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Silver nanoparticles inactivate sclerotial formation in controlling white rot disease in onion and garlic caused by the soil borne fungus *Stromatinia cepivora*

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Abstract Under Egyptian conditions, the white rot disease, caused by the soil borne fungus *Stromatinia cepivora*, causes a major problem in the production of onion and garlic crops. The current study aims to control this disease using silver nanoparticles (AgNPs), biologically synthesized by *Fusarium oxysporum*. In in vitro assays, carried out on Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) media, the biosynthesized AgNPs at various concentrations i.e. 40, 80, 120, 160 and 200 mg/L, showed a great and promising antifungal activity against the linear growth, mycelial biomass and scelerotial germination of *S. cepivora* isolates. With increasing of AgNPs concentration the antifungal activity was increased and the application of 200 mg/L produced maximum antifungal activity. It was noted that AgNPs at all concentrations inactivate sclerotial formation on the treated culture media and to our knowledge; this observation is considered a first report. The protein profile of AgNPs-untreated fungal biomass showed the presence of band at 28 k Dalton. This band was absent in the profile of AgNPs-treated fungal biomass and may be related to the formation of sclerotia. In the potted experiment, the chemical control using of tebuconazole recorded the highest efficiency in reducing the incidence of white rot disease. Significant reduction in the incidence of white rot was obtained with AgNPs applied as transplants/clove dipping or stems base spraying or dipping plus spraying. In general, the combined treatment was more effective than these methods used individually. In field experiments, the double treatment, which included the treatment of dipping and spraying, led to a significant reduction in the disease incidence, whether in the field with a low or high level of sclerotia. Under low inoculum levels, significant control was achieved with all methods used. However, the method of dipping only did not give significant control of garlic white rot under high inoculum levels. Application of AgNPs provides an improvement in the growth and yield of bulbs for both onion and garlic plants grown under field conditions. Therefore, AgNPs can be used as nanofungicide against white rot disease and as nanofertilizers for onion and garlic productions.

Keywords Onion · Garlic · White rot disease · Biogenic silver nanoparticles

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Introduction

The white rot disease causes severe damage to *Allium* fields throughout Egypt. The disease is characterized by above-ground symptoms include wilting, yellowing of older leaves and die-back of leaf tips (Abd-Elrazik et al., 1973). These symptoms progress down the leaf blades until they eventually collapse and decay. Root infection results watery decay that develops into mats of fluffy,

white mycelial grow at the base of the bulbs. Within these mycelial mats thousands of sclerotia are produced. These sclerotia are the source of primary inoculums of subsequent crops and can cause disease wherever crops of *Allium* spp. are grown. Colonized tissues of *Allium cepa* L. (onion) and *Allium sativum* L. (garlic) become so heavily infected that they senesce, resulting in unmarketable bulbs (Ulacio-Osorio et al., 2006). The causal agent of the disease is the phytopathogenic soil borne fungus *Stromatinia cepivora* (Berk.) Whetzel. It is a major threat for *Allium* crops and typically the limiting factor for production of onion and garlic in Egypt (Elshahawy et al., 2017a, 2017b, 2018a). The pathogen survives in soil and on the infected parts of host plant as sclerotia (0.3–0.6 mm in diameter) and does not produce spores in nature (Brix & Zinkernagel, 1992). Sclerotia can remain viable for over 30 years in the soil, even in the absence of hosts (Crowe et al., 1993). A very small number of sclerotia can cause significant disease (Crowe et al., 1980). Due to the formation of abundant sclerotia that can remain alive in soil for several years, white rot management is difficult (Crowe et al., 1993). Protection of *Allium* plants from this infection can be achieved by sequential applications of unenvironmental chemical fungicides such as triazole tebuconazole, vinclozolin and iprodione, but these frequently lose their effectiveness due to their microbial degradation in the soil (Entwistle & Hawling, 1984; Jackson et al., 1997). Soil fumigation with chemicals has been investigated to manage this disease (Merriman & Sutherland, 1978; Adams & Johnston, 1983), but the negative effects on human health and the environment have led to a reduction in use of certain chemicals, such as methyl bromide. Therefore, using other control strategies is safer to manage this disease. Biopesticides such as *Trichoderma* species, sclerotial mycoparasites and antagonistic bacteria have been evaluated for controlling white rot disease (Elshahawy et al., 2017a, 2017b, 2018a). To reduce the level of initial inoculum within the soil, an effective practice is to use sclerotia germination stimulants (Elshahawy et al., 2019, 2020). However, the effectiveness of these treatments depends on the density of viable sclerotia of *S. cepivora* (Elshahawy et al., 2018a).

The excessive use of chemical pesticides causes environmental as well as human health problems, which has led to the continuous search for alternatives to these pesticides. Due to technological advances in the synthesis of nano-sized silver particles, their use as an antimicrobial agent has become economical to produce. In recent years, the biosynthesis of AgNPs using microbial and plant agents has gained great interest due to the fact that these agents are environmentally friendly and controlled (Ahmad et al., 2002; Narayanan & Sakthivel, 2010; El-Naggar et al., 2014; Buhroo et al., 2017; Khalil et al., 2017; Darwesh et al., 2019a; Marrez et al., 2019; Rehman et al., 2019, 2020a, 2020b). Among the promising applications of AgNPs is their use in the control of plant diseases (Park et al., 2006; Jo et al., 2009; Min et al., 2009). Since silver nanoparticles displays multiple actions of inhibitory effect against microorganisms (Park et al., 2006), hence, it can be applied on plants in a more effective and safer way than using fungicides (Kim et al., 2012). Application of silver against *Bipolaris sorokiniana*, the cause of spot blotch disease of wheat, and *Magnaporthe grisea,* the cause of rice blast disease, has been tested by Jo et al. (2009). They found that from in vitro assays, the use of AgNPs led to significant inhibition of the growth of both fungi, and *M. grisea* was more sensitive to these treatments than *B. sorokiniana*. Marek et al. (2010) reported that significant inhibition of mycelial growth and spore germination of *Fusarium culmorum* at in vitro was observed for spores incubated with AgNPs. The treatment with silver nanoparticles significantly inhibited the growth of *Colletotrichum gloeosporioides*, the biological agent causing anthracnose disease on many fruits and vegetables (Aguilar-Méndez et al., 2011). Recently, the inhibitory effect of AgNPs has been studied against plant pathogenic fungi that inhabit soil under laboratory conditions only (Min et al., 2009; Elshahawy et al., 2018b). Park et al. (2006) found that the compound containing nanosized silica-silver inhibited the growth of plant pathogenic bacteria (*Pseudomonas syringae* and *Xanthomonas compestris* pv. *vesicatoria*) by 100% at a concentration of 100 ppm. While, this compound inhibited the growth of plant pathogenic fungi (*Magnaporthe grisea, Botrytis cinerea, Colletotrichum gloeosporioides, Pythium ultimum*, and *Rhizoctonia solani*) by 100% at a concentration of 10 ppm. AgNPs are now an environmentally acceptable alternative to chemical pesticides used in agriculture (Sharon et al., 2010). Despite that, there is very little research on the field application of using AgNPs (Elshahawy et al., 2018b). Therefore, the objectives of this work were to: (i) determine the effect of biogenic AgNPs on the in vitro growth of *S. cepivora*; (ii) evaluate the efficacy of biogenic AgNPs on the incidence of white rot disease

in onion and garlic under greenhouse conditions; (iii) confirm the efficacy of biogenic AgNPs to control white rot disease in onion and garlic under field conditions; and (iv) determine the effect of biogenic AgNPs on plant growth and bulbs yield of both crops grown under field conditions.

Materials and methods

Fungal isolates

Samples of onion and garlic plants showing typical symptoms of white rot disease were collected from white rot-infested field sites in El-Qalubia governorate, Egypt during 2015/2016 growing season. Small pieces from mats of fluffy, white mycelial grow at the base of the infected bulbs and roots of diseased onion and garlic plants were picked off and plated on PDA medium. Inoculated plates were incubated at 18–20 °C for 7–10 days and examined daily to observe growth of mycelium of the causal pathogen. Formation of distinguishable sclerotia was also noted and the cultures were purified using the hyphal tip technique. Ten isolates of *S. cepivora* were isolated from each crop. Identification of these isolates were carried out according to the morphological and cultural characters depend on the methods described previously (Gilman, 1957; Barnett & Hunter, 1972). Pure cultures of *S. cepivora* isolates were maintained on PDA slants and kept in a refrigerator at 4 °C as stock cultures for further tests. Pathogenic capabilities of these isolates showed varied degrees against onion and garlic plants (Elshahawy et al., 2017a, 2018b). Due to their aggressive pathogenicity, *S. cepivora* Sc2-isolated from infected onion plants and *S. cepivora* Sc8- isolated from infected garlic plants were selected as the main isolates for in vitro and greenhouse trials conducted in the present study. Pathogenic capabilities of these isolates were confirmed every year in pots under greenhouse conditions using the susceptible cultivar of onion and garlic plants (Elshahawy et al., 2019).

Biogenic AgNPs

AgNPs were synthesized biologically using *Fusarium oxysporum* strain conducted in previous study (Elshahawy et al., 2018b). In brief, fungal inoculates of *Fusarium oxysporum* strain were prepared on PDA medium at 28 ± 2 °C for 7 days in Petri dishes. The fungus was then grown into flask (250 mL) containing 100 mL of PDB medium and incubated in rotary shaker at 28 ± 2 °C for 3 days. After the full growth of cultures, mycelial mass of the fungus was removed using sterile filter papers. A 100 mL of supernatant (culture filtrate) were mixed with 100 mL of 1 mM $AgNO_3$ aqueous solution. The mixture was left at room temperature for 72 h. By the reduction of silver ions into silver nanoparticles, the AgNPs is produced. The obtained AgNPs was detected by spectrophotometer and transmission electron microscope and the size of AgNPs ranged between 10 and 30 nm and the shape of these particles was spherical.

Fungicide

Tebuconazole (commercialized as Folicur®, 25% a.i. Bayer Group Science, Germany) was used in greenhouse and field experiments for comparison. The chemical treatment was applied at the recommended rate of 1.0 ml Folicur / L.

Laboratory experiments

Antifungal effect of biogenic AgNPs

Linear growth assays The inhibitory effect of biogenic AgNPs was examined against the linear growth of onion isolate *S. cepivora* (Sc2) and garlic isolate *S. cepivora* (Sc8) in vitro using the pour plate method (Min et al., 2009). PDA medium (1 L; pH 6.5–6.8) containing 200 g potato, 20 g dextrose and 20 g agar was prepared and 99 ml distributed into 250 ml conical flasks. Potato dextrose agar medium was then autoclaved. Before solidification, aliquots of 1 ml distilled water containing AgNPs were added to PDA medium to obtain the desired concentrations of 40, 80, 120, 160 and 200 mg/L. After 48 h of incubation, agar plugs of uniform size (diameter, 5 mm) containing cultures (10-days old) of *S. cepivora* (Sc2) and *S. cepivora* (Sc8) isolates individually were inoculated simultaneously at the center of each Petri dish containing AgNPs, followed by incubation in darkness at 18 ± 2 °C for 10 days. Another set of Petri dishes, free of AgNPs were used as the control. Ten Petri dishes were used as replicates for each treatment as well as the control treatment. After 10 days of inoculations, the following parameters were measured:

(1) Average colony radius and percentages of growth reduction. The linear radial mycelial growth was measured to determine the inhibitor effect of nanoparticle. The percentages of fungal growth reduction were determined according to the following formula: Fungal growth reduction % = (– T)/ × 100, where is the diameter of mycelial growth in control plates and T is the diameter of mycelial growth in treated plates.
(2) Average number of formed sclerotia per cm^2 and percentages of sclerotial formation reduction. At the end of experiment, agar disc (3 mm) from AgNPs-treated and untreated plates were transferred to fresh PDA medium free from AgNPs and incubated for in darkness at 18 ± 2 °C for further 10 days. Growth rate (mm/day) was calculated from the radial growth between day 3 and 10 (mean of ten replicates).

Growth biomass assays The inhibitory effect of biogenic AgNPs was examined against the growth biomass (growth fresh and dry weight) of onion isolate *S. cepivora* (Sc2) and garlic isolate *S. cepivora* (Sc8) in vitro using poisoned PDB method (Min et al., 2009; Darwesh et al., 2020a). Potato dextrose broth medium was then autoclaved and aliquots of one ml distilled water containing AgNPs were added to PDB medium to obtain the desired concentrations of 40, 80, 120, 160 and 200 mg/L. After 48 h of incubation, agar plugs of uniform size (diameter, 5 mm) containing cultures (10-days old) of each of S. *cepivora* (Sc2) and *S. cepivora* (Sc8) isolates individually were inoculated, followed by incubation in darkness at 28 ± 2 °C for 14 days. Another set of conical flasks containing PDB free of AgNPs were used as the control. Ten conical flasks were used as replicates for each treatment as well as the control treatment. After 14 days of incubation, the growth of each isolates grown on inoculated flasks were filtered through Watman filter paper No.1 and weighted to obtain the fresh weight of growth biomass. Weighted growth was placed on pre-weighted Petri plates and dried at 70 °C until the consistent of the growth weight to obtain the dry weight of growth biomass. The percentages of growth biomass reduction were determined according to the following formula: Fungal growth reduction % = (– T)/ × 100, where is the weight of the growth in control treatment and T is the weight of the growth in AgNPs treatment.

Sclerotial germination assays The inhibitory effect of biogenic AgNPs was examined against the sclerotia of onion isolate *S. cepivora* (Sc2) and garlic isolate *S. cepivora* (Sc8) in vitro using sclerotial germination assays (Min et al., 2009). Sclerotia were collected from each of *S. cepivora* (Sc2) and *S. cepivora* (Sc8) cultures (60-day-old) and soaked in test tubes containing the concentration of AgNPs to be tested for 12 h at room temperature. The tested concentrations of AgNPs were 40, 80, 120, 160 and 200 mg/L. At the end of the dipping period, sclerotia were washed with sterile distilled water and thirty sclerotia from each treatment were transferred individually under aseptic conditions to Petri-dishes containing PDA medium. Water-soaked sclerotia were used for control treatment. Four replicates (dishes) were used for each treatment. Petri dishes bearing sclerotia were incubated in darkness at 18 ± 2 °C for 7 days and percentages of germinating sclerotia were determined.

Protein profile analysis of S. cepivora treated with AgNPs The effect of AgNPs on production of *S. cepivora* sclerotia was evaluated by analysis of total protein profile. The fresh biomass of *S. cepivora* treated with different AgNPs concentrations was collected and conducted to protein extraction method (Fido et al., 2004; Darwesh et al., 2020b). The fungal biomass was rapid freezed with liquid nitrogen and grounded to make dried powder. The dried samples were mixed with suitable amount of water-soluble protein extraction buffer in eppendorf tube and left in refrigerator overnight. The mixture was incubated at 65 °C for 60 min, vortexed for 15 s and centrifuged at 5000 rpm under cooling (4 °C) for 15 min. The supernatants containing water-soluble proteins were transferred to new eppendorf tubes and kept in a deep-freeze cabinet until use for electrophoretic analysis. To determine the relative molecular weight of extracted proteins, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a stacking and separating gel according to the method of Laemmli (1970) using Mini-gel electrophoresis (BioRad, USA). The molecular weight of the isolated proteins was estimated in comparison to standard molecular weight markers (standard protein markers, 11–245 kDa; Sigma, USA). The protein bands were visualized by staining with Coomassie Brilliant Blue G-250 (Sigma, USA) and documented using GelAnalyzer2010a program (Darwesh et al., 2019b).

Greenhouse experiment

Preparation of white rot pathogen inocula

Fungal mass production of each isolates used for soil infestation was obtained by growing the desired isolate on sand-barley medium (Elshahawy et al., 2017a). This natural medium was prepared by mixing sand and barley (1: 1, w: w and 40% water); then the mixture in glass bottles (500 mL capacity) with cotton plugs was sterilized three times (1 h each time) at 121 °C. The autoclaved medium was then inoculated individually with a 5-mm disk of each tested fungal isolate and then incubated in darkness at 18 ± 2 °C for three weeks. Autoclaved soil was infested individually at a ratio of 2% (w: w) with the tested isolate and mixed thoroughly to ensure equal distribution of fungal inoculum, then filled in sterilized plastic pots (30 cm diameter) and irrigated every second day for 1 week before sowing.

Effects of biogenic AgNPson white rot disease development

The effects of the three application methods of biogenic AgNPs (transplants/clove dipping or stems base spraying or dipping plus spraying) on the development of white rot disease on onion and garlic in soil artificially infested with *S. cepivora* (Sc2) or *S. cepivora* (Sc8) respectively were investigated. For each application method, AgNPs at 200 mg/L water was used. The dipping method was applied by dipping onion transplants or garlic cloves before sowing in biogenic AgNPs (200 mg/L) for 5 min. In stem base spraying method, stem bases of plants were sprayed with the same concentration three times intervals at 30, 60 and 90 days after planting. The experiment was carried out in pots under greenhouse conditions (the minimum and the maximum temperatures were 5–10 °C and 20–25 °C respectively) using susceptible cultivars of onion and garlic. For each crop of onion and garlic, the experiments were conducted with a completely randomized design (CRD) with five treatments (three of biogenic silver nanoparticles applications (dipping only, stem base spraying and dipping + spraying), chemical fungicide and infested control) each with four replicates. Each replicate consisted of a sterilized plastic pot (30 cm diameter) containing 5 kg of autoclaved loamy clay soil pre-infested with *S. cepivora* at the rate of 2% (*w*/w) 2 weeks before transplanting. The chemical treatment was applied according to the standard fungicide program. In this program, the seedlings and cloves were dipped and sprayed (two time-intervals with 6 weeks in between) with 1 ml/L Folicur. Before treatment, seedlings and cloves were superficially disinfected by dipping in sodium hypochlorite solution (0.25%) for 2 min and rinsed after surface-sterilization with sterile distilled water. For onion, five surface-disinfected 60-day-old onion transplants (cv. Giza 20) were transplanted in each pot. For garlic, five surface-disinfected garlic cloves (cv. Sides 40) were sown in each pot. Nitrogen fertilizer in form of urea (46% N) was added at the rate of 10 g/pot 30 days after planting and plants were irrigated when necessary. White rot disease evaluations were conducted periodically during the growing season based on top symptoms of white rot include wilting, yellowing of older leaves and die-back of leaf tips, and were confirmed by gently removing some soil from around the base of some plants to see watery decay mats of fluffy, white mycelial growth of the pathogen. At harvest, 100 days after planting, the percentage of dead plants due to white rot infection was calculated as follows:

$$\text{White rot infection } (\%) \frac{\text{No of dead plants due to white rot infection}}{\text{Total no.of plants}} \times 100$$

Field experiments

Selection of trials location

Field trials were located in El-Deer village, El-Qalubia governorate in which white rot disease was of high commercial interest. In this region, several fields with a well-established history of white rot disease were sampled preliminarily for inoculum density determinations according to the procedure of Utkhede and Rahe (1979). After that, two field sites were chosen. One of them was characterized by their low sclerotial density and had an average of 40 sclerotia per kg soil. The

second was characterized by high sclerotial density and had an average of 600 sclerotia per kg soil.

Effects of biogenic AgNPs on white rot disease development

Two field trials were used to estimate the efficiency of the three application methods of biogenic AgNPs (dipping only, stem base spraying and dipping + stem base spraying) for controlling white rot disease of onion and garlic plants. For each application method, AgNPs at 200 mg/L water was used. The field experiment was carried out during the growing season of 2018/ 2019 in clay soil naturally infested with sclerotia of *S. cepivora* at El-Qalubia governorate. The low sclerotial density trial had an average of 40 sclerotia per kg soil and the high sclerotial density second trial had 600 sclerotia per kg soil. For each crop in each trial, the experiments were conducted with a completely randomized design (CRD) with five treatments (three application methods of biogenic silver nanoparticles (dipping only, stem base spraying and dipping + stem base spraying), chemical fungicide and un-treated control). For each crop three replicated plots were used for each treatment as well as untreated controls. The plot area was 3.0 × 3.5 m (10.5 m^2) included 6 rows (each 3.0 m length and 50 cm width). The dipping method was applied by dipping onion transplants or garlic cloves before sowing in biogenic silver nanoparticles (200 mg/L) for 5 min. In stem base spraying method, stem bases of plants were sprayed with the same concentration three times intervals at 30, 60 and 90 days after planting. The chemical treatment was applied by dipping onion transplants or garlic cloves before sowing in fungicide formulation (1.0 ml Folicur /L) for 5 min. One month later, stem bases of both crops were sprayed (tow times) with the same concentration of Folicur at 6-weeks intervals. Garlic cloves cv. chinese that had been uniformly sized were hand planted 3 in. deep in rows spaced 10 cm × 10 cm within each row on the second week of October. Also, 60-days-old onion transplants (cv. Giza red) were transplanted in each row at spacing 10 cm × 10 cm within each row on the first week of December. Garlic and onion were grown to maturity under irrigation, fertilization and pest management practices standard with commercial production in the area. White rot disease evaluations were conducted periodically during the season of 2018/2019 based on top symptoms of white rot and were confirmed by gently removing some soil from around the base of some plants. At harvest, bulbs with symptoms of white rot were assessed by pulling and observing all garlic and onion bulbs in each plot. The percentage (%) of infected plants was calculated based on the number of plants with white rot symptoms in relative to the total number of plants.

Effect of biogenic AgNPs on plant growth and bulb yield

Effects of three application methods of biogenic AgNPs (dipping only, stem base spraying and dipping + stem base spraying) on plant growth were studied on onion and garlic grown under field conditions. The biogenic silver nanoparticles and chemical fungicide were applied as described in field experiments. Un-treated control plots for each crop were involved. Three replicates were used per treatment as well as the control. At 100 days after planting, some vegetative growth parameters: average plant height (cm), average number of leaves/plant and average plant biomass (g), of each crop was estimated. At the end of the experiment (150 days for onion and 180 days for garlic after planting), fresh weight of onion and garlic plants (bulbs with the tops of the plants) within each plot were weighed. Efficacy of treatments was calculated using the following formula: Efficacy (%) = Fresh weight of plants in control- Fresh weight of plants in treatment/ Fresh weight of plants in control × 100.

Statistical analysis

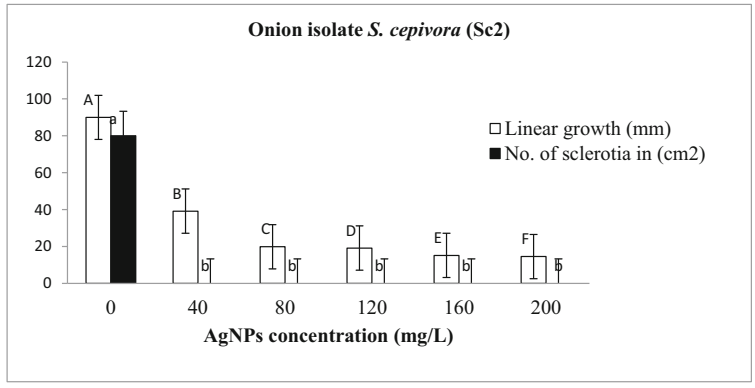
Data were entered into SPSS software version 14.0 and analyzed statistically by the analysis of variance (ANOVA) and the means were compared by Duncan's multiple range test at $P < 0.05$. Data from greenhouse and field experiments were analyzed separately for each crop and are presented as mean values ± standard errors (SE). Data for percentage of white rot disease incidence were statistically analyzed after arcsine square-root transformation; however, untransformed data are presented (Hussein et al., 2019a, 2019b).

Results

Laboratory trails

Inhibitory action of AgNPs at sequential concentrations of 40, 80, 120, 160 and 200 mg/L was analyzed using

the methods of linear growth assays on PDA, growth biomass assays in PDB and sclerotial germination assays in sterilized distilled water. The inhibitory effect of AgNPs at various concentrations against the linear growth of *S. cepivora* isolates were summarized in Figs. 1 and 2. The effect of AgNPs on *S. cepivora* isolates took the same approach and the antifungal effect of AgNPs is related to the concentration used. As the concentration of AgNPs increased, the radial growth of *S. cepivora* isolates decreased (Figs. 1 and 2). The lowest inhibition value was obtained from treating the PDA medium with AgNPs at a concentration of 40 mg / L of AgNPs, being 56.4 and 53.8% against *S. cepivora* (Sc2) and *S. cepivora* (Sc8), respectively. The highest inhibition value was obtained when treating the PDA medium with a concentration of 200 mg / L AgNPs, being 83.8 and 83.9% against *S. cepivora* (Sc2) and S. *cepivora* (Sc8), respectively. On the other hand, the synthesized AgNPs completely inhibited the formation of sclerotia on PDA poisoned with any tested concentrations (Figs. 1 and 2). It was evident that the synthesized AgNPs retuned the growth rate of both *S. cepivora* isolates when an agar-plug of their culture grown for 10 days on PDA poisoned with AgNPs was transferred to fresh PDA medium (Fig. 3). The inhibitory effect of AgNPs concentrations against *S. cepivora* isolates using the growth biomass assays in PDB are summarized in Figs. 4 and 5. As concentrations of the AgNPs increased, the weight of fresh and dray biomass of both *S. cepivora* isolates decreased. The lowest level of inhibition was observed against both isolates on PDB poisoned with 40 mg / L AgNPs, while the highest inhibition values were at 200 mg / L AgNPs. Finally, the inhibitory effect of AgNPs concentrations against *S. cepivora* isolates using the sclerotial germination assays are summarized in Fig. 6. As concentrations of the AgNPs increased, the germinated sclerotia of *S. cepivora* isolates decreased and from the concentration of 120 mg/L of AgNPs sclerotial germination were fully inhibited. To evaluate the anti-sclerotial-production effect of AgNPs, treated and untreated fungal biomass from each concentration was collected for protein extraction. The extracted proteins were electrophoresed using mini gel electrophoreses and the visualized proteins were analyzed using GelAnalyzer2010a program. The result showed that the main protein band



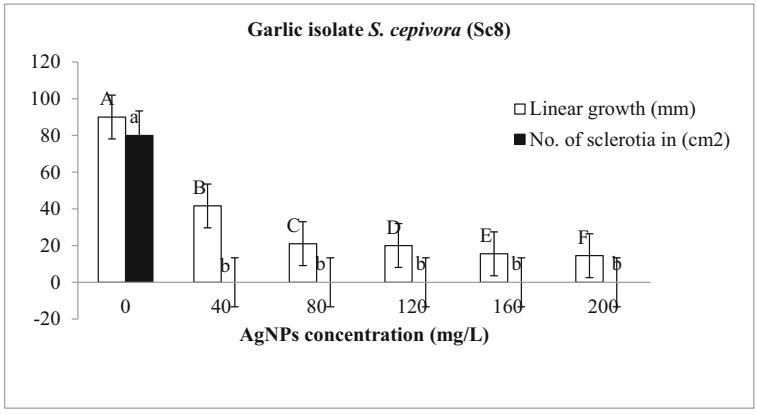


Fig. 1 Average linear growth (mm) and average number of sclerotia in (cm^2) of *Stromatinia cepivora* isolates in the presence of different concentration of AgNPs in vitro. *S. cepivora* isolates were grown for 10 days at 18 ± 2 °C on PDA medium amended with AgNPs. After that agar disc (5 mm) from each isolate was seeded. Fresh PDA medium free from AgNPs wad used as control. Linear growth (mm) and number of sclerotia in cm2 were calculated 10 days after inculcation. Values are mean of ten replications for each AgNPs concentration as well as the control. Bars with the same letters within each variable indicate that the means± standard errors are not significantly different at $P < 0.05$, according to Duncan's multiple range tests

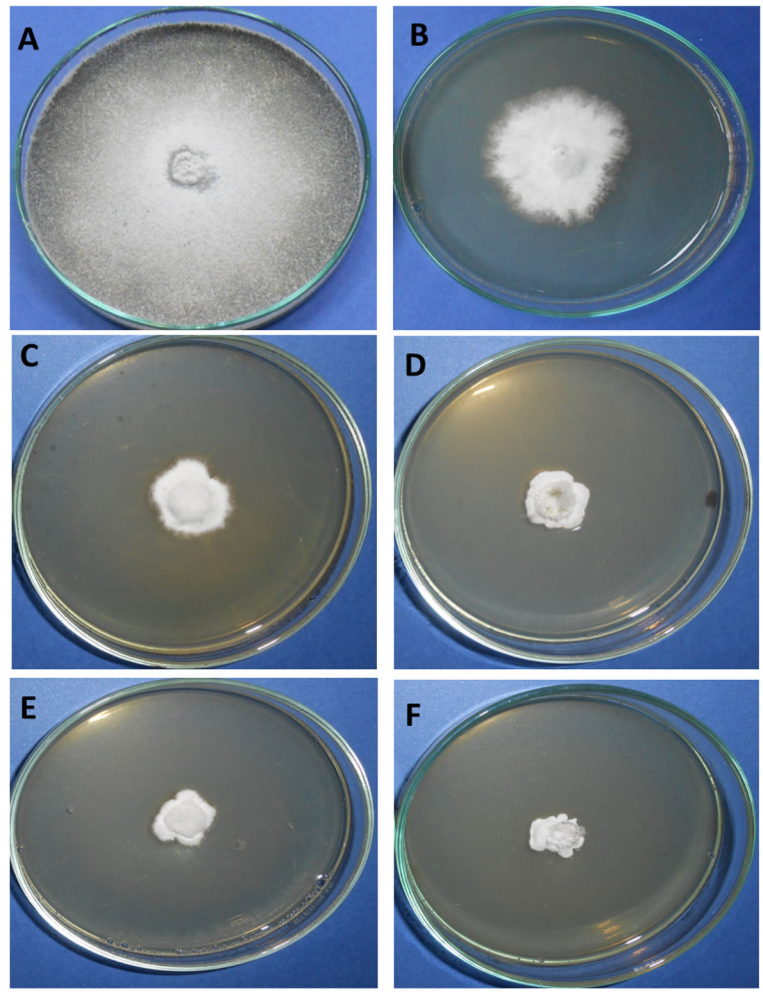


Fig. 2 The antifungal effect of AgNPs against *S. cepivora* (Sc2) using linear growth assays: **A** control, **B** AgNPs at the concentration 40 mg/L, **C** AgNPs (80 mg/L), **D** AgNPs (120 mg/L), **E** AgNPs (160 mg/L), **F** AgNPs (200 mg/L)

at 28 kDa was found in untreated fungal biomass (Fig. 7). This protein was disappeared with all AgNPs treatments. This confirmed the previous results, noted on PDA and PDB treated with AgNPs, the AgNPs at all concentrations inactivate sclerotial formation on the treated media and to our knowledge; this observation is considered a first report.

Greenhouse trail

Under greenhouse conditions the treatment with the fungicide tebuconazole gave the best result in reducing the incidence of white rot infection, as the infection percentages was 20% for onion and 35% for garlic, compared to 90 and 100% in the control treatment, respectively (Fig. 8). AgNPs treatments (200 mg/L) caused a significant decreasing in onion and garlic white rot compared to control treatment. In general, the combined treatment of transplants/clove dipping plus stems base spraying was the most effective treatment in this regard followed by stems base spraying whereas dipping only was the least effective one. The treatment, which included transplants/clove dipping plus stems base spraying, was the most effective in reducing the incidence of white rot by 77.8 in onion and by 70.0% in garlic (Fig. 8).

Field trails

In the field experiment, the success of the treatments was related to the inoculum density from sclerotia in the soil. As the percentage of plants infected with white rot increased in the field with high inoculum of sclerotia, while that percentage decreased in the field with low inoculum (Table 1). Where the treatment with AgNPs gave the best results in reducing the incidence of disease in the low-density field of the sclerotia of the pathogen

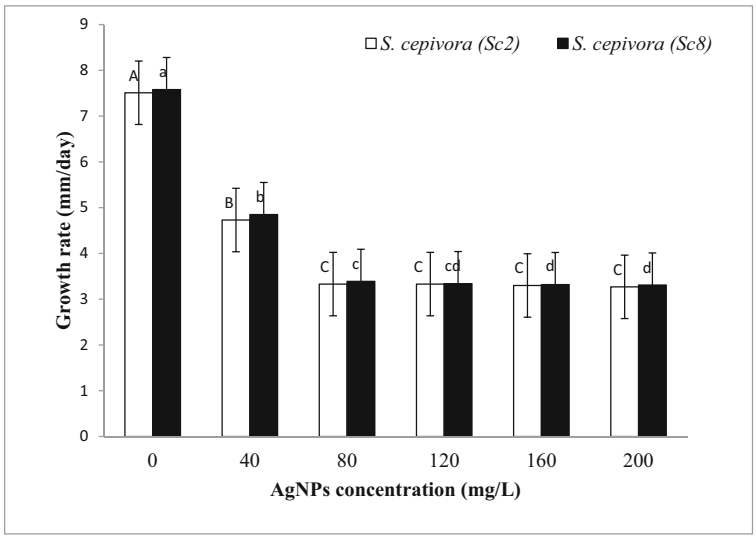


Fig. 3 Effect of AgNPs on the growth rate of *Stromatinia cepivora* isolates in vitro. *S. cepivora* isolates were grown for 10 days at 18 ± 2 °C on PDA medium treated with AgNPs. After that agar disc (5 mm) from each culture were transferred to fresh PDA medium free from AgNPs. Growth rate (mm/day) was calculated from the radial growth between day 3 and 10 (mean of ten replicates). Values are mean of ten replications for each AgNPs concentration as well as the control. Bars with the same letters within each variable indicate that the means± standard errors are not significantly different at $P < 0.05$, according to Duncan's multiple range tests

compared to the high-density field of the sclerotia. In the low-density field of the sclerotia of the pathogen, the treatment with the fungicide significantly was more effective in reducing the incidence of disease, as the percentage of infected plants was 9.25% in onion and 13.0% in garlic compared to 41.25% and 47.5% in

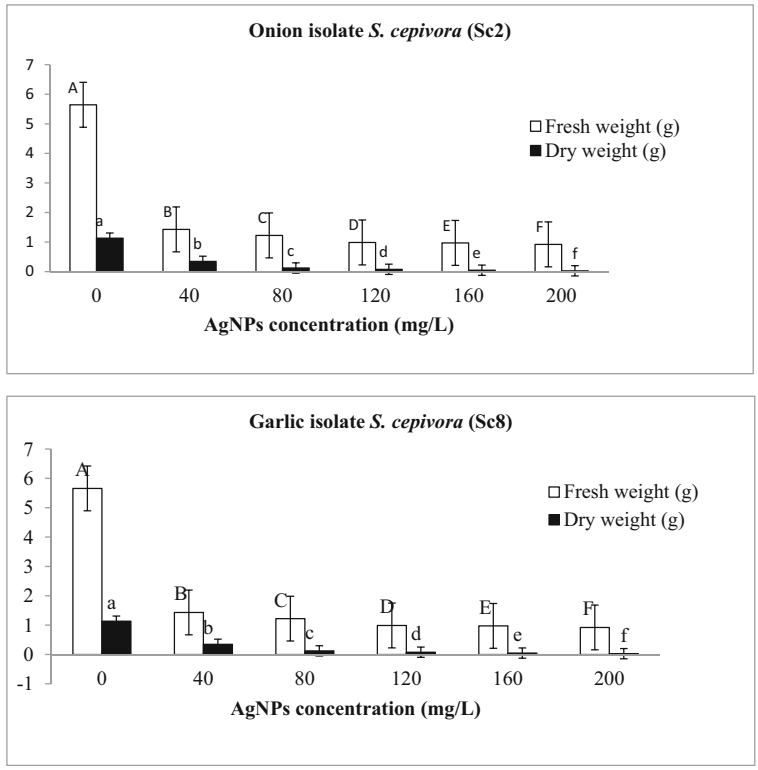


Fig. 4 Fresh weight (g) and dry weight (g) of growth biomass of *Stromatinia cepivora* isolates in the presence of different concentration of AgNPs in vitro. *S. cepivora* isolates were grown for 10 days at 18 ± 2 °C on PDB medium amended with AgNPs. After that agar disc (5 mm) from each isolate was seeded. Fresh PDB medium free from AgNPs used as control. Fresh and dry weights (g) of fugal biomass were calculated 10 days after inoculation. Values are mean of ten replications for each AgNPs concentration as well as the control. Bars with the same letters within each variable indicate that the means± standard errors are not significantly different at $P < 0.05$, according to Duncan's multiple range tests

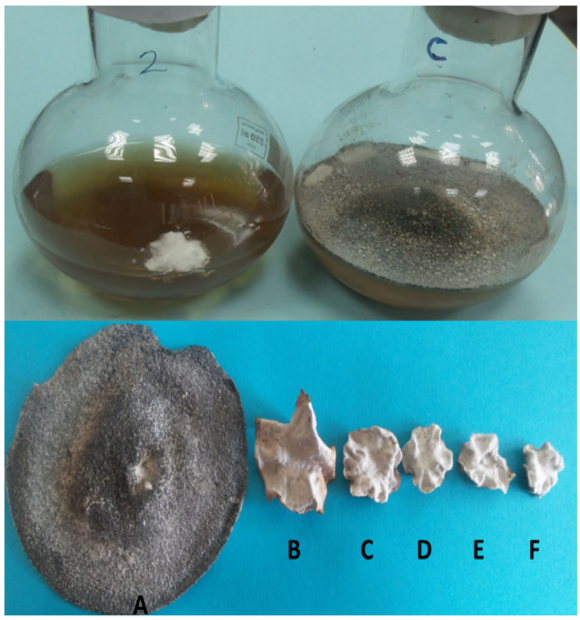


Fig. 5 The antifungal effect of AgNPs against *Stromatinia cepivora* (Sc2) using growth biomass assay: (A) control, (B) 40 mg/l of AgNPs, (C) 80 mg/l of AgNPs, (D) 120 mg/l of AgNPs, (E) 160 mg/l of AgNPs and (F) 200 mg/l of AgNPs

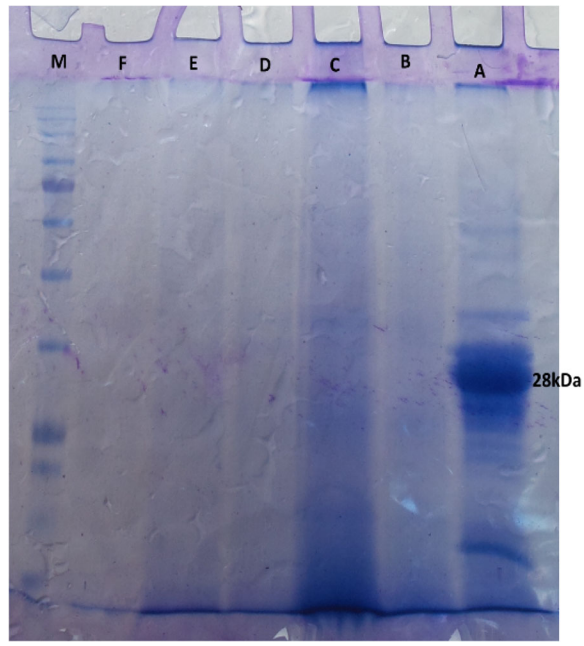


Fig. 7 Protein profile of *Stromatinia cepivora* treated by AgNPs and the electrophoresed proteins analyzed using GelAnalyzer2010a program (sclerotia protein: 28 kDa). Lane M: protein marker, Lane F: fungal biomass grown on PDB medium treated with 200 mg/L AgNPs. Lane E: fungal biomass grown on PDB medium treated with 160 mg/L AgNPs. Lane D: fungal biomass grown on PDB medium treated with 120 mg/L AgNPs. Lane C: fungal biomass grown on PDB medium treated with 80 mg/L AgNPs. Lane B: fungal biomass grown on PDB medium treated with 40 mg/L AgNPs. Lane A: fungal biomass grown on PDB medium free from AgNPs

control, respectively (Table 1). This was significantly followed by the treatment included transplants/clove dipping plus stems base spraying of AgNPs, where the percentage of infected plants was 16.0% in onions and 20.5% in garlic (Table 1). In general, the combined treatment of dipping plus stems base spraying significantly was the most effective treatment in this regard

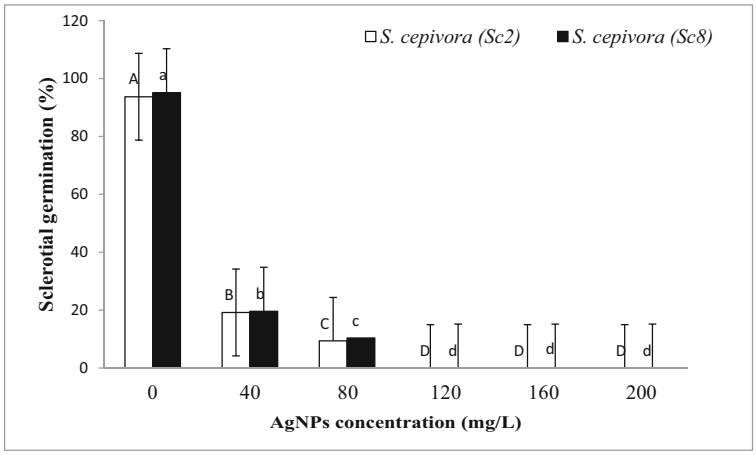


Fig. 6 Sclerotial germination (%) of *Stromatinia cepivora* isolates after they were soaked in different concentrations of AgNPs as well as distilled water in vitro. Values are mean of four replications for each AgNPs concentration as well as the control. Bars with the same letters within each variable indicate that the means± standard errors are not significantly different at $P < 0.05$, according to Duncan's multiple range tests. Percentages data of sclerotial germination were transformed into arcsine square root transformed data for analyses of variance; however, untransformed data are presented

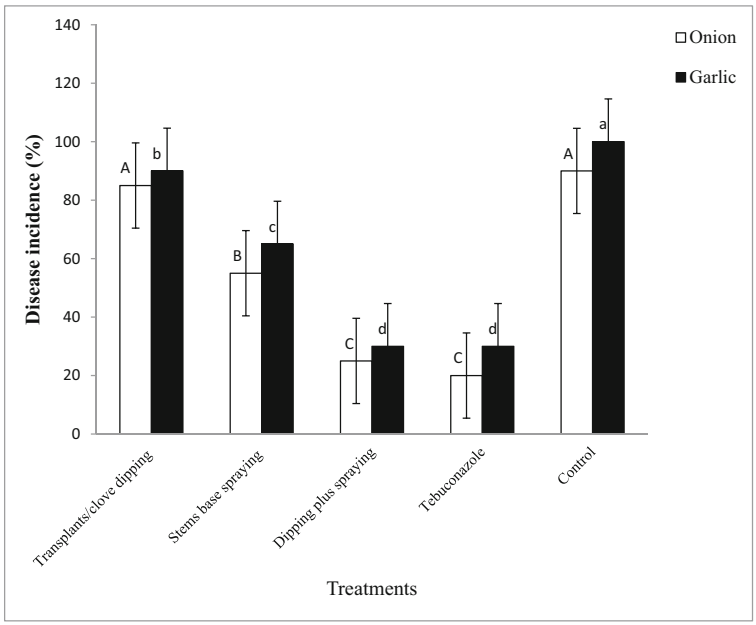


Fig. 8 Effects on white rot incidence (%) in onion and garlic due to application of AgNPs under greenhouse conditions. The dipping method was applied by dipping onion transplants or garlic cloves before sowing in biogenic AgNPs (200 mg/L) for 5 min. In soil drenching method, stem bases of plants were sprayed with the same concentration three times intervals at 30, 60 and 90 days after planting. Values are mean ± standard errors of five replicates for each treatment as well as the control. Percentages data of disease incidence (dead plants due to white rot infection) were transformed into arcsine square root transformed data for analyses of variance; however, untransformed data are presented

Table 1 Effects on white rot disease incidence (%) in onion and garlic due to application of silver nanoparticles (AgNPs) under field conditions

Treatment [(a)]	White rot incidence (%) and reduction (%)			
	Incidence (%)	Reduction (%)	Incidence (%)	Reduction (%)
Onion				
	Trial I (40 sclerotia/kg soil)		Trial II (600 sclerotia/kg soil)	
Transplants dipping	34.00±0.58 b[(b)]	17.6	70.75±1.11 a	1.4
Stem base spraying	29.25±0.48 c	29.1	66.00±0.41 b	8.0
Dipping+ spraying	16.00±0.71 d	61.2	44.75±1.25 c	37.6
Tebuconazole	9.25±0.75 e	77.6	28.00±0.41 d	60.9
Control	41.25±0.48 a	–	71.75±0.85 a	–
Garlic				
	Trial I (40 sclerotia/kg soil)		Trial II (600 sclerotia/kg soil)	
Clove dipping	35.50±0.65 b	25.3	83.50±0.50 a	2.9
Stem base spraying	30.75±0.48 c	35.3	78.00±1.08 b	9.3
Dipping+ spraying	20.50±1.04 d	56.8	66.25±1.55 c	22.9
Tebuconazole	13.00±0.58 e	72.6	59.25±0.48 d	31.1
Control	47.50±1.19 a	–	86.00±0.71 a	–

[(a)] Three application methods of AgNPs at 200 mg/L water were used. The dipping method was applied by dipping onion transplants or garlic cloves before sowing in biogenic silver nanoparticles (200 mg/L) for 5 min. In stem base spraying method, stem bases of plants were sprayed with the same concentration three times intervals at 30, 60 and 90 days after planting. Values are means of three replications. [(b)] Means ± standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$. Percentages data of disease incidence (dead plants due to white rot infection) were transformed into arcsine square root transformed data for analyses of variance; however, untransformed data are presented

followed by stems base spraying whereas dipping only was the least effective one. In the high-density field of the sclerotia of the pathogen, the treatment with the fungicide significantly was more effective in reducing the incidence of disease, as the percentage of infected plants was 28.0% in onion and 59.25% in garlic compared to 71.75 and 86.0% in control, respectively (Table 1). AgNPs treatment included transplants/clove dipping plus stems base spraying was the most effective treatment, decreasing disease incidence by 37.6% in onion and 22.9% in garlic. The treatment that included dipping transplants/clove in AgNPs only did not give a significant result in reducing the disease, while the stems base spraying treatment gave a less significant result.

Concerning the effect of treatments on plant growth and bulb yield, data showed that the treatment with AgNPs caused a significant increase in the growth and bulbs yield of onion and garlic plants during field experiments (Tables 2, 3 and 4). The effect of the treatments followed the same approach, but it was related to the sclerotial density in the soil. The average vegetative characteristics and bulbs yield of plants grown in soils with low sclerotial density were much better than those grown in soils with high sclerotial density. AgNPs was more effective for improving the growth and bulbs yield of onion and garlic plants in soils containing low sclerotial density than in those containing high sclerotial density. For onion plants grown in soil with low sclerotial density, double treatment of silver nanoparticles (transplants/clove dipping plus stems base spraying) gave the best results after the fungicide. This treatment caused a 19.8% increase in plant height, a 30.3% increase in average number of leaves/plant and a 34.4% increase in average plant biomass (Table 2). This treatment caused 23.2% increase in bulbs yield of onion (Table 4). For garlic plants grown in soil with low sclerotial density, double treatment of silver nanoparticles (transplants/clove dipping plus stems base spraying) gave the best results after the fungicide. This treatment caused a 14.0% increase in plant height, a 16.7% increase in average number of leaves/plant and a 31.0% increase in average plant biomass (Table 3). This treatment caused 24.9% increase in bulbs yield of garlic (Table 4).

Table 2 Effects on average plant height, average number of leaves/plant and average plant biomass of onion plants from application of silver nanoparticles (AgNPs) under field conditions at 100 days after planting

Treatment[(a)]	Onion plants grown in field		
	Plant height (cm)	Number of leaves/plant	Plant biomass (g)
	Trial I (40 sclerotia/kg soil)		
Transplants dipping	66.20±0.27 d[(b)]	7.75±0.25 a	84.08±0.50 c
Stem base spraying	70.78±0.24 c	8.00±0.00 a	95.65±0.03 b
Dipping+ spraying	73.88±0.43 b	8.25±0.25 a	96.28±0.25 b
Tebuconazole	79.68±0.54 a	8.50±0.29 a	105.30±2.05 a
Control	59.25±1.92 e	5.75±0.25 b	63.15±0.55 d
	Trial II (600 sclerotia/kg soil)		
Transplants dipping	57.63±0.10 c	6.75±0.25 b	52.53±0.05 c
Stem base spraying	57.85±0.06 c	6.75±0.25 b	52.63±0.09 c
Dipping+ spraying	62.43±0.23 b	7.25±0.25 ab	55.83±0.37 b
Tebuconazole	69.38±0.37 a	7.75±0.25 a	59.85±0.50 a
Control	46.00±0.82 d	5.25±0.25 c	45.20±0.26 d

[(a)] Three application methods of AgNPs at 200 mg/L water were used. The dipping method was applied by dipping onion transplants or garlic cloves before sowing in biogenic silver nanoparticles (200 mg/L) for 5 min. In stem base spraying method, stem bases of plants were sprayed with the same concentration three times intervals at 30, 60 and 90 days after planting. Values are means of three replications. [(b)] Means ± standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$

Discussion

White rot disease, caused by the soil borne fungus *S. cepivora*, has a great impact on onion and garlic productions worldwide due to reduction of yields and quality. Currently, the synthetic fungicides, with acceptable antifungal effect against *S. cepivora*, are the main option to control this disease. However, there is a need for new alternative methods of disease control according to the principles of sustainable agriculture. Researchers and scientists around the world work hard to develop new materials or derivatives for facing the pathogenic microbe's distribution (El-Baz et al., 2015; El-Shanshoury et al., 2020). These materials may be produced by microbial or plant sources (Ali et al., 2016; Kheiralla et al., 2016) Nanomaterials are considered

Table 3 Effects on average plant height, average number of leaves/plant and average plant biomass of garlic plants from application of silver nanoparticles (AgNPs) under field conditions at 100 days after planting

Treatment[(a)]	Garlic plants grown in field		
	Plant height (cm)	Number of leaves/plant	Plant biomass (g)
	Trial I (40 sclerotia/kg soil)		
Clove dipping	65.80±0.04 a[(b)]	7.00±0.00 ab	70.45±0.15 a
Stem base spraying	66.25±0.24 a	7.50±0.29 a	71.95±0.45 a
Dipping+ spraying	66.93±0.23 a	7.50±0.29 a	73.05±0.16 a
Tebuconazole	61.25±0.45 b	7.00±0.00 ab	52.73±0.11 b
Control	57.58±1.49 c	6.25±0.48 b	50.38±2.19 b
	Trial II (600 sclerotia/kg soil)		
Clove dipping	57.38±0.05 a	6.25±0.25	50.10±0.06 b
Stem base spraying	57.38±0.06 a	6.50±0.29	50.58±0.10 b
Dipping+ spraying	58.08±0.08 a	7.00±0.00	52.33±0.13 a
Tebuconazole	43.60±0.11 b	5.75±0.25	40.40±0.82 c
Control	37.65±0.52 c	5.25±0.25	30.80±0.68 d

[(a)] Three application methods of AgNPs at 200 mg/L water were used. The dipping method was applied by dipping onion transplants or garlic cloves before sowing in biogenic silver nanoparticles (200 mg/L) for 5 min. In stem base spraying method, stem bases of plants were sprayed with the same concentration three times intervals at 30, 60 and 90 days after planting. Values are means of three replications. [(b)] Means ± standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$

efficient and safer alternatives to the chemical methods of disease control (Mohamed et al., 2015; Abdelhameed et al., 2019; Panpatte & Jhala, 2019). Among them, AgNPs demonstrated biocidal activity against many plant pathogenic fungi and their application might have more advantages compared to conventional fungicides (Kim et al., 2012; Mendes et al., 2014; Mourad et al., 2019). In some cases, AgNPs showed better growth inhibition effect than certain synthetic fungicides against investigated fungi (Vrandečić et al., 2019).

In the current study inhibition assays using AgNPs in solid and liquid cultures media indicated that AgNPs was very effective against *S. cepivora* isolates in vitro. The results clearly demonstrated that the biologically synthesized AgNPs inhibited the linear growth, growth biomass and inhibited the formation of *S. cepivora* sclerotia in vitro. Min et al. (2009) reported that the application of silver nanoparticles is effective against sclerotium forming fungi. Also, Jung et al. (2010) found that liquid nano-silver significantly inhibited the growth of *Sclerotium cepivorum*. Lamsal et al. (2011) reported that AgNP at 100 ppm on PDA culture inhibited the growth of *Colletotrichum* sp., the causal agent of pepper anthracnose. Aguilar-Méndez et al. (2011) reported that silver nanoparticles significantly delay the mycelial growth of *Colletotrichum gloeosporioides* in vitro. Vrandečić et al. (2019) reported that AgNPs and selenium nanoparticles significantly inhibited the growth of *Macrophomina phaseolina, Sclerotinia sclerotiorum* and *Diaporthe longicolla*. The mechanism by which the AgNPs counteract the pathogenic fungi is due to the formation of free particles from the nanoparticles that lead to the destruction of the lipids present in the plasma membrane and thus the membrane loses its function and spoils (Kim et al., 2007). Kim et al. (1998) showed that the anti-silver effect against microbes is due to inactivation of the enzyme. McDonnell and Russell (1999) stated that Ag^+ affects the function of membrane-bound enzymes, such as those in the respiratory chain. AgNPs are highly reactive to fungal hyphae due to the production of Ag^+ ions which enhanced efficiency of silver nanoparticles against fungal pathogens (Morones et al., 2005). Lamsal et al. (2011) found that silver nanoparticles cause changes in structure of fungal hyphae, cell wall deformations, cell membrane damages and significant alterations in spore form and germination. Also, the production of reactive oxygen species (ROS), via the reaction of silver ions with oxygen, was causing damage to proteins, lipids, and nucleic acids of microbial cells (Storz & Imlay, 1999; Hwang et al., 2008). Stoimenov et al. (2002) and Sondi and Salopek-Sondi (2004) explained a new conceptualization of the counter activity of nano-silver based on that the membrane can degrade due to the formation of pits on the surface of the cell wall membrane of microorganisms. The presence of these pits on the membrane leads to an increase in the permeability of the membrane and a disturbance of the transport that becomes irregular, which leads to the death of cells.

Table 4 Effects on bulbs yield of onion and garlic plants results from the application of silver nanoparticles (AgNPs) under field conditions

Treatment	Bulbs yield (kg/plot) and efficiency of treatment (%)			
	Yield (kg/plot)	Efficiency (%)	Yield (kg/plot)	Efficiency (%)
Onion	Trial I (40 sclerotia/kg soil)		Trial II (600 sclerotia/kg soil)	
Transplants dipping	17.6±0.35 d	17.0	9.1±0.08 d	4.4
Stem base spraying	18.3±0.04 c	20.2	9.5±0.04 c	8.4
Dipping+ spraying	19.0±0.18 b	23.2	9.7±0.05 b	10.3
Tebuconazole	24.6±0.04 a	40.7	12.4±0.09 a	29.8
Control	14.6±0.15 e	–	8.7±0.09 e	–
Garlic	Trial I (40 sclerotia/kg soil)		Trial II (600 sclerotia/kg soil)	
Clove dipping	17.6±0.06 b	21.0	8.1±0.28 b	2.5
Stem base spraying	17.9±0.14 b	22.3	8.3±0.31 b	4.8
Dipping+ spraying	18.5±0.04 a	24.9	9.5±0.03 a	16.8
Tebuconazole	16.0±0.18 c	13.1	8.2±0.12 b	3.7
Control	13.9±0.24 d	–	7.9±0.36 b	–

[(a)] Three application methods of AgNPs at 200 mg/L water were used. The dipping method was applied by dipping onion transplants or garlic cloves before sowing in biogenic silver nanoparticles (200 mg/L) for 5 min. In stem base spraying method, stem bases of plants were sprayed with the same concentration three times intervals at 30, 60 and 90 days after planting. Efficacy of treatments was calculated using the following formula: Efficacy (%) = Fresh weight of plants in control- Fresh weight of plants in treatment/ Fresh weight of plants in control × 100. Values are means of three replications. [(b)] Means ± standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $P < 0.05$

In the present study, with increasing the concentration of AgNPs, the inhibition of linear growth and growth biomass of *S. cepivora* was increased. However, the formation of sclerotia of *S. cepivora* was inhibited completely even at low concentration of AgNPs. The higher density of AgNPs may play a role in this, as in this case the particles are able to saturate and adhere to the fragments of the pathogen and to deactivate the growth. Silver (Ag), in its ionic or nano form, is known for its high antimicrobial activity. However, silver in the nanometer size acquires new characteristics due to the morphological, size, structural and physiological changes (Nel et al., 2006; Abdel-Monem et al., 2020; Mourad et al., 2020). This is due to the intensity of the reaction that the silver nanoparticles acquire due to the generation of Ag^{+} ions compared to the non-reaction in the case of metallic silver (Morones et al., 2005). It was also found that the penetration of microbial cells by particles in the nanoscale is efficient, which makes the use of silver in low concentrations is highly efficient (Samuel & Guggenbichler, 2004). In this respect, Kim et al. (2007) reported that the antimicrobial properties of AgNPs depend on several aspects, such as morphology, size, and concentration. They added that, the spherical nanoparticles with diameters below 10 nm facilitate the interaction with the target microorganism by electronic effects at the cellular level. Nel et al. (2006) reported that the antifungal properties of AgNPs might come from their morphological, structural, and physiological forms.

Sclerotia of *S. cepivora* are one of the major concerns in onion and garlic production as sclerotia remain in dead plant tissue and soil where they survive for decades and cause infection in favorable conditions. In the present study, AgNPs at all concentrations i.e. 40, 80, 120, 160 and 200 mg/L inactivate sclerotial formation on the treated culture media. The protein profile of AgNPs-untreated fungal biomass showed the presence of band at 28 k Dalton. This band was absent in the profile of AgNPs-treated fungal biomass and may be related to the formation of sclerotia. Inhibition of fungal growth and sclerotial germination with AgNPs application against fungi that produce sclerotia such as *Rhizoctonia solani, Sclerotinia sclerotiorum* and *Sclerotinia minor* was reported in earlier reports (Min et al., 2009; Vrandečić et al., 2019). However, in our data, complete inhibition of sclerotia formation was demonstrated after treatment with very low amount of AgNPs at the concentration of 40 mg L^{-1}. This offers promise to disrupt the life cycle of *S. cepivora* in an environmentally friendly way by applying SeNPs. Our data also demonstrated that the

AgNPs inactivate the gene responsible for sclerotia formation. The reactive oxygen species (ROS) produced from the interaction of silver ions with oxygen causes damage to proteins, fats and DNA, which harms microbial cells (Storz & Imlay, 1999). Morones et al. (2005) found that silver nanoparticles disrupt the flow of ions by destroying the transport-related systems in the microbial cell. This imbalance in the flow of ions leads to a rapid accumulation of silver ions, which hinders cellular processes, including metabolism and respiration. Feng et al. (2000) reported that DNA of microorganism loses its ability to replicate due to the treatment with Ag^{+}. These resulting in inactivated expression of ribosomal subunit proteins, as well as certain other cellular proteins and enzymes essential to ATP production. Krishnaraj et al. (2012) reported that silver nanoparticles may kill fungal spores by destruction of membrane integrity. Vrandečić et al. (2019) reported that silver nanoparticles AgNPs and selenium nanoparticles SeNPs inhibited sclerotia formation of *Macrophomina phaseolina, Sclerotinia sclerotiorum* and *Diaporthe longicolla*.

In the present study, application of AgNPs, at 200 mg/L water, succeeded in reducing the incidence of white rot disease under greenhouse and field conditions. The dual treatment included transplants/clove dipping plus stems base spraying was the best in reducing the incidence of disease even at a high sclerotial level in the soil. AgNPs have several pathogen inhibitory effects and can therefore be used safely to control many plant pathogens (Kim et al., 2009; Min et al., 2009). The results obtained from the current study are consistent with those obtained by Elshahawy et al. (2018b), who found that treating tomato plants with AgNPs resulted in protecting these plants from the sudden death disease caused by the oomycete *Pythium aphanidermatum*. They added that all the application methods they used were effective in reducing disease. Nano silver has been tested against many pathogens of plants. Jo et al. (2009) found that silver ions and nanoparticles, when applied 3 h prior to pathogen inoculation, reduced the severity of spot blotch disease of wheat and rice blast disease of rice caused by *Bipolaris sorokiniana* and *Magnaporthe grisea*, respectively. Sharon et al., 2010 reported that nano-silver particles with an average size of 1.5 nm in diameter gave effective results in protecting rose plants from powdery mildew disease caused by *Sphaerotheca pannosa* var. *rosae*. Lamsal et al. (2011) reported that AgNP at concentrations below 50 ppm was enough to inhibit infection incited by *Colletotrichum* sp. under field conditions. In fact, many studies indicate that silver particles in the nanoscale are effective in their antimicrobial effect. Our data clearly demonstrated that AgNPs improved the growth and bulb yield of onion and garlic under field conditions. This may be due to the role of silver nanoparticles in preventing the damage caused by white rot (Sharon et al., 2010). Salama (2012) found that application of Ag-NPs at 60 ppm showed an increase in fresh weight and dry weight of common bean and corn seedlings compared to untreated controls. El-Batal et al. (2016) reported that foliar application of Ag-NPs (5–60 ppm) significantly increased fresh and dry weight of common bean seedlings. Recently, Jurkow et al. (2020) reported that foliar exposure of lettuce seedlings to Ag-NPs at 40 ppm significantly increased the total antioxidant capacity, the concentration of chlorophyll a as well as fresh and dry weight of shoots.

Conclusions

In this study, we developed a new method for controlling white rot disease in onion and garlic crops using AgNPs as an alternative to chemical fungicides. In vitro, the biosynthesized AgNPs showed a promising antifungal activity against *S. cepivora* isolates by inactivating sclerotial formation on the treated media and to the best of our knowledge this observation represents the first report of this activity. Under greenhouse conditions, all application methods of AgNPs significantly reduced the incidence of white rot and the combined treatment of transplants/clove dipping plus stem base spraying was more effective than these methods used individually. Under field conditions, the potential of AgNPs for reducing white rot in onion is influenced by the level of *S. cepivora* inocula. When evaluating new control agents, the growth characteristics and yield of the crop under study must be taken into account. The application of AgNPs to onion and garlic plants led to a significant increase in the growth and yield of bulbs compared to the untreated plants.

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Author's contribution Osama M. Darwesh and Ibrahim E. Elshahawy designed the experiments. Osama M. Darwesh and Ibrahim E. Elshahawy carried out the experiments and performed the data analysis. Osama M. Darwesh and Ibrahim E. Elshahawy wrote and revised the manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest. The research did not involve Human Participants and/or Animals, hence informed consent is not applicable.

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