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Biosynthesis and characterization of silver nanoparticles using marasmius palmivorus MG717877.1 and their antifungal activity

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***Abstract*---**Silver nanoparticles (AgNPs) were produced utilizing Marasmius palmivorus MG717877.1 in the current investigation. The fungal cell filtrate was used to accomplish external production of silver nanoparticles (AgNPs) from silver nitrate (AgNo3) solution. The aqueous silver (Ag) ions in a 1mM AgNo3 aqueous solution were decreased when it was exposed to fungal cell filtrate, resulting in highly stable AgNPs. When challenged with 1mM AgNo3 solution, the fungal biomass acquired AgNPs on its surface, inside the cytoplasmic membrane, and within the cytoplasm in 72 hours. UV was used to characterize the AgNPs that had been produced. For M.palmivorus MG717877.1, the greatest absorbance of AgNPs was recorded at 400nm in the visible spectrum. Furthermore, the FTIR and SEM analyses of silver nanoparticles on these fungus were used to characterize silver nanoparticles. In addition, we tested the antifungal activity of M.palmivorus MG717877.1 at various concentrations, including 25, 50, 100, and 150 mg/ml. The findings revealed that silver nanoparticles produced in a concentration-dependent manner have strong antifungal activity on isolates. At 150 mg of AgNPs, the highest decrease was seen for this isolate.

***Keywords*---**silver nanoparticles (AgNPs), marasmius palmivorus, biosynthesis, characterization, antifungal activity.

Introduction

Nanotechnology has fast gained much importance as a result of its growing applications in electronics and medicine (Boisselier and Astruc, 2009).Since our own biological system is really a complex of nano-machines, nanotechnology has endless promise in biomedical applications. Nanotechnology is a field of study that focuses on developing new methods for designing, synthesizing, manipulating, and applying particles with dimensions less than 100 nanometers. Nanoparticles are the technical term for these tiny particles. Nanoparticles are important components in nanotechnology. Many nano-structured materials and gadgets start with them. Nanoparticles have exceptional physicochemical features due to their incredibly small size and high surface area to volume ratio. These characteristics enable them to be used in a variety of industries, including electronics and photonics, catalysis, data storage, chemical sensing and imaging, environmental cleanup, medication delivery, and biological labeling, hence increasing their commercial worth (Prabhu and Poulose, 2012).A nanoparticle is defined as a particle with a single dimension of 100 nanometers or less. Reducing agents that are toxic to the environment or biologically dangerous are commonly used in the chemical synthesis of nanoparticles. Inorganic minerals are produced by organisms either intracellular or extracellularly (Shankar *et al.,* 2003).

Because of its environmentally friendly characteristic, little cost, and non-poison material through the synthesis process, biologically generated AgNPs have recently piqued the interest of researchers (Horky *et al.,* 2018). This method, however, makes use of a variety of complicated natural bases, such as fungus and bacteria. Fungi are frequently used in nanoparticle manufacturing methods according to the active complex process that occurs in the source, such as polysaccharide, vitamin, and protein. Using agents and conventional stabilizers, these combinations help in the production of nanoparticles from Ag+ (El-Adlyand Shabana,2018). In several respects, fungi have an advantage over other microbes. In comparison to plant materials and bacteria, fungal mycelia mesh can endure flow pressure, agitation, and other conditions in bioreactors or other chambers. These are meticulously grown, easy to handle, and simple to fabricate. Reductive proteins secrete more extracellularly, which can be easily handled in downstream processing.

Furthermore, because the nanoparticles precipitated outside the cell are free of unwanted biological components, they can be employed directly in a variety of applications (Narayanan and Sakthivel, 2010). Nanoparticles come in a variety of shapes and sizes, including spheres, cylinders, platelets, tubes, and more. As a result, they are created with surface alterations that are particular to the applications for which they will be utilized. The fungus mycelium is exposed to the metal salt solution during the production of metal nanoparticles by the fungus. Metal nanoparticles are synthesized using three different methods: chemical, physical, and biological. The synthesis of radiation-induced AgNPs is a straightforward, clean process that utilizes radiolysis of an aqueous solution and

is an effective way to reduce metal ions (El-Batal *et al.,*2013)..In present study, we investigated the biosynthesis of AgNPs using *Marasmius palmivorus* MG717877.1 and its underlying mechanisms, the properties of obtained AgNPs were characterized by UV spectrum, SEM, FTIR, and investigate the effect silver compounds for inhibition of pathogenic fungi.

Materials and Methods

Fungal strains

Marasmius palmivorus MG717877.1 was received from the University of Babylon's. Advanced Mycology Unit, Department of Biology, College of Science.

Growth media
Potato Dextrose Agar (PDA)

PDA medium was formed by adding 39 gram of medium in one liter of distilled water and boiling it with regular agitation until it was fully dissolved, according to Indian production business HEMIDIA. Chloramphenicol was added to the medium at a concentration of 250 mg/L before being divided into conical flasks (250ml). For 15 minutes, it was sterilized in an autoclave at 121° C. The medium was then cooled to 45-50˚C., fully homogenized, and placed into sterile petri plates.

Potato dextrose broth (PDB)

PDB medium was obtained by adding 24 gram of medium in one liter of distilled water and boiling it with continuous stirring until it was fully dissolved, according to Indian production company HEMIDIA. Chloramphenicol was added to the medium at a concentration of 250 mg/L before being separated into conical flasks (250ml). Sterilized for 15 minutes in an autoclave at 121°C. The medium was then cooled to 45-50 ° C.

Growth and maintenance of fungal isolates

M.palmivorus MG717877.1 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, which were obtained from the edge of recently generated colonies, were injected separately into the medium. Tubes were incubated at 25°C for 5 days before being maintained at 5°C in the refrigerator.

Biomass preparation

M.palmivorus MG717877.1 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 D.W to remove any residual media from the fungal cells.

AgNP production

For AgNP formation, 50 ml of cell extract was mixed 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. All liquids were kept in the dark during the work 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 each time.

Characterization of AgNPs
UV-Visible spectroscopy

At 24 hours, the UV-Vis spectra of the dye solution was used to evaluate the decrease of silver ions, and their absorbance was recorded at 380, 400, and 420 nm. A UV-Vis spectrophotometer (Shimodzu, UV-2150) was used to record the spectra of the surface Plasmon resonance of AgNPs in the dye solution at wavelengths ranging from 200 to 800 nm.

FTIR analysis

An FTIR spectrometer was used to investigate the chemical composition of the synthesized silver nanoparticles" (perkin-Elmer LS-55-Luminescence spectrometer). The mixtures were dried at 75°C, and the dried particles were analyzed using the KBr pellet technique in the range 4000–400 cm-1.

SEM Analysis

Using an 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.

Antifungal activity of silver nanoparticles on

PDA was treated with various concentrations of silver nanoparticles (25, 50, 100, and 150 mg/L) in an in vitro assay. Prior to plating in a Petri plate, 5 mL of silver nanoparticles in varied concentrations were put into PDA. At 25°C, PDA-containing Ag NPs were incubated. After two days of incubation, 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 cell (control plate).
(m): the growth of fungal cell (plate with silver nanoparticles).

Results and Discussion

Characterization of Ag NPs
UV–vis spectrum of Ag NPs

Marasmius palmivorus MG717877.1 was cultured in liquid media. The biomass was used in order to receive mycelia free cell filtrate. The color of the culture altered with the mix of silver nitrate with free cell filtrate, as observed by eye observation in the culture (Figure 1). The coluor of cell filtrate containing silver nitrate changed from non color to yellow, then purple, after 10 minutes. In contrast to free cell silver nitrate, which did not change color, this color shift is attributable to the creation of AgNPs in the solution. The color density is affected by the size of the generated silver nanoparticles. The creation of AgNPs was validated by UV–vis spectroscopy, which detected the SPR property in the absorption spectra band (Zhang *et al.,* 2016). The biochemical features of fungal extracts, as well as the concentration used in AgNPs synthesis, determine the absorption peak (Kamar *et al.,* 2017, Yaser *et al.,* 2017). The SPR peak for *M.palmivorus* MG717877.1 was discovered at 400 nm in our research (Figure 2). As a result, we established that these isolates have a higher capacity for reducing Ag ions into Ag nanoparticles, prompting us to pursue additional study into silver nanoparticle manufacturing. With the progression of time, the strength of the absorption peak increases. UV absorption peak of silver nanoparticles synthesized from *T.viride* and *T. harzianum* was observed at 400 nm, 440nm respectively. (Tripathi *et al.,*2013, shelar and chavan, 2015). The six-day-old isolates produced a high amount of silver nitrate during a 24-hour incubation period, which was similar to what Devi *et al.,*2013 found. Biogenic SNPs appear to be less cytogenotoxic in vivo than chemically produced nanoparticles. Furthermore, human cells were discovered to be more resistant to the detrimental effects of SNPs when compared to other organisms. SNPs are harmful to mammalian cells in both in vivo and in vitro tests, implying that greater human exposure to SNPs poses a risk. Some research have revealed broad application of SNPs in the technological and medical domains (Lima *et al.,* 2012).

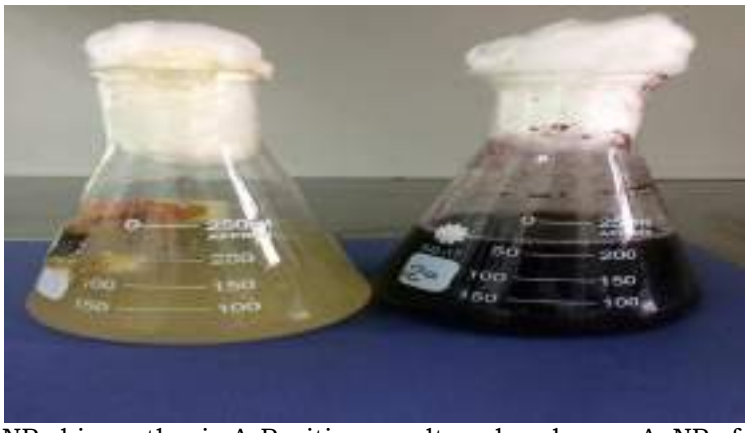

Figure 1. AgNPs biosynthesis A-Positive results color change AgNPs formation, B-control, potato dextrose broth with fungi (Negative control).

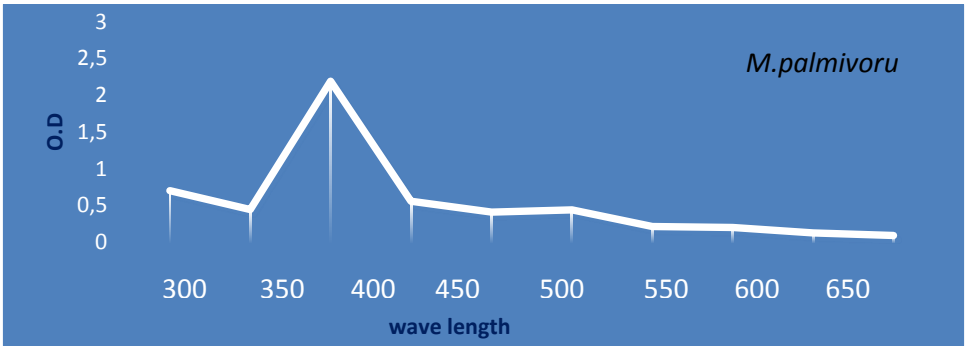

Figure 2. UV-Vis spectra recorded after the exposure of 1mM silver nitrate solution in crude cell filtrate of *M.palmivorus*

FTIR analysis

The biomolecules for capping and efficient stability of the metal nanoparticles produced were identified using FTIR measurements. Figure 3 shows the FTIR spectra of silver nanoparticles for isolate *M.palmivorus* MG717877. This fungus was demonstrated. The stretching vibrations of primary and secondary amines were given to the bands detected at 3400 cm-1 and 2924 cm-1, respectively. The stretch molecule vibration is represented by the bands at 1645 cm-1 and 1508 cm-1. The C-N structural properties of aromatic and aliphatic amines can be allocated to the two bands at 1317 cm-1 and 1076 cm-1. The role of proteins in the creation of silver nanoparticles is supported by this FTIR spectrum. Amino acid residues and peptides' carbonyl groups have a strong capacity to attach to silver (Balavijayalakshmi and Ramalakshmi, 2017). Proteins can also attach to nanoparticles via free amine or cysteine groups in proteins, according to the findings. Proteins on the surface of silver nanoparticles may act as a stabilizing capping agent (Gole *et al.*,2001). The absorption peaks found in representative nanoparticle spectra are about 2360 cm-1 (aromatic – CH stretching), 1683.9.66 cm-1 (- NHCO of amide), and 825.16 cm-1 (C – CI). The role of proteins in the production of silver nanoparticles is supported by this FTIR spectrum. The existence of distinct functional groups of phytochemicals in plants utilized to synthesize AgNPs was confirmed by FTIR analysis. In the current study, phytochemicals were thought to operate as a reducing agent for the conversion of Ag+ to Ag0, as well as a capping and stabilizing agent for AgNPs. The conclusions back up Ondari and Nalini's (2014) earlier findings. The phytochemicals phenols, aldehydes, aromatic groups of amino acid residues, and proteins were thought to have a great potential to bond with metal and operate as a reducing, capping, and stabilizing agent of green produced AgNPs. It is also said to be in charge of preventing the agglomeration of green produced AgNPs. As a result, the phytochemicals/biomolecules found in the fungal sources were found to serve a dual role in the creation and stabilization of green produced AgNPs.

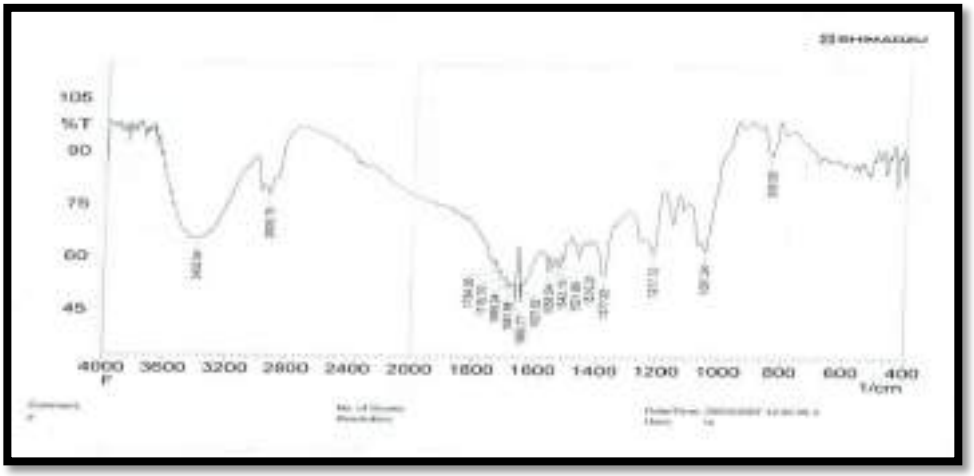

Figure 3. Detection of various functional groups by FTIR from *M.palmivorus* MG717877.1

Scanning Electron Microscopy studies

Silver nanoparticle formations are observed in the SEM micrograph. Round nanoparticles in the size range of 20-50nm were found in this micrograph. Even within the aggregates, the nanoparticles were not in direct contact, indicating that the nanoparticles had been stabilized by a capping agent. The SEM analysis of *M.palmivorus* MG717877.1, as well as nanoparticles generated using the fixing technique, is presented in fig4. As a result, we might speculate that the enzymes responsible for the creation of 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 in shape (Birla *et al.,*2013). Silver nanoparticles in the filtrate were found to be circular in shape, widely dispersed in solution without coagulation, and have an average size of about 5-50nm, according to SEM micrographs. Scanning electron microscopy was used to evaluate the shape and size characteristics of the nanoparticles generated. SEM micrographs revealed the formation of polydispersed nanoparticles with diameters ranging from 5 to 50 nanometers (Nakamura *et al.,*2019)

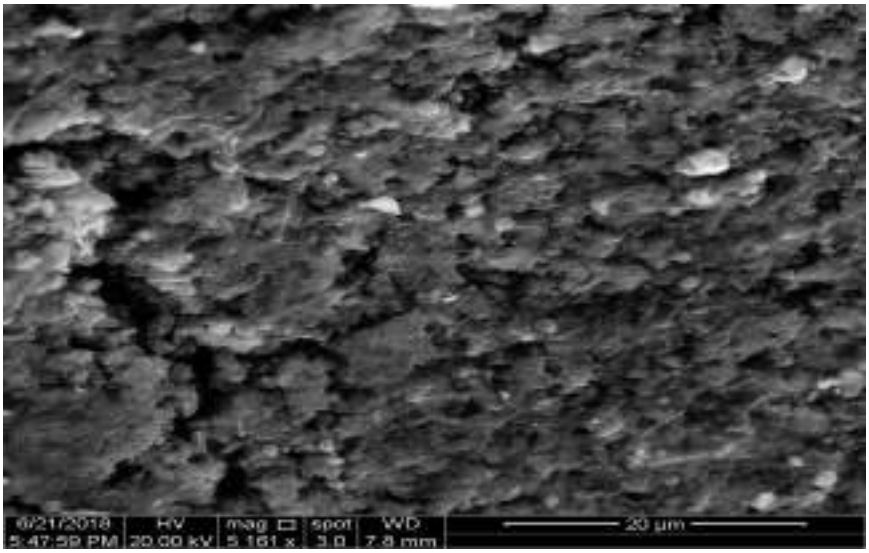

Figure 4. Scanning Electron Microscopy

Antifungal Activity

PDA was used to assess the inhibitory effect of silver nanoparticles at various concentrations (Figure 5). The greatest level of suppression was seen for *M.palmivorus* MG717877.1 at 100 and 150 mg/ml, respectively, as compared to other concentrations that showed inhibition but at lower concentrations 100 and 150 mg/ml for *M.palmivorus* MG717877.1. Silver nanoparticles were observed to be highly influence against pathogenic fungus in this investigation. The findings revealed that silver nanoparticles have the right to block *M.palmivorus* MG717877.1 from growing (Table 1). All fungus showed the impact in a concentration-dependent manner. At a level of 150 mg/ml AgNPs, these isolates displayed the greatest inhibition. As the concentration of silver nanoparticles increased, so did the inhibition. The high intensity with which AgNPs solution could state and agglutinate to fungal hyphe and deactivate destructive organisms is most likely responsible for silver nanoparticles' antifungal activity. Ag suppresses microorganisms through a variety of mechanisms, including DNA losing its replication capacity (Jo et al., 2009), which results in the inactive production of ribosomal subunit proteins in place of other enzymes and cellular proteins required for adenine triphosphate synthesis (Jebril *et al.,* 2020). Additionally, it is thought that silver ions predominantly affect the functioning of membrane-bound enzymes, such as those in the respiratory chain. Similar results were previously published by Kaviya *et al.,*2011, who used the same methodology to investigate the effect of SNPs on bacteria and fungi. Qian *et al.,* 2013 observed SNPs antifungal activity against several fungi as *candida* spp, *Aspergillus* spp, and *Fusarium* spp, presenting the concentration that effect on fungal species are 150 mg/ ml. Recently, Ghojavand et al., 2020 showed SNPs possess antifungal activity effects through apoptosis. The treatment induced in isolates of fungi an accumulation of ROS, reducteon in the mitochondral membrane potential, phosphatidylserine externalization DNA and nuclear fragmentotion and the activation of metacapases.

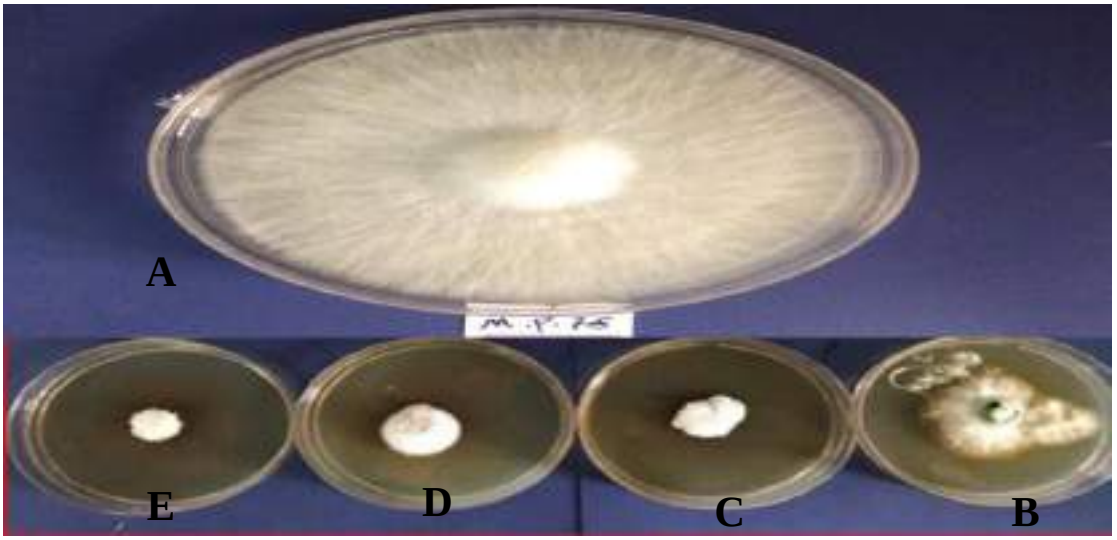

Figure 5. Growth inhibition of extracellular silver nanoparticles against pathogenic fungi

Table 1
Antifungal activety of extracellolar silver nanaparticles against fungi (Inhibition %)

Fungus	Concentration (mg/ml)			
	25mg	50mg	100mg	150mg
M.palmivorus	85%	87.5%	98%	100%

Conclusions

Furthermore, The fungus *Marasmius palmivorus*MG717877.1 demonstrated the ability to synthesize Ag-NPs outside of the cell. Ag-NPs can be made quickly utilizing cell free filtrate. This shows that biological nanoparticle synthesis is rapid and suited for large-scale manufacture. U.V,FTIR, and SEM was used to characterize the Ag-NPs. Nanotechnology demonstrates a cutting-edge and unique technique to developing and testing new antifungal approaches based on metallic nanoparticles' antifungal characteristics. Antifungal activity of Ag-NPs against T. rubrum was impressive.

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