Potential antifungal effects of silver nanoparticles (AgNPs) of different sizes against phytopathogenic *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) strains

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Abstract

Fusarium species are the primary fungal pathogen affecting agricultural foodstuffs both in crop yield and economic loss. Due to these problems, control of phytopathogenic fungi has become one of the critical problems around the World. Nanotechnology is a new technology with potential in many fields, including agriculture. This study focused on determining potential effects of silver nanoparticles (AgNPs) with different nanosizes (3, 5, 8 and 10 nm) and at different concentrations (12.5–100 ppm) against phytopathogenic *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) strains. The maximum antifungal activity was achieved by decreasing nanosize and increasing concentration of AgNPs. Mycelium growth abilities were decreased about 50%, 75% and 90% by AgNPs treatment with 3 nm sizes at 25 ppm, 37.5 ppm and 50 ppm concentrations, respectively. The productivity of fungal biomass in the liquid growth media was found to be too limited at the 25–37.5 ppm of AgNPs concentrations with all sizes. In addition, both septation number and dimensions of micro- and macroconidia were found to be gradually decreased with the application of silver nanoparticles. This work showed that the low concentration of AgNPs could be used as potential antifungal agents and applied for control of phytopathogens.

Keywords Metal-based nanoparticles · Plant pathogens · Antimicrobial · Fungal inhibition

1 Introduction

Around the world, the yield and quality of agricultural products continue to be gradually decreased every year due to plant diseases. Plant diseases are a worldwide problem related to food security. *Fusarium* is one of the most significant and widespread wilt pathogens causing diseases in agricultural products [1]. *Fusarium* species can also produce mycotoxin in several kinds of cereals, fruits, and vegetables [2]. *F. oxysporum* known as potential plant pathogenic fungi have been able to grow and survive in soil and on various organic materials for a long time [3]. *F. oxysporum* is a soilborne pathogen that attacks the roots

of the plant and considered as a limiting factor for tomato production [4, 5].

The different types of chemicals, pesticides and fungicides have been applied to eliminate fungal contamination. These treatments can also be responsible for environmental pollution and health hazards [6, 7]. Additionally, the use of agrochemicals may support the development of resistant pathogens while reducing the adverse effects of plant diseases [8]. They cause undesirable effects on non-target organisms due to overuse of pesticides. The most important of these effects is that the pesticides are included in the food chain and leave chemical residues on non-target organisms. For these reasons, alternative and

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potential antifungal agents instead of these chemicals should be investigated.

Recently, several metallic nanoparticles (silver, zinc, copper, titanium etc.) have been investigated as antimicrobial agents for the control of microorganisms including phytopathogens [9, 10]. Among them, silver is well known for its antimicrobial activity since ancient times [11–13]. Also, silver nanoparticles (AgNPs) prevent resistance development of pathogens by increasing antimicrobial activity [14]. Due to these properties, it can be used safely as an alternative to synthetic fungicides in the control of plant pathogens. It has been reported that the antimicrobial effect of nanoparticles (metal/metal oxide, metalloid and non-metal nanomaterials) on test pathogens are based on size and dose dependent [15, 16]. The size of nanoparticles can effect microstructure and mechanical properties [17]. Nanoparticles tend to attack a wide variety of biological processes, including cell membrane structure and function in microorganisms [18]. Similarly, AgNPs also inhibit the expression of proteins associated with ATP production [19]. These properties of AgNPs have attracted many practitioners worldwide to use it in various applications such as water purification [20], food packaging [21], antibacterial, antifungal, anti-inflammatory, anticancer, and therapeutic agents [14, 22–24].

In this study, potential antifungal effects of the silver nanoparticles against *Fusarium oxysporum* f. sp. *radicis-lycopersici* strains isolated from the eastern Mediterranean region of Turkey was aimed to determine. Then, the effect of both various sizes (3–10 nm) and different concentrations (12.5–100 ppm) of silver nanoparticles in determining their antifungal activity was investigated. In addition, the morphological characterization (dimension and separation) of microconidia and macroconidia of FORL strains exposed with silver nanoparticle was determined.

2 Material and methods

2.1 Fungal strains

In this work, six different *Fusarium oxysporum* f. sp. *radicis-lycopersici* Jarvis Shoemaker isolates (AY5, H2, JR, SC, TA4, and TR1) obtained from Eastern Mediterranean Agricultural Research Institute (Adana, Turkey) were used in the experiments. Among them, 5 FORL strains (AY5, H2, SC, TA4, and TR1) were originated from the eastern Mediterranean region of Turkey and the other one (JR) was originated from Japan [1]. These strains were maintained on Potato Dextrose Agar (PDA; Merck, Darmstadt, Germany) plates containing 4 g/L potato extract, 20 g/L glucose, and 15 g/L agar.

2.2 Silver nanoparticles

Four different sizes of AgNPs nanoparticles (3 nm, 5 nm, 8 nm, and 10 nm) used in this experiment were provided by Nanografi Inc. (Jena, Germany). A stock solution of AgNPs at a concentration of 1000 ppm was prepared and then added to growth media at different initial AgNPs concentrations (12.5–100 ppm). All AgNPs solutions were freshly prepared and used.

2.3 Culture conditions

The fungal strains were precultured on PDA (Merck, Darmstadt, Germany) medium in a 90 mm diameter petri dish at 25 °C for 3 days in an incubator (Binder, Germany) under dark conditions. Additionally, the fungal strains were incubated in Potato Dextrose Broth (PDB containing 4 g/L potato extract and 20 g/L glucose; Merck, Darmstadt, Germany) medium at 25 °C and 150 rpm for 7 days under dark conditions (Edmund Buhler, Germany). To determine the antifungal activities of AgNPs, fungal strains were performed using PDA and PDB for mycelial and fungal growth inhibitions, respectively.

2.4 Mycelial growth inhibition (MGI)

The mycelial growth inhibition (MGI) of *Fusarium* strains was evaluated using by agar diffusion method on PDA [25]. PDA was sterilized by heating at 121 °C for 15 min. Then the stock nanoparticle solution was transferred into the PDA to be obtained different initial AgNPs concentrations (to make up 12.5, 25, 37.5, and 50 ppm).

The agar plates were prepared by flooding pre-grown agar plates with 20 mL distilled water, and fungal spores were brought into suspension using by a sterile L-shape plastic spreader. PDA plates were inoculated with 100 µL spore suspension and spread with new sterile L-shape plastic spreader. The new plates were incubated at 25 °C for 3 days in an incubator (Binder, Germany) under dark conditions.

The pre-grown agar plates were punched using by a sterile cork borer (8 mm size) and transferred to the centre of each fresh PDA agar treated with AgNPs nanoparticles. Then, the cultures were incubated at 25 °C for 7 days, under the dark conditions. After the incubation, the colony diameters were measured in millimetres. FORL strains were also grown in PDA without nanoparticles and evaluated as a positive control.

The percentage of mycelial growth inhibition (MGI) was calculated according to the following formula [25];

$$MGI(\%) = \left[\frac{dc - dt}{dc} \right] \times 100$$

where dc (mm) is the mean fungal growth diameter for the controls and dt (mm) is the mean fungal growth diameter for each group treated with AgNPs.

2.5 Fungal growth inhibition (FGI)

The fungal growth inhibition (FGI) was determined by the broth-dilution method using PDB [26]. The assay was performed using 96 well polystyrene plates. Each well was filled with PDB treated with AgNPs with different sizes (3 nm, 5 nm, 8 nm, and 10 nm) and at different concentrations (from 12.5 to 100 ppm).

Fusarium strains were pre-grown on PDA at 25 °C for 3 days under the dark conditions. The pre-grown agar plates were treated with 20 mL sterile distilled water, and spore suspensions were obtained using by sterile L-shape plastic spreader. The spore suspensions (final concentration was set as 2.5×10^4 spores/mL) were inoculated into the PDB medium supplemented with AgNPs. PDB treated with only AgNPs (without fungal growth) were used as a blank, PDB incubated with only FORL strains (without nanoparticles) were also used as a positive control. The plates were incubated at 25 °C for 7 days in the incubator (Binder, Germany) under dark conditions. After the incubation, the fungal growth was measured at 600 nm using an ELISA plate reader.

The fungal growth inhibition (FGI) was calculated by the following formula with some modifications [26];

$$FGI(\%) = \left[\left(\frac{(Cg - Cb) - (Tg - Cb)}{(Cg - Cb)} \right) \right] \times 100$$

where Cg is the average growth in the control PDB (fungal growth density), Tg is the average growth in the treated group with AgNPs, and Cb is the blank.

2.6 Morphological characterization

The morphological characterization of six FORL strains was investigated by growing in both PDB and PDB supplemented with AgNPs (at 37.5 ppm and 50 ppm concentrations). The fungal strains were cultivated at 25 °C for 7 days under dark conditions. After the incubation, the dimensions of microconidia and macroconidia were measured using by an ocular micrometer, and the number of septation of conidia was recorded [27].

2.7 Statistical analysis

All experiments were carried out at least three times, and the obtained results were statistically analyzed using

Minitab17®. Analysis of variance (ANOVA) using general linear models was carried out, and significant differences were considered at p -value < 0.05 within a 95% confidence interval. Pairwise comparisons among groups of data were also carried out according to the Tukey's test. All error bars and intervals represent standard deviation.

3 Results and discussion

3.1 Antifungal activities

Previous studies showed that metallic nanoparticles such as silver, titanium, tin, zinc, and copper having high antimicrobial activity, low toxicity to humans, chemical stability, and inhibition activity against various phytopathogenic microorganisms [28–30]. Therefore, in this work, the AgNPs nanoparticles at different concentrations with various nanometer sizes were tested against the six *F. oxysporum* FORL strains.

3.1.1 The mycelial growth inhibition (MGI)

The mycelial growth inhibition (MGI) of AgNPs at different concentrations with different sizes were analyzed on PDA. The MGI results were given in Fig. 1. The data of the study showed that higher AgNPs concentrations (50 ppm) at all sizes had the highest antifungal activity against all tested strains (Figs. 1, 2). Antifungal activities of higher than 50 ppm concentrations (75 ppm and 100 ppm) were also tested, the results were determined to be similar, and there were no statistically different (p -value > 0.05; data not shown). Similarly, higher concentrations of silver nanoparticles (100 ppm) have been reported to have most significant inhibition against various plant pathogens [30, 31]. When the low sizes of nanoparticles even at low concentrations were applied, the inhibition ratios were increased. When the 3 nm size of AgNPs was applied, fungal growth of FORL strains was decreased by 20–62% and 50–76% at 12.5 ppm and 25 ppm concentrations, respectively (Table 1). In addition, in the 3 nm size of AgNPs treatments, MGI ratios were determined to be at least 75% by 37.5 ppm treatment and 99% with 50 ppm treatment. According to MGI ratios, it can be said that the size combined with concentration of nanoparticles have been an important parameter. When the nanometer size was increased to 5 nm, the MGI ratios were generally increased in low concentrations (i.g. 12.5 and 25 ppm); however, MGI ratios were tended to decrease in higher concentrations (i.g. 37.5 ppm and 50 ppm of AgNPs) (Fig. 1). In summary, the mycelium growth was reduced by 50% and 75% with AgNPs performed at 25 ppm concentrations with 3 nm or 5 nm sizes and 37.5 ppm concentrations with 3 nm size,

Fig. 1 Mycelial growth inhibition (MGI, %) of *Fusarium oxysporum* f. sp. *radicle-lycopersici* (FORL) strains after application of AgNPs at different concentrations (12.5 ppm, 25 ppm, 37.5 ppm, and 50 ppm) with different nanoparticle sizes (3 nm, 5 nm, 8 nm, and 10 nm).

*Values are averages of 3 individual experiments. Error bars indicate sample standard deviations, which were estimated individually for each average

respectively. Similarly, it has been reported that nanoparticles with smaller sizes have more effective antimicrobial activity against human pathogens (*Aeromonas* spp., *Klebsiella pneumonia*, *E. coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Yersinia enterocolitica*), but their antimicrobial effects decreased as the nanosize were increased [32, 33]. Smaller nanoparticles, like 5 nm, had shown more antimicrobial activity by both increasing membrane permeability and causing cell membrane damage [34].

3.1.2 The fungal growth inhibition (FGI)

Previous studies showed that antifungal activities were determined in broth dilution bioassay [35, 36]. Therefore, in this work, silver nanoparticles at different concentrations (12.5–100 ppm) with different sizes (3–10 nm) were tested in PDB (Table 1). It has been shown that increasing concentrations and decreasing nanometer size of AgNPs resulted in higher antifungal activities for *F. oxysporum* isolates. The FGI ratios were determined as low at 12.5 ppm concentration of AgNPs with all sizes. The fungal growth of five of the six strains were inhibited at least 90% at a

concentration of 25 ppm. However, the fungal growth of AY-5 strain was reduced ranged from 24 to 32% at 12.5 ppm and 25 ppm concentrations, and about 70% at higher than 25 ppm concentrations. For inhibition of *Fusarium* strains, silver nanoparticles at the 25–50 ppm concentrations could be used (Table 1).

The antimicrobial activities of silver nanoparticles may be the result of a loss of replication activity that inactivates the cellular proteins and enzymes of the pathogens [19, 37]. The antimicrobial capacity can also be increased by modifying the surface with the mediations of various extractions or different combinations with antimicrobials [38–40]. Fernández et al. [38] demonstrated that AgNPs biosynthesized from *Rhodotorula glutinis* had shown more effective antifungal activity. Gajbhiye et al. [39] tested synergistic effects of silver nanoparticles combination with fluconazole against pathogenic fungi. It was concluded that these combinations did not improve the antifungal activity against *Fusarium semitectum*; however, antifungal activity was enhanced against *Trichoderma* sp. and *Candida albicans*. Except for silver-based nanoparticles, other metallic nanoparticles, especially copper, having potential

Fig. 2 Inhibition effect of silver nanoparticles (AgNPs) with 3 nm size against *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) strains on PDA

antifungal activity have been investigated against *Fusarium* species. Saharan et al. [41] synthesized and evaluated Cu–chitosan nanoparticles against pathogenic fungi. Cu–chitosan nanoparticles, at the concentration of 0.12%, inhibited mycelium growth of *Alternaria solani* and *Fusarium oxysporum* by 70.5% and 73.5%, respectively.

3.2 Morphological characterization

The morphological characterization of conidia of six different FORL strains was listed in Table 2. The average length of microconidia and macroconidia were

varied from 3.59 to 5.52 μm and from 9.85 to 15.98 μm , respectively. The maximum antifungal activities were determined at 37.5–50 ppm concentrations of AgNPs in both agar diffusion and broth dilution bioassays. Then dimensions of micro- and macro-conidia of FORL strains were measured after AgNPs treatments. These dimensions were decreased to 3.07–4.94 μm (p -value < 0.05) for microconidia and 8.28–14.12 μm (p -value < 0.05) for macroconidia. Similarly, septation numbers of both microconidia and macroconidia were decreased via AgNPs treatments compared to the control (Table 2). Our findings results were correlated with previous results.

Table 1 Fungal growth inhibition (FGI, %) of different size (3 nm, 5 nm, 8 nm, and 10 nm) with various concentrations (12.5 ppm, 25 ppm, 50 ppm, 75 ppm, and 100 ppm) of silver nanoparticles against *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) strains in Potato Dextrose Broth medium

Strains (ppm)	AgNP sizes	AgNP concentrations			
		3 nm	5 nm	8 nm	10 nm
AY5	12.5	28 ± 2 ^{abc}	29 ± 2 ^{abc}	32 ± 2 ^c	23 ± 2 ^c
	25	29 ± 4 ^c	32 ± 3 ^c	27 ± 4 ^c	24 ± 1 ^c
	50	71 ± 2 ^c	68 ± 4 ^c	70 ± 1 ^c	66 ± 1 ^{ac}
	75	69 ± 4 ^c	70 ± 5 ^c	71 ± 2 ^c	67 ± 1 ^c
	100	68 ± 3 ^c	71 ± 6 ^c	73 ± 3 ^c	73 ± 2 ^c
H2	12.5	50 ± 2 ^{bc}	49 ± 2 ^{ac}	48 ± 3 ^{bc}	48 ± 2 ^c
	25	94 ± 4 ^c	96 ± 5 ^c	96 ± 4 ^a	98 ± 5 ^{ab}
	50	96 ± 2 ^c	97 ± 1	98 ± 2	98 ± 4 ^b
	75	97 ± 3	97 ± 2	98 ± 1 ^{ac}	98 ± 1
	100	96 ± 4	97 ± 3	98 ± 5	99 ± 2
JR	12.5	38 ± 3 ^c	40 ± 4 ^c	30 ± 3 ^{ac}	36 ± 4 ^{ac}
	25	91 ± 4 ^c	90 ± 2	92 ± 4	89 ± 2 ^{ac}
	50	91 ± 2	90 ± 1	92 ± 1	90 ± 1 ^a
	75	92 ± 1	89 ± 5	93 ± 2	92 ± 5
	100	91 ± 2	91 ± 2	93 ± 4	93 ± 3
SC	12.5	40 ± 4 ^{bc}	15 ± 3 ^c	14 ± 2 ^c	3 ± 1 ^c
	25	91 ± 1 ^c	93 ± 4	93 ± 3	93 ± 2 ^c
	50	93 ± 2 ^c	95 ± 1	94 ± 4	94 ± 4
	75	92 ± 4	96 ± 2 ^c	95 ± 5 ^c	96 ± 3
	100	91 ± 3	95 ± 4	96 ± 6	96 ± 2 ^c
TA-4	12.5	54 ± 4 ^c	56 ± 3 ^{abc}	47 ± 4 ^a	45 ± 2 ^c
	25	96 ± 2 ^c	91 ± 2 ^a	87 ± 2 ^{ab}	92 ± 4 ^c
	50	97 ± 1 ^c	92 ± 4	92 ± 1	94 ± 5
	75	97 ± 5	93 ± 2	94 ± 4 ^c	94 ± 1
	100	97 ± 2	91 ± 6	95 ± 2	94 ± 2
TR1	12.5	31 ± 2 ^{bc}	37 ± 3 ^c	38 ± 2 ^{ac}	19 ± 3 ^c
	25	93 ± 1 ^c	94 ± 2	95 ± 1	94 ± 2
	50	94 ± 4	94 ± 2 ^c	95 ± 3	94 ± 1
	75	94 ± 2	95 ± 2	96 ± 4 ^c	94 ± 2
	100	95 ± 3	96 ± 3	96 ± 2	95 ± 2

Values are averages of 3 individual experiments. Data are given as mean values ± standard deviations, which were calculated individually for each average. Means followed by a different letter(s) in the identical column differ significantly ($p=0.001$) according to Tukey's test. a = $p < 0.05$, b = $p < 0.01$, c = $p < 0.001$

Kheiri et al. [42] reported that chitosan and chitosan-based nanoparticles reduced the conidial germination of *Fusarium graminearum*. Chitosan-AgNP composite also reduced conidial germination and spore germination of *Colletotrichum gloeosporioides* [43]. Similarly, the treatments of chlorogenic acid, caffeic acid and coumarin could inhibit the germination of *Fusarium oxysporum* f. sp. *niveum* (FON) conidia [44, 45].

4 Conclusion

Metal-based nanoparticles have been extensively studied for having antimicrobial activities. The AgNPs exhibited high antifungal and antibacterial activity with several previous reports [39, 46]. Generally, a high concentration of AgNPs showed high antifungal activity against *Fusarium* strains. Different applications can be performed to use nanoparticles at lower concentrations. Nanoparticles size is also another important parameter affecting their antimicrobial efficiencies. For this, antifungal activities of nanoparticles in different concentrations and sizes were investigated.

Silver NPs exhibit high antifungal activity against plant pathogenic *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) strains at the concentrations around 37.5–50 ppm with low size. The results indicated that low AgNPs concentrations show efficient antifungal activity against *Fusarium* FORL species. As a result of the study, it can be said that silver nanoparticles can be used as plant protection in cultivating various agricultural products like tomato plants. However, further studies are needed to understand the toxicity of AgNPs before developing them for using in agricultural applications. Similarly, the different types and different sizes of metal-based nanoparticles might be tried for investigation of their antifungal activities against plant pathogenic fungi. Silver nanoparticles synthesized with natural phytochemicals, polypeptides or by several microorganisms might be also tested.

Table 2 Average dimensions and septation numbers of both microconidia and macroconidia of *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) strains after treatment with AgNPs at 37.5 ppm and 50 ppm concentrations

Strains	AgNPs concentrations	Microconidia		Macroconidia	
		Average dimensions		Average dimensions	
		Length (μm) × width (μm)		Length (μm) × width (μm)	
AY5	–	3.64 ± 0.35 × 2.83 ± 0.26	1–2	9.85 ± 0.94 × 3.10 ± 0.24	2–3
	37.5 ppm	3.38 ± 0.40 × 2.36 ± 0.03	0–1	9.73 ± 0.87 × 3.02 ± 0.12	1–2
	50 ppm	3.07 ± 0.37 × 2.00 ± 0.23	0	8.28 ± 0.93 × 2.98 ± 0.34	1–2
H2	–	4.33 ± 0.72 × 2.36 ± 0.12	1–2	15.98 ± 0.97 × 2.67 ± 0.31	3–4
	37.5 ppm	4.26 ± 0.83 × 2.09 ± 0.27	1–2	15.74 ± 0.73 × 2.65 ± 0.11	2–3
	50 ppm	3.89 ± 0.10 × 1.89 ± 0.13	0–1	14.12 ± 1.29 × 2.53 ± 0.07	1–2
JR	–	3.59 ± 0.39 × 2.19 ± 0.39	1–2	10.32 ± 0.52 × 2.76 ± 0.08	2–3
	37.5 ppm	3.36 ± 0.41 × 2.07 ± 0.20	0–1	10.70 ± 0.11 × 2.56 ± 0.36	1–2
	50 ppm	3.37 ± 0.39 × 1.97 ± 0.27	0–1	9.02 ± 0.61 × 2.37 ± 0.46	0–1
SC	–	5.52 ± 0.19 × 2.23 ± 0.14	1–2	13.05 ± 0.11 × 2.81 ± 0.16	3–4
	37.5 ppm	5.19 ± 0.40 × 2.18 ± 0.28	0–1	12.25 ± 0.43 × 2.73 ± 0.33	2–3
	50 ppm	4.94 ± 0.15 × 2.08 ± 0.34	0	11.45 ± 0.54 × 2.61 ± 0.10	2–3
TA-4	–	3.69 ± 0.43 × 2.29 ± 0.35	1	12.32 ± 0.26 × 2.68 ± 0.13	3–4
	37.5 ppm	3.59 ± 0.29 × 1.95 ± 0.28	0–1	11.26 ± 0.24 × 2.54 ± 0.19	2–3
	50 ppm	3.47 ± 0.42 × 1.56 ± 0.12	0–1	9.36 ± 0.56 × 2.41 ± 0.21	1–2
TR1	–	3.82 ± 0.70 × 2.21 ± 0.51	1–2	10.73 ± 0.30 × 2.93 ± 0.86	2–3
	37.5 ppm	3.64 ± 0.53 × 2.19 ± 0.38	1–2	10.22 ± 0.84 × 2.81 ± 0.44	1–2
	50 ppm	3.63 ± 0.56 × 2.13 ± 0.43	0–1	10.20 ± 0.42 × 2.78 ± 0.53	1–2

Values are averages of 3 individual experiments. Data are given as mean values ± standard deviations, which were calculated individually for each average

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Author contributions IA: Designed and performed research, analyzed data, wrote the manuscript, MU: Designed research, analyzed data, TS: Designed and performed research, analyzed data, wrote the manuscript.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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