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Original Article

Silver Nanoparticles in Antifungal Applications: A Comparison of Biosynthesized and Physical Synthesized Nanoparticles on Paper-Biodegrading Fungi

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Abstract

Background: Paper substrates are highly susceptible to fungal degradation, as fungi produce enzymes that decompose paper materials. This study investigates and compares the antifungal efficacy of silver nanoparticles (AgNPs) synthesized through various physical and biological methods. The fungal strains tested, *Penicillium* sp. and *Aspergillus* sp., were isolated from paper samples in a previous study, chosen for their more prevalence in contaminated paper.

Methods: Five types of AgNPs, synthesized using both biological and physical methods, were evaluated. The biological methods involved the use of extracts from *Juglans regia* green husk, *Malva sylvestris* leaves, and cyanobacterial cells. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the AgNPs were determined using standard protocols.

Results: The MIC values of the AgNPs synthesized from different sources exhibited varying degrees of antifungal activity against the tested fungi. The AgNPs' effectiveness ranked as follows: (1) AgNPs produced by *Nostoc pruniforme*, (2) AgNPs produced by *M. sylvestris*, (3) AgNPs produced by *Nostoc* IBRC-M5064, (4) AgNPs produced by *J. regia*, and (5) physically synthesized AgNPs.

Conclusion: These findings highlight the potential of biologically synthesized AgNPs as environmentally friendly biocidal agents for preventing and controlling paper biodegradation.

Keywords: Paper, Fungi, Nanoparticles, Biocides, Biodegradation

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Introduction

Paper substrates are among the most important materials susceptible to various forms of biodeterioration, which can result in irreversible damage to manuscripts and artworks.[1] Microorganisms present in the environments of libraries and archives not only cause deterioration of materials, but can also negatively affect the health of staff members.[2,3] Among microorganisms, fungi play a major role in the deterioration of paper due to their ability to secrete key enzymes, such as cellulase.[4]

Various physical and chemical methods are used to eliminate fungal contamination from paper, including active ingredients such as alcohols, aldehydes, phenolic derivatives like thymol, alkylating agents such as ethylene oxide, azoles, essential oils, and nanoparticles (NPs).[3] However, traditional biocides have been phased out in European Union countries due to their carcinogenic and irritating properties.[2,5] Around 2001, nanomaterials were first applied to restoration work, led by the University of Florence. Nanotechnology proved successful in repairing and cleaning surfaces, particularly on canvases, sculptures, ancient wood, and architectural monuments, which led to its use in controlling deterioration caused by biological factors. Nanomaterials commonly used in medicine, such as nano-titanium oxide, nano-silver, nano-zinc oxide, and nano-compound mixtures, were repurposed for this purpose. These biocidal nanostructured materials can form a protective layer due to their photocatalytic properties. Over the years, there has been continued progress in developing eco-friendly strategies to prevent biological colonization on cultural heritage materials. Today, nanomaterials are specifically designed with properties that emphasize photocatalytic action and self-cleaning effectiveness. Therefore, there is a critical need to

replace toxic chemical materials with safer alternatives.[6]

NPs can be synthesized through chemical, physical, or biological methods. Biological methods, a type of green synthesis for silver nanoparticles (AgNPs), involve natural sources such as plants, fungi, bacteria, algae, and their extracts to form NPs. These methods are considered environmentally friendly as they typically avoid the use of hazardous chemicals and energy-intensive processes associated with conventional synthesis techniques. Biological synthesis offers several advantages, including cleanliness, eco-friendliness, and safety.[7] Numerous studies have shown that biosynthesized NPs possess effective antimicrobial properties.[8-10] Bacteria, fungi, plants, and algae have all been used for the green synthesis of NPs.[11-18]

In our study, the antimicrobial effects of NPs using *Penicillium* and *Aspergillus* were assessed. These two fungi were selected owing to their high prevalence in the prestigious Astan e Quds Razavi library and numerous reports of contamination related to museums and archives.

Fungi have the potential to produce compounds known as mycotoxins and volatile organic acids. Exposure to these compounds can cause mild symptoms such as headaches, nausea, and sneezing, as well as severe complications, including cancer and allergic reactions in sensitive individuals. These symptoms may be observed in personnel working in libraries and archive centers who are exposed to fungal structures and spores. Therefore, it is crucial to eliminate these contaminating fungi.[19-21]

The aim of our study was to investigate and compare the antimicrobial effects of AgNPs prepared using biological methods employing plant extracts and cyanobacteria, with NPs prepared using physical methods.

Materials and Methods

AgNPs Biosynthesis

AgNPs were prepared using the aqueous extract of dried *Juglans regia* green husk following the method of Abbasi et al. Briefly, 150 µL of walnut extract was mixed with 9 mL of water and 1 mL of silver nitrate (10 mM). This solution was stored at room temperature in the dark.[22]

AgNPs were also synthesized using *Malva sylvestris* leaf extracts according to the method described by Feizi et al. In this case, 400 µL of the extract was added to 9 mL of distilled water, followed by the addition of 1 mL of silver nitrate (10 mM).[23]

For AgNPs synthesized from cyanobacteria extract, the method employed by Aletayeb et al was used.[24] Briefly, the culture medium containing cyanobacterial cells was centrifuged for 20 minutes at 1400 relative centrifugal force (rcf). Subsequently, 20 g of biomass was added to 50 mL of sterile distilled water and kept at room temperature for 5 days. After this period, the biomass was centrifuged again at 4400 rcf for 20 minutes. The supernatant was then distributed into several tubes, to which silver nitrate (10 mM) was added, achieving a final concentration of 5 mM.

Colloidal AgNPs were purchased from Payam Avaran Nano Technology Fardanegar Company (PNF). These NPs were produced using a physical method involving the electric explosion of silver wire in a liquid medium.

Preparation of the Most Concentrated Colloidal Nanoparticles

The colloidal solution of AgNPs, prepared in AgNPs Biosynthesis section, was washed three times with distilled water and then centrifuged at 10 000 rcf at 4 °C for 30 minutes. The nanoparticle sediment was dried for 5 minutes and then weighed. Next, the sediment was dissolved in the minimum amount of distilled water to achieve the highest concentration. To do this, a small volume of distilled water was added to the sediment and sonicated for 10 minutes using a sonicator (25 W, 35 J). The solution was then allowed to stand for 15 minutes. If precipitation occurred after this time, the solution was considered supersaturated, and additional distilled water was added. These steps were repeated until the colloidal solution reached a saturation state, at which point no sedimentation was observed after 15 minutes.

Culture of Fungi

The effectiveness of AgNPs was assessed on five strains, including *Penicillium* 3092, *Penicillium* 4116-01, *Penicillium* 4116-02, *Aspergillus* 4116, and *Aspergillus* 3092, which were isolated from documents and books in our previous study.[25] These fungi were the most frequently isolated species in that study. The fungi were stored on potato dextrose agar (PDA, Biolife, Italy) in the microbial bank. Fresh cultures of the fungi were prepared and incubated at 28–30 °C for 48–72 hours.

Evaluation of Antifungal Activity of the AgNPs

Preparation of the Fungal Spore

According to the CLSI-M38A protocol, a fungal suspension was prepared.[26] Briefly, the surface of a 7-day fungal colony was covered with 1 mL of 0.85% saline containing sterile Tween 80 and then transferred to a sterile microtube (1.5 mL). The microtubes were kept motionless for 3 to 5 minutes to allow heavy particles and any fungal mycelia to precipitate. Next, the supernatant was transferred to another sterile microtube and vortexed for about 20 seconds. The optical density (OD) was measured using a spectrophotometer at a wavelength of 530 nm and adjusted to between 0.9 and 0.15, which corresponds to a concentration of $0.4–5 \times 10^6$ CFU/mL and is considered a spore stock suspension. To confirm this concentration, cell counting was also performed using a Neubauer slide. A spore working suspension was prepared by diluting the spore stock suspension 1:50 in Sabouraud Dextrose Broth (SDB).

Minimum Inhibitory Concentration and Minimum Fungicide Concentration Determination

The minimum inhibitory concentration (MIC) is defined

as the lowest concentration of a material that can inhibit the growth of the target fungus, while the minimum fungicide concentration (MFC) is the lowest concentration that kills the desired fungus. To assay the MIC, 100 µL of the SDB medium was added to all wells of a microtiter plate. Then, 100 µL of the NPs was added to the first well and serially diluted through to well 10. Subsequently, 100 µL of the prepared spore working suspension was inoculated into all wells except the negative control well. The positive control wells contained 100 µL of the culture medium and 100 µL of fungal suspension, while the negative control well contained 100 µL of the culture medium and 100 µL of NPs. The experiment was performed with three replicates. For NPs derived from cyanobacteria, the antifungal effects of the bacterial extract were also tested.

To determine the MFC, 10 µL from wells without any fungal growth was cultured on the surface of PDA plates and incubated at 30 °C for 24 to 48 hours.

Results and Discussion

The microscopic and macroscopic images of the fungal isolates used in this study have been presented in Figure 1.

The synthesis of metallic NPs involves two stages: production and coating for stabilization and prevention of agglomeration. In chemical and physical methods, these stages are performed separately. However, in the biological method, both stages are carried out simultaneously because biomolecules induce the production of metallic NPs and interact with the metallic part as capping agents to stabilize it. Essentially, metallic NPs comprise both the metallic component and the coating part.[22-24]

The metallic part of AgNPs can include Ag, Ag_2S, AgO/Ag_2O/Ag_2O_3/Ag_3O_4/Ag_4O_4, AgCl, Ag_3PO_4, AgBr and/or AgI.[24] The characteristics, including initial concentration, type, shape, size, and capping biomolecules (capping agents) of AgNPs used in this study, are shown in Table 1.

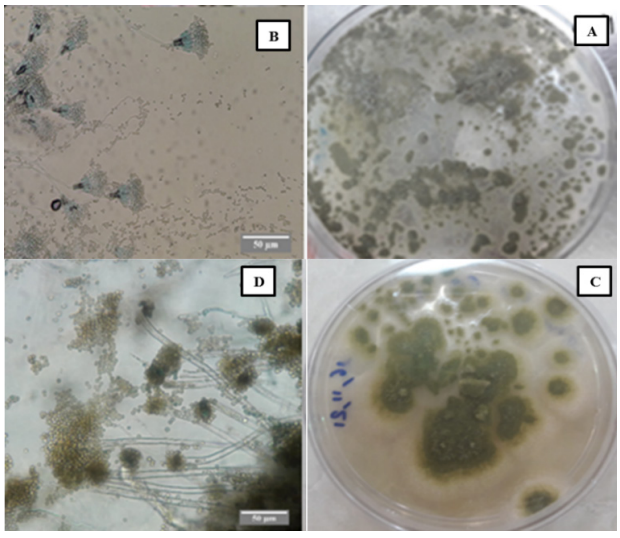

Figure 1. The Macroscopic and Microscopic Images of Fungi. A) Colony of *Penicillium* 4116 on PDA medium, B) Microscopic image of *Penicillium* 4116 with its conidiophore, C) Colony of *Aspergillus* on PDA medium, and D) Microscopic image of *Aspergillus* 3092 with its conidiophore

Antifungal Activity of AgNPs

The effects of five AgNPs produced through biological and physical methods were evaluated on five fungal strains isolated from paper. The results of the MIC and MFC of AgNPs have been summarized in Table 2 and illustrated in Figures 2 and 3.

According to the findings presented in Table 2, the biologically produced NPs exhibited a stronger antifungal effect than those synthesized through physical methods. Specifically, the biologically prepared AgNPs demonstrated fungicidal activity at concentrations ranging from 33 to 520 µg/mL.

The results of the antifungal tests for NPs revealed that the AgNPs synthesized by *M. sylvestris* extract exhibited greater effectiveness compared to those derived from *J. regia* extract against the tested fungi. The MIC and MBC for all tested fungi ranged from 135 to 260 µg/mL for AgNPs synthesized from *J. regia* extract, whereas for the AgNPs synthesized from *M. sylvestris* extract were notably lower, ranging from 46.5 µg/mL.

The MIC of the AgNPs produced from *J. regia* extract was lower for Aspergillus strains than Penicillium strains, indicating that Aspergillus strains are more sensitive to these NPs than Penicillium strains. Regarding AgNPs synthesized by cyanobacteria, the results

showed that NPs derived from *N. pruniforme* cyanobacterium were more effective than those produced by *Nostoc* IBRC-M5064 cyanobacterium.

In this study, the AgNPs produced by *N. pruniforme* cyanobacterium extract demonstrated the highest effectiveness. The MIC and MFC of the AgNPs produced by *N. pruniforme* for all fungi were found to be 16 and 33 µg/mL, respectively. The study aimed to evaluate the impact of different types of AgNPs derived from plant extracts, cyanobacteria, and NPs synthesized through physical methods against *Aspergillus* and *Penicillium* strains isolated from paper. Among the various fungal species isolated from contaminated paper materials, Aspergillus niger and *Penicillium rubrum* were found to be the most abundant.[27] Therefore, in this study, *Aspergillus* and *Penicillium* strains were selected as the most frequently isolated strains from biodeteriorated papers.

Based on the obtained MIC values in our experiments, the order of effectiveness of AgNPs is as follows:

AgNP produced by *N. pruniforme* > AgNP produced by *M. sylvestris* > AgNP produced by *Nostoc*-IBRC-M5064 > AgNP produced by *J. regia* > physically produced AgNPs.

The results of the MIC in this study indicate that, apart from the type of fungal species, the method of AgNPs production and their characteristics play a significant role in their antifungal properties. Tasca and Antiochia reported that the biologically synthesized AgNPs with very small diameters exhibit higher antimicrobial effects against a wide range of pathogenic microorganisms.[28] Barberia-Roque et al evaluated the antimicrobial effect of the AgNPs obtained from plant extracts to control

Table 1. The Properties of the Biological Produced AgNPs

The Biological Source for AgNPs Synthesis	Initial Concentration (µg/ mL)	Type	Shape and Size	Capping Agent	Ref.
Juglans regia	4166	Ag	Spherical 90% particles with a diameter of 69/09 nm (DLS) TEM=3-50 nm	Phenolic compounds, tannins, flavonoid, reducing sugars, protein, ascorbic acid and carotenoid	[22]
Malva sylvestris	11900	Ag/AgCl	Spherical 90% particles with a diameter of 126.72 nm (DLS) TEM=10-50 nm	Phenolic compounds, tannins, flavonoid, reducing sugars, protein, amino acids, chlorophyll, ascorbic acid and carotenoid	[23]
Nostoc sp IBRC-M5064	540	AgCl	Spherical average diameter 386.46 nm (DLS)	Carbohydrate, tannins, flavonoid, reducing sugars, protein, amino acid, vitamin C, chlorophyll and carotenoid	[24]
Nostoc pruniforme	540	$AgCl/Ag_3PO_4$	Spherical average diameter 258.48 nm (DLS)	Carbohydrate, tannins, flavonoid, reducing sugars, protein, amino acid, vitamin C, chlorophyll and carotenoid	[24]
Physically produced AgNPs	4545	Ag	Spherical>20 nm		

Table 2. The Results of MIC and MFC of Different AgNPs Against the Tested Fungi

Fungi	AgNPs Synthesized From *Juglans regia* Extract (µg/mL)		AgNPs Synthesized From *Malva sylvestris* Extract (µg/mL)		AgNPs Synthesized From Nostoc IBRC-M5064 Extract (µg/mL)		AgNPs Synthesized from *Nostoc pruniforme* Extract (µg/mL)		Physically Produced AgNPs (µg/mL)	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Penicillium 4116-02	260	520	46.5	93	67	134	16	33	1100	-
Aspergillus 3092	135	520	46.5	186	33	134	16	33	-	-
Aspergillus 4116	135	260	46.5	93	67	134	16	33	1100	1100
Penicillium 3092	260	260	46.5	46.5	33	67	16	33	1100	-
Penicillium 4116-01	260	260	46.5	46.5	67	134	16	33	568	562

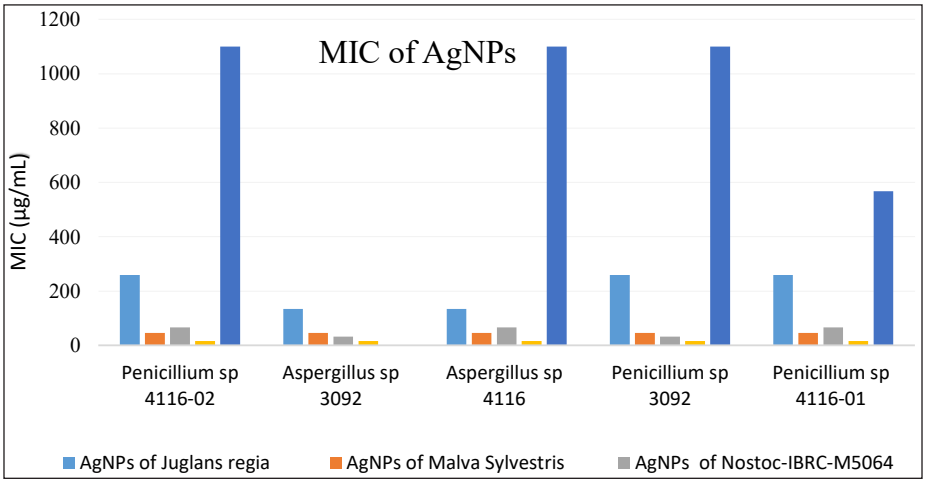

Figure 2. Comparison of the MIC of Different Produced NPs Against the Fungi Isolated from Paper

biodeterioration. They stated the MIC values of 3.3 and 67.5 µg/mL, depending on the microbial strain, with the most effective strain exhibiting the lowest MIC value.[10] In the current study, NPs produced with *N. pruniforme* extract were found to be more effective than other NPs against fungal strains. This enhanced effectiveness can be attributed to the unique characteristics of AgNPs, including their size and shape. Interestingly, this NP exhibited equal MIC values for all fungi, suggesting that they may possess similar antifungal mechanisms against all fungal cells.

Gutarowska et al conducted a study investigating the MIC and minimum bactericidal concentration (MBC) of AgNPs on 32 bacterial and fungal strains isolated from museums and archives. Their results, similar to ours, indicated differences in the sensitivity of various cells to NPs, even at the level of species and strains. In their study, the MIC and MBC for Aspergillus and Penicillium strains were reported to be 22.5 and 45 ppm, respectively.[14]

Pietrzak et al conducted a study investigating and comparing the disinfection of archival documents using three methods: essential oil, AgNPs, and low temperature. Their results revealed that all three methods were less effective against fungi compared to bacteria. Specifically, in this study, AgNPs demonstrated a reduction in fungal growth ranging from 29 to 89%.[2] The results of the MIC of different AgNPs in the present study indicated that NPs produced using biological or physical methods exhibited

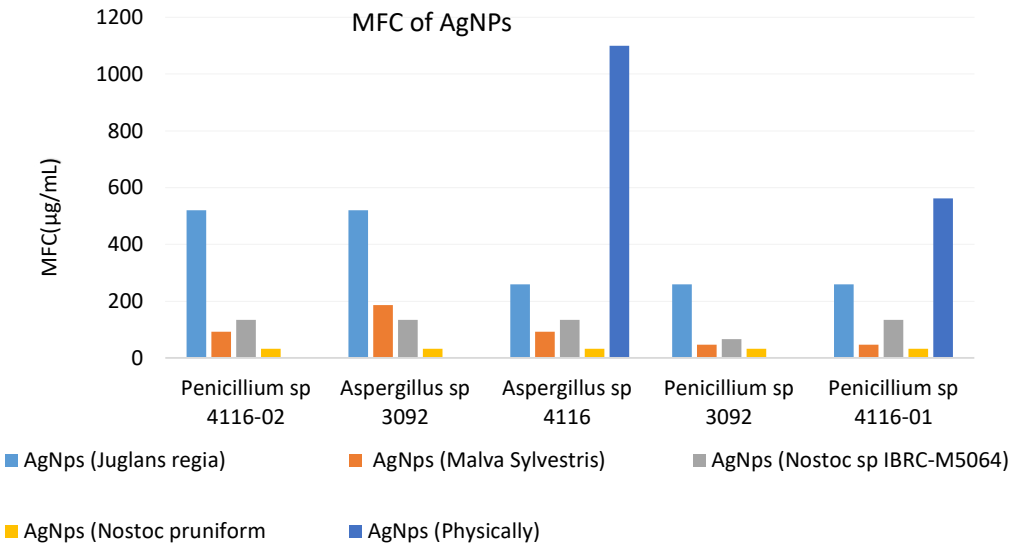

Figure 3. Comparison of the MFC of Different NPs Against the Fungi Isolated from Paper

distinct antifungal properties.

Conclusion

In this study, AgNPs were prepared using plant extracts and cyanobacterial biomass. The antifungal effects of AgNPs obtained from biological methods were compared to those obtained from physical methods against Aspergillus and Penicillium strains. The results showed that the antifungal activity of AgNPs prepared by biological methods was more effective than those prepared by physical methods in inhibiting the growth of biodeteriogenic fungi. Among the biological methods, the AgNPs derived from *N. pruniforme* extract exhibited the most significant impact. These findings suggest that the biological synthesis of NPs could be a promising method for inhibiting the growth of biodeteriogenic fungi in paper, thereby contributing to conservation strategies and control efforts. However, further investigations are needed to ensure that NPs do not have any adverse effects on substrates and do not cause cytopathic symptoms in personnel involved in conservation work.

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Project administration: Parisa Mohammadi, Parinaz Ghadam.
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Validation: Parisa Mohammadi, Parinaz Ghadam.
Visualization: Akram Kheiri.
Writing–original draft: Akram Kheiri.
Writing–review & editing: Akram Kheiri, Parisa Mohammadi, Parinaz Ghadam.

Competing Interests

None declared.

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