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Eco-friendly fungal nanoparticles derived from *Penicillium notatum* and their antifungal activity against environmentally hazardous fungi

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ABSTRACT

In this research, the focus was on finding methods to eliminate dangerous fungi that pose a threat to human health and the environment. The study utilized the fungus *Penicillium notatum* to biosynthesize silver nanoparticles. The silver nanoparticles were characterized using a variety of methods, including scanning electron microscopy and ultraviolet-visible spectroscopy, which shed light on their optical and morphological properties as well as their crystallinity, to assess the effectiveness of the biosynthesis process. Researchers used nanomaterials added to SDA media to evaluate the bioactivity of the generated nanoparticles against two environmentally hazardous fungi, *Aspergillus ochraceus* and *Cladosporium* cladosporioides. The biogenic silver nanoparticles, which ranged in size from 20 to 22 nanometers, exhibited polydispersity. Especially, nanoparticles exhibited significant antifungal action, effectively inhibiting the growth of Aspergillus ochraceus and *Cladosporium cladosporioides.* The research presents an innovative, straightforward, and environmentally friendly approach to producing biofunctionally valuable biogenic nanoparticles. Using *Penicillium notatum* to synthesize silver nanoparticles allows for a natural way to produce these particles, which can be used in many ways to fight harmful fungi and other related issues. This eco-friendly method holds promise for addressing health concerns associated with dangerous fungi, contributing to the development of sustainable and effective antifungal strategies. By harnessing the natural processes of fungi, this study offers a practical means of producing biogenic nanoparticles, paving the way for more developments in the area of nanotechnology and its uses in environmental remediation, agriculture, and healthcare.

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Introduction

The Greek word "nano," which signifies "midget" in English, is the origin of the English term "nano." Based on this, the nanometer (nm) fairly represents a fraction of 10^{-9} meters. To put this into perspective, the cold virus is roughly 100 nm in size, and one nanometer equals approximately the length of three aligned carbon atoms. The typical diameter of human hair is around 10,000 nanometers (Hulkoti & Taranath, 2014).

The use of these materials defines the branch of research known as nanotechnology, which is applied when at least one dimension of a material is less than

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100 nm (Madkour, 2019). Due to the unique properties that nanoparticles (NPs), or nanoscale particles, possess, nanotechnology is considered one of the most significant technologies across multiple sectors. Metal nanoparticles with varied shapes and sizes exhibit distinct properties from the minerals from which they are derived (Rao & Gan, 2015). In recent years, interest in the production of nanomaterials has grown. Nanoparticles are used in diverse fields, including biological, medical, environmental, agricultural, and industrial sectors (Singh et al., 2016).

Microorganism metabolites such as viruses, bacteria, fungi (including yeast), algae, or plant extracts—are used in the biosynthesis of nanoparticles. This approach offers the advantage of being eco-friendly, requiring no energy, and being both cost-effective and fast. According to Ribeiro et al. (2020), silver nitrate ($AgNO_3$) has gained significant attention from researchers due to its excellent properties, including high conductivity, chemical stability, and notable biological activity. Most importantly, $AgNO_3$ exhibits antifungal, antibacterial, and antiviral properties without causing toxicity, making it valuable for both medical and industrial applications (Abdel-Azeem et al 2020a, Bruna et al., 2021; Srivastava et al. 2021, Crisan et al., 2021; Gezaf et al. 2022, Mossa et al. 2024). Furthermore, silver nitrate nanostructures have shown selective targeting of cancer cells as well as prokaryotic cells, exhibiting antibacterial activity against both Gram-negative and Gram-positive bacteria, in addition to antifungal properties (Gibała et al., 2021).

Penicillium is a diverse genus with worldwide distribution. Its species play essential roles as decomposers of organic material and are responsible for destructive rots in the food industry due to the production of a wide range of mycotoxins (Visagie et al., 2014). Their primary ecological role is the breakdown of organic compounds. However, as pathogens of food crops before and after harvest, these fungi produce severe rots (Zhao et al., 2022). According to Kadhim Al-Ziadi et al. (2021), food contamination with ochratoxin A (OTA), a toxin produced by fungi, poses a serious threat to food safety and animal health through its transmission along the food chain to humans.

The toxic effects of OTA on male reproductive efficiency have been documented, with evidence of expanded seminal linings, suppressed spermatogenesis, and degeneration of Leydig cells in testicular tissue. In addition, the lumen of the epididymal tubules was found devoid of sperm, with epithelial damage and edema in the epididymal tubules (Al-Husseini et al., 2019).

Aspergillus was isolated from the pulmonary system, where its virulence factors were found to be more significant than those of other taxa isolated from the eyes, indicating a considerable risk to patients (Hashim et al., 2024).

The genus *Cladosporium* is one of the known agents of human chromoblastomycosis (Torres-Guerrero et al., 2018). Moreover, Cladosporium cladosporioides is a pathogen that causes superficial skin diseases in humans (Wang et al., 2022).

A common fungus found in various environments is *Penicillium notatum*. These species are present in air, food, soil, and rain (Hashim et al., 2024). They play an essential role in breaking down organic matter and contribute to the natural biomass cycle (Oliveira et al., 2023).

The objective of this study is to produce silver nanoparticles (SNPs) using cell-free filtrates (CFFs) of *Penicillium notatum* cultures and silver nitrate ($AgNO_3$), and to confirm their biosynthesis through scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), and ultraviolet-visible (UV-Vis) spectroscopy. Additionally, the study aims to evaluate the bioactivity of the synthesized nanoparticles against two hazardous pathogens.

Materials and Methods

Isolation and identification of the fungi used in this study

Penicillium notatum was cultivated in a liquid medium of potato dextrose broth (PDB) at 28°C for 14 to 21 days, following the protocol outlined by De Man et al. (1960). The source of this fungal isolate was the laboratory of the Environmental Research and Pollution Prevention Unit at the Faculty of Science, University of Al-Qadisiyah. The identity of the isolate was confirmed by using the relevant taxonomic keys, specifically those provided by Jawetz et al. (1997) and Pitt (1980).

The research was conducted between November 2020 and January 2021 at the University of Al-Qadisiyah. Fungi were collected from the indoor air of student clubs and classrooms within the College of Science. The methodology involved exposing dishes containing potato dextrose agar (PDA), supplemented with 250 mg/L of chloramphenicol, to indoor air for 5 minutes at a height of 1.5 meters above ground level. Subsequently, the Petri dishes were incubated at 25°C for one week. Fungi were identified based on their phenotypic criteria macroscopic using the relevant keys such as Jawetz et al. (1997), Guarro et al. (2012), and Abdel-Azeem et al. (2020b).

Mycogenic of silver nanoparticles

Extracellular silver nanoparticles were synthesized by mixing 50 ml of cell-free filtrate (CFF) obtained from a 14-day culture of *Penicillium notatum* (filtered using a 0.2 µm pore diameter membrane filter) with 50 ml of an aqueous solution containing 1 mM silver nitrate ($AgNO_3$). The mixture was agitated on an orbital shaker for 10 minutes at 70°C under dark conditions. A control flask containing only the cell-free supernatant (CFF) without $AgNO_3$ was included alongside the experimental flasks (Al-Ziadi et al., 2015).

Visual and spectrophotometric analysis of silver nanoparticles

The color change of the reaction mixture was visually observed after the addition of $AgNO_3$ and upon completion of the reaction. The transition from colorless to brown indicated the formation of silver nanoparticles, a phenomenon attributed to the activation of surface plasmon vibrations (Singh & Raja, 2011). Optical Density (OD) measurements of the AgNPs solution were performed using a Carl-Zeiss Jena Spectrophotometer after incubation under dark conditions for 24 hours. Deionized water was used as a blank, and absorbance was measured at a wavelength of 320 nm.

Scanning electron microscopy (SEM) analysis

Scanning Electron Microscopy (SEM) was used to characterize the morphology and size of the silver nanoparticles. The analysis was conducted at the Electron Microscopy Unit, College of Science, University of Kufa. AgNPs were ground or sonicated to create a colloidal suspension, and a drop of this suspension was applied to the specimen holder. The samples were then dried and examined under SEM following the protocol described by Caroling et al. (2013). Imaging was performed under low vacuum conditions with an accelerating voltage of 12.5–15 kV, a spot size of 5, working distances of 5–10 mm, and various magnification levels.

Energy dispersive spectroscopy (EDS) analysis

Energy Dispersive Spectroscopy (EDS) was performed at the College of Science, University of Kufa, to analyze the elemental composition of the silver nanoparticles. Both point and mapping compositional analyses were conducted under low vacuum conditions using an accelerating voltage of 12.5–15 kV, a spot size of 5, and working distances of 5–10 mm, following the method of Caroling et al. (2013).

Antifungal activity of silver nanoparticles

The biosynthesized silver nanoparticles were evaluated for antifungal activity against the most common air isolates of *Aspergillus ochraceus* and *Cladosporium cladosporioides* using the food poisoning technique described by Dixit et al. (1976). Three milliliters of biosynthesized AgNPs at a concentration of 50 mg/ml were added to Petri dishes containing Sabouraud Dextrose Agar (SDA) along with the test fungi. SDA plates without AgNPs were used as controls. All plates were incubated at 25°C for one week, after which the diameter of fungal colonies was measured to assess growth inhibition.

Results and Discussion

Synthesis of AgNPs by Penicillium notatum indicated visually

A noticeable change in color signaled the creation of AgNPs, or *Penicillium notatum*-mediated silver nanoparticles. Molds are considered more suitable candidates for widespread green nanoparticle production because this production is typically extracellular, a desirable strategy due to its simple final processing and high yield. *Penicillium notatum* cell-free filtrate was incubated in a water bath for 10 minutes at 70°C after treatment with 1 mM $AgNO_3$. A color change was observed in the cell filtrate of the fungus under investigation. The solution changed from colorless to a dark brown tone during the formation of AgNPs (Fig. 1), which is characteristic of silver nanoparticles. According to researchers, the macroscopic evidence for the creation of silver nanoparticles is a visible change in color (Barabadi & Honary, 2016; Danagoudar et al., 2021).

According to our research, proteins and extracellular enzymes in *Penicillium notatum* metabolites are capable of producing AgNPs. These enzymes reduce silver ions, leading to the brown color of the solution through the electron transport process. The role of bioproduction of silver nanoparticles has been confirmed by many studies, which demonstrate that the process occurs via nitrate reduction. It has been shown that nitrate reductase activity may play a role in the biogenic production of silver nanoparticles by *Aspergillus flavus* (Zomorodian et al., 2016). Similarly, the ability of *Fusarium oxysporum* to produce the enzyme NADH-dependent nitrate reductase, which reduces hydrated silver ions to AgNPs, has been linked to its high potential for silver nanoparticle synthesis.

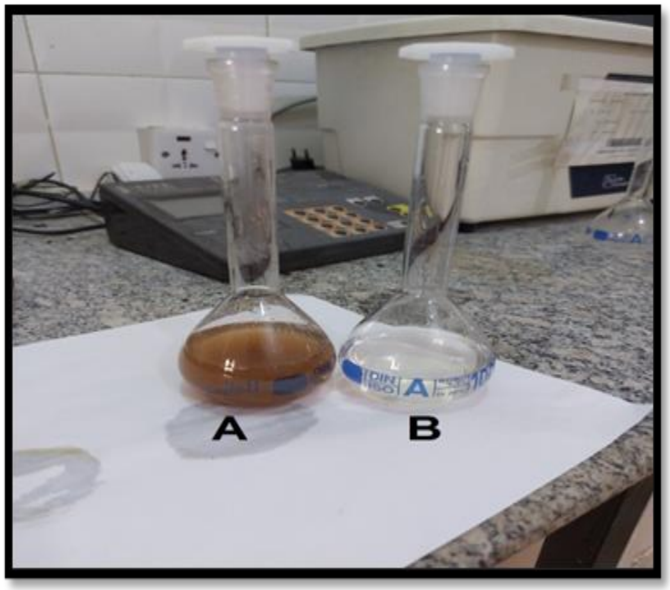

Fig 1. Figure X. Visual observation of color change in the reaction mixture containing *Penicillium notatum* filtrate and 1 mM silver nitrate in a 1:1 ratio. (A) Control (filtrate without $AgNO_3$), (B) Reaction mixture showing dark brown color indicating the formation of silver nanoparticles (AgNPs).

The surface plasmon resonance (SPR) optical phenomenon, which can be described as the oscillation of free electrons in AgNPs with a specific energy that absorbs and scatters a particular wavelength of visible light, is responsible for the observed color change (Ahmad et al., 2003). The appearance of a brown color can be considered the initial indication of silver nanoparticle formation, as the control extract without silver nitrate did not exhibit any color change.

Among the isolates obtained, *Aspergillus ochraceus* and *Cladosporium cladosporioides* exhibited the highest occurrence rates. These specific fungi were selected for further investigation due to their potential risk to human health, attributed to their production of mycotoxins, as documented by Alwatban et al. (2014) and Al Hallak et al. (2023).

UV-visible spectrophotometric analysis

The UV-Visible absorption spectrum was used as the first characterization method to confirm the production of AgNPs. The absorbance was measured at 320 nm, with the maximum peak observed at 320 nm (Fig. 2), which is consistent with the surface plasmon resonance (SPR) phenomenon, resulting from the collective electron oscillation in the conduction band (Ashraf et al., 2020).

The appearance of a peak at 320 nm indicated that active substances from the *Penicillium notatum* cell-free filtrate were released, mediating the reduction and encapsulation of silver nanoparticles. The enzymatic activity within the aqueous solution led to the bioreduction of Ag^{+} to Ag^{0} ions, resulting in a colloidal suspension and the characteristic brown color.

The mycogenic production of AgNPs by Alternaria sp. was previously observed using UV-Visible spectroscopy, with absorption peaks appearing in the 350–500 nm range (Theint et al., 2020). A UV-Visible spectrophotometer is a reliable method to monitor the reduction of silver nitrate to AgNPs. Due to the SPR phenomenon, AgNPs can absorb visible light, with this absorption influenced by their size and shape (Rahimi et al., 2019).

Microorganism-mediated nanoparticle synthesis is considered a safe, non-toxic, and environmentally friendly approach (Banu & Balasubramanian 2014). Fungi are a promising option for the bioproduction of AgNPs due to their high metal tolerance, ease of handling, and ability to produce abundant extracellular proteins that stabilize nanoparticles (Netala et al., 2016).

Scanning Electron Microscopy (SEM) Analysis

SEM micrographs revealed that the size of AgNPs produced from the crude extracts of *Penicillium* notatum ranged between 50 and 200 nm, with an average particle size of 20–22 nm (Fig. 3). The frequency distribution showed that most biosynthesized AgNPs had diameters smaller than 25 nm.

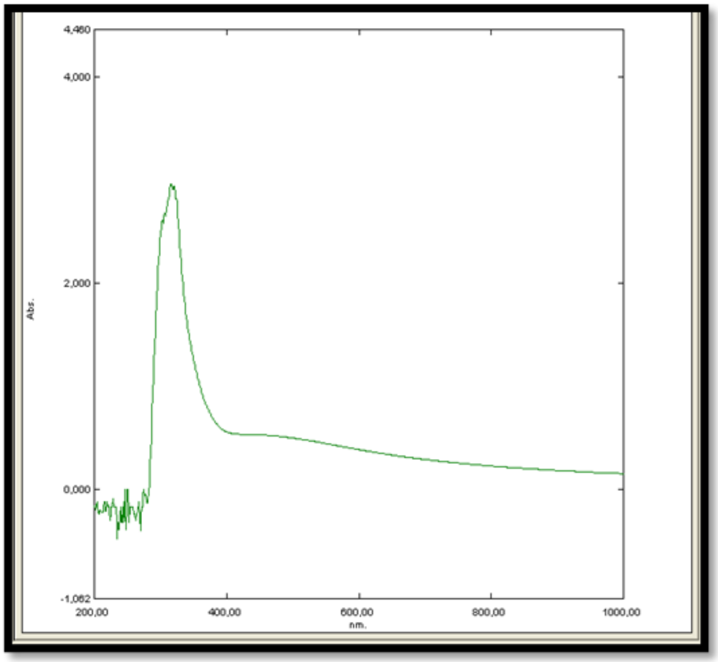

Fig 2. UV-Visible spectrophotometric study of silver nanoparticles produced via biosynthesis using a 1:1 ratio of *Penicillium notatum* filtrate and 1 mM silver nitrate ($AgNO_3$).

SEM was employed to examine the dimensions and morphology of biogenic AgNPs, revealing that the AgNPs produced by Penicillium notatum were well-dispersed and spherical. These findings align with those of Dada et al. (2019), who reported that the composition of the reaction environment significantly affects the physical properties of nanoparticles (Luo et al., 2018).

Further studies on Penicillium oxalicum demonstrated that the presence of iron (Fe) enhances the expression of genes responsible for AgNP production (Theint et al., 2020).

Energy dispersive spectroscopy (EDS) analysis

AgNPs were quantitatively analyzed using Energy Dispersive X-ray Spectroscopy (EDS), which identifies optical absorption peaks associated with silver. EDS spectrum analysis confirmed the presence of elemental silver, indicating the successful reduction of silver ions to silver metal.

The strongest signal detected corresponded to silver, followed by oxygen and other elements contributing weaker signals (Fig. 4). The elemental composition of AgNPs synthesized by Penicillium notatum was 15.68% silver and 23.79% oxygen. The optical absorption peak specific to silver nanoparticles appeared at 3 keV.

The presence of oxygen peaks, though weak, suggests that the AgNPs are capped by biomolecules through oxygen interactions. Additional minor peaks were likely caused by biomolecules in the cell-free filtrate (Bonnia et al., 2016).

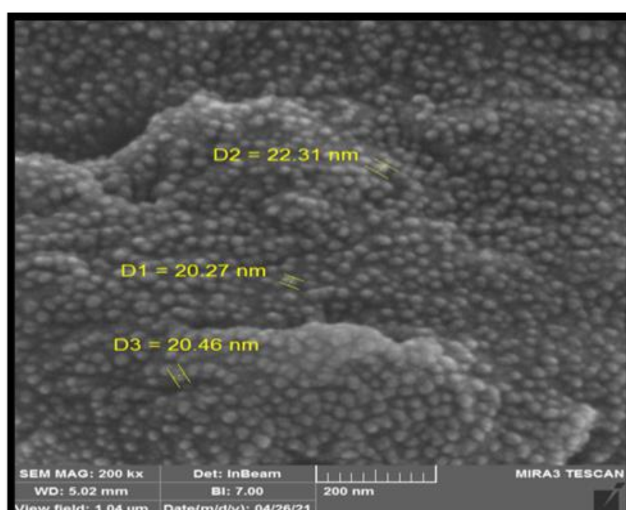

Fig 3. Silver nanoparticles produced using Penicillium notatum filtrate, as observed under SEM.

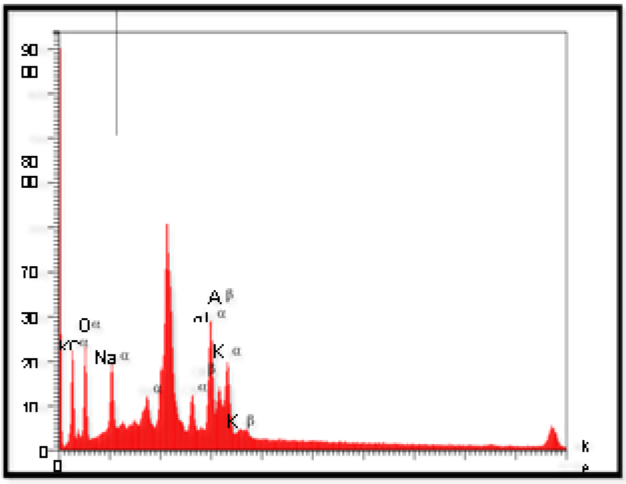

Fig 4. EDS spectrum analysis of mycogenic silver nanoparticles produced by Penicillium notatum, showing elemental composition and confirming the formation of AgNPs.

Antifungal activity of silver nanoparticles

Silver nanoparticles synthesized from the crude extract of Penicillium notatum inhibited the growth of all tested fungal species (Fig. 5). The inhibition zone produced by AgNPs against *Aspergillus ochraceus* and *Cladosporium cladosporioides* measured 20.56 mm and 20 mm, respectively (Figs. 6 and 7).

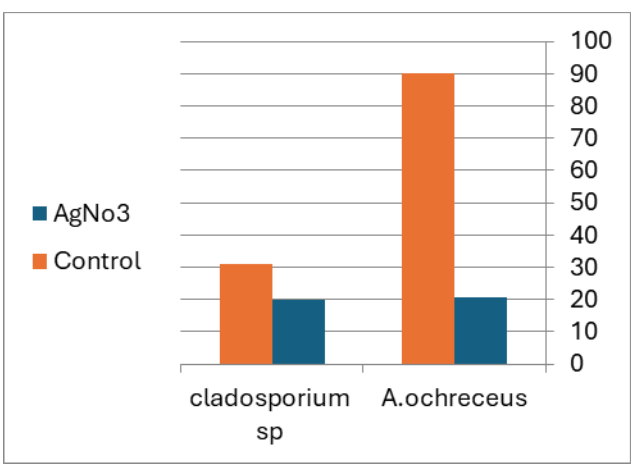

Fig 5. Biosynthesized AgNPs using Penicillium notatum filtrate showing inhibition of pathogenic fungal growth. C1: Treatment with AgNPs; C2: Control (without AgNP addition).

AgNPs are widely used in energy generation, optical devices, electronics, tissue engineering, catalysis, and antimicrobial applications (Jaidev et al., 2010). For these uses, it is essential to control particle composition, size, and shape. Environmentally friendly methods for producing metallic nanoparticles are increasingly sought after.

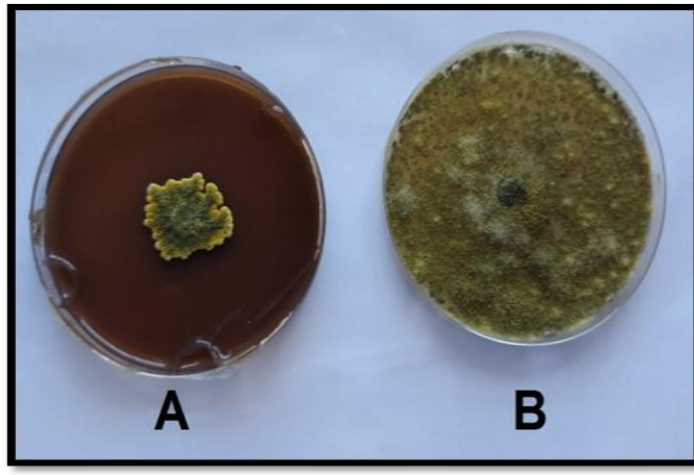

Fig 6. Inhibition of Aspergillus ochraceus growth by biosynthesized AgNPs produced using Penicillium notatum filtrate, where (A) shows growth after treatment and (B) is the untreated control.

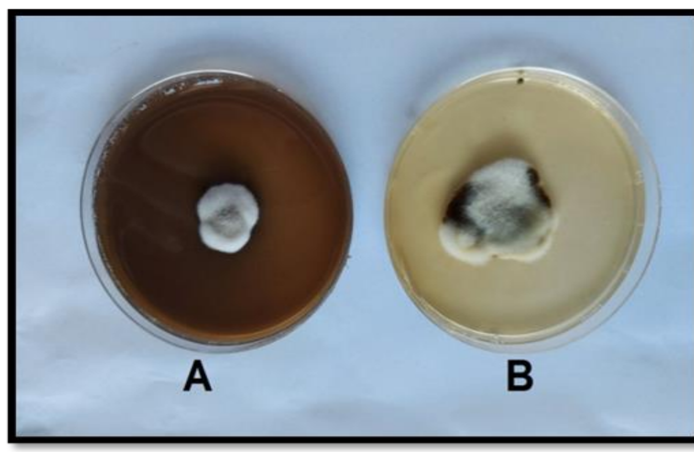

Fig 7. Inhibition of Cladosporium cladosporioides growth by biosynthesized AgNPs produced using Penicillium notatum filtrate, where (A) shows growth after treatment and (B) is the untreated control.

Biological systems, particularly fungi, offer promising alternatives for metal nanoparticle synthesis, as they can reduce metal ions to insoluble metal forms, decreasing toxicity and bioavailability. Fungi have demonstrated a unique ability to biosynthesize metal nanoparticles, aided by their rich biodiversity and production of redox-active biomolecules (Sastry et al., 2003; Kitching et al., 2015; Abed Ali et al., 2023).

Researchers are increasingly exploring the use of AgNPs to inhibit the growth of pathogenic fungi associated with mycoses. In this study, biosynthesized AgNPs induced significant morphological alterations in the hyphae of the tested fungi. The dark brown color associated with AgNP presence diminished, likely due to nanoparticle adhesion to or internalization within fungal hyphae.

These results align with those of Kotzybik et al. (2016), who demonstrated that AgNPs adhered to the cell surfaces of Penicillium verrucosum and penetrated the hyphae, forming aggregates within the cytoplasm. The characteristic color change persisted unless fungal growth was entirely suppressed.

Morphological abnormalities, such as dichotomous branching and altered hyphal structures, were observed, consistent with previous reports linking such phenotypes to disrupted polarity establishment in filamentous fungi (Harris & Momany, 2004; AL-Ziadi et al., 2024).

Fungi remain attractive candidates for the biosynthesis of AgNPs due to their high protein output, ease of handling, environmental safety, and ability to produce stable nanoparticles (Gezaf et al., 2022).

Conclusion

In conclusion, silver nanoparticles were successfully produced using crude extracts of *Penicillium notatum*, which demonstrated effective inhibition of *Aspergillus ochraceus* and *Cladosporium cladosporioides*. To the best of our knowledge, this research presents a simple, innovative, and environmentally friendly approach for the biosynthesis of functionally valuable biogenic nanoparticles.

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Conflict of interest

The authors of this paper do not have any competing interests.

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