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# Antifungal Activities of Silver Nanoparticles (AgNPs) Against Selected Phytopathogenic Fungi

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**Abstract:** The widespread use of antimicrobial agents has contributed to the rapidly increasing threat of antimicrobial resistance. One advocated alternative to combat multidrug resistance is the use of nanoparticles, such as silver nanoparticles. This study aimed to assess the antifungal activity of silver nanoparticles against *Trichoderma* sp., *Fusarium oxysporum*, *Lasiodiplodia* sp. and *Hymenoscyphus fraxineus*. The test fungi were obtained from pure cultures stored in the laboratory. $AgNO_3$ crystals were purchased from a local vendor and phytotosynthesized using home-grown *Moringa* leaves. This was further characterized using ultraviolet-visible light spectrophotometry, with maximum absorbance recorded at 510 nm. Antimicrobial activity against the fungal isolates was assessed using the food poisoning method. Plates without AgNPs served as the negative control. AgNPs demonstrated significant inhibition of mycelial radial growth in the pathogens, ranging from 64.83 ± 4.98 mm (100% concentration) to 75.00 ± 0.00 mm (25% concentration) for *Trichoderma* sp., 11.67 ± 0.88 mm (25% concentration) to 18.00 ± 0.29 mm (100% concentration) for *F. oxysporum*, 47.50 ± 3.82 mm (25% concentration) to 55.33 ± 3.03 mm (75% concentration) for *Lasiodiplodia* sp., and 10.00 ± 0.00 mm (25%, 50%, and 75% concentrations) to 35.17 ± 15.33 mm (100% concentration) for *H. fraxineus*. *Hymenoscyphus fraxineus* was the most inhibited pathogen by the AgNP treatment, with percentage inhibition of mycelial radial growth ranging from 53.11% (100% concentration) to 86.67% (25%, 50%, and 75% concentrations), while *Trichoderma* sp. was the least inhibited by the AgNP treatment, with percentage inhibition of mycelial radial growth ranging from 8.16% (25% concentration) to 20.61% (100% concentration). AgNPs have great potential for combating plant diseases caused by these pathogens, especially *H. fraxineus* and *F. oxysporum*, which are associated with ash dieback and *Fusarium* wilt, respectively. AgNPs should be tested for antimicrobial activity against several plant and human pathogens, and their synergistic activity with other plant extracts or biomolecules should be further investigated.

## 1. Introduction

Nanotechnology is a rapidly developing field of science that encompasses the synthesis and development of various nanomaterials, the manipulation of matter on an atomic, molecular, and supramolecular scale, and the understanding and control of matter at dimensions approximately between 1 and 100 nanometers (Mousavi *et al*., 2011; Arivalagan *et al*., 2011). At the nanoscale, the matter presents altered properties that are novel and very different from those observed at the macroscopic level (Gutierrez *et al*., 2011; Aldwayan *eta l*., 2013).

Nanoparticles (NPs) are particles that are at least one dimension long and range from one to one hundred nanometers (1.0 nm = $10^{-9}$ m). Gold (Au), silver (Ag), silica (Si), and carbon-based materials

are just a few of the substances that may be used to create nanoparticles. Applications of these ultrafine particles in research, technology, and medicine are becoming more and more numerous. Given their well-established antibacterial properties and several proposed applications, silver nanoparticles (AgNPs) are particularly intriguing (Martinez-Abad *et al*., 2012; Derbalah *et al*., 2012). There have been suggestions for ways to maintain the efficacy of antibiotics. According to Huh and Kwon (2011), nanoantibiotics—nanomaterials having antibacterial properties—are one possibility for combating pathogens that are resistant to many drugs.

Silver nanoparticles (AgNPs) are currently the most promising nanoantibiotics due to their antiviral and antibacterial properties (Panáček *et al*., 2009; Basumiman *et al*., 2023; Rodrigues *et al*., 2024; Sati *et al*., 2025). AgNPs' broad mode of action against various pathogens, including bacteria, fungi, and viruses, as well as their antibacterial efficacy irrespective of the microorganism's susceptibility to standard antibiotics, such as efflux pumps and formation of biofilm, allow AgNPs to overcome the microorganism's resistance to standard antibiotics (Rudramurthy *et al*., 2016).

Furthermore, the use of AgNPs offers benefits beyond their antibacterial and antiviral properties. Both chemical techniques and biosynthesis may be used to create AgNPs, and they are easy, inexpensive, and ecologically benign to generate. AgNPs exhibited a multi-level mode of action on bacterial cells that affected metabolic processes: A) disruption of cell walls and membranes and an increase in cell permeability; B) penetration of AgNPs and intracellular damage that disrupted metabolic pathways; C) damage to biomolecules (DNA, proteins); and D) generation of reactive oxygen species (Li *et al*., 2010; Cui *et al*., 2013).

The creation, synthesis, manufacturing, characterization, and use of devices, systems, and architectures of nanoparticles with sizes ranging from 1 to 100 nm are all included in the nano drug delivery system (Brosset, 2013). The widespread use of antimicrobial drugs has been linked to the development and rapid spread of bacterial resistance, which has been observed in zoonotic enteropathogens, animal diseases, and commensal bacteria, such as *Escherichia coli* (*E. coli*). It is known that bacterial membranes are impacted by zinc oxide nanoparticles (ZnONPs) and silver nanoparticles (AgNPs) (Pelgrift and Friedman, 2013). Human erythrocytes and other eukaryotic cells are not cytotoxically affected by AgNPs, which are efficient antibacterial agents at low doses. AgNPs have demonstrated antibacterial activity at extremely low concentrations and have the potential to inhibit the development of microorganisms resistant to antibiotics. Methicillin-resistant strains of bacteria, as well as Gram-positive and Gram-negative bacteria, are susceptible to the strong bactericidal and antibacterial effects of AgNPs (Shahverdi *et al*., 2007).

## 2. Methodology

### 2.1 *Purchase of Silver Nitrate*

Silver nitrate ($AgNO_3$) crystals were purchased from a local vendor in Nigeria.

### 2.2 *Source of fungi*

The fungi used in this study, namely *Trichoderma* sp., *Fusarium oxysporum*, *Lasiodiplodia* sp. and *Hymenoscyphus fraxineus* were obtained from pure culture stored in the laboratory and were characterized and identified following the protocols outlined by Barnett and Hunter (1998).

### 2.3 *Sterilization of materials*

All glasswares used in this study were washed in soap and rinsed with purified water. They were wrapped with aluminium foil paper and sterilized at 160 $^oC$ for 1 hr in a hot-air oven before use. The media used was also sterilized at 121 $^oC$ for 15 min in an autoclave.

### 2.4 *Synthesis of silver nanoparticles using Moringa oleifera leaves*

Double-distilled water (DDW) was used to thoroughly wash 20g of home-grown *Moringa* oleifera leaves. The finely chopped leaves were steeped for 48 hours in DDW before being filtered through a Whatman No. 1 filter paper and the filtrate was obtained in a 250 ml Erlenmeyer flask and kept at 4 ºC for subsequent research. 10 ml of a 1 mM $AgNO_3$ solution was mixed with 90 ml of extract to synthesize silver nanoparticles (Sathyavathi *et al*., 2011).

### 2.5 *Characterization of silver nanoparticles*

A UV-visible spectrophotometer was used to track the bioreduction of Ag+ in solution. The absorbance was measured between 300 and 900 nm at regular intervals, including 1, 24, and 48 hours following synthesis (Obiazikwor and Shittu, 2018).

### 2.6 *Preparation of potato dextrose agar*

As directed by the manufacturer, the potato dextrose agar was made. A conical flask coated with cotton wool and aluminium foil paper was used to dissolve 39.0g of potato dextrose agar powder in 1 litre of distilled water. After being agitated and autoclaved for 15 minutes at 121ºC, this was chilled to 45–50ºC. A sterile Petri plate was filled with 20 millilitres of the medium, which was then left to harden.

### 2.7 *Isolation of fungi*

A pure culture of the fungi isolates was obtained by repeated subculture which was done on each plate, and a piece of mycelium was transferred with the aid of a sterilized wire lop into the center of freshly prepared potato dextrose agar and then incubated at room temperature and the fungal mycelia growth was measured after 72 hours using graduated millimetre (mm) according to the methods of Obiazikwor and Shittu (2018).

### 2.8 *Identification of fungal isolates*

The approach outlined by Barnett and Hunter (1972) was used to identify the fungal isolates by microscopic analysis and conventional morphological parameters of the colony. The colony length comprises the colonies' breadth and length, colour, wrinkles, furrows, and any additional pigments, as well as whether or not aerial mycelium is present. Additionally, the macromorphological features were assessed (Diba *et al*., 2007).

### 2.9 *Antifungal activity of silver nanoparticles*

The silver nanoparticles synthesized using *Moringa oleifera* and silver nitrate solution were used in different concentrations. Different concentrations corresponding to 25, 50, 75 and 100% of silver nanoparticles were used. Plates without silver nanoparticles served as negative controls. The PDA was poisoned with different concentrations of AgNP, and agar plugs of the pathogens were introduced at the middle of each Petri dish. The radial growth inhibition of the fungi mycelia was measured for a period up to three days (Mahdizah *et al*., 2015). Altaf et al. synthesized mixed ligand silver(I) complexes exhibiting antimicrobial, antifungal, and antituberculosis activities (Altaf *et al*., 2013)

### 2.10 *Determination of the percentage mycelial growth inhibition*

The percentage mycelia inhibition was determined according to the equation:

Percentage growth inhibition= R-r/R= 100

Where; R= Linear growth rate of fungus on control Petri dishes

r= Linear growth of fungus on Petri dishes with AgNP (Elgorban *et al*., 2015).

### 2.11 *Statistical analysis*

All of the data were statistically analyzed using ANOVA and were presented as means ± standard errors of three measurements. A significance level of $P < 0.05$ was used (Oboh *et al*., 2016).

## 3. Results and Discussion

**Table 1** shows the cultural and morphological characteristics of the fungal isolates. The pure cultures obtained from the laboratory were used to culturally characterize the fungal isolates. *Trichoderma* sp. displayed a white woolly colony with green patches, *Fusarium oxysporum* displayed a white colony turning violet, *Lasiodiplodia* sp. displayed a black fluffy colony with a well-defined margin, while *Hymenoscyphus fraxineus (Chalara) displayed* irregular growth with white cottony colony turning yellow. Further identification of the isolates was done using microscopy. Colonies of *Trichoderma* sp. showed septate hyphae with green conidiospore. Colonies of *Fusarium* oxysporum showed septate hyphae with cream coloured chlamydospore. Colonies of *Lasiodiplodia* sp. showed non septate hyphae with brown ascospore and thick-walled conidia. Colonies of *Hymenoscyphus fraxineus* showed septate hyphae with grey ascospores and short cylindrical phialoconidia. **Figure 1** shows the characterization of AgNP biosynthesized by the reduction of $AgNO_3$ solution with *Moringa oleifera* leaf extract after 48 hours. Maximum absorbance was recorded at 510 nm.

**Table 1**: Cultural and morphological characteristics of test fungi

| Characteristics | Isolate 1 | Isolate 2 | Isolate 3 | Isolate 4 |
|---|---|---|---|---|
| **Cultural** | White woolly colony, with green patches | White colony, turning violet | Black, fluffy colony with well-defined margin | Irregular growth, white cottony colony, later yellow |
| **Microscopic** | | | | |
| Nature of hyphae | Septate | Septate | Non septate | Septate |
| Colour of spore | Green | Cream | Brown | Grey |
| Type of spore | Conidiospore | Chlamydospore | Ascospore | Ascospores |
| Special structure | Green patches with concentric rings | Microscopores | Thick-walled conidia | Short cylindrical phialoconidia |
| Tentative isolate | *Trichoderma* sp | *Fusarium oxysporum* | *Lasiodiplodia* sp | *Hymenoscyphus fraxineus* |

**Table 2** shows the *in vitro* antifungal activity of silver nanoparticles (AgNP) against *Trichoderma* sp., *Fusarium oxysporum, Lasiodiplodia* sp. And *Hymenoscyphus fraxineus* (Chalara) as represented by their mycelial radial growth inhibition (mm). The mycelial radial growth of the test fungi is as follows; *Trichoderma* sp., ranged from 64.83 ± 4.98 mm (100% conc.) to 75.00 ± 0.00 mm (25% conc.), *Fusarium oxysporum* ranged from 11.67 ± 0.88 mm (25% conc.) to 18.00 ± 0.29 mm (100% conc.), *Lasiodiplodia* sp. ranged from 47.50 ± 3.82 mm (25% conc.) to 55.33 ± 3.03 mm (75% conc.), while *Hymenoscyphus fraxineus* ranged from 10.00 ± 0.00 mm (25%, 50% and 75% conc.) to 35.17 ± 15.33 mm (100% conc.). The silver nanoparticles had the highest inhibition against *Hymenoscyphus fraxineus* while the least inhibition was observed in *Trichoderma* sp. **Table 3** shows the percentage (%) mycelial growth inhibition of AgNP against *Trichoderma* sp., *Fusarium oxysporum, Lasiodiplodia*

sp. and *Hymenoscyphus fraxineus* (Chalara). The percentage mycelial radial growth inhibition of the fungi is as follows; *Trichoderma* sp. ranged from 8.16% (25% conc.) to 20.61% (100% conc.), *Fusarium oxysporum* ranged from 36.49% (75% conc.) to 69.81% (100% conc.), *Lasiodiplodia* sp. ranged from 22.62% (75% conc.) to 33.57% (25% conc.), while *Hymenoscyphus fraxineus* ranged from 53.11% (100% conc.) to 86.67% (25%, 50% and 75% conc.). *Hymenoscyphus fraxineus* had the highest percentage inhibition while *Trichoderma* sp. displayed the least percentage inhibition.

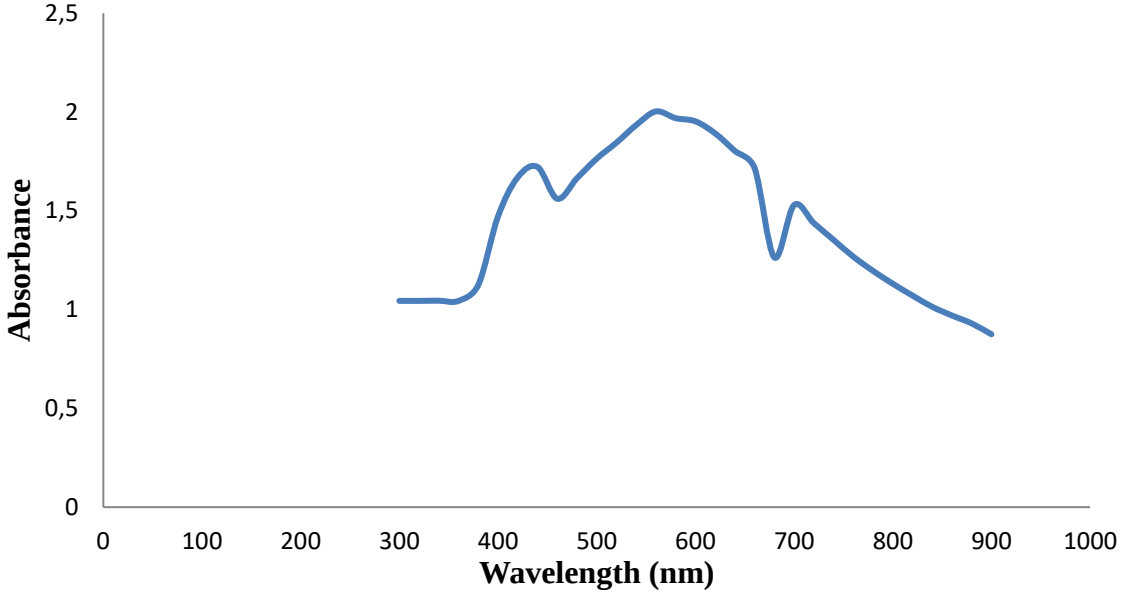


**Figure 1:** Characterization of AgNP biosynthesized by the reduction of $AgNO_3$ solution with *Moringa oleifera* leaf extract after 48 hours.

**Table 2:** Antifungal activity of AgNP against the selected fungal isolates represented by the mycelial radial growth inhibition (mm)

| Test organisms | Concentrations (%) | | | | |
|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | Control |
| *Trichoderma* sp. | 75.00 ± 0.00 | 66.60 ± 1.67 | 65.00 ± 0.00 | 64.83 ± 4.98 | 81.67 ± 1.67 |
| *Fusarium oxysporum* | 11.67 ± 0.88 | 14.50 ± 2.00 | 16.83 ± 1.01 | 18.00 ± 0.29 | 26.50 ± 5.63 |
| *Lasidiplodia* sp. | 47.50 ± 3.82 | 48.33 ± 1.76 | 55.33 ± 3.03 | 50.83 ± 0.83 | 71.50 ± 7.52 |
| *Hymenoscyphus fraxineus* | 10.00 ± 0.00 | 10.00 ± 0.00 | 10.00 ± 0.00 | 35.17 ± 15.33 | 75.00 ± 0.00 |

Key: *Values are means of triplicates, *Mean ± Standard error

**Table 3:** Percentage (%) mycelial growth inhibition of AgNP against the selected fungal isolates

| Test organisms | Concentrations (%) | | | |
|---|---|---|---|---|
| | 25 | 50 | 75 | 100 |
| *Trichoderma* sp. | 8.16% | 18.45% | 20.41% | 20.61% |
| *Fusarium oxysporum* | 55.90% | 45.98% | 36.49% | 69.81% |
| *Lasidiplodia* sp. | 33.57% | 32.41% | 22.62% | 28.90% |
| *Hymenoscyphus fraxineus* | 86.67% | 86.67% | 86.67% | 53.11% |

Key: *Values are means of triplicate

It is crucial to manage fungal infections in crops. There has been a recent push to improve management techniques and reduce hazards to human safety. *Moringa* synthesis of silver nanoparticles in this study is advantageous as it eliminated the production of toxic waste, such as ammonia, usually produced by chemical synthesis, which has been reported to be harmful to human health and the environment (Javad and Naser, 2016). biomaterial nanocomposites based on HAp and pectin from Opuntia Ficus-Indica exhibited a strong antimicrobial activity against both Gram-positive (Staphylococcus aureus and Bacillus cereus) and Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacteria in addition to various fungi (e.g., Aspergillus fumigatus, Penicillium funiculosum, and Trichoderma viride) (Saidi *et al*., 2022). The biomaterial membrane based on hydroxyapatite, chitosan, and glycerol offers promising potential for both industrial and biological applications (Akartasse *et al*., 2022).

A major factor in the rapidly growing threat of chemical agent-induced antimicrobial resistance is the widespread usage of antimicrobial agents. Using nanoantibiotics, or nanoparticles with antibacterial qualities, is one way to combat resistant organisms (Huh and Kwon, 2011). AgNPs are carriers that researchers have employed to transport different loads of big biological molecules to certain destinations. It may be possible to give a specific target location and minimize side effects by focusing on high cargo density and concentration of nanoparticles (Tokumaru *et al*., 1974). Silver or silver salts have gained popularity in recent years as essential elements for limiting microbial growth. Numerous studies, including Rudramurthy *et al*. (2016), have emphasized the antimicrobial potential of silver nanoparticles. They have explained that the benefits of AgNPs include their ability to overcome the microorganism's resistance to conventional antimicrobials and their broad mode of action against various pathogens, including bacteria, fungi, and viruses.

In this study, the antifungal activities of *Moringa oleifera* synthesized silver nanoparticles (AgNPs) against *Trichoderma* sp., *Fusarium oxysporum*, *Lasiodiplodia* sp. and *Hymenoscyphus fraxineus* were investigated. Firstly, no concentration-dependent rise or decline in the mycelial radial growth (in mm) could be successfully established against the test fungi. *Hymenoscyphus fraxineus* was the most inhibited fungi by the AgNP treatment, with percentage inhibition of mycelial radial growth ranging from 53.11% (100% conc.) to 86.67% (25%, 50% and 75% conc.). *Trichoderma* sp. was the least inhibited by the AgNP treatment with percentage inhibition of mycelial radial growth ranging from 8.16% (25% conc.) to 20.61% (100% conc.). These findings are similar to those obtained by Mallmann *et al*. (2015), who recorded that silver nanoparticles had high antimicrobial activity against *C. albicans* and *C. tropicalis* and documented that the AgNPs activity compares favorably with the activity of amphotericin B, which is a powerful antifungal agent.

The most significant finding in this study is the high inhibition of *Hymenoscyphus fraxineus* by the silver nanoparticles treatment across all the concentrations used, with percentage inhibition of mycelial radial growth as high as 86.67% (in 25%, 50% and 75% conc.). There is however paucity of literature regarding the susceptibility of *Hymenoscyphus fraxineus* to AgNP treatment. The high inhibitory activities recorded against *Hymenoscyphus fraxineus and Fusarium oxysporum* in this study is in line with the research conducted by Javad and Naser (2016), who found that while silver nanoparticles did not completely prevent *Fusarium oxysporum* colonies from forming, they did reduce the number of *F. oxysporum* colonies by 65% after an hour of exposure and around 76% of mycelial development after five hours. Meanwhile, at variance with this study, Abdel-Azeem *et al*. (2020) reported that *F. oxysporum* showed complete resistance to the mycogenically and chemically synthesized AgNPs tested against it, although significant activity was obtained against *Candida albicans* and *Aspergillus* sp. A similar result to the lack of activity obtained in this study against *Trichoderma* sp. was reported by

Mahdizadeh *et al*. (2015), who documented that silver nanoparticles had minimal effect on *Trichoderma harzianum*, which was the least inhibited among the six fungal isolates tested in their study. The result also differs from that of Petica *et al*. (2008), who reported that silver nanoparticles have effective antifungal properties against *Trichoderma*. However, Mahdizadeh *et al*. (2015) suggested that the cause of the difference can be attributed to the nano-silver type and the strain of fungus used.

## Conclusion

This study shows that *Moringa*-synthesised silver nanoparticles (AgNP) have a significant antifungal effect against *Fusarium oxysporum* and *Hymenoscyphus fraxineus*. According to these results, silver nanoparticles may be helpful in the field and can regulate plant fungal diseases. It is essential to evaluate silver nanoparticles for antimicrobial action against a variety of different plant fungi and human infections. Additionally, it is necessary to look into how well they work in combination with other plant extracts or biomolecules.

**Disclosure statement:** *Conflict of Interest:* The authors declare that there are no conflicts of interest.
*Compliance with Ethical Standards***:** This article does not contain any studies involving human or animal subjects.

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