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Original Article

Silver Nanoparticles that Synthesis by Using *Trichophyton rubrum* and Evaluate Antifungal Activity

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Abstract

Using microorganisms to make this nanomaterial is a new research technique. In a culture medium, *Trichophyton rubrum* was permitted to biosynthesis silver nanoparticles. This study used *Trichophyton rubrum*, *a* dermatophytes fungus, to make silver nanoparticles. These species' clinical strains were produced in a medium containing mineral salt and cultured for 5-7 days at 25°C. Each culture's cell-free filtrate was taken and used to make AgNps in the presence of 1 mM AgNO3. The reduction of Ag+ ions in metal nanoparticles was virtually studied by observing the colour of the solution, which changed to a reddish-light brown after 72 hours. SEM was used to establish the presence of AgNo3. The presence of AgNPs was confirmed by SEM, which revealed that they are primarily spherical and 100nm in size. Furthermore, the findings showed that silver nanoparticles have antifungal activity against both infections in a concentration-dependent manner. At (150 ppm) of AgNPs, the growth decreased.

Keywords: Silver Nanoparticles, *Trichophyton rubrum*, Evaluate Antifungalx

1. Introduction

Fungi have a great variety of species, including 6 million, and play an essential role in agriculture, ecology, and human health; some are known as pathogens that cause lethal infectious diseases (1). Identifying and describing new fungal infections continues (2). *Dermatophytes sp.*, a type of fungi that causes skin illnesses, have the highest frequency among humans, with an estimated 20-25% of the population affected (3, 4). This study used new metal nanoparticles to control microbes such as fungi. Both pathogenic and nonpathogenic fungi have been found to produce nanoparticles of various elements (5). Several nanoparticles such as; silver, gold, zirconium, silica, titanium, iron (magnetite), and platinum have been synthesized using fungal systems or myconanofactories. Many fungal strains, including *Fusarium oxysporum*, could synthesize (AgNPs) extracellularly (6). Fungi are essential to many other microorganisms. Compared to plant materials and bacteria, fungal cells can flow through pressure, agitation, and other conditions in bioreactors or other chambers. These are meticulously grown, easy to handle, and simple to fabricate. Traditional chemical procedures may quickly produce AgNPs of varied sizes and forms; however, the bulk has a substantial environmental impact (7). For large-scale production of AgNPs, chemical techniques are often less expensive. Nonetheless, in most synthetic procedures, toxic materials and reducing agents are used frequently (8). Microorganisms make nanoparticles through various processes. However, several researchers give the critical idea that microbes generate molecules in high amounts

and participate in decreasing metal ions, thus altering nanoparticle size (9). There are a few properties of biological nanoparticle synthesis over chemical nanoparticle creation. Fungi could be valuable since they have metal bioaccumulation potential, secrete high amounts of enzymes, and are easy to scale up nanoparticle production. As a result, nanotechnology science has proven to be cost-effective, easier, and more environmentally friendly. Several research has found that AgNPs have substantial antifungal activity against pathogenic fungi, with the majority focusing on the effects of AgNPs on Candida species (10). However, there have been few studies on the direct influence of AgNPs on dermatophytes. The antifungal efficiency of AgNPs in bio-stabilized substances was tested against dermatophytes and some fungal species. In the chemical approach, the generation of AgNPs (Chem-AgNPs) has been shown to have antifungal efficiency against several genera of *Trichophyton mentagrophytes*. The impact of *Klebsiella pneumonia* producing biologically generated AgNPs (Bio-AgNPs) against *Trichophyton rubrum* was investigated by Tran, Nguyen (7). The disc diffusion test was utilized by Dias, Oliveira (3) to demonstrate that Bio-AgNPs exhibit strong antifungal effectiveness against *T. rubrum*. In this study, the biosynthesis of silver nanoparticles using *Trichophyton rubrum* and the underlying mechanisms were investigated, and the properties of AgNPs produced using SEM and UV spectrum was described. The efficiency of silver compounds in reducing pathogenic fungi was evaluated.

2. Materials and Methods

2.1. Fungal Strains

The Dermatophytes Fungus (*Trichophyton rubrum)* was obtained from the advanced Mycology Unit, Department of Biology, College of Science, the University of Babylon.

2.2. Growth Media

Sabrauad dextrose agar (SDA) medium was prepared according to Indian production company HEMIDIA. Potato dextrose agar (PDA) medium was prepared according to the Indian production business HEMIDIA.

2.2.1. Growth and Maintenance of Fungal Isolates

Trichophyton rubrum isolates were cultivated on Petri dishes with PDA and cultured for 5 days at 25 °C. PDA was put into glass tubes (capacity 50 ml) in 20 ml increments and allowed to set. This isolate, obtained from the edge of recently generated colonies, was injected separately into the medium. Tubes were incubated at 25°C for 5 days before being maintained at 5°C in the refrigerator.

2.2.2. Biomass Preparation

Trichophyton rubrum mycelia were placed into 250mL Erlenmeyer flasks with 100mL PDB medium and cultivated on a rotary shaker for 96 hours at 28°C (120 rpm). Mycelia were then extracted and treated with sterilized DW to remove any residual media from the fungal cells.

2.3. AgNP Production and Characterization

For AgNP formation, 50 ml of cell extract was treated with 50 ml AgNO3 solution (5 mM), and a sample solution without AgNO3 was used as a control. At 28 °C, the produced solutions were incubated for 3 days. The study kept all liquids in the dark to prevent any photochemical reactions. Before being collected for further analysis, the AgNPs were separated twice by centrifugation at 10,000 rpm for 10 minutes.

2.3.1. SEM Analysis

Using a scanning electron microscope, Bio-AgNPs were morphologically described and elementally examined. A fraction of dried silver nitrate (AgNPs) and Bio-AgNPs was inserted into the substance holder of the SEM apparatus and evaluated.

2.4. Antifungal Activity and Statistical Analysis

PDA was treated with various concentrations of silver nanoparticles (25, 50, 100, and 150 mg/L) in an in vitro assay. Before plating in a Petri plate, five mL of silver nanoparticles in varied concentrations were put into SDA. At 25°C, SDA-containing Ag NPs were incubated. After two days of incubation, a piece of culture (5 mm) containing fungi was put in the middle of each plate containing Ag NPs, followed by five days

of incubation at 25°C. For the computation of the inhibition rate, the following formula was used (%).

$$\text{Inhibition rate} = \frac{M - m}{M} \times 100$$

(M): the growth of fungal cells (control plate).
(m): the growth of fungal cells (plate with silver nanoparticles).

3. Results and Discussion

3.1. Characterization of Ag NPs

3.1.1. Silver Reduction

After adding Ag and filtered cell-free cultures in the dark condition, the colour of the samples progressively changed from virtually colourless to reddish-light brown. During the incubation period, the colour intensity increased. The operation was stopped after 72 hours, and the particles were analyzed further. The virtual investigation of the reduction of Ag+ ions into metal nanoparticles resulted in the hue of the three solutions changing to a reddish-light brown following incubation (Figure 1). When incubated in the same conditions as the control sample (without silver ions), the colour of the cell filtrates did not change. The creation of SNP was suggested by the emergence of a reddish-brown colour in the reaction (11). Even after 72 hours of incubation, no precipitation was found in the solutions.

3.1.2. Scanning Electron Microscopy Studies

Silver nanoparticle aggregates were visible in the scanning electron micrograph. Ball shape nanoparticles in approximately 20-50nm were found in this micrograph. Even within the aggregates, the nanoparticles were not in direct contact, indicating that a capping agent had stabilized the nanoparticles. The SEM analysis of *T. rubrum* with nanoparticles generated by the fixing technique is shown in figure 2. Most nanoparticles are found on the surface of the fungal cell wall. As a result, it might assume that the enzymes responsible for creating silver nanoparticles may be found in fungi's cell walls. SEM examination was used to investigate the morphological characteristics of produced silver nanoparticles. According to SEM analysis, the majority of the particles are spherical SEM micrographs of silver nanoparticles in the filtrate revealed that they are spherical, well spread in solution without aggregation, and have a size distribution of about 5-50nm. The formed and size characteristics of the nanoparticle were studied further using scanning electron microscopy. Baker, Pradhan (12) observed that nanoparticles in solution at low concentrations were entirely poisonous to fungus. Antifungal activity depends on size, dose, and against fungal growth. After Ag ion treatment, deoxyribonucleic acid (DNA) loses the ability to replicate, characterize ribosomal subunit proteins, some other cellular proteins and enzymes required for ATP production, which loses activity, according to research on the inhibitory influence of silver ions on microorganisms (13). The level of fungal growth inhibition discovered in this study was related to the nanoparticle concentration in the medium that consisted of the interaction between nanoparticles and the fungus cell walls (14).

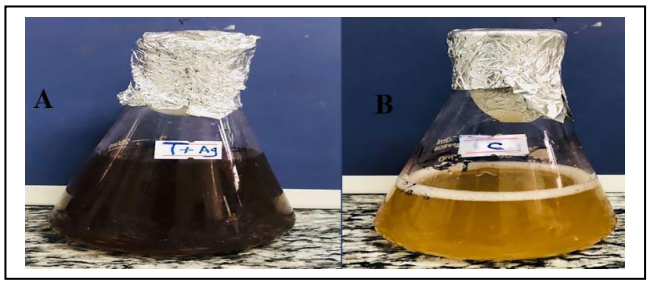

Figure 1. AgNPs biosynthesis A-Positive results in colour change AgNPs formation, B-control, potato dextrose broth with fungi (Negative control)

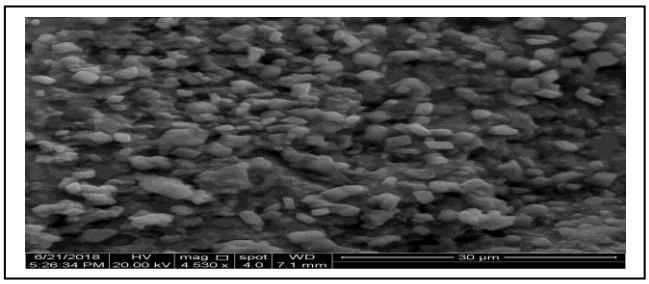

Figure 2. Scanning electron microscopy

3.2. Antifungal Activity

In SDA, the inhibitory effect of silver nanoparticles at different concentrations was investigated (Figure 3). At 150 mg/ml, the highest reduction of fungal growth was observed. Compared to other concentrations that demonstrated inhibition but were less than 100 and 150 mg/ml. This study revealed AgNPs to be particularly effective against pathogenic fungi. The researchers discovered that silver nanoparticles could block *T. rubrum* from proliferating (Table 1). All fungi showed the impact in a concentration-dependent manner. This isolate displayed the most inhibition at a dosage of 150 mg/ml AgNPs. The inhibition decreased as the number of nanomaterials raised. The great intensity with which AgNPs solution could state and agglutinate to fungal hyphae and deactivate dangerous organisms is most likely the reason for silver nanoparticles' extraordinary antifungal effectiveness. Ag ion inhibits microorganisms through a number of mechanisms, including DNA losing its capacity to replicate.

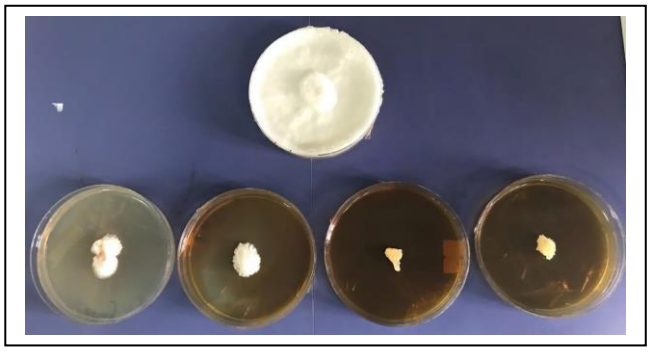

Figure 3. Growth inhibition of extracellular silver nanoparticles against dermatophytes pathogenic fungi

Table 1. Antifungal activity of extracellular silver nanoparticles against fungi (Inhibition %)

Organisms	Concentration (mg/ml)			
	25mg	50mg	100mg	150mg
T. rubrum	85%	87.5%	98%	100%

Furthermore, biosynthesized Ag-NPs have been shown to have antifungal action and produced Ag-NPs on the surface of the fungus Rhizopusoryzae; in addition, they inhibited the growth of (gram-positive and gram-negative) bacterial strains, in addition to other fungi like; Saccharomyces *cerevisiae* and *Candida albicans* (3). The manufacture of Ag-NPs that reduce the growth of *Candida albicans* has also been produced using a banana peel extract (12). Furthermore, silver ions are expected to strongly influence tissue activities, such as those in the mitochondrial membrane (15). Against a range of pathogenic fungi, the effect of mixing AgNPs with an antifungal agent, such as fluconazole, on the mixture outcome has been examined (16). The fungi were able to produce AgNPs and had antifungal properties (16, 17). Tran, Nguyen (7) that demonstrated the same methodology to assess the effect of SNPs on bacteria and fungi, observed similar results. SNPs have antifungal efficacy against candida spp., *Aspergillus spp., and Fusarium spp*., according to Qian, Yu (18). The concentrations that affect fungal species are 150 mg/ml. SNPs have been shown to have antifungal efficacy through apoptosis, according to Hwang *et al.*, 2012. The therapy resulted in a raised in (ROS), a decrease in mitochondrial membrane potential, phosphatidylserine externalization, DNA and nuclear fragmentation, and the activation of metacaspases in fungus isolates.

Furthermore, the dermatophytes fungus *T. rubrum* demonstrated the ability to synthesize Ag-NPs outside the cell. Ag-NPs can be made quickly utilizing cell-free filtrate. The results showed that biological nanoparticle synthesis is rapid and suited for large-scale manufacture. The SEM was used to characterize the Ag-NPs. Nanotechnology demonstrates a unique, cutting-edge technique for developing and testing new antifungal approaches based on metallic nanoparticles' characteristics. The antifungal activity of Ag-NPs against *T. rubrum* was impressive.

Authors' Contribution

Study concept and design: L. Y. M.
Acquisition of data: L. Y. M.
Analysis and interpretation of data: M. F. A.
Drafting of the manuscript: R. A. L.

Critical revision of the manuscript for important intellectual content: A. K.
Statistical analysis: L. Y. M.
Administrative, technical, and material support: L. Y. M.

Conflict of Interest

The authors declare that they have no conflict of interest.

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