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Application of Eco-Friendly Silver Nanoparticles as an Antifungal Agent Against *Fusarium* spp. Infecting Pepper Plants (*Capsicum annuum* L.)

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Abstract : One of the most destructive fungal diseases affecting pepper plants (*Capsicum annuum* L.) is *Fusarium* wilt, caused by *Fusarium* species. leading to significant yield losses worldwide. Traditional chemical fungicides, while effective in treating these diseases, pose environmental and health risks. In recent years, silver nanoparticles (AgNPs) have emerged as a promising, eco-friendly alternative agents for treating fungal diseases, due to their strong antifungal properties and low toxicity to plants. This study investigates the efficacy of eco-friendly AgNPs in reducing the severity of Fusarium infection in pepper plants. The AgNPs were sprayed on plants at concentrations of 20, 40 and 60 mg/L Resulted showed enhancements in pepper plant growth parameters and a positive influence on their chlorophyll levels, relative water content, and enzymatic activities associated with stress resilience compared to the untreated control group. In general, plant performance under the AgNPs treatments exhibited a significant and gradual improvement of the measured traits with increasing concentrations up to 40 mg/L, followed by a subsequent decline at 60 mg/L, reaching its lowest point with the application of AgNPs at 80 mg/L. However, particularly noteworthy is the superior efficacy observed with AgNPs application at a concentration of 40 mg/L, followed by 20 mg/L, and then 60 mg/L. The application of AgNPs at 80 mg/L exerted further adverse effects compared to the control group. In conclusion, the AgNPs may be used to mitigate environmental pollution and promote ecosystem health.

keywords: Silver nanoparticles, Pepper, Pathogen, *Fusarium*, Sustainable agriculture

1.Introduction

In modern agriculture, the effective management of plant diseases caused by phytopathogen is a critical factor in sustaining crop productivity and ensuring global food security. However, conventional disease control strategies, particularly the widespread use of chemical pesticides, are often associated with significant environmental and public health concerns [1]. As a result, there is increasing interest in developing alternative strategies that offer both high efficacy and environmental sustainability. [2]. In the realm of nanotechnology, the advancement of eco-friendly methods for synthesizing nanoparticles is becoming an increasingly important area of research [3]. Green nanotechnology is gaining growing attention, with the goal of producing commercially viable, eco-friendly, and safer products that possess distinctive optical, chemical, photochemical, and electrical characteristics. [4]

The eco-friendly biosynthesis of nanoparticles has gained significant traction due to the increasing demand for environmentally responsible methods of material production [5]. As the application of nanoparticles continues to expand, it becomes increasingly important to assess and understand their potential environmental effects [6].

The study by [7] investigated the use of nano-chitosan and a synthetic bactericide on chili pepper plants infected with *Xanthomonas campestris*. Among the various nanomaterials, silver nanoparticles (AgNPs) have attracted significant interest due to their strong antimicrobial activity. Their large surface area-to-volume ratio greatly increases their reactivity and effectiveness against a wide range of pathogens [8].

Pepper (*Capsicum annuum L.)* is a widely grown vegetable crop that is vulnerable to numerous diseases, particularly those caused by pathogenic organisms. These diseases can cause substantial yield reductions and financial losses for growers [9]. Among these, *Fusarium* species are particularly harmful, often resulting in *Fusarium* wilt a severe disease characterized by wilting, leaf yellowing, and eventually plant death. The fungus targets the plant's vascular tissues, disrupting the transport of water and nutrients, which leads to the observed wilting symptoms. Furthermore, *Fusarium* infections can negatively impact both the yield and quality of pepper fruits [10].

This study seeks to explore the potential of silver nanoparticles (AgNPs) as antimicrobial agents for controlling fungal diseases in pepper plants. It focuses on assessing the impact of AgNPs on plant growth, physiological traits, and disease resistance. The primary goal is to support the advancement of sustainable strategies for disease management in agriculture. Evaluating the effectiveness and safety of AgNPs in managing fungal infections in pepper is crucial not only for improving crop yield and quality but also for reducing the environmental risks linked to traditional control methods. Consequently, this research carries important implications for promoting sustainable agricultural practices and ensuring food security.

2. Materials and methods

The trials for this article were conducted at the experimental farm of Mansoura University, Dakahlia governorate, Egypt to assess the efficacy of AgNPs in mitigating the detrimental effects of pathogen infections on pepper plants, thereby contributing to sustainable disease management practices. The methodology involved the application of various concentrations of AgNPs to both infected and non-infected pepper plants, followed by comprehensive monitoring of plant health parameters.

1. Source of the causal organism

Fusarium oxysporum isolates were collected from the mycological laboratory, Botany Department, Faculty of Science, Mansoura University.

2. Experimental design and treatments

These experiments were executed under a split plot design with three replicates for each experiment. The main factor was the infection with pathogen *Fusarium oxysporum* and its comparison with non-infection, while the main factor was spraying four different rates of AgNPs (20, 40, 60 and 80 mg/L) compared control in treated plants.

Table 1. Properties of the soil before the implementation the experiment

Characteristics	Values
Clay,%	48
Silt,%	30
Sand,%	22
Textural class	Clayey
N, mg Kg^{-1}	40
P, mg Kg^{-1}	5.9
K, mg Kg^{-1}	183.5
O.M, %	1.02
EC dSm^{-1} (suspension 1: 5)	3.5
pH (suspension 1:2.5)	8.05

3.Properties of the soil before the implementation of the experiment. The soil used for cultivation of plants in pots underwent analysis following the methodology outlined by [11], which provides a comprehensive description of their properties as presented in Table 1.

4. Silver nanoparticles synthesis and characterization

The initial confirmation of the synthesis of AgNPs is made by observation of the color shift of the reaction mixture from pale white to yellowish brown. Several characterization techniques have been employed to establish the presence of AgNPs in the solution [12]. It is the most crucial phase in the biosynthesis of AgNPs since it explains the existence of any biomolecules that might be linked to these particles in addition to the size and shape of the

generated AgNPs. The silver nanoparticles are characterized using a variety of molecular techniques, such as the following: 1- UV-visible spectroscopy; 2- X-ray diffraction technique (XRD); 3- Fourier transform infrared spectroscopy (FTIR); 4- Atomic force microscopy (AFM); 5- Scanning electron microscopy (SEM); 6- Transmission electron microscopy (TEM); and 7- Energy Dispersive X- ray Spectroscopy (EDX).

For the initial characterization of created AgNPs, UV-visible spectroscopy is a very beneficial and trustworthy method that is also utilized to demonstrate the production and stability of AgNPs [13]. AgNPs have unusual optical properties that greatly affect how they interact with certain light wavelengths [14]. According to various studies, characterization is best done by absorbance band at a wavelength of bout 200-800 nm when the particle size is between 2-100 nm [15]. The free motion of the electrons inside these bands produces the surface plasmon resonance absorption bands. Depending on the size of the particles, the chemical environment, and the dielectric medium, AgNPs absorb differently [14]. This peak is attributed to a surface plasmon for several metal nanoparticles with diameters ranging from 2 to 100 nm as has been frequently reported by Almatroudi[16].

Kleemann [17] proposed a technique that determines the presence of AgNPs by measuring the absorbance of bio-reduced solution at wavelengths between 200 and 800 nm [17]. When AgNPs brown solution containing reduced silver nitrate in aqueous extracts of fungus are exposed to light, a high absorption peak is thought to develop in the wavelength region between 390 and 420 nm, whereas the control does not (pale white solution containing silver nitrate in deionized Milli-Q water). This indicates how endophytic fungus water extracts have less silver nitrate.

The shape and size of nanoparticles, which are both important elements, play a huge role in determining how well they perform. These variables are affected by the type of microorganisms used, the pH of the medium, and other factors. As a result, a wide range of nanoparticles are produced by various fungus species. Electron microscopy can be used to measure and in large part determine the size and shape of nanoparticles. By using transmission electron microscopy (TEM) or scanning electron microscopy (SEM) as proposed by [18]. The TEM offers information on the size and morphology of nanoparticles. The magnification of TEM is primarily responsible for determining the ratio of the distance between the objective lens and the specimen to that between the objective lens and its image plane [19]. TEM can provide superior spatial resolution and the ability to run more analytical procedures than SEM [20].

The size of nanoparticles has been the subject of numerous investigations using microscopic methods. The SEM is used to determine the shape and topology of metal nanoparticles. The surface picture of the specimen is produced in the SEM by scanning the surface with an accelerated electron beam. Backscattered and secondary electrons are collected by the detector and analyzed to create pictures or images. The diameters of several nanoparticles at the micro (10-6) and nano (10-9) scales were determined using SEM [21].

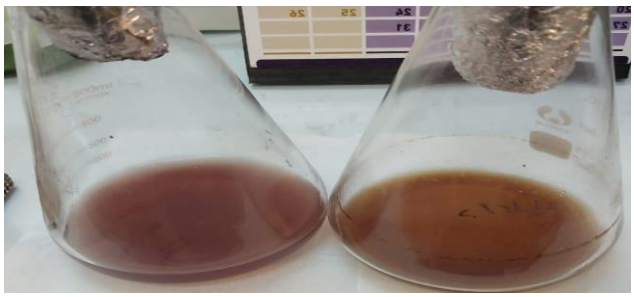

Fig 1: Color changes from pink to brown during the biosynthesis of AgNPs. Flask (A, left) is a control (cell free filtrate without silver ions), and flask (B, right) is a test flask (cell free filtrate with silver ions) after 72 hours incubation.

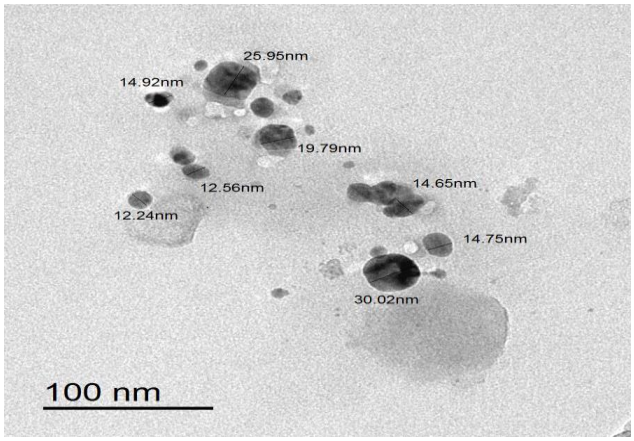

Fig 2. Transmission electron microscopy (TEM) imaging of the prepared AgNPs.

5.Transplanting and experimental setup

Pepper seeds were planted in pots filled with soil contaminated with Fusarium at a concentration of 5% on December 26, 2023. Two seedlings were transplanted per pot, and each treatment was replicated with four pots. Standard agricultural practices, as recommended by the Ministry of Agriculture and land Reclamation (MALR) protocol, including both organic and mineral fertilization, were followed for pepper cultivation. The sweet pepper plants were sprayed with 30 ml per pot, once 35 days after transplanting using a hand sprayer until saturation was achieved, Additionally, the AgNPs treatments were applied to the soil as a drench 10 days after the foliar application, with a dosage of 60 ml per treatment.

6.Measurements of plant traits, enzymatic antioxidants, and other constitutions

6.1. Measurements After 60 days

Plant height (cm), number of leaves plant^{-1}, leaves fresh weight (g plant^{-1}), leaves dry weight (g plant^{-1}), leaf area (cm^2 Plant^{-1}), root length (cm), root fresh weight (g plant^{-1}), root dry weight (g plant^{-1}) and relative water content (%) were measured. Also, the photosynthetic pigments chlorophyll a & chlorophyll b and carotene (mg g^{-1}) were determined according to Lichtenthaler [22].

The samples underwent washing, slicing into small pieces, and subsequent storage until the extraction process commenced. Extraction involved immersing the samples in methanol for two days at room temperature, followed by filtration. The residue underwent two additional rounds of extraction with methanol. The filtrates obtained were evaporated using a rotary evaporator at a pressure of 45°C. The crude methanolic extracts were then refrigerated at 4°C until analysis commenced. The enzymatic antioxidants measured include peroxidase (Unit.min^{-1} g^{-1} protein), catalyse (Unit.min^{-1} g^{-1} protein) and poly phenol oxidase (Unit.min^{-1} g^{-1} protein) according to Alici & Arabaci [23] , while the non-enzymatic antioxidants include phenol (mg GAE.100g^{-1}), flavonoid (mg QE.100g^{-1}) Additionally, other biochemical parameters such as proline (µmol g^{-1} F.W), glycine betaine (µmol g^{-1} D.W), saponin (%), DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity (%), total antioxidant activity (mg AAE /g) were measured by Cosmulescu et al. [24].

The proline content in the leaf tissues of the sweet pepper was extracted and analyzed following the method outlined by Bates et al. [25]. Glycine betaine content was determined following the method described by Grieve & Grattan(1983) [26]. Saponin content was assessed spectrophotometrically using the protocol outlined by Makkar & Becker (1996) [27] . DPPH was analyzed following the procedure outlined by Dasgupta et al. [28]. Total antioxidant capacity of the pepper methanolic extracts was assessed using the phosphomolybdate method, as described by Arefin et al. [29].

6.2.Measurements the harvest time

The measured yield traits include at this stage include, fruit length (cm), fruit diameter (cm), fruit dry matter (%), average fruit weight (g), number of fruits plant^{-1}, fruit yield (ton ha^{-1}) were measured. Also, fruit quality parameters such as carbohydrates (%), total sugar (%), vitamin C (mg 100g^{-1}), total dissolved solids (TDS %) and acidity (%) were determined according to the standard methods as described by [30].

6.Statistical analysis

Statistical Analysis was done using the one-way analysis of variance, (ANOVA) as described by [31] and [32].

3. Results

3.1.Plant performance after 60 days from transplanting

3.1.1. Shoot and root parameters, photosynthetic pigments and leaf RWC

Table 2 demonstrates the effect of different pathogen treatments and concentrations of AgNPs treatments and their combinations on shoot growth parameters in both infected and non-infected pepper plants. Overall, as the concentration of AgNPs increases until 60 mgL^{-1}, there is a trend of improvement in shoot growth parameters such as plant height, number of leaves per plant, fresh and dry weights of leaves, and leaf area. This suggests that AgNPs may have a positive impact on the growth of pepper plants, particularly at higher concentrations. However, it is interesting to

note that this trend varies between infected and non-infected plants, indicating potential interactions between AgNPs and pathogen infection.

Table 2. Data illustrating the effects of various concentrations of AgNPs on the shoot growth parameters of the infected and non-infected pepper plants

Treatments		Plant height, cm	No. of leaves plant^{-1}	Leaves fresh weight, g plant^{-1}	Leaves dry weight, g plant^{-1}	Leaf area, cm^2 Plant^{-1}
1. Fungal Pathogen treatments						
Non infected with Pathogen		49.50a	16.67a	182.81a	29.76a	310.33a
Infected with pathogen		45.24b	14.67b	177.71b	27.99b	290.53b
LSD at 5%		**1.16**	**2.16**	**0.88**	**0.36**	**1.49**
2. AgNPs concentrations						
0.0 mg L^{-1} (Control)		45.00d	14.33c	177.23c	27.85c	289.50c
20 mg L^{-1}		49.78b	16.83ab	183.21ab	29.97a	311.67ab
40 mg L^{-1}		50.97a	17.67a	184.60a	30.37a	317.17a
60 mg L^{-1}		47.53c	15.83b	180.45b	28.93b	301.33b
80 mg L^{-1}		43.58e	13.67c	175.80c	27.25d	282.50c
LSD at 5%		**0.72**	**1.12**	**3.03**	**0.48**	**11.26**
3. Interaction of Fungal Pathogen treatments and AgNPs concentration						
Non infected with fungal pathogen	0.0 mg L^{-1} (Control)	46.85	15.67	179.46	28.67	298.67
	20 mg L^{-1}	51.68	17.33	185.39	30.63	320.33
	40 mg L^{-1}	52.78	18.33	187.06	31.16	324.67
	60 mg L^{-1}	50.50	17.00	183.98	30.25	315.00
	80 mg L^{-1}	45.71	15.00	178.16	28.07	293.00
Infected with fungal pathogen	0.0 mg L^{-1} (Control)	43.14	13.00	174.99	27.03	280.33
	20 mg L^{-1}	47.88	16.33	181.04	29.30	303.00
	40 mg L^{-1}	49.16	17.00	182.14	29.59	309.67
	60 mg L^{-1}	44.57	14.67	176.92	27.61	287.67
	80 mg L^{-1}	41.45	12.33	173.44	26.42	272.00
LSD at 5%		**1.01**	**1.58**	**4.30**	**0.68**	**15.92**

Means within a row followed by a different letter (s) are statistically different at a 0.05 level

Table 3 illustrates the influence of the applied concentrations of AgNPs on root growth parameters in infected and non-infected pepper plants. Similar to the findings in Table 2, there is a general trend of increased root growth parameters with higher concentrations of AgNPs (until 60 mg L^{-1}). This suggests that AgNPs may promote root development in pepper plants, which is crucial for nutrient uptake and overall plant health. However, as with shoot growth parameters, the effects of AgNPs on root growth appear to vary depending on the presence of pathogen infection.

Table 3. Data illustrating the effects of various concentrations of AgNPs on the root growth parameters of the infected and non-infected pepper plants

Treatments		Root length, cm	Root fresh weight, g plant^{-1}	Root dry weight, g plant^{-1}
1. Fungal Pathogen treatments				
Non infected with fungal pathogen		14.01a	11.38a	5.38a
Infected with fungal pathogen		12.56b	10.10b	4.90b
LSD at 5%		**0.24**	**0.11**	**0.09**
2. AgNPs concentrations				
0.0 mg L^{-1} (Control)		12.50d	10.00d	4.86d
20 mg L^{-1}		14.13b	11.53b	5.43b
40 mg L^{-1}		14.56a	11.89a	5.56a
60 mg L^{-1}		13.28c	10.72c	5.16c
80 mg L^{-1}		11.96e	9.58d	4.68d
LSD at 5%		**0.22**	**0.16**	**0.08**
3. Interaction of Fungal Pathogen treatments and AgNPs concentration				
Non infected with fungal pathogen	0.0 mg L^{-1} (Control)	13.20	10.58	5.07
	20 mg L^{-1}	14.70	12.04	5.63
	40 mg L^{-1}	15.10	12.41	5.77
	60 mg L^{-1}	14.32	11.63	5.52
	80 mg L^{-1}	12.75	10.25	4.92
Infected with fungal pathogen	0.0 mg L^{-1} (Control)	11.79	9.41	4.66
	20 mg L^{-1}	13.56	11.01	5.24
	40 mg L^{-1}	14.01	11.36	5.36
	60 mg L^{-1}	12.24	9.80	4.80
	80 mg L^{-1}	11.18	8.91	4.44
LSD at 5%		**0.30**	**0.24**	**0.11**

Means within a row followed by a different letter (s) are statistically different at a 0.05 level

Table 4 presents the data on the impact of different concentrations of AgNPs on photosynthetic pigments and relative water content in pepper plants infected and non-infected with fungal pathogens. The results indicate that AgNPs can influence the photosynthetic pigments of pepper plants, with varying effects on chlorophyll a, chlorophyll b, and carotene levels. It was most effective at the concentrations of 40 mg L^{-1} in both infected and non-infected plants. Additionally, the AgNPs treatments seem to affect the relative water content of pepper plants slightly differently depending on the presence of pathogen infection.

Table 4. Data illustrating the effects of the AgNPs concentrations on the photosynthetic pigments and relative water content of the infected and non-infected pepper plants

Treatments		Chlorophyll a, mg g^{-1}	Chlorophyll b, mg g^{-1}	Carotene, mg g^{-1}	Relative water content, %
1. Fungal Pathogen treatments					
Non infected with fungal pathogen		0.813a	0.614a	0.303a	83.79a
Infected with fungal pathogen		0.759b	0.581b	0.287b	82.77b
LSD at 5%		**0.001**	**0.032**	**0.004**	**0.13**
2. AgNPs concentrations					
0.0 mg L^{-1} (Control)		0.755c	0.579d	0.286d	82.69bc
20 mg L^{-1}		0.818a	0.618b	0.305b	83.87ab
40 mg L^{-1}		0.832a	0.625a	0.309a	84.26a
60 mg L^{-1}		0.786b	0.598c	0.296c	83.30abc
80 mg L^{-1}		0.740e	0.569e	0.281e	82.28c
LSD at 5%		**0.014**	**0.006**	**0.003**	**1.50**
3. Interaction of Fungal Pathogen treatments and AgNPs concentration					
Non infected with fungal pathogen	0.0 mg L^{-1} (Control)	0.783	0.594	0.294	83.13
	20 mg L^{-1}	0.839	0.632	0.312	84.30
	40 mg L^{-1}	0.854	0.638	0.315	84.64
	60 mg L^{-1}	0.825	0.623	0.307	84.18
	80 mg L^{-1}	0.764	0.586	0.289	82.70
Infected with fungal pathogen	0.0 mg L^{-1} (Control)	0.727	0.565	0.279	82.25
	20 mg L^{-1}	0.796	0.604	0.297	83.44
	40 mg L^{-1}	0.809	0.612	0.303	83.88
	60 mg L^{-1}	0.747	0.574	0.285	82.43
	80 mg L^{-1}	0.715	0.553	0.274	81.86
LSD at 5%		**0.113**	**0.009**	**0.005**	**2.12**

Means within a row followed by a different letter (s) are statistically different at a 0.05 level

Generally, the data in the Tables numbered 2, 3, and 4, indicate a notable decrease in the detrimental impact of pathogens following treatment with AgNPs (20, 40 and 60 mg L^{-1}), highlighting the considerable antimicrobial potential of this nanomaterial. Moreover, AgNPs exhibited minimal phytotoxic effects, suggesting their potential as safe and effective antimicrobial agents in agriculture. Spraying AgNPs at concentrations of 20, 40 and 60 mg L^{-1} resulted in enhancements in plant growth parameters and exerted a positive influence on chlorophyll levels and relative water content associated with stress resilience compared to the untreated control group. Particularly noteworthy was the superior efficacy observed with AgNPs application at a concentration of 40 mg L^{-1}, followed by 20 mg L^{-1}, and then 60 mg L^{-1}. Conversely, the application of AgNPs at 80 mg L^{-1} exhibited adverse effects compared to the control group. In essence, plant performance under AgNPs treatments showed a significant and gradual improvement with increasing concentrations up to 40 mg L^{-1}, followed by a subsequent decline at 60 mg L^{-1}, reaching its lowest point with the application of AgNPs at 80 mg L^{-1}.

3.1.2.Enzymatic and non-enzymatic antioxidants

Table 5 presents data on the effect of various concentrations of AgNPs on enzymatic and non-enzymatic antioxidants in the leaves of both infected and non-infected pepper plants. The measured enzymatic antioxidants include peroxidase, catalase, and polyphenol oxidase activities, while the non-enzymatic antioxidants include phenol and flavonoid contents. Additionally, other biochemical parameters such as proline, glycine betaine, saponin content, DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity, and total antioxidant activity are also included in the analysis.

Overall, the results show that the application of AgNPs at different concentrations influences the levels of both enzymatic and non-enzymatic antioxidants in pepper plants. There are significant differences observed in the antioxidant levels between infected and non-infected plants, as well as between different concentrations of AgNPs. For example, higher concentrations of AgNPs generally result in increased enzymatic and non-enzymatic antioxidant activities, suggesting a potential role of AgNPs in enhancing the antioxidant defense system of pepper plants. However, the effects of AgNPs on antioxidant activities vary depending on the presence of pathogen infection. These findings highlight the complex interactions between AgNPs, pathogen infection, and antioxidant defense mechanisms in pepper plants.

Table 5. Data illustrating the effects of the AgNPs concentrations on the enzymatic and non-enzymatic antioxidants in leaves of infected and non-infected pepper plants.

Treatments		Peroxidase, Unit.min^{-1}.g^{-1} protein	Catalyse, Unit.min^{-1}.g^{-1} protein	Poly phenol oxidase,Unit.min^{-1}.g^{-1} protein	Phenol, mg GAE.100g^{-1}	Flavonoid, mg QE.100g^{-1}
1. Fungal Pathogen treatments						
Non infected with fungal pathogen		7.17a	0.226a	7.63a	43.31a	86.75a
Infected with fungal pathogen		6.52b	0.211b	6.51b	42.34b	85.12b
LSD at 5%		**0.08**	**0.003**	**0.14**	**0.08**	**0.12**
2. AgNPs concentrations						
0.0 mg L^{-1} (Control)		6.46d	0.210d	6.42d	42.25cd	85.06bc
20 mg L^{-1}		7.23b	0.228b	7.73b	43.41ab	86.88a
40 mg L^{-1}		7.43a	0.231a	8.06a	43.74a	87.30a
60 mg L^{-1}		6.85c	0.219c	7.07c	42.83bc	86.01ab
80 mg L^{-1}		6.26e	0.203e	6.07e	41.91d	84.44c
LSD at 5%		**0.12**	**0.003**	**0.10**	**0.77**	**1.53**
3. Interaction of Fungal Pathogen treatments and AgNPs concentration						
Non infected with fungal pathogen	0.0 mg L^{-1} (Control)	6.72	0.216	6.93	42.68	85.75
	20 mg L^{-1}	7.52	0.234	8.19	43.84	87.64
	40 mg L^{-1}	7.74	0.237	8.52	44.13	88.01
	60 mg L^{-1}	7.30	0.229	7.89	43.60	87.10
	80 mg L^{-1}	6.57	0.213	6.61	42.29	85.26
Infected with fungal pathogen	0.0 mg L^{-1} (Control)	6.19	0.204	5.91	41.81	84.36
	20 mg L^{-1}	6.94	0.222	7.27	42.98	86.11
	40 mg L^{-1}	7.12	0.225	7.59	43.34	86.58
	60 mg L^{-1}	6.39	0.208	6.24	42.05	84.92
	80 mg L^{-1}	5.95	0.194	5.53	41.53	83.63
LSD at 5%		**0.16**	**0.004**	**0.14**	**1.09**	**2.17**

Means within a row followed by a different letter (s) are statistically different at a 0.05 level

Continued Table (5)

Treatments		Proline, µmol g^{-1} F.W	Glycine betaine, µmol g^{-1} D.W	Saponin, %	DPPH, %	Antioxidant activity, mg AAE /g
1. Fungal Pathogen treatments						
Non infected with fungal pathogen		7.62b	18.24b	1.09a	30.76a	236.07a
Infected with fungal pathogen		8.33a	19.82a	0.98b	28.95b	230.47b
LSD at 5%		**0.23**	**0.32**	**0.04**	**0.55**	**2.14**
2. AgNPs concentrations						
0.0 mg L^{-1} (Control)		8.39b	19.95b	0.97c	28.79d	229.88cd
20 mg L^{-1}		7.55d	18.18d	1.11a	30.95b	236.52ab
40 mg L^{-1}		7.34e	17.60e	1.15a	31.44a	238.28a
60 mg L^{-1}		7.98c	19.04c	1.04b	29.90c	233.27bc
80 mg L^{-1}		8.59a	20.39a	0.92d	28.20e	228.40d
LSD at 5%		**0.10**	**0.28**	**0.05**	**0.47**	**3.57**
3. Interaction of Fungal Pathogen treatments and AgNPs concentration						
Non infected with fungal pathogen	0.0 mg L^{-1} (Control)	8.10	19.28	1.03	29.56	232.22
	20 mg L^{-1}	7.22	17.43	1.14	31.71	238.93
	40 mg L^{-1}	7.03	16.85	1.19	32.24	240.86
	60 mg L^{-1}	7.45	17.96	1.13	31.23	237.34
	80 mg L^{-1}	8.28	19.69	0.98	29.04	231.01
Infected with fungal pathogen	0.0 mg L^{-1} (Control)	8.69	20.61	0.91	28.02	227.54
	20 mg L^{-1}	7.88	18.92	1.07	30.19	234.12
	40 mg L^{-1}	7.65	18.35	1.10	30.65	235.71
	60 mg L^{-1}	8.50	20.12	0.95	28.57	229.21
	80 mg L^{-1}	8.90	21.10	0.85	27.35	225.79
LSD at 5%		**0.14**	**0.39**	**0.07**	**0.67**	**5.04**

Means within a row followed by a different letter (s) are statistically different at a 0.05 level

3.2.Quantitative and qualitative yield at harvest time

Table 6 displays the data regarding how different concentrations of AgNPs affect the physical characteristics of fruits in pepper plants both infected and non-infected with the Fusarium pathogen. The characteristics assessed encompass fruit length, diameter, dry matter, average fruit weight, number of fruits plant^{-1}, fruit yield. Meanwhile, the results indicate that the application of AgNPs at different concentrations influences the physical and quality characteristics of pepper fruits. Generally, higher concentrations of AgNPs (until 40 mg L^{-1}) lead to increased fruit length, diameter, dry matter content, average fruit weight, number of fruits per plant, fruit yield, carbohydrate content, total sugar content, vitamin C content and total dissolved solids (TDS). However, there are variations in these effects depending on the presence of pathogen infection and the concentration of AgNPs. Particularly noteworthy was the superior efficacy observed with AgNPs application at a concentration of 40 mg L-1, followed by 20 mg L^{-1}, and then 60 mg L^{-1}. Conversely, the application of AgNPs at 80 mg L^{-1} exerted adverse effects on fruit yield and its components compared to the control group. In essence, fruit yield and its components under the AgNPs treatments showed a significant and gradual improvement with increasing concentrations up to 40 mg L^{-1}, followed by a subsequent decline at 60 mg L^{-1}, reaching its lowest point with the application of AgNPs at 80 mg L^{-1}.

Table 6. Data illustrating the effect of various concentrations of AgNPs on the fruits physical traits of the infected and non-infected pepper plants.

Treatments		Fruit length, cm	Fruit diameter, cm	Fruit dry matter, %	Average fruit weight, g	No. of fruits plant^{-1}	**Fruit yield, ton ha^{-1}**
1. Fungal pathogen treatments							
Non infected with pathogen		6.44a	4.60a	18.25a	43.81a	25.87a	38.55a
Infected with pathogen		5.24b	3.99b	17.40b	42.05b	23.67b	33.89b
LSD at 5%		0.02	0.28	0.17	0.63	2.28	**3.11**
2. AgNPs concentrations							
0.0 mg L^{-1} (Control)		5.05d	3.88c	17.31c	41.98c	23.83bc	34.05bc
20 mg L^{-1}		6.62b	4.68a	18.36a	43.97a	26.00a	38.88a
40 mg L^{-1}		7.01a	4.86a	18.61a	44.45a	26.33a	39.81a
60 mg L^{-1}		5.83c	4.31b	17.80b	43.01b	24.50b	35.89b
80 mg L^{-1}		4.70e	3.74c	17.05c	41.23d	23.17c	32.53c
LSD at 5%		0.23	0.20	0.27	0.63	1.17	**1.95**
3. Interaction of fungal pathogen treatments and AgNPs concentration							
Non infected with pathogen	0.0 mg L^{-1} (Control)	5.53	4.11	17.70	42.76	25.00	36.37
	20 mg L^{-1}	7.19	5.01	18.71	44.67	26.67	40.51
	40 mg L^{-1}	7.54	5.14	18.95	45.21	27.00	41.51
	60 mg L^{-1}	6.78	4.76	18.44	44.26	26.00	39.10
	80 mg L^{-1}	5.17	3.98	17.45	42.14	24.67	35.34
Infected with pathogen	0.0 mg L^{-1} (Control)	4.56	3.65	16.92	41.20	22.67	31.75
	20 mg L^{-1}	6.04	4.36	18.01	43.28	25.33	37.27
	40 mg L^{-1}	6.48	4.58	18.27	43.69	25.67	38.13
	60 mg L^{-1}	4.88	3.85	17.15	41.75	23.00	32.65
	80 mg L^{-1}	4.23	3.50	16.65	40.32	21.67	29.70
LSD at 5%		0.32	0.29	0.39	0.89	1.65	**2.76**

Means within a row followed by a different letter (s) are statistically different at a 0.05 level

Table 7 presents findings regarding the influence of various concentrations of AgNPs on the quality attributes of fruits in both infected and non-infected pepper plants. These attributes encompass carbohydrate content, total sugar content, vitamin C content, total dissolved solids (TDS) and acidity.

Concerning acidity levels (%), It was found that the acidity values took an opposite direction to the values of all the fruit yield traits studied. Among pepper plants not infected with fungal pathogen, those subjected to AgNPs at 80 mg L^{-1} exhibited the highest acidity level (0.310%), while the lowest acidity level (0.289%), was observed in plants not infected with fungal pathogen and treated with the control (0.0 mg L^{-1}). Among pepper plants infected with fungal pathogen, those treated with AgNPs at 80 mg L^{-1} also displayed the highest acidity level (0.316%), while the lowest acidity level (0.303%), was observed in plants infected with fungal pathogen and treated with the control (0.0 mg L^{-1}).

Table 7. Effect of various concentrations of AgNPs on the fruit's quality traits of the infected and non-infected pepper plants.

Treatments		Carbohydrates, %	Total sugar, %	Vitamin C, mg $100g^{-1}$	TDS %	Acidity, %
1. Fungal Pathogen treatments						
Non infected with pathogen bacteria		19.95a	8.16a	88.19a	6.17a	0.289b
Infected with pathogen bacteria		19.00b	7.45b	86.99b	5.47b	0.303a
LSD at 5%		**0.24**	**0.09**	**1.32**	**0.07**	**0.004**
2. AgNPs concentrations						
0.0 mg L^{-1} (Control)		18.95c	7.39d	87.00bc	5.41d	0.304b
20 mg L^{-1}		20.05a	8.23b	88.22ab	6.22b	0.287d
40 mg L^{-1}		20.31a	8.41a	88.53a	6.41a	0.283e
60 mg L^{-1}		19.49b	7.83c	87.63abc	5.85c	0.297c
80 mg L^{-1}		18.59c	7.14e	86.58c	5.19e	0.310a
LSD at 5%		**0.37**	**0.15**	**1.52**	**0.10**	**0.003**
3. Interaction of Fungal Pathogen treatments and AgNPs concentration						
Non infected with pathogen bacteria	0.0 mg L^{-1} (Control)	19.35	7.71	87.59	5.69	0.297
	20 mg L^{-1}	20.44	8.50	88.64	6.52	0.283
	40 mg L^{-1}	20.78	8.70	89.11	6.73	0.276
	60 mg L^{-1}	20.17	8.35	88.50	6.33	0.286
	80 mg L^{-1}	19.04	7.52	87.13	5.54	0.304
Infected with pathogen bacteria	0.0 mg L^{-1} (Control)	18.55	7.08	86.40	5.12	0.311
	20 mg L^{-1}	19.65	7.95	87.80	5.91	0.292
	40 mg L^{-1}	19.84	8.13	87.96	6.09	0.290
	60 mg L^{-1}	18.81	7.31	86.75	5.37	0.308
	80 mg L^{-1}	18.15	6.77	86.03	4.84	0.316
LSD at 5%		**0.52**	**0.21**	**2.16**	**0.15**	**0.005**

Means within a row followed by a different letter (s) are statistically different at a 0.05 level

4. Discussion

The effects of AgNPs on pepper plant growth, fruit traits, and overall yield can be linked to several physiological and biochemical mechanisms. Silver nanoparticles are known to function as biostimulants, promoting plant development by enhancing various cellular processes. This stimulation can result in greater shoot and root biomass. The underlying mechanisms may involve the modulation of gene expression associated with plant growth hormones, nutrient absorption, and photosynthetic activity [7].

Silver nanoparticles (AgNPs) also exhibit strong antimicrobial activity, which can help reduce the negative impact of pathogen infections on plant growth. By inhibiting pathogen activity or lowering their populations, AgNPs may lessen the stress experienced by plants, enabling them to divert more energy and resources toward growth and development. This may account for the observed variations in growth performance between infected and non-infected plants treated with AgNPs [33].

In addition, AgNPs have been found to trigger antioxidant responses in plants. They can enhance the production of enzymatic antioxidants such as peroxidase, catalase, and polyphenol oxidase, along with non-enzymatic antioxidants like phenols and flavonoids. This elevated antioxidant activity strengthens the plant's ability to manage oxidative stress caused by pathogen attacks or adverse environmental conditions, ultimately contributing to better growth and higher productivity [34].

AgNPs can directly engage with plant cells and organelles, influencing key physiological processes including cell division, metabolic activity, and signal transduction pathways. These direct interactions may play a role in the observed changes in growth, development, and the biochemical profile of pepper plants treated with AgNPs [8].

AgNPs were found to enhance the weight of 100 seeds, a key indicator of yield productivity, across two generations of pea (*Pisum sativum*). However, at lower concentrations, AgNPs also

caused genotoxic effects in the parent plants, which were passed on to subsequent generations. This suggests that AgNPs may have the potential to induce genetic variation, which could be valuable for pre-breeding studies in plants [35].

AgNPs have the potential to boost the absorption and assimilation of vital nutrients through plant roots, leading to enhanced nutrient levels within the plant and supporting better growth and development. Moreover, AgNPs can impact soil microbial populations and modify the physicochemical characteristics of the soil, which may indirectly affect nutrient availability for plant uptake [36].

Silver nanoparticles (AgNPs) are believed to influence plant water dynamics, including absorption, transport, and retention of water. By enhancing the efficiency of water uptake and helping maintain proper hydration, AgNPs can improve plant tolerance to drought stress and support key physiological functions like photosynthesis. This may account for the observed variations in relative water content between plants treated with AgNPs and those left untreated [37].

5. Conclusion

This study sheds light on the potential of AgNPs, as an effective and environmentally sustainable solution for managing fungal diseases in pepper plants. The results demonstrate that AgNPs treatments significantly reduce the harmful effects of pathogenic fungus, while exhibiting minimal phytotoxicity, highlighting their promise as safe and efficient antimicrobial agents in agriculture. Furthermore, AgNPs treatments at concentrations of 20, 40, and 60 mg/L positively influence plant growth performance, chlorophyll content, relative water content, and enzymatic activities related to stress tolerance compared to untreated control groups. Particularly noteworthy is the superior efficacy observed with AgNPs application at a concentration of 40 mg/L, suggesting its optimal dosage for enhancing plant health and disease resistance. However, it is important to note that the application of AgNPs at a higher concentration of 80 mg mg/L resulted in adverse effects on plant performance, indicating the need for careful consideration of dosage levels to avoid potential harm. Moving forward, it is recommended to further optimize AgNPs formulations and application methods to maximize their efficacy while minimizing any potential adverse effects. Overall the results indicated a strong potential of AgNPs as valuable tools in disease management strategies. By embracing innovative approaches like AgNPs treatments, crop yield and quality may be improved and the reliance on conventional pesticides may be reduced, thereby promoting environmental stewardship and ensuring food security for future generations. Overall, the multifaceted effects of AgNPs on pepper plants' growth and fruit characteristics stem from their diverse modes of action at the cellular, physiological, and molecular levels that deserves further investigations.

6. References

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