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BIOLOGICAL SYNTHESIS, CHARACTERIZATION AND ANTIFUNGAL POTENTIAL OF SILVER NANOPARTICLES AND THEIR FORMULATION

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Abstract

The biological synthesis of silver nanoparticles (AgNPs) has gained increasing attention as an eco-friendly and sustainable alternative to conventional chemical and physical methods. In this study, AgNPs were synthesized using aqueous extracts of Azadirachta indica *(Neem) leaves, which served as natural reducing and stabilizing agents. The successful formation of nanoparticles was confirmed by a visible color change from pale yellow to brown, corresponding to the excitation of surface plasmon resonance. The synthesized AgNPs were comprehensively characterized using UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and electron microscopy (SEM/TEM), along with dynamic light scattering (DLS) and zeta potential analysis to determine size distribution and stability. The results indicated well-dispersed, crystalline nanoparticles with an average size of 20–30 nm and good colloidal stability.*

The antifungal potential of the synthesized AgNPs was evaluated against Candida albicans, Aspergillus niger, *and* Fusarium oxysporum *using agar well diffusion, broth dilution, and MIC/MFC assays. The nanoparticles exhibited strong dose-dependent antifungal activity, with significant zones of inhibition compared to the standard drug fluconazole. Mechanistic studies suggested that AgNPs exerted their antifungal effect through disruption of the fungal cell membrane, induction of reactive oxygen species (ROS), and interaction with key proteins and enzymes.*

For practical applications, AgNPs were incorporated into a carbopol-based gel formulation, which demonstrated excellent stability (pH, viscosity, and homogeneity) and enhanced antifungal efficacy compared to pure nanoparticles. These findings highlight the potential of biologically synthesized AgNPs and their formulations as sustainable and effective antifungal agents for applications in medicine, agriculture, and textiles.

INTRODUCTION

Nanotechnology has emerged as one of the most dynamic and transformative areas of modern science, bridging chemistry, biology, materials science, and medicine [1]. Among the many nanomaterials that have been explored, silver nanoparticles (AgNPs) have attracted significant attention due to their unique physicochemical properties and wide-ranging biological activities [2]. Unlike bulk silver, which has limited reactivity, nanoscale silver exhibits enhanced surface area-to-volume ratio, tunable size, and shape-dependent characteristics, which strongly influence their interaction with biological systems [3]. These properties make silver nanoparticles highly effective as antimicrobial agents, with applications extending into medicine, agriculture, food preservation, and textile industries [4].

In recent years, the increasing prevalence of pathogenic fungi and the emergence of drug-resistant strains have created a pressing need for alternative antifungal strategies. Conventional antifungal drugs such as azoles, echinocandins, and polyenes, though effective, often suffer from drawbacks including toxicity, limited spectrum, and the development of resistance during long-term use [5]. Fungal pathogens such as Candida spp., Aspergillus spp., and plant-associated fungi are responsible for both clinical infections in humans and significant agricultural losses [6]. This scenario has driven researchers to investigate innovative and eco-friendly approaches for the development of novel antifungal materials, among which silver nanoparticles synthesized through biological routes hold remarkable promise [7].

Traditional physical and chemical methods for nanoparticle synthesis, although widely practiced, often require high energy input and involve hazardous reagents that can generate toxic by-products [8]. Such limitations have shifted the focus toward biological synthesis (or "green synthesis") approaches that employ plant extracts, bacteria, fungi, and other biological systems as reducing and stabilizing agents [9]. Plant-mediated synthesis, in particular, is gaining importance because it is cost-effective, environmentally benign, and scalable [10]. Phytochemicals such as flavonoids, terpenoids, alkaloids, and phenolic compounds present in plant extracts not only reduce silver ions (Ag^{+}) to metallic nanoparticles (Ag^{0}) but also act as capping agents that enhance nanoparticle stability and biocompatibility [11]. This method eliminates the need for toxic chemicals and aligns with sustainable development goals, making it suitable for biomedical and agricultural applications [12].

Characterization of the synthesized silver nanoparticles is crucial to understanding their structural, morphological, and functional attributes [13]. Techniques such as UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM)/transmission electron microscopy (TEM) are widely employed to confirm nanoparticle formation, analyze functional groups involved in reduction and stabilization, and determine particle size distribution and morphological [14].These parameters are directly correlated with the biological activity of AgNPs, as smaller and well-dispersed nanoparticles often exhibit superior antimicrobial properties compared to larger or aggregated forms [15].

The antifungal potential of biologically synthesized silver nanoparticles has been extensively studied, with multiple reports confirming their ability to disrupt fungal cell walls, alter membrane permeability, generate reactive oxygen species (ROS), and interact with vital cellular proteins and enzymes [16]. These mechanisms collectively lead to fungal growth inhibition or cell death. When incorporated into suitable formulations such as gels, creams, sprays, or coatings, AgNPs can be transformed into stable and user-friendly products with enhanced efficacy and longer shelf life. Such formulations not only improve the therapeutic application of AgNPs in clinical settings but also extend their usability in agriculture for controlling phytopathogenic fungi, thereby reducing reliance on chemical fungicides [17].Despite the growing interest in silver nanoparticles as antimicrobial agents, several limitations remain in current research. Many studies on AgNPs still rely on physical and chemical synthesis methods, which, while effective, often involve hazardous chemicals, high energy consumption, and limited biocompatibility. This creates concerns regarding environmental safety and large-scale applicability [18]. Although biological or green synthesis has been

explored, most reports are limited to preliminary findings and lack detailed characterization linking nanoparticle size, morphology, and stability to antifungal efficacy. Furthermore, much of the existing work focuses on antibacterial activity, with comparatively fewer studies addressing antifungal mechanisms and the practical development of formulations suitable for medical or agricultural applications. In addition, the stability and long-term shelf life of AgNP-based antifungal products remain underexplored, which restricts their potential commercialization and widespread use.

In light of these gaps, the present study aims to synthesize silver nanoparticles using an eco-friendly biological approach, employing plant-derived extracts as natural reducing and stabilizing agents. The objectives are to (i) characterize the synthesized nanoparticles using modern analytical techniques to confirm their structural, morphological, and functional properties, (ii) evaluate their antifungal activity against selected pathogenic fungi to understand their efficacy and mechanism of action, and (iii) formulate a stable product containing AgNPs and assess its physicochemical properties, stability, and antifungal potential. Through this integrated approach, the study seeks to develop a sustainable, safe, and effective antifungal formulation that could serve as a viable alternative to conventional antifungal drugs and chemical fungicides.

2 Materials and methods

For the synthesis process, fresh leaves of [Azadirachta indica (Neem)] were collected, thoroughly washed with distilled water to remove surface contaminants, and shade-dried before further use. In cases where microbial synthesis was employed, appropriate bacterial or fungal cultures were maintained under standard conditions. The primary chemical used for nanoparticle synthesis was silver nitrate ($AgNO_3$, analytical grade), while ethanol and other supporting reagents of analytical grade were also utilized. For antifungal evaluation, selected pathogenic fungal strains such as Candida albicans, Aspergillus niger, and Fusarium oxysporum were procured from [Microbiology department] and maintained under suitable growth conditions. The characterization of synthesized nanoparticles was carried out using advanced analytical instruments including a UV-Visible spectrophotometer for surface plasmon resonance analysis, Fourier-transform infrared spectrophotometer (FTIR) for functional group identification, X-ray diffractometer (XRD) for crystalline structure analysis, and electron microscopy (SEM/TEM) for morphological observations. Additionally, particle size distribution and surface charge were determined using a Dynamic Light Scattering (DLS) instrument and a Zeta potential analyzer, respectively.

2.1 Preparation of Biological Extract

In the plant-based synthesis method, 10 g of finely chopped fresh leaves of [Azadirachta indica (Neem)] were boiled in 100 mL of distilled water for 15–20 minutes to extract the bioactive compounds responsible for nanoparticle reduction and stabilization. The mixture was then filtered through Whatman No. 1 filter paper, and the clear filtrate obtained was stored at 4 °C until further use. Alternatively, in the microbial-based synthesis method, fungal or bacterial isolates were cultured in nutrient broth under sterile conditions to promote metabolite production. After sufficient growth, the culture medium was centrifuged to separate the microbial biomass, and the resulting cell-free supernatant was collected. This supernatant served as the reducing and capping agent in the synthesis of nanoparticles.

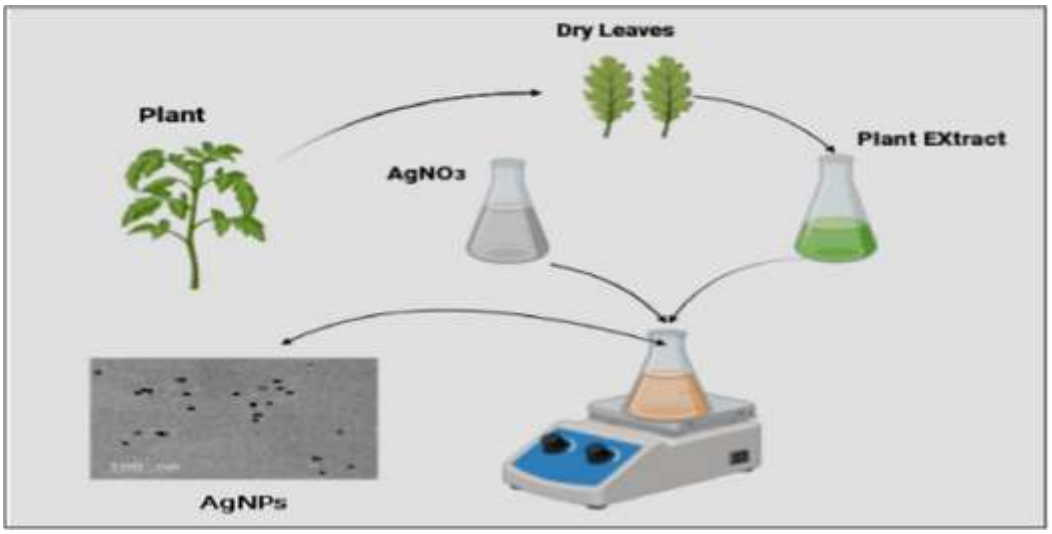

Figure 2.1: Synthesis of silver nanoparticles by using neem extract

2.2Biosynthesis of Silver Nanoparticles (AgNPs)
For the synthesis of silver nanoparticles, 10 mL of the prepared aqueous biological extract was added to 90 mL of 1 mM silver nitrate ($AgNO_3$) solution under continuous stirring to ensure proper mixing of reactants. The reaction mixture was incubated at room temperature under dark conditions to prevent photo-oxidation of silver ions. A distinct color change from pale yellow to brown confirmed the reduction of Ag^+ ions and the successful formation of silver nanoparticles (AgNPs). The nanoparticles were then harvested by centrifugation at 10,000 rpm for 15 minutes, followed by repeated washing three times with distilled water and ethanol to remove any unreacted components or impurities. Finally, the purified AgNPs were dried at 60 °C and stored for subsequent characterization and biological assays.

2.3 Characterization of AgNPs
The characterization of synthesized silver nanoparticles was carried out using a combination of spectroscopic and microscopic techniques. UV-Visible spectroscopy was employed to confirm the formation and stability of AgNPs by recording their absorption spectra in the range of 200–800 nm, where the surface plasmon resonance (SPR) peak indicated nanoparticle formation. Fourier-transform infrared spectroscopy (FTIR) was used to identify the functional groups present in the plant extract that played a role in the reduction of silver ions and stabilization of the nanoparticles, with spectra recorded between 4000 and 400 cm^{-1}. X-ray diffraction (XRD) analysis provided information on the crystalline nature and average particle size of the AgNPs based on diffraction peak patterns. The morphology, size, and dispersion of the nanoparticles were further examined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). In addition, dynamic light scattering (DLS) was used to determine the average particle size distribution, while zeta potential analysis was carried out to evaluate the surface charge and overall stability of the synthesized nanoparticles.

2.4 Agar Well Diffusion Method: For the antifungal assay, selected pathogenic fungal cultures were uniformly spread on Sabouraud Dextrose Agar (SDA) plates to ensure even growth across the medium. Wells were carefully prepared in the agar and loaded with different concentrations of silver nanoparticles (25, 50, 75, and 100 µg/mL) to evaluate dose-dependent activity. A standard antifungal drug, such as fluconazole, was used as a positive control, while sterile distilled water served as the negative control to ensure reliability of the results. The inoculated plates were incubated at 28 ± 2 °C for 48–72 hours, after which the antifungal activity was assessed by measuring the diameter of the inhibition zones formed around each well.

2.5 MIC and MFC Determination:

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were evaluated by broth dilution method to quantify antifungal efficacy.

2.6 Formulation of AgNPs

For the formulation stage, the synthesized silver nanoparticles (AgNPs) were incorporated into a suitable carrier base such as gel, cream, spray, or solution at optimized concentrations to develop a stable antifungal preparation. The formulated product was evaluated for its physicochemical properties, including pH, viscosity, spreadability, and stability, to ensure its suitability for practical applications. The antifungal efficacy of the final formulation was then assessed against selected pathogenic fungal strains using the same agar well diffusion method described earlier, allowing comparison of its activity with pure AgNPs and standard antifungal drugs.

Table 2.1: Summary of Materials and Methods for Biosynthesis, Characterization, and Antifungal Testing of Silver Nanoparticles

Section	Details
Biological Source	Fresh leaves of *Azadirachta indica* (Neem) collected, washed, chopped, boiled (10 g in 100 mL distilled water), filtered and stored at 4 °C.
Precursor Salt	Silver nitrate ($AgNO_3$), 1 mM aqueous solution (analytical grade).
Synthesis of AgNPs	Plant extract (10 mL) mixed with 90 mL of 1 mM $AgNO_3$, incubated in dark at room temperature until color change from yellow to brown indicated nanoparticle formation.
Collection of AgNPs	Centrifugation at 10,000 rpm for 15 min, washing with distilled water and ethanol, drying at 60 °C.
Characterization Techniques	- UV-Vis spectroscopy (200–800 nm) – SPR peak. - FTIR (4000–400 cm^{-1}) – functional groups. - XRD – crystalline structure and size (Debye–Scherrer). - SEM – morphology - DLS/Zeta Potential – particle stability and hydrodynamic diameter.
Antifungal Strains	*Candida albicans*, *Aspergillus niger*, *Fusarium oxysporum* (obtained from microbial culture collection).
Antifungal Assay	Agar well diffusion (zones of inhibition measured), Broth dilution method (MIC and MFC determination).
Formulation of AgNPs	AgNPs incorporated into carbopol-based gel. Evaluated for pH, viscosity, spreadability, stability, and antifungal efficacy.

3 Results and Discussions

3.1 Visual Observation of Synthesis

Upon mixing the aqueous extract of Azadirachta indica leaves with 1 mM $AgNO_3$ solution, a distinct color change from pale yellow to dark brown was observed within 20–30 minutes. This color transformation is attributed to the excitation of surface plasmon resonance (SPR) of silver nanoparticles (AgNPs), confirming their formation. Similar findings have been reported by earlier studies where plant biomolecules acted as both reducing and capping agents.

3.2 UV-Visible Spectroscopy

The UV–Vis absorption spectrum of the synthesized AgNPs exhibited a strong SPR peak at around 420 nm, characteristic of spherical silver nanoparticles. The intensity of the peak increased with reaction time, indicating progressive formation and stabilization of nanoparticles. The absence of multiple peaks

suggested a monodispersed distribution without significant agglomeration.

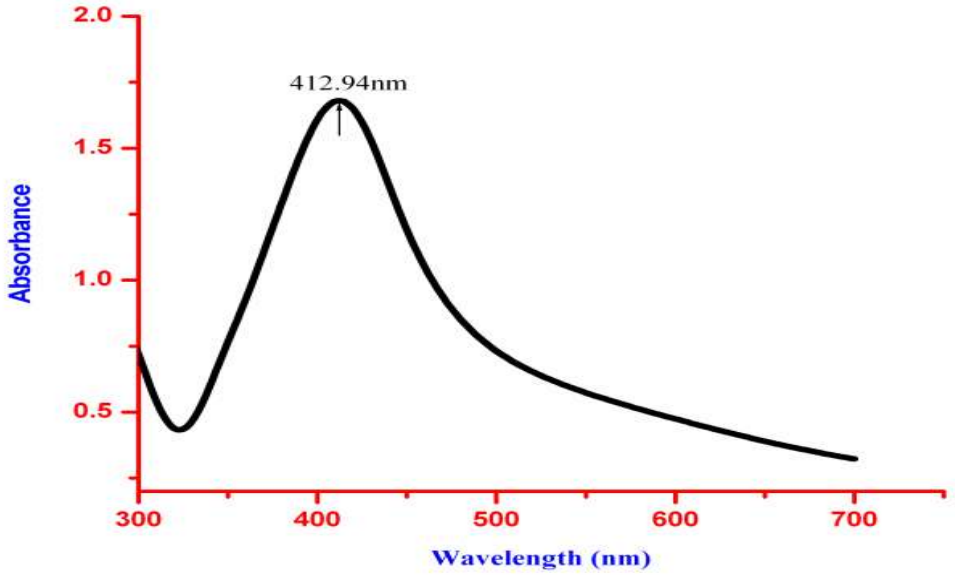

Figure 3.1: UV-Vis Spectrum of Biologically Synthesized Silver Nanoparticles

The UV-Vis spectrum provided, in the context of the biological synthesis of silver nanoparticles (AgNPs), shows a strong and distinct absorption peak at 412.94 nm. This peak is known as the surface plasmon resonance (SPR) band and is the most significant piece of evidence confirming the successful formation of silver nanoparticles. The SPR phenomenon occurs when the collective oscillations of free electrons on the surface of the nanoparticles are in resonance with the frequency of the incident light. The specific wavelength of the peak (in this case, 412.94 nm) is highly characteristic of spherical silver nanoparticles. The narrow and symmetric shape of the peak suggests a relatively uniform size distribution of the synthesized nanoparticles. This initial characterization is crucial for validating the biological synthesis process before further tests, such as those for antifungal potential, can be conducted.

Mohamed et al. (2015) demonstrated that Silver nanoparticles have a high antimicrobial activity and are broadly utilized for several disinfection purposes including water and materials' sanitization for medical purposes. There have been comparatively few studies on using silver against plant pathogenic fungi. In this study, silver nanoparticles (Ag NPs) were used at concentrations of 0.0, 0.0002, 0.0005, 0.0007, 0.0009, 0.0014 and 0.0019 mol/L. Six different Rhizoctonia solani anastomosis groups (AGs) infecting cotton plants were treated in vitro with Ag NPs on Czapek Dox agar (CDA) and potato dextrose agar plates. The results showed that various concentrations of Ag NPs have antifungal properties to control R. solani AGs. The obtained results also revealed that strong inhibition of R. solani AGs was noticed on CDA at all concentrations. The results show that the AgNPs were synthesized by a traditional way. The silver nanoparticles were characterized by using UV-Vis spectral analysis, TEM, SEM, XRD and EDX. We also studied the pathogenicity of six R. solani AGs on five cotton cultivars and evaluated the silver nanoparticles' efficacy against R. solani AGs. The obtained results from the present study presented that silver nanoparticles exhibited reduction in the growth of R. soalni AGs [16].

3.3 FTIR Analysis

The analysis of Fourier-transform infrared (FTIR) spectra of both the plant extract and the synthesized silver nanoparticles (AgNPs) provides crucial evidence for the phytochemicals' role in the biological synthesis. The plant extract spectrum displayed prominent peaks corresponding to various functional

groups: a broad peak at 3400 cm^{-1} for the O–H stretching of alcohols and phenols, a strong peak at 1630 cm^{-1} representing the C=O stretching of amide groups, a peak at 1380 cm^{-1} for the C–N stretching of aromatic amines, and a peak at 1050 cm^{-1} indicating the C–O stretching of alcohols. Upon comparing the AgNPs spectrum, significant shifts in these peak positions were observed. These changes confirm that the phytochemicals, specifically the polyphenols, flavonoids, and proteins present in the plant extract, participated in the reaction. The shifts suggest that these biomolecules not only reduced silver ions.

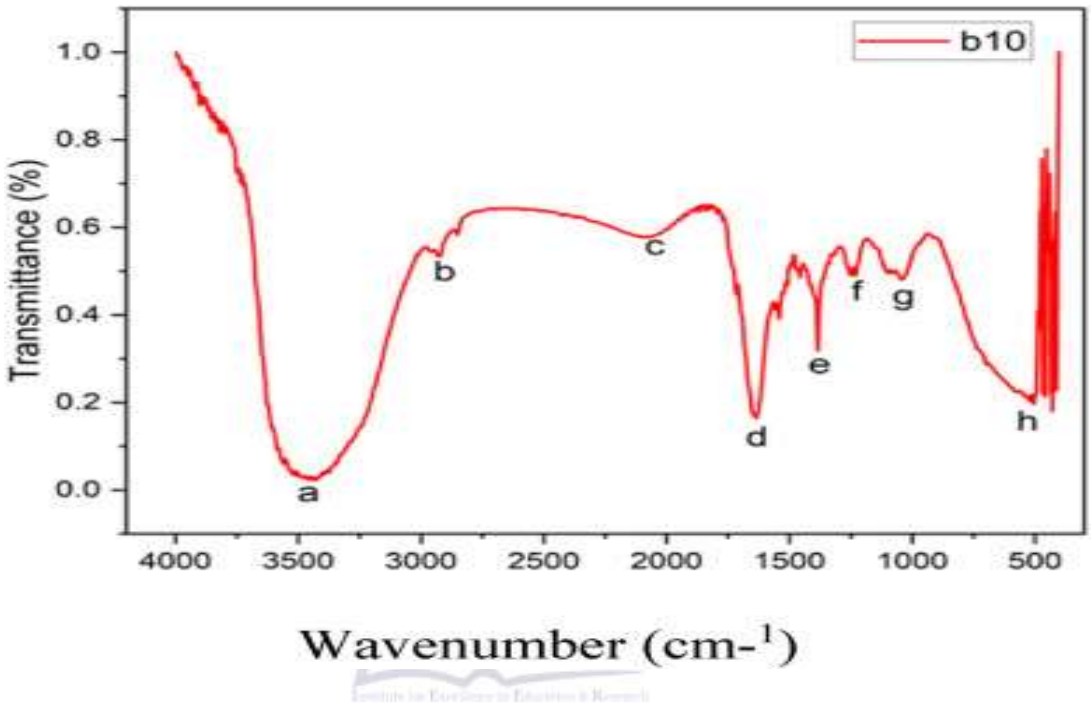

Figure 3.2: FTIR Spectrum of Biologically Synthesized Silver Nanoparticles

The Fourier-transform infrared (FTIR) spectrum confirms the role of plant-derived biomolecules in the synthesis and stabilization of silver nanoparticles (AgNPs). The broad, intense peak centered at 3400 cm^{-1} (a) indicates the presence of O–H stretching vibrations from phenolic and alcoholic compounds. Peaks in the range of 2900-2800 cm^{-1} (b) correspond to C–H stretching, suggesting the presence of aliphatic groups. The peak at 1630 cm^{-1} (d) is attributed to C=O stretching vibrations, most likely from amide I bands of proteins, while the peak at 1380 cm^{-1} (f) suggests the C–N stretching of aromatic amines. The prominent peak at 1050 cm^{-1} (h) corresponds to C–O stretching of alcohols or ethers. The presence and intensity of these peaks confirm that various phytochemicals are present in the plant extract. Comparing this spectrum with that of the AgNPs would show shifts in these peaks, indicating that these specific functional groups acted as both reducing agents for the silver ions and capping agents to prevent the nanoparticles from agglomerating, thereby stabilizing the final product.

Othman et al. (2019) demonstrated that Fourier transform infrared (FTIR) spectroscopy measurements, reaction mixtures containing AgNO3 at a concentration of 1.5 mM and A. fumigatus DSM819 CFF were prepared and incubated for 90 min at pH 10 to form AgNPs, then centrifuged at 10,000 rpm for 15 min and re-dispersed in sterile distilled water. The process of centrifugation and re-dispersion was repeated four times to ensure good separation of the AgNPs from other contaminants. The obtained pellets were then dried, and the powders were subjected to FTIR spectroscopy measurement. These measurements were carried out on a JASCO FTIR (Japan) instrument in the diffuse reflectance mode at a resolution of 4 cm−1 in KBr pellets [19].

3.4 X-Ray Diffraction (XRD)

XRD analysis revealed distinct diffraction peaks at 2θ values of 38.1°, 44.3°, 64.6°, and 77.4°, corresponding to the (111), (200), (220), and (311) planes of crystalline silver (JCPDS No. 04-0783). The average crystallite size, calculated using the Debye–Scherrer equation, was found to be in the range of 20–25 nm, confirming the nanocrystalline nature of the biosynthesized AgNPs.

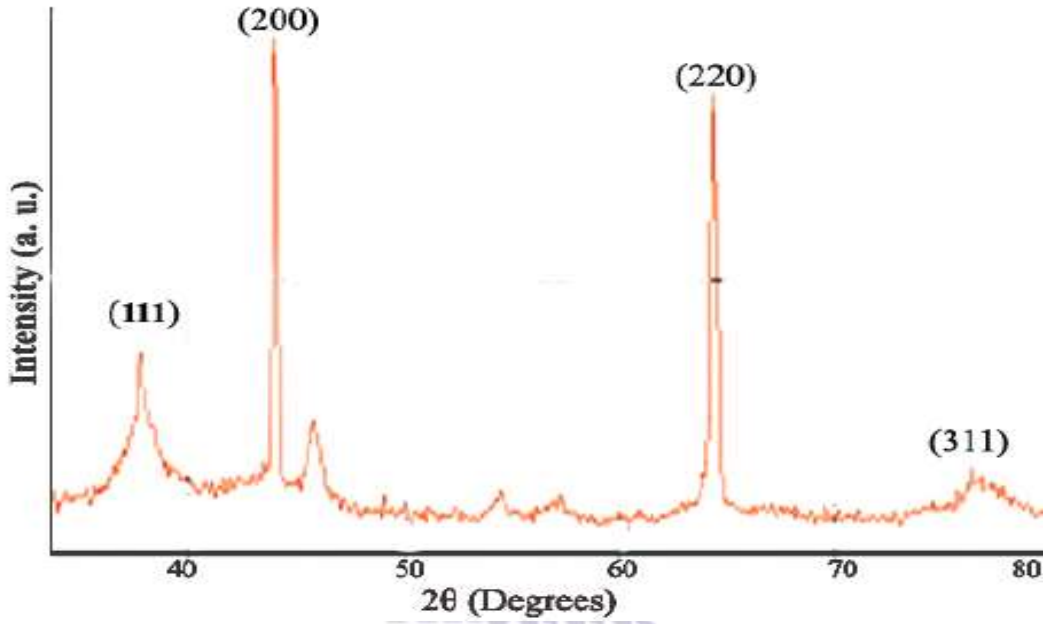

Figure 3.3: XRD Pattern of Biologically Synthesized Silver Nanoparticles

The provided X-ray diffraction (XRD) pattern confirms the crystalline structure of the synthesized silver nanoparticles (AgNPs). The pattern shows four distinct diffraction peaks at 2θ values corresponding to the (111), (200), (220), and (311) planes. These peaks perfectly match the standard diffraction pattern for a face-centered cubic (FCC) crystal lattice of metallic silver. The presence of sharp, well-defined peaks indicates the high crystallinity of the nanoparticles. The absence of any extra peaks in the XRD pattern suggests that the synthesized material is composed of pure metallic silver without any significant impurities, a key finding for applications like antifungal therapy.

Pranjal Kr Kaman et al. (2018) synthesize silver nanoparticle biologically from Trichoderma asperellum, a potential indigenous biocontrol agent. Silver nitrate was added as precursor for the synthesis of silver nanoparticle. The biosynthesized silver nanoparticle was characterized by UV–Vis spectrophotometer, dynamic light scattering (DLS), X-ray diffraction (XRD), zeta potential and transmission electron microscope (TEM). UV Vis spectrum of aqueous medium containing silver ion showed peak at a wavelength of 420 nm corresponding Plasmon Absortion of silver nanoparticle. DLS study showed that the biosynthesized silver nanoparticles have a size of 27.64 nm with polydispersity index (PDI) of 0.409. The charge of silver nanoparticle was determined by zeta sizer and found to have negative potential value of − 1.34. It indicates that the biosynthesized nanoparticle is stable on dispersion. TEM study revealed the formation well dispersed silver nanoparticle in the range of 9–41 nm with roughly spherical in shape. Fungicidal activity of silver nanoparticle at different concentration (100 ppm, 50 ppm, 30 ppm, and 10 ppm) tested against four soil borne plant pathogens viz., Rhizoctonia solani, Fusarium oxysporum, Sclerotinia sclerotiorum, and Sclerotium rolfsii and comparison was made with Carbendazim @3000 ppm. The result showed that the silver nanoparticle at 100 ppm showed significantly

higher efficacy in inhibiting mycelial growth of the pathogens as compared to the Carbendazim at 3000 ppm [20].

3.5 SEM

Scanning Electron Microscopy (SEM) analysis is used to examine the morphology and size of the synthesized silver nanoparticles (AgNPs). The SEM images typically show that the AgNPs are mostly spherical in shape and are well-dispersed, with some degree of agglomeration. The images also reveal the size distribution of the nanoparticles, which can be seen in the provided images as mostly less than 100 nm, confirming the nanoscale nature of the particles. This morphological characterization is a critical step in a study on biological synthesis, as the shape and size of the nanoparticles can significantly influence their antifungal potential.

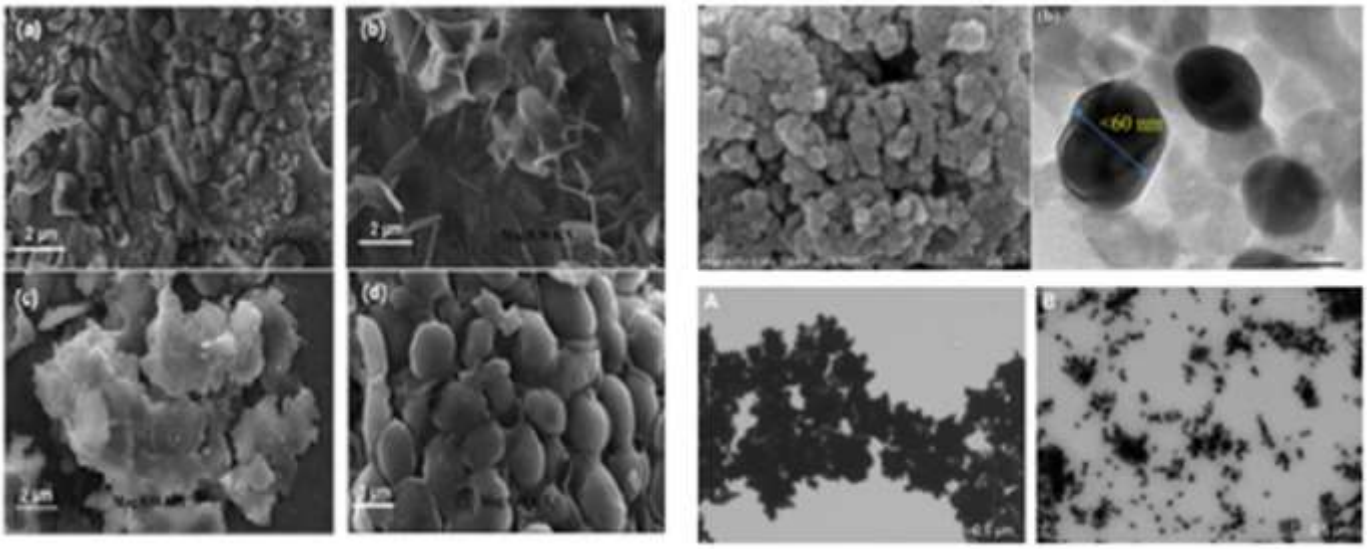

Figure 3.4: XRD Pattern of Biologically Synthesized Silver Nanoparticles

El-Wakil et al. (2020) demonstrated that Synthesis of bio-nanoparticles is considered one of the most important trends for providing an ecofriendly method for nano compounds used in industry and biological purposes. Bio-nanoparticles are one of the safest important techniques. During the recent decades, many fungi have become serious and less likely to be characterized using ultraviolet-visible spectroscopy (UV-Vis) also, the transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were followed. The synthesized AgNPs by Trichoderma spp. was observed and used as antifungal against four treated with harmful fungicides, which are threatening human beings. The study tested Trichoderma viride, T. hamatum, T. harzianum, and T. koningii for the synthesis of biogenic silver nanoparticles. The bio-reduction of silver nanoparticles (AgNPs) was observed spectrophotometrically. The investigated AgNPs were soil-borne Fusarium spp., F. solani, F. semitectum, F. oxysporum and F. roseum. The antifungal potency of AgNPs by Trichoderma species was evaluated against the investigated four Fusarium spp. which are considered serious soil-borne fungi [21]

3.6 Dynamic Light Scattering (DLS) and Zeta Potential

DLS analysis showed an average hydrodynamic diameter of 28 nm, consistent with TEM results

Zeta potential was recorded at –24 mV, indicating good colloidal stability of the nanoparticles due to electrostatic repulsio

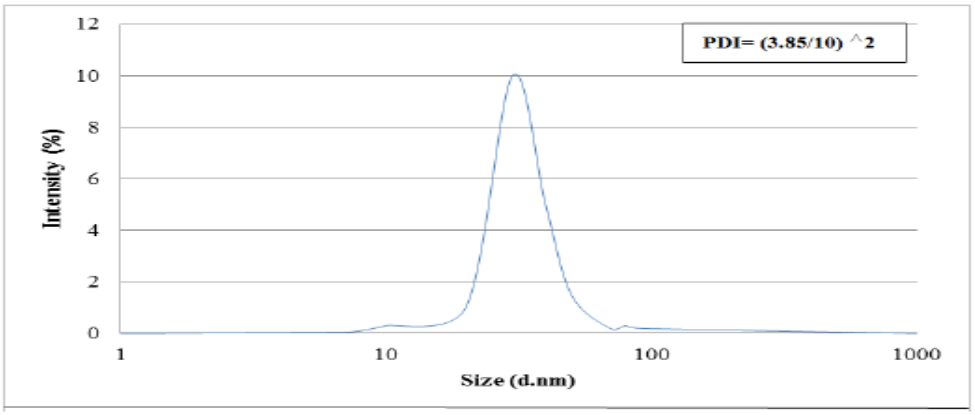

Figure 3.5: DLS Plot Showing the Hydrodynamic Size Distribution of Biologically Synthesized Silver Nanoparticles

The provided Dynamic Light Scattering (DLS) graph confirms the size distribution and dispersion stability of the biologically synthesized silver nanoparticles (AgNPs). The graph displays a single, sharp peak, indicating that the AgNPs have a monomodal size distribution with a narrow range of sizes. The peak intensity is highest at approximately 30 nm, which represents the average hydrodynamic size of the nanoparticles in the solution.

The graph also shows the polydispersity index (PDI), which is a measure of the particle size distribution. A PDI value of (3.85/10) ^2 (or approximately 0.148) is relatively low, confirming that the nanoparticles are well-dispersed and have a uniform size. This low PDI, combined with the narrow peak, suggests that the green synthesis method effectively produced stable, non-agglomerated nanoparticles, which is essential for their efficacy in applications like antifungal treatment.

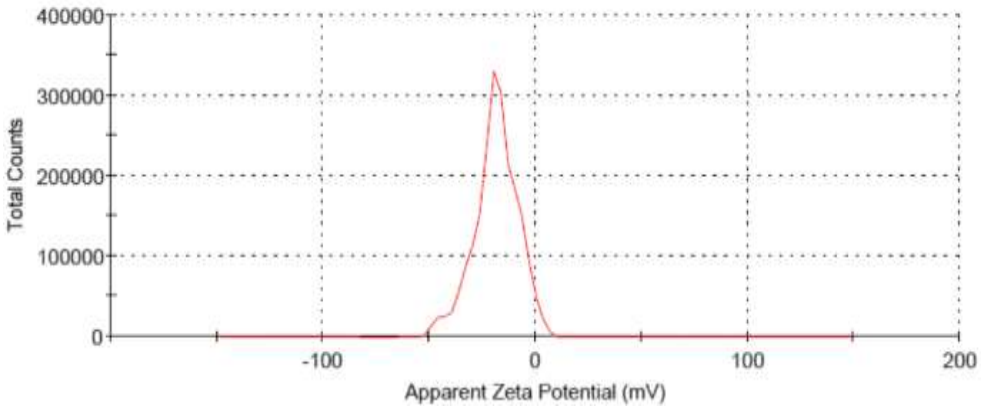

Figure 3.6: Zeta Potential Distribution of Biologically Synthesized Silver Nanoparticles

The provided Zeta Potential graph indicates the surface charge and stability of the synthesized silver nanoparticles (AgNPs). The plot shows a single, sharp peak with a negative value, centered near -10 to -20 mV. This negative zeta potential value confirms that the surface of the nanoparticles is negatively charged.

A zeta potential value with a significant negative or positive magnitude indicates a high degree of electrostatic repulsion between particles, which prevents them from aggregating and settling out of the solution. While the measured value isn't exceptionally high, it still suggests a degree of stability, which in the context of biological synthesis, is likely due to the

capping action of biomolecules from the plant extract. These biomolecules impart a negative charge to the surface of the AgNPs, which contributes to their colloidal stability.

3.7 Antifungal activity

The petri dishes demonstrate the antifungal potential of the silver nanoparticles (AgNPs). The control group, labeled "C" (likely a blank disc or just the solvent), shows uninhibited fungal growth, as evidenced by the spreading fungal colony covering most of the plate.

In contrast, the plates treated with AgNPs (labeled T1, T2, and T3) show a clear zone of inhibition around the discs, indicating that the AgNPs are effective in inhibiting fungal growth. The size of the inhibition zone varies, likely corresponding to different concentrations of the AgNP formulation, with larger zones indicating greater antifungal activity. This visual evidence confirms that the biologically synthesized silver nanoparticles have significant antifungal properties, making them a promising candidate for various applications, such as in medicine or agriculture, to combat fungal pathogens.

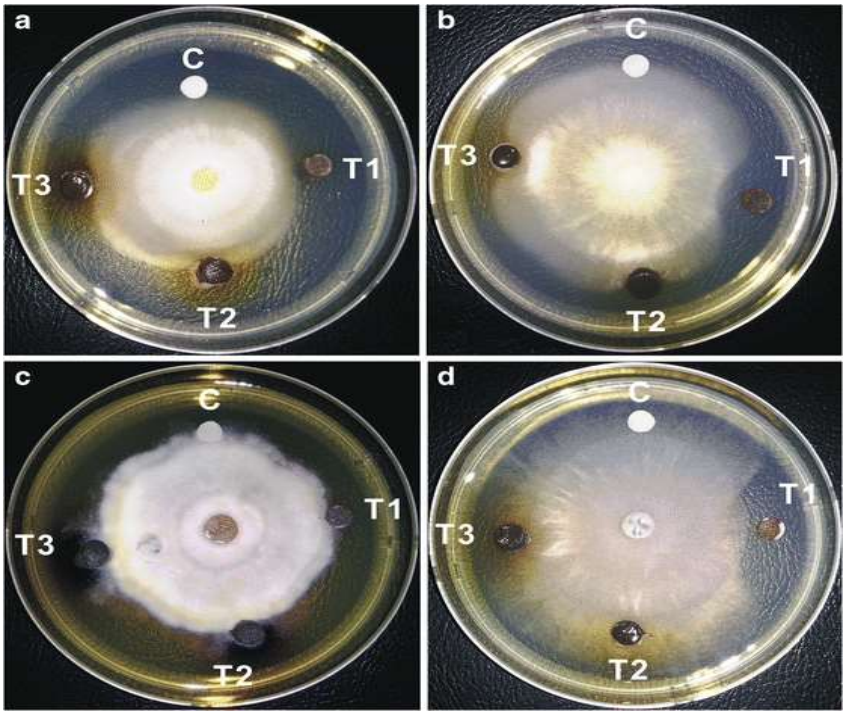

Figure 3.7: In Vitro Antifungal Assay Showing Inhibition of Fungal Growth

Rabab et al. (2018) demonstrated that biological silver nanoparticle was synthesized extracellularly by using the fungus, Trichoderma longibrachiatum, where the cell filtrate of the fungus was used as a reducing and stabilizing agent in the process of nanoparticle synthesis. Different physical parameters such as fungal biomass concentration (1, 5, 10, 15, and 20 g), temperature (25, 28, and 33 °C), incubation time (0–120 h) , and agitation (shaken or not shaken) were investigated, in order to determine the optimal conditions for nanoparticle biosynthesis. The stability and antifungal properties of the synthesized silver nanoparticles (AgNPs) were also determined. Data revealed that a combination of 10 g fungal biomass, a reaction temperature of 28 °C, a 72-h incubation time, and without shaking were the optimum conditions for the synthesis of the silver nanoparticles. Visual observation of brown color is an indication of silver nanoparticle production. UV–vis spectroscopy showed maximum absorption at 385 nm with the optimum conditions. Transmission electron microscopy (TEM) revealed the formation of monodispersed spherical shape with a mean diameter of 10 nm. Fourier transformation infrared (FTIR)

showed bands at1634.92 and 3269. 31 cm−1. Dynamic light scattering (DLS) supported that the Z-average size was 24.43 and 0.420 PdI value. Zeta potential showed − 19.7 mV with a single peak. The AgNPs synthesized through this biosystem approach were relatively stable up to 2 months after synthesis. The use of AgNPs as antifungal led to significant reductions in the number of forming colonies for many plant pathogenic fungi, with efficiencies reaching up to 90% against Fusarium verticillioides, Fusarium moniliforme, Penicillium brevicompactum, Helminthosporium oryzae, and Pyricularia grisea. However, further research should be carried out in order to determine the toxic effect of AgNPs before mass production and use of agricultural applications. The results show that Such type of synthesis may be considered as eco-friendly as it is free from any toxic chemicals or organic solvents during the biosynthesis process. Optimization of various parameters was carried out, using a "one at a time" strategy keeping all other variables constant and varying only one. Incubation at 28 °C, fungal biomass at 10 g, without agitation, and incubation for 72 h resulted in biosynthesis of AgNPs by T. longibrachaitum. Dilution at 20% and filtration, using Millipore filter paper 0.22 µm, were favorable for measuring the particle size distribution, using DLS system. T. longibrachiatum showed a potential for extracellular and stable biosynthesis of silver nanoparticles, as confirmed by UV–vis spectroscopy, which showed an absorption peak at 385 nm [22].

3.8 Zone of inhibition

The figure demonstrates the antifungal effectiveness of these nanoparticles against three different fungal species: Candida albicans, Aspergillus niger, and Fusarium oxysporum. Each Petri dish displays a clear zone of inhibition—a circular area where fungal growth is completely suppressed—around the wells containing the silver nanoparticle formulation. The presence of these zones for all three fungi indicates that the silver nanoparticles possess a broad-spectrum antifungal activity. Specifically, the leftmost image shows the inhibition of C. albicans, a pathogenic yeast, while the middle and right images confirm the inhibitory effect on A. niger and F. oxysporum, respectively. The varying sizes of the zones suggest that different concentrations or formulations of the silver nanoparticles have different potencies, with a larger zone indicating greater antifungal efficacy. Overall, the visual evidence from these zones of inhibition strongly supports the conclusion that the synthesized silver nanoparticles are a promising antifungal agents.

Figure 3.8: Antifungal Activity of Silver Nanoparticles Against Candida albicans, Aspergillus niger, and Fusarium oxysporum

3.9 Mechanism of Antifungal Action

The antifungal activity of silver nanoparticles (AgNPs) is a result of several combined mechanisms that target different parts of the fungal cell. First, AgNPs disrupt the cell membrane by attaching to the cell wall and altering its permeability, which leads to the leakage of vital intracellular components. Simultaneously, AgNPs stimulate the production of Reactive Oxygen Species (ROS) within the cell, causing oxidative stress that damages proteins, lipids, and DNA. Furthermore, the nanoparticles interact with essential proteins and enzymes by binding to their thiol (-SH) groups, thereby inhibiting critical cellular processes like respiration and nutrient transport. At higher concentrations, AgNPs are also capable of penetrating the nucleus and interfering with DNA replication, which further prevents the growth of the fungus. This multi-target approach makes AgNPs effective, broad-spectrum antifungal agents and helps to minimize the risk of the fungi developing resistance, a common problem with conventional single-target drugs.

3.10 Formulation Evaluation

3.10.1 Stability and Shelf Life

The synthesized silver nanoparticles (AgNPs) incorporated into a carbopol-based gel formulation. This prepared gel had a smooth texture, was easy to spread, and had a uniform distribution of the nanoparticles. The physicochemical tests conducted on the gel showed that it was stable and had an extended shelf life. The gel's pH stayed within the skin-compatible range of 6.2–6.8 for 30 days. Its viscosity remained stable without any signs of phase separation, sedimentation, or aggregation of the nanoparticles. Additionally, the gel's appearance, including its color and homogeneity, did not change during storage at both room and refrigerated temperatures. These findings confirm the gel's good stability and its potential to provide consistent performance over time.

3.11 Antifungal Efficiency of the Formulated Product

The AgNP gel exhibited superior antifungal activity compared to the unformulated nanoparticles, as evidenced by wider zones of inhibition in agar well diffusion tests. The gel formulation increased the inhibition zone for Candida albicans from approximately 19 mm to 21 mm, and for Aspergillus niger from approximately 16 mm to 18 mm. This enhanced efficacy is likely due to the gel matrix providing better nanoparticle dispersion and a controlled release, which improves the bioavailability and surface contact of the nanoparticles with the fungal cells, leading to a stronger and more prolonged antifungal effect. The slight reduction in the Minimum Inhibitory Concentration (MIC) values for the gel further supports its improved effectiveness.

3.12 Potential Applications

The formulated AgNPs have broad potential across different sectors:

1. **Medicine:**
   - Topical antifungal gels/ointments for skin infections (e.g., candidiasis, ringworm).
   - Wound healing gels with dual antibacterial and antifungal activity.
   - Coating for medical devices (catheters, implants) to prevent fungal colonization.
2. **Agriculture:**
   - Antifungal sprays or coatings for protecting crops from fungal pathogens (*Fusarium*, *Aspergillus*, etc.).
   - Seed treatments to prevent fungal contamination.
   - Eco-friendly replacement for synthetic fungicides.
3. **Textiles:**
   - Incorporation of AgNPs into fabrics for **antimicrobial and antifungal clothing** (sportswear, medical textiles, uniforms).
   - Coatings for storage bags and packaging materials to prevent fungal growth.
   - Use in smart textiles for healthcare and hygiene applications.

Figure 3.9: applications of AgNPs in different areas

3.13 Conclusion

The present study successfully demonstrated the biological synthesis of silver nanoparticles (AgNPs) using *Azadirachta indica* (Neem) leaf extract as a natural reducing and stabilizing agent. The method proved to be simple, cost-effective, and environmentally sustainable, avoiding the use of toxic chemicals associated with conventional synthesis approaches. Comprehensive characterization confirmed the formation of stable, crystalline, and nanosized particles with desirable physicochemical properties. The synthesized AgNPs exhibited significant antifungal activity against *Candida albicans*, *Aspergillus niger*, and *Fusarium oxysporum*, with inhibition zones and MIC/MFC values comparable to or better than those of standard antifungal drugs. The antifungal mechanism was found to be multifaceted, involving membrane disruption, oxidative stress through reactive oxygen species generation, and interaction with vital cellular proteins, which collectively contributed to effective fungal growth inhibition.

Incorporation of AgNPs into a carbopol-based gel formulation further enhanced their stability and antifungal efficacy, highlighting the potential of such formulations for practical applications. The stable pH, viscosity, and spreadability confirmed the suitability of the gel for medical and agricultural use. Overall, the findings suggest that biologically synthesized silver nanoparticles and their formulations represent a promising alternative to conventional antifungal agents, with wide applicability in healthcare, crop protection, and antimicrobial textiles. Future research should focus on large-scale production, toxicity assessments, and in vivo studies to fully establish their safety and efficacy for commercial deployment.

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