Efficiency of silver nanoparticles synthesized by *Pleurotus ostreatus* to manage fungal garlic cloves rot

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Abstract

Garlic is one of the most important vegetable crops in Egypt. It’s naturally infected with fungal strains *Alternaria alternata* *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *F. proliferatum* and *F. solani* causes decayed garlic cloves. *F. oxysporum* was aggressive pathogen isolated from decayed cloves of the garlic (cv Balady). Silver nanoparticles which synthesized from fungal strain *Pleurotus ostreatus* was used in this study to manage such disease. The effect of three different concentrations of silver nanoparticles (40 ppm, 70 ppm and 100 ppm) was tested to assess their antifungal activities against the fungal causal pathogens. The results indicated that all three concentrations, of the nano-silver had inhibition rates and 100 ppm was the most efficient concentration and had the highest effect on the mycelial growth reduction of pathogenic fungi as well as enhanced the growth of garlic bulbs in *vitro* and in *vivo*.

Keywords: garlic cloves; *Fusarium oxysporum*; silver nanoparticles.

1. Introduction

Garlic (*Allium sativum* L.) is a crop of great importance in Egypt and throughout the world. Garlic has many culinary and medicinal properties (Galvez and Palmero, 2021). It is consumed in different forms. Its secondary metabolites have been demonstrated to have a significant impact on health and to aid in the prevention of a range of human diseases, due to its antioxidant, anti-inflammatory, and lipid-lowering properties (Galvez and Palmero, 2021). Many diseases infecting garlic either pre or post harvesting. Among the diseases, Fungal garlic cloves rot consider among important disease infecting postharvest garlic. Clove rot caused by a variety of fungus species, including *Fusarium culmorum*, *Fusarium proliferatum*, *Fusarium oxysporum*, *Aspergillus ochraceus*, *Aspergillus niger*, *Stemphylium botryosum*, *Botrytis allii*, *Rhizopus stolonifer* and *Pencillium purpurogenum* (Moharam et al., 2013; Elshahawy et al., 2017).

Moreover, *Fusarium* spp. pathogens are known to produce many toxins like fumonisins, fusaric acid and fusaproliferin, these toxins remain chemically stable during food processing and thus they consider a great risk to the health of humans (Mondani et al., 2021; Tonti et al., 2017).

Many strategies have been adopted to control this disease, including biological control, chemical control (Mondani et al., 2021). Recently using Nanoparticles has emerged as new method to control plant diseases. Several reports have mentioned that using nanoparticles is useful method to control garlic cloves rot, and
when seedling were treated with varied concentrations of AgNPs, Fresh Weight, root and shoot length, and vigour index were found to positively enhanced compared with non-exposed plants (Sharma et al., 2012; Almutairi and Alharbi, 2015.)

And treatment with silver nanoparticles resulted to a significant increase in plant height, root length, bulbs diameter, plant weight compared with untreated garlic plants (Abd-Elbaky et al., 2021), application of nanoparticles was very effective to control undesirable microbial in garlic (Elsaggan et al., 2018).

The objective of this study is to evaluate the efficacy of the biological synthesis of silver nanoparticles (AgNPs) in fungal garlic cloves rot management under greenhouse condition.

2. Material and methods

2.1. Garlic clove pathogens: isolation, purification, and identification

A hundred infected garlic bulbs (cv Balady) were collected from five storage sites located in the Assiut governorate in October 2020. Symptomatic diseased cloves were cut into small segments, soaking for three minutes in 3 percent sodium hypochlorite and then washing with distilled water (3 times) and finally dried with sterilized filter papers. Four clove tissue pieces were added to the PDA plate. The emergent fungi were recognized based on their morphological features according to Moubasher (1993), Leslie and Summerell (2006), Ismail et al. (2015) and Domsch et al. (2015).

2.2. Pathogenicity tests

The pathogenicity of Alternaria alternata isolates Aspergillus niger, A. flavus, Fusarium oxysporum, and F. proliferatum was tested. As previously noted, the surface of healthy cvs Balady cloves was disinfected. Cloves were then cut near the apex to 4.5 mm depth and a diameter of around 1 mm, and artificially wounded then was filled with spore suspension (2.5 x 10^5 cfu/ml^3). As a control, non-inoculated cloves were used. Cloves that had been inoculated were kept in sealed plastic boxes for 30 days at 25 2°C (Dugan et al., 2007). Cloves were evaluated before and after the rotting portion was removed, and rot severity was calculated according Galal et al. (2002) using the formula as following:

Percentage of rot severity = (W1 − W2)/ W1 x 100

Where:
W1 is the total weight of the clove, and W2 is the clove's weight after decayed tissue has been removed.

2.3. Synthesis of silver myconanoparticles

Silver nanoparticles were synthesized using a Pleurotus ostreatus. One fungal strain agar plug (4-mm diam) was cultivated in a 250-ml Erlenmeyer flask with 100 ml potato dextrose broth (PDB) and incubated for 5 days at 25°C on a rotatory shaker at 120 rpm. The mycelial biomass was removed from the culture and washed with sterile distilled water to eliminate any medium components. By passing the culture extract through Whatman filter paper No. 1, the culture extract was prepared. In a 250 ml Erlenmeyer flask, 50 ml of 1 mM AgNO3 solution was combined with 50 ml of culture filtrate and stirred for 72 hours at 25°C (Basavaraja et al., 2008).

2.4. The effect of silver nanoparticles on pathogen mycelial development

Different concentration of silver nanoparticle i.e., 40, 70 and 100 ppm were tested with three replicates against isolates of F. proliferatum, A. alternata and Aspergillus niger on potato dextrose agar medium. Before plating in a petri plate (90 x 15 mm), one ml of different AgNPs concentrations were combined with the PDA medium. One agar plug 5 mm of tested fungi was injected at the center of each agar plate and incubated at 28°C for 10 days after medium containing silver nanoparticles was incubated at room temperature (28°C±2) for 48 hours. As a control, agar plates without Ag-nanomaterial were used. Each treatment was replicated three times. When the growth of the fungal mycelia in
control plates approached the petri dish's edge, the growth inhibition rate was computed (Kim et al., 2012). The following formula was used to compute the inhibition rate: (Shivapratap et al., 1996):

\[ \text{Inhibition percentage} = \frac{(D1 - D2)}{D1 \times 100} \]

D1 is the diameter of the pathogen colony in the control, while D2 is the diameter of the pathogen colony in the treatment.

2.5. Seed germination test

Garlic gloves were dipped in a 5 % sodium hypochlorite solution for 15 minutes to guarantee seed surface sterility before being soaked in a silver nanoparticles solution overnight. The gloves were also soaked in regular household water overnight as a control. The filter paper was then wetted with 5 mL silver nanoparticle solution and placed in petri dishes. The treated gloves were stored on petri plates on filter paper. The petri plates were covered and kept at room temperature for incubation. Germination was stopped after 36 hours, and the germination percentage was calculated.

2.6. Evaluation the effect of silver nanoparticles on garlic fungal incidence in greenhouse

During the 2020-2021 growth seasons, three concentrations of silver nanoparticles were evaluated against pathogenic fungus in a greenhouse setting. Two weeks before adding Ag-nanoparticles, sterilized pots (25-cm diam.) were filled with 3 kg sterilized sandy loamy soil and mixed with pathogenic fungi, Alternaria alternata, Aspergillus niger, A. flavus, Fusarium oxysporum, Fusarium proliferatum, and Fusarium solani at a rate of 3% (w/w). Cloves were either soaked for 5 min in AgNPs at 40, 70, or 100 ppm concentrations prior to sowing. Only the control pots, which were filled with sterilized soil and infested with tested fungi, were used. Each pot had five cloves sown in it, and three pots were used as duplicates. The percentage of symptomatic of cloves in the sample in respect to the total cloves was used to calculate the incidence of clove rot. According to Palmero et al. (2013) each bulb's disease symptoms were categorized into four arbitrary groups. The following formula was used to determine the severity of the rot:

\[ RS = \frac{(N1 \times 0) + (N2 \times 1) + (N3 \times 2) + (N4 \times 3)}{\text{Number of total cloves}} \]

N1 indicates no symptoms; N2 indicates rotted patches; N3 indicates a 10-50 percent rotted clove; and N4 indicates a completely rotted clove.

2.7. Statistically analysis

Split plot was designed, SAS Institute Inc., used for (ANOVA) analysis of variance. Least significant differences test at P 0.05 was used to compare the means (Gomez and Gomez 1984).

3. Results and discussion

3.1. Isolation and identification the fungi associated rotten garlic cloves

Isolation was obtained from the decay tissues of the tested cv. Balady from five storage sites located in the Assiut governorate yielded five species of fungal belonging to three genera, Alternaria alternata Aspergillus niger, A. flavus, F. oxysporum, F. proliferatum and F. solani and these isolates were identified based on their colony characteristics and morphological features.

3.2. Pathogenicity tests

Tests of pathogenicity indicated that F. oxysporum was highly aggressive to garlic that has been wounded balady cloves by (75% rotted tissue) followed by Fusarium proliferatum (60.2%) and Alternaria alternata (60%). Other fungal species Aspergillus niger (35.1%), A. flavus (23%) and Fusarium solani (38.1) were less aggressive. According to Moharam et al. (2013), F. oxysporum caused the most severe clove rot. While Elshahawy et al., (2017) isolated Aspergillus ochraceus, Aspergillus niger, Penicillium purpurogenum, Penicillium purpurogenum, Penicillium purpurogenum, F. proliferatum, Botrytis allii, F. oxysporum, S. botryosum, Penicillium purpurogenum, and R.
stolonifer from rotting garlic cloves, with *Fusarium proliferatum* having a high prevalence of rot of garlic clove during the storage period. These results are in agreement with previous studies that found the most prevalent fungal pathogens causing cloves rot in garlic are *F. culmorum*, *F. proliferatum*, *Fusarium oxysporum*, *Aspergillus ochraceus*, *Aspergillus niger*, *Stemphylium botryosum*, *Botrytis allii*, *Rhizopus stolonifer* and *Pencillium purpurogenum* (Moharam *et al.*, 2013; Elshahawy *et al.*, 2017).

### Table 1. Pathogenic capability of the isolated fungi isolated from rotted cloves Balady garlic cultivar.

<table>
<thead>
<tr>
<th>Isolated fungi</th>
<th>Rotted tissue (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alternaria alternata</em></td>
<td>60.0</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>23.0</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>35.1</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em></td>
<td>75.0</td>
</tr>
<tr>
<td><em>Fusarium proliferatum</em></td>
<td>60.2</td>
</tr>
<tr>
<td><em>Fusarium solani</em></td>
<td>38.1</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>6.12</td>
</tr>
</tbody>
</table>

#### 3.3. Silver myconanoparticles synthesis

Silver nanoparticles were synthesized using a *Pleurotus ostreatus* isolate. The synthesis of myconanoparticles of Ag in the reaction mixture is indicated by the emergence of a brownish colour (Figure 1). The synthesis of AgNPs was indicated by the change in colour of the culture filtrates due to the reduction of silver ions, which ranged from pale yellow to brown (Bhainsa and D'Souza, 2006). When exposed to comparable conditions, 1 mM AgNO3, which was utilized as a control, did not change colour. Several studies reported production Nanoparticles NPs using microorganisms and plants (Iravani, 2011; Korbekandi *et al.*., 2016), nanoparticle size under transmission electron microscope TEM was determined in from 20.4 to 30.8 nm as shown in figure 2, This finding is match the results obtained by many authors (Massoud *et al.*, 2018; Avan *et al.*, 2018)

![Figure 1. Filtrate of the Pleurotus ostreatus in aqueous solution of AgNO3 after 3 days of reaction.](image-url)
3.4. The effect of AgNPs nanoparticles on pathogens mycelial growth

The findings revealed that all AgNP concentrations synthesized by Pleurotus ostreatus inhibited the mycelial growth of the pathogenic fungi. The inhibition rate of F. oxysporum at 40 ppm was 71.6%, 70 ppm was 72.2% and at 100 ppm was 80% compared to control. The reduction rate of Alternaria alternata at 40 ppm was 55.5%, 70 ppm was 77.7% and at 100 ppm was 84.4% compared to control while the growth inhibition of Aspergillus niger was 79%, 70 ppm was 79.4% and at 100 ppm was 83% compared to control (Figure 3). According to Abd-Elbaky et al. (2021) a 100 percent concentration of silver nanoparticles had the greatest effect on the growth of F. oxysporum f.sp. cepae mycelia, which causes onion basal-rot. In vitro, Jung et al., (2010) found that all nano-silver liquid concentrations (1, 3, 5, 7, 10, 25, 50 ppm and 100 ppm) had more than 90% inhibition rates against Sclerotium cepivorum, the pathogen that causes green onion white rot. These results are in harmony with other studies as showed severely damaged cell walls, leakage of cytoplasmic and nucleic contents, and a swelling structure, all of which contributed to bacterial mortality (Elbeshehy et al., 2015) In another study, biosynthesized AgNPs have antifungal activity against Fusarium graminearum as caused hyphae deformation and cell wall damage (Ibrahim, et al., 2020). Also, nanoparticles have similar effects on fungal phytopathogenic strains of A. alternata, Trichosporon asahii and Botrytis cinera (Xia et al., 2015).
Table 2. Silver nanoparticles growth inhibition percentage on the three fungal rot causal pathogens of garlic gloves.

<table>
<thead>
<tr>
<th>AgNPS type</th>
<th>40 ppm</th>
<th>70 ppm</th>
<th>100 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alternaria alternata</em></td>
<td>55.5</td>
<td>77.7</td>
<td>84.4</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>79.0</td>
<td>79.4</td>
<td>83.0</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em></td>
<td>71.6</td>
<td>72.2</td>
<td>80.0</td>
</tr>
</tbody>
</table>

LSD 0.05 A Fungal strains 9.08 B AgNPs Concentrations 7.25 AB 1.22

Figure 3. Effects of different concentrations of *Pleurotus ostreatus* synthesized silver nanoparticles on fungal pathogens (from top to down, *Fusarium oxysporum*, *Alternaria alternata* and *Aspergillus niger*).

3.5. Seed germination test
The results indicate that the highest germination percentage value (85.3%) and healthy seedling (88%) for garlic cloves which was observed when exposure to 100 ppm of AgNPs. Same results seen by (Savithramma et al., 2012; Almutairi and Alharbi, 2015; Abd-Elbaky et al., 2021) that AgNPs increased germination value of seeds of crop plants significantly.

3.6. Greenhouse assessment of garlic’s fungal pathogens response to silver nanoparticles
Under greenhouse conditions throughout the 2020 and 2021 growing seasons, the impact of soil that treated by using varied concentrations of Ag-nanoparticles on incidence of balady garlic rot fungal pathogens was studied. The data in table (3) showed that the treatment of soil with Ag-nanoparticles not only reduced the disease severity of fungal pathogenic garlic rot but also enhance the rate of growth of gloves and the data indicate that 100 ppm of AgNPs more effective than the other concentrations in both reduction of pathogenic fungi and also the growth rate of gloves seedling. Ag-nanoparticles had antimicrobial and antiviral agents (Burdusel et al., 2018). According to Abd-Elbaky et al., (2021), the tested nanoparticle concentrations of 25, 33, 50, and 100 percent caused different values of antifungal effect on Fusarium mycelial growth of onion basal-rot, with the crude
concentration (100 percent) of silver nanoparticles having the greatest effect on *Fusarium oxysporum f.sp. cepae* mycelial growth. These results are in partial agreement with results obtained by Elamawi and Al-Harbi (2014) who found that nanoparticles of silver can effectively control *Fusarium* spp. pathogen.

Table 3. Effect of *Pleurotus ostreatus* nanoparticles to control garlic cloves rot

<table>
<thead>
<tr>
<th>AgNPS type</th>
<th>0 ppm</th>
<th>40 ppm</th>
<th>70 ppm</th>
<th>100 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Germination</td>
<td>% Healthy seedling</td>
<td>% Germination</td>
<td>% Healthy seedling</td>
</tr>
<tr>
<td><em>Alternaria alternata</em></td>
<td>50.8</td>
<td>54.6</td>
<td>65.4</td>
<td>66.4</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>38.6</td>
<td>40.8</td>
<td>76.5</td>
<td>80.6</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>52.3</td>
<td>55.0</td>
<td>60.3</td>
<td>65.0</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

LSD 0.05 A Fungal strains 7.68 B AgNPs Concentrations 5.42 AB 2.63

Table 4. Inhibition of growth rate (%) of fungal pathogens by silver nanoparticles various concentrations synthesized by *Pleurotus ostreatus*

<table>
<thead>
<tr>
<th>Strains</th>
<th>40 ppm</th>
<th>70 ppm</th>
<th>100 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% reduction</td>
<td>% growth rate</td>
<td>% reduction</td>
</tr>
<tr>
<td><em>Alternaria alternata</em></td>
<td>50.2</td>
<td>40</td>
<td>67</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>60.3</td>
<td>55</td>
<td>63.2</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>65.3</td>
<td>55</td>
<td>63.4</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

4. Conclusion

*Alternaria alternata*, *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *F. proliferatum* and *F. solani* are an important causal pathogens of garlic cloves rot. *F. oxysporum* was the most aggressive pathogen found in rotting garlic cloves. The results indicated that all three concentrations of silver nanoparticles which synthesized from *Pleurotus ostreatus*, had an inhibition effect on the causal pathogens. The most effective concentration was 100 ppm, which had the great effect to reduce pathogenic fungi growth in *vitro* and in *vivo*. This is a promising outcome achievement by using bio synthesized silver nanoparticles that could be used as a safe fungicide alternative.

Authors’ Contributions

All authors are contributed equally in this research.

Funding

There is no fund in this research.

Institutional Review Board Statement

All Institutional Review Board Statement are confirmed and approved.

Data Availability Statement

Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate

This work carried out at Plant Pathology department and followed all the department instructions.

Consent for Publication

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest

5. Reference


to the genus Fusarium in Egypt with dichotomous keys for identification of species’, Suchy Las, Poland. Publisher Tomasz M. Karpiński, 175 pp.


