

Synthesized Nanoparticles from *Pleurotus Ostraeus* and Study the Effect Against *Fusarium* and *Alternaria*

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ABSTRACT

Objective: The aim of the research is to produce silver nanoparticles (AgNps) using aqueous extract of *Pleurotus ostreatus* and evaluate the effectiveness of these nanoparticles against fungal isolates. **Methods:** The investigation was carried out on fungi that were isolated from rotting fruit. Fungi were classified based on their microscopic and morphological characteristics. For the biosynthesis of silver nanoparticles, a quick and eco-friendly method has been created. A practical, environmentally friendly technique producing stabilised silver nanoparticles (AgNps). These were created using an aqueous extract of *Pleurotus ostreatus* with an average diameter of 30 ± 60 nm and a spherical shape. At 10-3 M of silver nitrate, the reaction is carried out. The produced AgNps were verified by colorimetric transformation from dark brown to grey. Atomic force microscopy (AFM), scanning electron microscope (SEM), and ultraviolet-visible spectroscopy were used to study the properties. Using the well diffusion method, the inhibitory effect of AgNps against fungi was studied by examining the effects of different concentrations (100, 75, 50, and 25). **Results:** According to the results, AgNps and the extract have strong and comparable antifungal activity. The results showed that the concentration of 100 $\mu\text{g/mL}$ of AgNps was the highest level of inhibition zone identified, with the inhibition zones reaching (23.33 ± 4.41) for *Fusarium* and at a dose of 25 $\mu\text{g/mL}$ AgNps, the lowest amount of inhibition was found. The zone of inhibition of *Alternaria* was observed to be 6.00 ± 1.15 . The aqueous extract at a concentration of 100 $\mu\text{g/ml}$ showed the largest inhibition zone, with *Fusarium* inhibition zones reaching 12.33 ± 1.45 . At a concentration of 25 $\mu\text{g/ml}$ of aqueous extract, the smallest inhibition zone was observed, with inhibition zones for *Alternaria* of 4.67 ± 0.33 . **Novelty:** For the biosynthesis of silver nanoparticles, a quick and eco-friendly method has been created. A practical, environmentally friendly technique producing stabilised silver nanoparticles (AgNps).

INTRODUCTION

Recently, there has been a lot of interest in the biosynthesis of nanomaterials. The possibility for greater productivity and reduced costs over chemical approaches could be an additional benefit of fully utilizing biological synthesis. In order to synthesize metal nanoparticles like gold, silver, cadmium, zirconia, and silica titanium, a new aspect of the metal-microbial interaction has been investigated recently [1]. By taking advantage of their special physical, chemical, and biological properties, metal nanoparticles have been synthesized from bacteria, yeasts, fungi, and other biological sources and applied to a variety of fields such as drug delivery, biosensors, bioimaging, antimicrobial activity, food preservation, etc [2]. Compounds with antifungal activity either kill fungus locally or inhibit their growth without typically endangering the tissues around them. Nowadays, chemically altered natural substances make up the majority of antifungal medications [3]. The silver cations produced by Ag nanoparticles, which serve as reservoirs for the Ag^+ bactericidal agent, are undoubtedly the cause of strong bactericidal

activity [4]. There are multiple pathways linked to the bactericidal activity of nanoparticles. Additionally, there's a chance that microbial cells and silver nanoparticles will interact directly. By blocking respiratory chain enzymes or interfering by obstructing permeability to phosphate and protons, oxidative phosphorylation can be linked to respiratory electron transport, which can be investigated in relation to silver ions. disruption of transmembrane electron transmission, oxidation of cell constituents, disruption of, penetration of the cell membrane or reactive oxygen species (ROS), or dissolution of harmful heavy metal ions [5].

RESEARCH METHOD

Identification of fungi:

Various fungal colonies were seen on (SDA) at 37°C for a period of 5–10 days. These colonies displayed distinct characteristics, including Dense, dark green colonies, compact, white or yellow colonies. Initially white to pale yellowish, the colonies turn creamish-yellow over the day. darker and more grayish. Pale gray, Colony light brown to gray in color. Smooth radial furrows in colonies that range in hue from white to cream to grayish-brown. Throughout the incubation period, various fungal colonies were.

Preparation of hot aqueous extracts of mushrooms

After the mushroom powder was oven-dried, it was soaked in distilled water at a 1:10 (w/v) ratio and heated to $60 \pm 2^\circ\text{C}$ for 30 minutes while stirring. After that, the powdered boiled mushroom was covered and kept for half an hour. After that, the leftover material was filtered through gauze and centrifuged again at 10,000 rpm for 30 minutes at 4°C. After that, supernatants were gathered and filtered using Whatman No. 1 filter paper. Subsequently, extract powders were freeze-dried using a freeze-drier and kept in storage at $4 \pm 2^\circ\text{C}$.

Biosynthesis of silver nanoparticles

Biosynthesis of silver nanoparticles (AgNPs). A silver nitrate stock solution (1×10^{-3} M, AgNO₃) was generated using triple-distilled deionized water and served as the basis for all subsequent dilutions. Using sterile distilled water, an aqueous extract solution of 10 mg/ml is made and filtered through a 0.2 μm syringe filter. The aqueous extract of *P. ostreatus* was diluted with sterile distilled water to make a total volume of 10 ml, ranging from 2 to 7 ml, based on the outcome of an initial trial. Subsequently, the solution is mixed with 5 milliliters of 1×10^{-3} M aqueous AgNO₃ solution and subjected to UV light at 365 nm for seven days (Long UV). Following a 24-hour incubation period, the mixed solution's light yellow color changed to dark yellow, signifying the creation of silver nanoparticles [6].

Characterisation of AgNPs

When AgNPs formed, the AgNO₃ solution's hue changed from pale yellow to a dark brownish yellow tone. The excitation of surface plasmon vibration in the NPs is what causes the color shift [7]. The *P. ostreatus* extract's active molecules converted the silver metal ions into AgNPs. AgNPs were proven to have formed by strong absorption

peaks at 400–470 nm wavelengths, which are characteristic absorption bands of spherical AgNPs

Characterization of AgNPs:

Ultraviolet-visible spectroscopy:

An absorption spectroscopy instrument is called an Ultraviolet-Visible spectrophotometer (UV-Vis). Because of their surface plasmon, the samples were examined using UV-VIS double beam spectrophotometers at 300–600 wave lengths [8].

Atomic force microscopy (AFM)

Via an atomic force microscope (Veeco) operating in typical air conditions, the surface shape of the nanoparticles was seen. Spread out on a little slide, the analyzed materials were investigated using the instrument's contact mode [9].

Scanning electron microscopy (SEM)

The samples' morphological characteristics were assessed using SEM analysis utilizing the JEOL Jsm6480 LV. The substance was analyzed after the samples were spread out on a slide and coated with platinum using an auto fine coater [10].

Estimation of antifungal activity of silver nanoparticle:

Inoculum preparation

The inoculum used in the susceptibility testing of filamentous fungus was made using the serial dilution method of the suspension of pathogenic fungi.

Agar Well Diffusion Method:

Using the pour plate method, 6 cm Petri dishes were homogeneously mixed with SDA medium at varied concentrations of 75, 75, 50, and 25 mg/ml of silver nanoparticles and 1 ml of suspension of 10⁻¹ and 10⁻³ dilution of pathogenic filamentous fungi. Then, a sterile cork borer was used to create a 5 mm well in the media. 100µl of each silver nanoparticle concentration was added to different wells. For one day, the plates are incubated with the fungal species at a temperature of 35 ± 2 °C. The diameter of the inhibitory zone was measured for each repetition, and the average diameter was determined [11].

Sabouraud Dextrose Agar (SDA)

The media was prepared by adding 65 grams of media material to 1000 milliliters of distilled water (D.W.) in a glass flask, and then using a magnetic stirrer to agitate the mixture for 30 minutes in order to combine the material with the D.W. After that, the objects were sterilized in the autoclave. Pour into petri dishes, then leave to solidify. The fungus were grown in these media in order to examine their characteristics and the impact of nanoparticles and *Pleurotus ostreatus* aqueous extract on the fungi.

Sabouraud Dextrose Broth

Using a glass beaker, media was prepared by adding 65 grams of media per 1,000 milliliters of D.W., stirring the mixture for 30 minutes using a magnetic stirrer, and then sterilizing the mixture using an autoclave. Fill the tubes with liquid. This medium has been relatively used to preserve mushrooms for a long time.

Sample collection

Samples were collected in sterile conditions and the entire research was divided into three main stages. It included collecting samples of rotting fruits, then growing them on Sabouraud dextrose agar, and determining their identity using a microscope. Finally, the sensitivity of the aqueous extract and silver nanoparticles against fungi was tested.

RESULTS AND DISCUSSION

Alternaria

The filamentous fungus *Alternaria* species belongs to the dematiaceous family that, because of melanin, creates mold with a black pigment [12]. Certain species are common diseases and agents. Additionally linked to allergic sinusitis and rhinitis is this fungus [7]. Apart from being a filamentous fungus that affects a range of economically significant plants and can withstand a wide range of temperatures, *Alternaria spp.* can also produce conjugated mycotoxins, which are primarily coupled to sulfates or glucosides and exist in the plant host [13]. Furthermore, the hydrolysis of these conjugated mycotoxins during metabolism releases the free toxin, which may be harmful to human health [14].

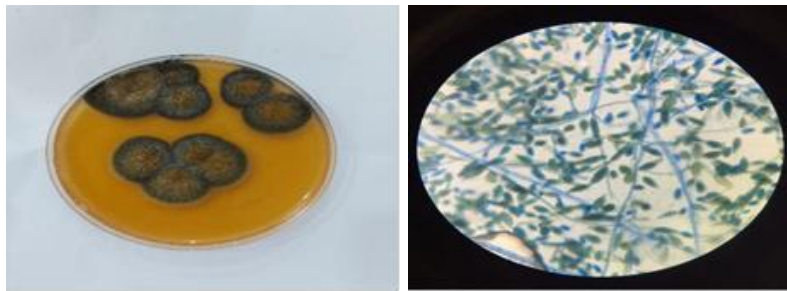


Figure 1. *Alternaria spp.* grown on SDA after seven days of incubation at 28 ± 2 °C: (A) Top view: *Alternaria* (B) microscopic feature of *Alternaria spp* (40X).

Fusarium

Serious fungi that cause massive economic losses are *Fusarium spp* [7]. Thus, hunger and undernourishment the two most significant medical problems that humanity is currently facing are being caused by these fungus. Actually, it's well known that some species of *Fusarium* can produce a wide range of mycotoxins in large amounts [13], endangering not just the health of humans but also that of animals. Mycotoxins are pathogenicity factors, although they can also operate as virulence factors on occasion [14]. This means that mycotoxins have the potential to either increase the virulence of a fungus or damage the organs of the host, particularly its defense systems. Their ability to infect a human host is what they emphasize most. However, only a small number of *Fusarium* species are capable of infecting humans, at least under ideal circumstances in these hosts [15]. *Fusarium* molds are classified as ascomycetes, although they can also be classified as hyalohyphomycetes because their hyphae lack pigment. *Fusarium* species are known to exist in at least 70 identifiable and well-characterized taxa, while there may be many more that are still poorly understood [16].



Figure 2. *Fusarium* grown on SDA after seven days of incubation at 28 ± 2 °C: (A) Top view: *Fusarium* (B) microscopic feature of *Fusarium* (40X).

Synthesis and Characterization of Silver Nanoparticles:

1. Visual Observation and UV-Vis Spectral Study:

Following the initial detection of silver nanoparticles in the chemical reaction vessel during the incubation periods, the reaction mixture changed from colorless to pale yellow, indicating an initial reduction. Over time, this color changed to brownish yellow, light brown, and deep brown, or grey. The next step to characterize the biosynthesized AgNps was to use a UV-vis spectrophotometer. The size and structure of the nanoparticles in the aqueous suspension were investigated using UV-visible spectroscopy. The UV-Vis spectrophotometer verified the formation and stability of the generated AgNps in sterile distilled water within the wavelength range of 300–800 nm. After combining *Pleurotus ostreatus* extract with AgNO_3 , the reduction response of Ag^+ ion to Ago was observed by measuring the UVVis spectrum for the reaction media. The obtained UV-Vis spectra following the reaction's completion were displayed in (Figure 3).

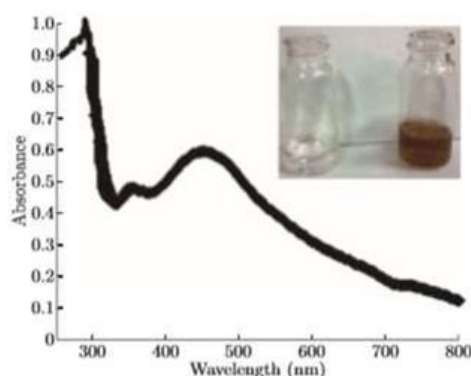


Figure 3. UV-Vis spectrophotometry of silver nanoparticles by *Pleurotus ostreatus*.

Characterization by Scanning Electron Microscopy (SEM):

Utilizing a scanning electron microscope, the form of the environmentally friendly silver nanoparticles was examined. In the micrograph, the surface-deposited silver nanoparticles can be easily observed at a high magnification of 100,000 kx. The *Pleurotus ostreatus* has a remarkable capacity to produce silver nanoparticles, according to SEM analysis. These particles were uniformly dispersed, mostly spherical, and had an average size of slightly less than 50 nm. (Figure-4).

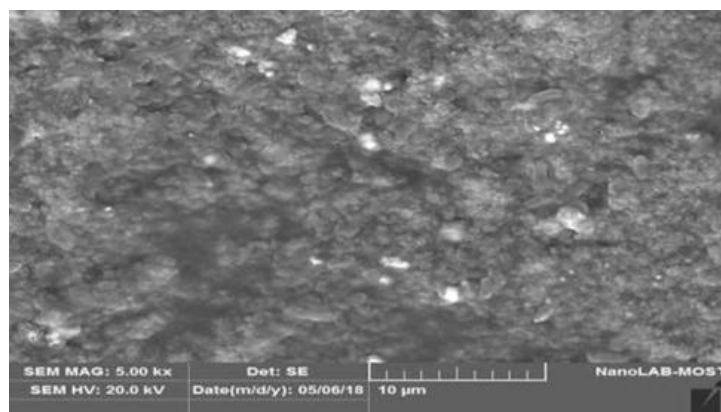


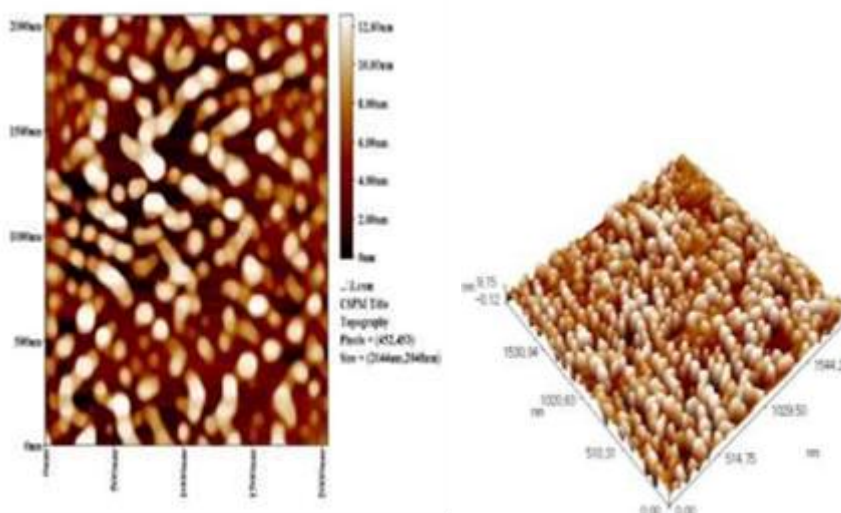
Figure 4. SEM image of AgNPs synthesized from *Pleurotus ostreatus* extract at 5.00 kx of magnification power.

Characterization by Atomic Force Microscopy (AFM)

The average diameter of AgNPs as well as their two- and three-dimensional morphology were detected using atomic force microscopy, which was utilized as a check method to characterize the biosynthesis of AgNPs. Based on the study's findings, Table 1 and Figure 5 a, b, and c illustrate the average diameter of the AgNPs that the *Pleurotus ostreatus* plant biosynthesizes, which is 50.28 nm.

Table 1. The accumulation size of silver nanoparticles biosynthesized by *Pleurotus ostreatus* extract.

Diameter(nm)<	Volume (%)	Cumulatio n(%)	Diameter(nm)<	Volume (%)	Cumulatio n(%)	Diameter(nm)<	Volume (%)	Cumulatio n(%)
30.00	0.70	0.70	65.00	4.90	20.98	100.00	8.39	64.34
35.00	0.70	1.40	70.00	6.99	27.97	105.00	12.59	76.92
40.00	2.10	3.50	75.00	5.59	33.57	110.00	3.50	80.42
45.00	1.40	4.90	80.00	6.99	40.56	115.00	4.90	85.31
50.00	4.20	9.09	85.00	2.10	42.66	120.00	6.29	91.61
55.00	2.10	11.19	90.00	8.39	51.05	125.00	8.39	100.00
60.00	4.90	16.08	95.00	4.90	55.94			



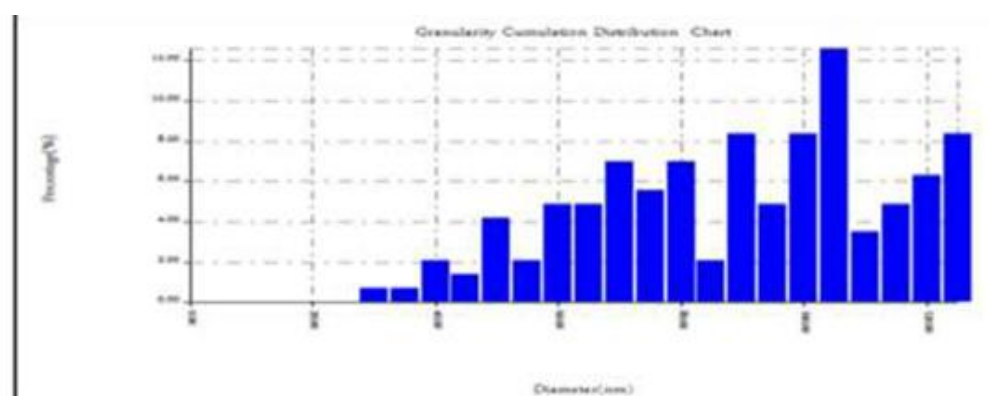


Figure 5. Biosynthesized AgNPs by *Pleurotus ostraeatus* extract under AFM (a) 2D image of silver Nanoparticles synthesis (b) 3D image of silver nanoparticles synthesis (c) Granularity distribution chart of silver nanoparticles synthesis.

Antifungal activity of AgNPs

Table 2 shows the fungal growth inhibition zone (mm) following AgNp treatment at concentrations of 100, 75, 50, and 25 $\mu\text{g}/\text{ml}$. The results showed that the highest level of inhibition zone for *Fusarium* occurred at (23.33 ± 4.41) at a concentration of 100 $\mu\text{g}/\text{mL}$ of AgNPs. On the contrary, the lowest level of the inhibitory zone for *Alternaria* fungus is (6.00 ± 1.15) at a concentration of 52 $\mu\text{g}/\text{mL}$ of AgNPs. It was observed that in all cases, the level of inhibition increased as the concentration of AgNPs increased. They investigated a high reduction in fungal growth as a result of the greater concentration of AgNPs, similar to the study conducted by [14]. AgNPs' ability to prevent fungal growth is attributed to [15]:

- Lipopolysaccharide liberation.
- Variations in the cell tissue layer's permeability protein membrane.
- Dissipation of the proton motive force causes the membrane potential to break down.
- Production of free radicals is accountable for the membrane's damage.

Table 2. Growth inhibition zone of fungi by different concentrations of AgNPs.

Fungi	Concentration				LSD value
	100 mg/ml	75mg/ml	50mg/ml	25mg/ml	
<i>Fusarium</i>	23.33 ± 4.41	20.00 ± 1.15	15.00 ± 2.88	7.67 ± 1.45	9.111 *
<i>Alternaria</i>	20.00 ± 1.15	18.00 ± 1.15	14.33 ± 2.33	6.00 ± 1.15	5.011 *

Antifungal activity of aqueous extract:

The results obtained indicate that the highest level of inhibition zone was detected at a concentration of 100 $\mu\text{g}/\text{ml}$ of extract, where the inhibition zones for *Fusarium* reached 12.33 ± 1.45 . For *Fusarium*, at a concentration of 25 micrograms/ml of alcohol, the lowest level of inhibition zone was detected. For *A. alternaria*, the inhibition zones are (4.67 ± 0.33) . Table 3 provides a summary of the findings. The summary is recognized for

its ability to prevent the growth of germs. It has been documented that the damage damages the mitochondrial DNA in yeast cells [17], deactivating some enzymes, like dehydrogenase and hexokinase. Nonetheless, certain fungal strains can adapt to and tolerate its appearance. Elevated extract concentrations [18]. To get this effect, do the following: direct application of aqueous extract to surfaces, yet as of right now, modeling is unknown. Aqueous extract's impact on fungal growth [19].

Table 3. Growth inhibition zone of fungi by different concentrations of aqueous extract.

Fungi	Concentration				LSD value
	100 mg/ml	75mg/ml	50mg/ml	25mg/ml	
<i>Fusarium</i>	12.33 ± 1.45	10.67 ± 0.67	6.67 ± 0.67	5.33 ± 0.33	2.876 *
<i>Alternaria</i>	11.00 ± 2.08	8.33 ± 1.20	8.33 ± 1.20	4.67 ± 0.33	4. 415 *

CONCLUSION

Fundamental Finding : The fungi were isolated from rotting fruits and vegetables, and the effectiveness of biotinylated silver nanoparticles prepared from aqueous extract of *Pleurotus ostreatus* was tested. And the *Pleurotus ostreatus* aqueous extract is used as an antifungal, and it has been shown that silver nanoparticles are more effective than the aqueous extract in inhibiting the growth of fungi. **Implication :** And the *Pleurotus ostreatus* aqueous extract is used as an antifungal, and it has been shown that silver nanoparticles are more effective than the aqueous extract in inhibiting the growth of fungi. **Limitation :** The fungi were isolated from rotting fruits and vegetables. **Future Research :** Further studies are needed to investigate the mechanism of antifungal activity of silver nanoparticles and to evaluate their effectiveness against a wider range of fungal species under different environmental conditions.

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