Original Article

Silver Nanoparticles Produced Extracellularly by *Sclerotinia sclerotiorum*

Luma Amer Yasir[1], Liqaa Yehya Mohsen[2], Jumana Najem Abdul Sahib[1]

[1]Department of Microbiology, College of Medicine, Mustansiriyah University, Baghdad, Iraq, [2]Department of Microbiology, College of Science, University of Babylon, Babylon, Iraq

Abstract

Background: *Sclerotinia sclerotiorum* is one of the most important fungal species capable of manufacturing silver nanoparticles (AgNPs), which are formed through the biosynthesis process extracellular from silver nitrate solution ($AgNo_3$) to the culture medium using a fungal cell filter; this study aims to explore the biosynthesis of AgNPs using *S. sclerotiorum*. **Materials and Methods:** AgNPs were produced by adding $AgNo_3$ to the culture and then incubating for 72 h at 37°C. The nanoparticles were formed inside the cytoplasm and on the outer surface of the *S. sclerotiorum* fungus. These molecules were characterized using ultraviolet radiation, and the highest absorbance of AgNPs for *S. sclerotiorum* fungus was measured at 450 nm. AgNPs were also characterized using infrared analysis (Fourier transform infrared) and scanning electron microscope (SEM) to determine the size and crystal structure of these molecules. **Results:** *S. sclerotiorum* is a well-known phytopathogenic fungus with significant potential beyond its role as a plant pathogen. Various isolates of this fungus have diverse applications in agriculture, biotechnology, and nanotechnology. Notably, these isolates have demonstrated potential in reducing silver ions (Ag^+) to (AgNPs), highlighting their promise in nanoparticle synthesis. In addition, the intensity of the absorption peak associated with the nanoparticles was found to increase over time, further supporting their potential for research and application. **Conclusion:** AgNPs produced by *S. sclerotiorum* is predominantly spherical, ranging from 5 to 50 nm, and are stabilized by a capping agent. These nanoparticles, found mainly on the fungal cell walls, likely result from fungal enzyme activity. Their antifungal properties are due to their interference with microbial DNA and disruption of metabolic processes, highlighting their potential as effective antifungal agents.

Keywords: Nanoparticles, *sclerotinia*, silver

Introduction

One of the most significant fields of study in the modern world is nanotechnology, that is involved in many medical, health, engineering, and physical applications. Nanomaterials are industrially distinctive and indispensable in many fields, especially related to industries because of their compositional properties that depend on size. Nanotechnology applications vary in many fields, especially agriculture, food industries, and medicine.[1]

Many studies have reported that the most important applications of nanomaterials are in general fields, such as communication technologies, cognitive sciences, social psychology, physical sciences, biotechnology, computational sciences, and other social sciences. It is also crucial in resolving a lot of industrial issues such as food and medical products.[2] The size of nanoparticles typically ranges from 1 to 100 nm for 3D crystals, varying based on their biological, chemical, and physical properties. Additionally, the size range of nanoparticles differs significantly from that of bulk materials, as well as from the properties of individual molecules and atoms. Many chemicals are used to stimulate microorganisms (fungi and bacteria) to produce nanomaterials in the culture medium. These materials consist of carbon, biomolecules, polymers, organic compounds, metals, metal oxides, silicates, and nonoxide ceramics. Depending on the origin of the chemicals, these nanomaterials are formed in many distinctive shapes that make them easy to use in biological and medical applications.[3]

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Address for correspondence: Dr. Luma Amer Yasir, Department of Microbiology, College of Medicine, Mustansiriyah University, Baghdad, Iraq.
E-mail: luma.amer77@gmail.com

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Experiments conducted on some eukaryotic organisms, including fungi, have proven that they have the ability to manufacture nanoparticles. It has the ability to form these nanomaterials as receptors outside the cell to help it survive when it is exposed to environmental stresses such as temperature changes, predators, and toxic substances such as metal ions.[4] Numerous researches have indicated that bacteria, actinomycetes, fungi, and viruses can biosynthesize various metal nanoparticles, including those made of gold, silver, gold–silver alloys, selenium, tellurium, platinum, palladium, silica, titanium, zirconium, quantum dots, and magnetite.[5-8] Metal nanoparticles can be synthesized using three primary methods: chemical, physical, and biological. The focus on silver nanoparticles (AgNPs) stems from their distinctive properties, such as their shape and size-dependent optical, electrical, and magnetic characteristics, making them useful in numerous applications such as antimicrobial agents, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetics, and electronic components. To synthesize and stabilize AgNPs, various physical and chemical techniques have been employed, as detailed by Thakur *et al*.[9]

Adding some mineral or salt solutions to the development medium of fungal mycelium stimulates some fungi to produce silver or gold nanoparticles, as exogenous metabolites or enzymes. In this process, extracellular enzymes and fungal metabolites catalyze the reduction of harmful metal ions into nontoxic solid metal nanoparticles. *Fusarium oxysporum*, *Trichoderma reesei*, and *Trichoderma viride* were among the fungi shown to contain hydrogenases when suspensions of washed cells, grown either aerobically or anaerobically in media containing glucose and salts amended with nitrate, were examined. Nitrate reductase was necessary for the reduction of ferric iron.[5]

The antifungal capabilities of both ionic and nanoparticulate forms of silver show promising potential for managing fungal plant pathogens that produce spores. Compared to synthetic fungicides, silver poses a lower toxicity risk to humans and animals. The advantage of silver lies in its multiple mechanisms of action, targeting a wide array of microbial biological pathways. This characteristic is particularly crucial for preventing resistance development, a growing concern in the chemical management of plant fungal diseases, as highlighted by Kim *et al*.[10]

In the current study, we explored the biosynthesis of AgNPs using *Sclerotinia sclerotiorum*.

Materials and Methods

Methods

Fungal strains

S. sclerotiorum was obtained from the unit of advanced/Mycology/Department of Biology/College of Science/University of Babylon from January 2023 to March 2023.

Growth media

Potato dextrose agar

Potato dextrose agar (PDA) medium was prepared according to Indian production HEMIDIA.

Potato dextrose broth

Potato dextrose broth medium was prepared according to Indian production HEMIDIA.

Growth of fungal isolates

The test was conducted by first pouring 20 mL of PDA culture medium into each Petri dish, as well as into glass tubes with a volume of 50 mL, also containing 20 mL of the medium according to Indian production HEMIDIA. Both the dishes and the tubes were inoculated with isolates of *S. sclerotiorum*. These were then incubated at 25°C for five days. After the incubation period, the isolates were stored in a refrigerator until further use.

Biomass preparation

A 250 mL Erlenmeyer flasks containing 100 mL PDA medium (broth) per flask were inoculated with mycelium *S. sclerotiorum.* They were incubated at 28°C in a shaking incubator for 96 h, finally the fungal mycelium was separated from the culture using a centrifuge and washed with sterile distilled water to get rid of any remaining medium inside the fungal cells.

Biosynthesis of silver nanoparticles

In this test, 50 mL of *S. sclerotiorum* mycelium was mixed with 5 mL of silver nitrate solution ($AgNO_3$) solution at experiment tubes to form the reaction mixture used in the biosynthesis of AgNPs. Then, tubes were incubated at 28°C in the dark to prevent any photochemical reactions for 3 days. Finally, the AgNPs were separated using a centrifuge at 10,000 rpm for 10 min to purify them.

Silver nanoparticles characteristics

Ultraviolet-visible spectroscopy

The absorbance of nanosilver ions was measured at 380, 400, and 420 nm, using the ultraviolet (UV)–vis spectrum of the reaction mixture over a 24-h period. A UV-Vis spectrophotometer (Shimodzu, UV-2150) was used to measure the surface plasmon resonance (SPR) spectra of AgNPs in the reaction mixture at wavelengths ranging from 200 to 800 nm.[11]

Infrared analysis (Fourier transform infrared)

Fourier transform infrared (FTIR) spectrometer (Perkin-Elmer LS-55-Luminescence Spectrometer) was used to analyze the chemical makeup of the produced AgNPs. Using the KBr pellet technique, the solutions were dried at 75°C, and the dried powders were characterized in the 4000-400 cm^{-1} range.[12]

Scanning electron microscopy analysis

Using a scanning electron microscope (SEM) (JSM-6480 LV), the morphological characteristics of artificially created AgNPs derived from neem plant extract were investigated. SEM slides were produced by spreading the solutions across them 24 h after

$AgNO_3$ was added. The samples were coated with a tiny coating of platinum to make them conductive. After that, the samples were examined in the SEM at a 20 KV accelerating voltage.[13]

Results

S. sclerotiorum was cultivated in a solid medium obtained from the Unit of Advanced Mycology, Department of Biology, College of Science, University of Babylon, as shown in Figure 1.

The process involved harvesting the biomass to obtain a mycelia-free cell filtrate. A significant observation was the color change in the filtrate after adding silver nitrate. The solution transitioned from colorless to yellow within 10 min and eventually turned purple, as shown in Figure 2.

This color transformation is indicative of the formation of (AgNPs) in the solution. In contrast, the $AgNO_3$ in its free cell form exhibited no such color alteration. It was also observed that the density of the color correlated with the size of the synthesized AgNPs. UV-visible spectroscopy, a highly effective technique for structural characterization of AgNPs, was employed in this study.

In our experiments, the SPR peak for *S. sclerotiorum* was identified at 450 nm, as depicted in Figure 3.

Infrared test (Fourier transform infrared) analysis

Fourier transform infrared (FTIR) analysis was conducted to identify the biomolecules responsible for capping and effectively stabilizing the synthesized metal nanoparticles. The FTIR spectrum of AgNPs associated with the isolate *S. sclerotiorum* is displayed in Figure 4. This spectrum revealed six distinct peaks at 1066.6, 1384.9, 1660, 1683, 2362, and 3578 cm^{-1}. The peak at 2362 cm^{-1} is indicative of the NH stretch vibration typical of primary and secondary amides in proteins. Peaks at 1660 and 1683 cm^{-1} correspond to the carbonyl stretch, attributed to the amide I bond in proteins. The 1384 cm^{-1} peak is associated with amino and amino methyl stretching groups in proteins, whereas the band at 1066 cm^{-1} aligns with C-N stretching vibrations in aromatic and aliphatic amines. Furthermore, the peak at approximately 3578.07 cm^{-1} is related to the C-OH group of phenols.

The FTIR analysis confirmed the presence of various functional groups of phytochemicals in plants used for the synthesis of AgNPs. These phytochemicals are believed to serve as reducing agents for converting Ag+ to Ag0 and as capping and stabilizing agents for AgNPs.

Aggregates of AgNPs are seen in the SEM micrograph. There are spherical nanoparticles in the 20–50 nm size range seen in this image. Even inside the aggregates, the nanoparticles were not in direct contact, suggesting that a capping agent had stabilized them. The SEM examination of *S. sclerotiorum* and the nanoparticles made using the fixing approach is depicted in Figure 5, which also demonstrates that the majority of the nanoparticles are on the fungal cell wall's surface.

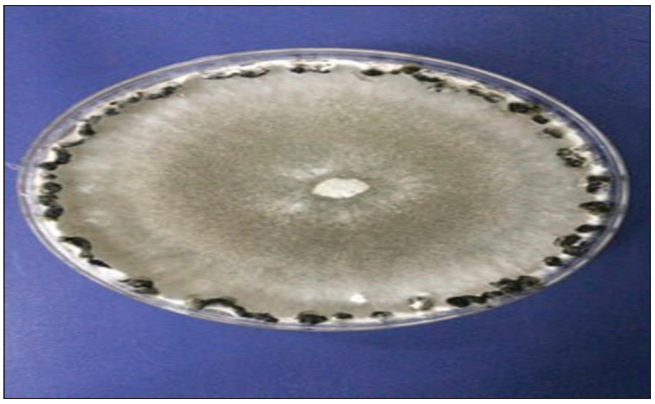

Figure 1: Plate of *Sclerotinia sclerotiorum* on potato dextrose agar

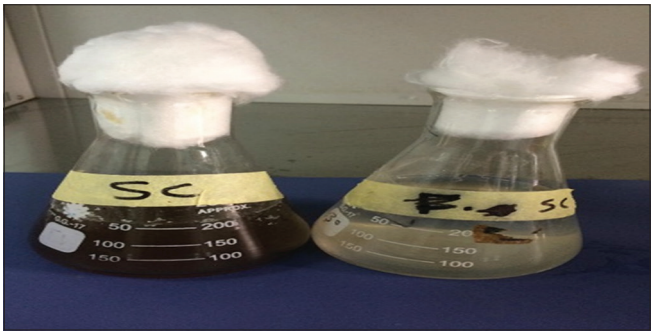

Figure 2: The change of color from light yellow to purple of live cell filtrate of *Sclerotinia sclerotiorum* after the addition of silver nitrate solution ($AgNO_3$) solution (1 mM) with control without $AgNO_3$

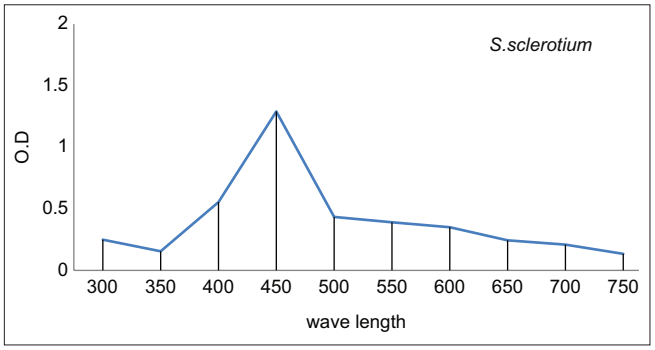

Figure 3: Ultraviolet-Vis spectra recorded after the exposure of 1 mM silver nitrate solution in crude cell filtrate of *Sclerotinia sclerotiorum*

Discussion

In this experiment, the SPR peak for *S. sclerotiorum* was identified at 450 nm, as depicted in Figure 3. Building on the findings of Kim *et al.,* we identified the surface plasmon peak, which is a result of electrons stimulated by an interactive electromagnetic field.[10] In our experiments, the SPR peak for *S. sclerotiorum* was identified at 450 nm,

This discovery confirms the potential of these isolates in reducing Ag ions to Ag nanoparticles, paving the way for further research in the synthesis of these nanoparticles. In addition, the intensity of the absorption peak was found to increase over time.

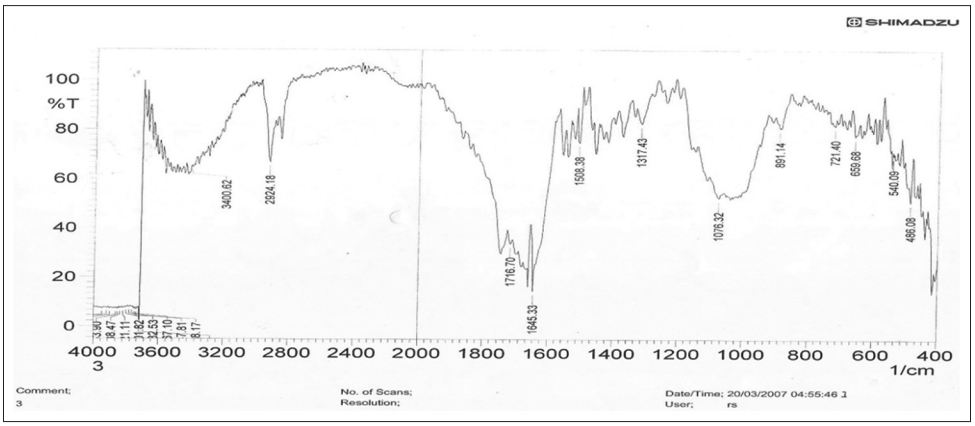

Figure 4: Detection of various functional groups by Fourier transform infrared from *Sclerotinia sclerotiorum*

The process of metal ion reduction occurs on the cell wall surface, facilitated by enzymes, as stated by Jo *et al*.[14] The maximum synthesis of silver was observed at a wavelength of 500 nm, whereas the minimum peak occurred at 400 nm.[14] This observation aligns with previous studies on AgNPs synthesized from *T. viride* and *Trichoderma harzianum*, which exhibited UV absorption peaks at 400 nm and 440 nm, respectively.[15,16] It was also found that 6-day-old isolates produced a high amount of $AgNO_3$ after 24 h of incubation, a finding consistent with the research conducted by Shelar and Chavan.[17] In the context of cytogenotoxicity, biogenic AgNPs are generally less harmful *in vivo* compared to their chemically synthesized counterparts. Furthermore, human cells have demonstrated greater resilience to the toxic effects of AgNPs compared to other organisms. Although *in vivo* and *in vitro* studies have shown that AgNPs can be toxic to mammalian cells and increased human exposure to AgNPs poses potential risks, there are extensive applications of AgNPs in technological and medical fields, as highlighted by Goal *et al*. in 2023 and Li *et al*. in 2020.[18,19]

According to FTIR, analysis was conducted to identify the biomolecules responsible for capping and effectively stabilizing the synthesized metal nanoparticles. The FTIR spectrum of AgNPs associated with the isolate *S. sclerotiorum.* This spectrum revealed six distinct peaks at 1066.6, 1384.9, 1660, 1683, 2362, and 3578 cm^{-1}. The peak at 2362 cm^{-1} is indicative of the NH stretch vibration typical of primary and secondary amides in proteins. Peaks at 1660 and 1683 cm^{-1} correspond to the carbonyl stretch, attributed to the amide I bond in proteins. The 1384 cm^{-1} peak is associated with amino and amino methyl stretching groups in proteins, whereas the band at 1066 cm^{-1} aligns with C-N stretching vibrations in aromatic and aliphatic amines. Furthermore, the peak at approximately 3578.07 cm^{-1} is related to the C-OH group of phenols,[20] emphasize that the carbonyl groups in amino acid residues and peptides possess a strong affinity for binding to silver. Balaji *et al*., reported that proteins can bind to nanoparticles through free amine groups or cysteine residues and potentially act as capping agents for stabilization.[21] The lyophilized nanoparticle samples were also analyzed via FTIR to identify biomolecules involved in reducing Ag+ ions in the cell filtrate. The observed spectra presented absorption peaks around 2360 cm^{-1} (aromatic-CH stretching), 1683.9 cm^{-1} (-NHCO of amide), and 825.16 cm^{-1} (C-Cl). The absorbance wavelengths of these bands typically range from 400 to 450 nm, with a peak at a longer wavelength indicating the presence of larger nanoparticles, as noted by Gola *et al*.[22]

The FTIR analysis confirmed the presence of various functional groups of phytochemicals in plants used for the synthesis of AgNPs. These phytochemicals are believed to serve as reducing agents for converting Ag+ to Ag0 and as capping and stabilizing agents for AgNPs. This study aligns with earlier findings which suggested that phytochemicals such as phenols, aldehydes, aromatic groups in amino acid residues, and proteins have a strong capacity to bind with metals, acting as reducing, capping, and stabilizing agents for green synthesized AgNPs. Moreover, these phytochemicals are reported to prevent agglomeration of green synthesized AgNPs.[15,23,24]

Aggregates of AgNPs are seen in the SEM micrograph. There are spherical nanoparticles in the 20–50 nm size range. Even inside the aggregates, the nanoparticles were not in direct contact, suggesting that a capping agent had stabilized them. The SEM examination of *S. sclerotiorum* and the nanoparticles made using the fixing approach, which also demonstrates that the majority of the nanoparticles are on the fungal cell wall's surface. These findings suggested that the fungus cell wall may contain the enzymes needed to synthesize AgNPs. Using SEM examination, the morphological characteristics of the produced AgNPs were examined. The majority of the particles appear to be spherical in form, according to SEM examination.[25,26]

Our SEM results showed that the AgNPs were of a spherical shape and uniformly distributed, not aggregated in solution, with an average size of 5–50 nm. The development of polydisperse nanoparticles with a size range of 5–50 nm was also seen in the SEM [Figure 5], and this was consistent with a study conducted by the researcher,[27] who indicated that

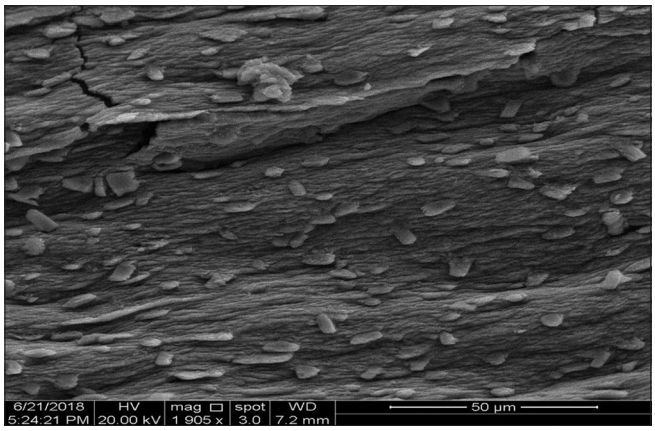

Figure 5: Scanning electron microscopy for nanoparticles on the fungal cell wall

AgNPs of spherical structure have the ability to inhibit the growth of fungi. As mentioned by the researcher, it was noted that the antifungal effect was stronger against the development of fungi and that it depends on size and dose. Many studies conducted on the inhibitory effect of silver ions on inhibiting the growth of microorganisms have indicated that they have the ability to interfere with the DNA of microorganisms and destroy it.[28,29] They also have the ability to interfere with the vital metabolism of fungi and the synthesis of inactive ATP molecules.[28] Furthermore, depending on the above findings, we demonstrated the ability of AgNPs to inhibit fungal growth through the interaction of the nanoparticles with the fungal cell walls.[30-32]

Conclusion

The phytochemicals or biomolecules present in the studied plant sources play a dual role in both the formation and stabilization of green synthesized AgNPs.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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