04± 0.59
T5	Tomato seeds soaked in AgNPs solution and inoculated with *F. oxysporum*	0.07± 0.54
T6	Tomato seeds soaked in AgNPs solution, inoculated with *G. mosseae* and the pathogenic	0.07± 0.65
Significant at level P≤0.05		0.228 *

Total chlorophyll content in plants (mg/g)

Chemical analysis of total chlorophyll content in plants revealed a notable increase for mycorrhiza-inoculated plants, with an average content of 28.53 mg/g, compared to plants that were not inoculated with mycorrhiza. Similarly, plants treated with an AgNPs solution exhibited a significant rise in total chlorophyll content, averaging 28.04 mg/g, compared to those that did not receive the AgNPs treatment. Table 4 illustrates the effect of these treatments on the average total chlorophyll content.

In terms of the interaction between mycorrhiza inoculation and AgNPs solution treatment in the presence of the pathogenic fungus *F. oxysporum*, there was a significant increase in the average total chlorophyll content of the plants compared to untreated controls.

When compared to plants infected with *F. oxysporum*, mycorrhiza-treated plants that received the AgNPs solution showed a total chlorophyll content of 27.74 mg/g, which was significantly higher. Additionally, plants treated solely with the AgNPs solution had a total chlorophyll content of 16.58 mg/g, also showing a significant increase compared to those infected with the pathogenic fungus.

Table 4. Effect of AgNPs solution and mycorrhiza on total chlorophyll content of tomato plants.

Treatment	Description	Total chlorophyll content of tomato plants (mg/g)
T1	Tomato seeds only	1.36± 27.46
T2	Tomato seeds inoculated with *G. mosseae*	1.54± 28.53
T3	Tomato seeds inoculated with the pathogenic fungus *F. oxysporum*	1.07± 18.25
T4	Tomato seeds soaked in AgNPs solution prepared using *F. solani*	1.26± 28.04
T5	Tomato seeds soaked in AgNPs solution and inoculated with *F. oxysporum*	0.76± 16.58
T6	Tomato seeds soaked in AgNPs solution, inoculated with *G. mosseae* and the pathogenic	1.06± 27.74
Significant at level P≤0.05		4.029 *

Infection percentage of the pathogenic fungus *F. oxysporum*

The results indicated that all treatments exhibited lower severity of root infection by *F. oxysporum* compared to untreated plants, as shown in Table 5. Plants treated with the AgNPs solution did not show any significant differences in infection severity when compared to the control plants in the absence of the pathogenic fungus. However, in plants inoculated with mycorrhiza alone, a mild infection rate of 10.2% was observed, which was lower than the infection rate in untreated and infected plants.

Furthermore, the combined treatment with mycorrhiza and AgNPs in the presence of the pathogenic fungus significantly reduced infection severity. The lowest infection severity was observed in the combined treatment group (8.64%), followed by plants treated with the AgNPs solution alone (9.05%), compared to plants infected with *F. oxysporum* but untreated with mycorrhiza or AgNPs solution.

Mycorrhizal infection

The results of assessing mycorrhizal infection percentage, following the staining of tomato root sections at the conclusion of the experiment, revealed a 100% success rate of mycorrhizal colonization in the tomato roots. These findings align with those of Ali and Muhsen (2015), who observed that *Glomus* sp. mycorrhiza enhances infection rates in tomato plants, promotes symbiotic relationships, and boosts plant growth and disease resistance. The fungal infection of *G. mosseae* in tomato root cells at 40× magnification is shown in Figure 5.

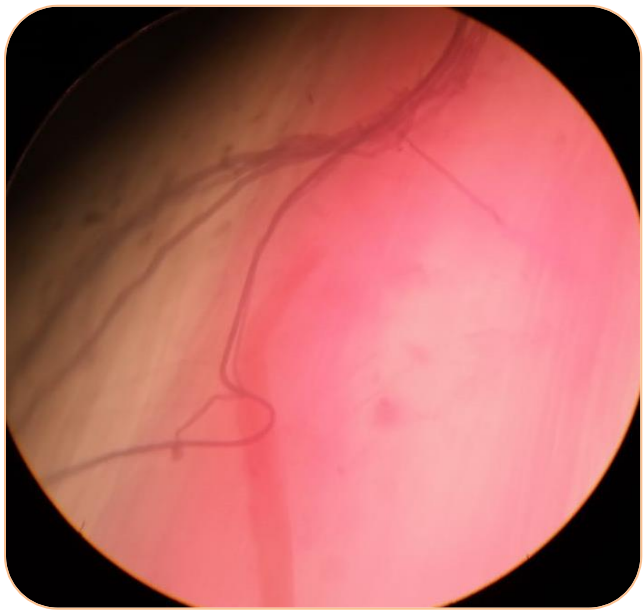

Figure 5. Fungal infection of *G. mosseae* in tomato root cells.

Table 5. Effect of AgNPs solution and mycorrhiza on percentage of infection with the pathogenic fungus *F. oxysporum*.

Treatment	Description	Percentage of infection of pathogenic fungus *F. oxysporum*
T1	Tomato seeds only	0.00± 0.00
T2	Tomato seeds inoculated with *G. mosseae*	0.63±10.02
T3	Tomato seeds inoculated with the pathogenic fungus *F. oxysporum*	2.72±41.12
T4	Tomato seeds soaked in AgNPs solution prepared using *F. solani*	0.00± 0.00
T5	Tomato seeds soaked in AgNPs solution and inoculated with *F. oxysporum*	0.71±9.05
T6	Tomato seeds soaked in AgNPs solution, inoculated with *G. mosseae* and the pathogenic	0.57±8.64
Significant at level P≤0.05		* 7.537

DISCUSSION

Tomatoes are among the most important crops globally, serving as a staple food for millions of people worldwide. Due to their widespread consumption, tomato cultivation is prevalent in many countries (Alnuaimy et al., 2016). However, tomato plants are highly susceptible to various pests and pathogens throughout their growth cycle, which can significantly impact both their growth and yield. One of the most damaging pathogens is the fungus *F. oxysporum*, which causes substantial crop losses. This fungus not only affects tomato plants but also exhibits resistance to chemical pesticides, making it particularly challenging to manage. *F. oxysporum* can persist in agricultural soils by forming chlamydospores, which further complicates control efforts. As a result, farmers often resort to excessive and indiscriminate use of

chemical pesticides to mitigate this issue, leading to negative consequences for the agricultural environment, including soil degradation and ecosystem imbalance (Gomaa et al., 2021).

Characterization of AgNPs solution synthesized by *F. solani*

UV-visible spectrophotometry

In this study, AgNPs synthesized by *F. solani* were characterized using UV-visible spectrophotometry. The analysis revealed a strong absorbance peak at 437 nm, indicating the successful synthesis of AgNPs. These findings align with previous studies, such as those by Sathiya et al. (2015), who reported a similar absorbance peak for AgNPs in the range of 340-560 nm, with a maximum absorbance at 440 nm. Moreover, the results are consistent with those of Abd El-Aziz et al. (2015), further validating the spectroscopic evidence of AgNP formation.

AFM

AFM analysis showed that the size of the biogenically synthesized AgNPs produced by *F. solani* ranged from 0 to 39.27 nm. This size distribution is in agreement with the findings of Shafiq et al. (2016), who reported a size range of 1 to 95.5 nm for AgNPs synthesized from *Fusarium graminearum* using AFM. The small size of these nanoparticles is particularly advantageous, as smaller particles tend to have a greater surface area, enhancing their antifungal activity. Biogenically synthesized AgNPs, owing to their small size, hold great promise as effective antifungal agents in agriculture (Mwangi, 2023).

SEM

The sizes of the biologically synthesized AgNPs were also examined using SEM, which revealed a size range of 26.71 to 51.05 nm, with an average size of 38.88 nm. These SEM results are consistent with the findings from the AFM analysis and corroborate previous research. For instance, Mwangi (2023) documented the sizes of silver nanoparticles produced by *Fusarium* species to be within the range of 12 to 20 nm. The similarity between the SEM and AFM findings further supports the reliability of the size characterization of these biologically synthesized nanoparticles.

Measurements of plant growth rate

The results of this study demonstrate that the pathogenic fungus *F. oxysporum* caused a significant reduction in all plant growth parameters compared to untreated tomato plants, as shown in Figure 6. This reduction is attributed to the fungus penetrating plant root tissues by secreting cell wall-degrading enzymes, which disrupt essential physiological processes, including nutrient absorption critical for plant growth and development. As a result, germination rates decreased, and plant length and weight were significantly reduced. Moreover, total chlorophyll content was lower in infected plants compared to untreated ones.

Figure 6. Plant growth rate A: (Plant+ mycorrhiza), B: (Plant+Nano+ Pathogenic fungus+mycorrhiza), C: (control), D: (Plant+Pathogenic fungus).

This decline in chlorophyll content is likely due to the secretion of fungal toxins that impair enzymatic activity, thereby affecting key physiological processes such as respiration, protein synthesis, and nutrient transport, which are essential for photosynthesis (Narayanan and Hyun, 2014). These findings align with previous research by Hassana and Hassan (2020).

In this study, AMF of the genus *G. mosseae* were used. The results showed a notable increase in plant height, average dry weight, and total chlorophyll content in tomato plants inoculated with mycorrhiza compared to untreated plants. This improvement can be attributed to the ability of mycorrhiza to enhance biomass production, potentially due to an increase in defensive enzyme activities, including peroxidase, polyphenol oxidase, chitinase, phenylalanine ammonia-lyase, and phenolic compounds (Ali and Muhsen, 2015).

Interestingly, inoculation with both mycorrhiza and *F. oxysporum* resulted in a significant increase in all plant growth parameters compared to untreated plants. However, plants inoculated with mycorrhiza alone exhibited a slight root infection by *F. oxysporum*, though the infection was far less severe than in plants without mycorrhizal inoculation. Mycorrhizae are known to stimulate plant defense mechanisms by inducing biochemical and physiological changes, such as increased secretion of defensive enzymes like β-1,3-glucanase, chitinase, and chitosonase. Some studies have also shown that mycorrhizae boost plant production of hydrogen peroxide and phenolic compounds, which enhance resistance to fungal and bacterial infections (El-Khallal, 2007; Parmar and Subramanian, 2012).

The enhanced growth rate observed in mycorrhiza-inoculated plants has been extensively documented and is attributed to improved nutrient absorption from the soil, which stimulates metabolic processes. This leads to the production of growth regulators such as gibberellins, auxins, and cytokinins, thereby increasing plant biomass and chlorophyll content (Koza et al., 2022). The findings are consistent with those of Hashem et al. (2021), who demonstrated that mycorrhiza can reduce Fusarium wilt in tomatoes by enhancing defense mechanisms of plant.

Moreover, plants treated with AgNP solution derived from *F. solani* showed a significant increase in all growth parameters compared to untreated plants. This improvement is possibly due to an increase in defensive enzyme activities, similar to those observed with mycorrhiza (Hassana and Hassan, 2020). When AgNPs were used alongside *F. oxysporum*, no infection was observed, highlighting the potential of plant-extract-derived AgNPs as a safe, cost-effective alternative to chemical fungicides. This finding is consistent with that of Gomaa et al. (2021), who also reported the effectiveness of silver nanoparticles in controlling tomato wilt disease.

Silver nanoparticles inhibit pathogenic fungi by disrupting protein synthesis and degrading fungal hyphal walls, leading to the release of cellular contents and eventual cell death (Yuri and Agustino, 2017). Ansari et al. (2023) further demonstrated that plant-mediated silver nanoparticles significantly increased tomato plant biomass, chlorophyll content, carotenoids, flavonoids, soluble sugars, and proteins, highlighting their potential as a cost-effective, non-toxic alternative for applications in agriculture, biomedicine, and beyond.

Various nanomaterials, such as FeNPs, enhance phosphorus solubility in the rhizosphere and promote the decomposition of organic matter. SiO2NPs also improve mycorrhizal colonization in tomato plants. In sandy soils, AgNPs boost the mycorrhizal infection rate through interactions with mycorrhizal fungi. These effects are influenced by factors such as plant type, mycorrhizal species, experimental environment, and nanomaterial type (Feng et al., 2013; Wang et al., 2022).

Furthermore, combined treatment with mycorrhiza and AgNPs in the presence of pathogenic fungi significantly reduced infection severity and enhanced all plant growth parameters compared to untreated plants. Numerous studies have shown that AM fungi treated with AgNPs increase phosphorus uptake, bacterial diversity in the rhizosphere, and plant biomass while inhibiting Ag accumulation in plants (Cao et al., 2020). Moreover, Metwally and Abdelhameed (2024) reported improved enzyme activity and nutrient uptake in pea plants treated with nano ZnFe2O4 and mycorrhiza.

Numerous studies have highlighted the low toxicity and high antifungal properties of silver nanoparticles, which inhibit fungal spore formation and colony growth. They have significant potential as a sterilization agent and a viable alternative to chemical pesticides, which pose long-term environmental risks (Gibała et al., 2021; Li et al., 2022; Ansari et al., 2023).

CONCLUSION

The present study demonstrated that inoculation with arbuscular mycorrhizal fungi, particularly *Glomus*

mosseae, significantly enhanced plant growth parameters and total chlorophyll content. This improvement can be attributed to the ability of the fungus to boost plant defense mechanisms and enhance nutrient uptake efficiency. Furthermore, treatment with the silver nanoparticle solution proved highly effective, leading to substantial improvements in plant growth and completely eliminating *Fusarium oxysporum* infection. The interaction between mycorrhizal fungi and silver nanoparticles further underscored the potential of this approach as an eco-friendly alternative to chemical fungicides.

The antimicrobial and antifungal properties of silver nanoparticles (AgNPs) highlight their potential for controlling plant pathogens, offering a sustainable alternative that reduces reliance on harmful chemical pesticides. Based on the findings of this study, it is recommended to use AgNP solutions prepared with fungal synthesis, applying low concentrations tailored to the specific needs of the plant and farming conditions. This approach aims to enhance tomato crop production while minimizing pathogen presence.

ACKNOWLEDGMENTS

We extend our sincere gratitude to the Department of Biological Sciences, College of Science, University of Baghdad, for providing the necessary support, laboratory facilities, and assistance in the isolation and diagnosis processes.

AUTHORS' CONTRIBUTIONS

ZR, and AF designed, formulated and laid out the study; ZR, TH, and TR conducted the experiments; AF collected, arranged and analyzed the data; TH, and TR provided technical assistance; ZR and AF supervised the work; ZR and AF wrote the manuscript; TR proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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