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Eco-Friendly Biosynthesis of Silver Nanoparticles Using *Fusarium Proliferatum*: A Promising Agent for Bioremediation and Antimicrobial Applications

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Abstract

The metallic nanoparticles production has attracted much attention lately because of their growing use in the medicinal sciences and engineering. They may be modified with specific functional groups to interact with medications and antibodies in an efficient manner, these nanoparticles are particularly interesting because of their special nanotechnology capabilities. Mostly between 10 and 100 nm in size, among other traits metallic nanoparticles have surface plasmon resonance and special optical qualities**.**

The subject of this study is Fusarium proliferatum, That was isolated from a soil sample, in order to find out its potential as a biocatalyst for the manufacture of silver nanoparticles (AgNPs) and its potential to withstand multidrug-resistant (MDR) bacteria. The evaluation was The fungus's ability to endure different heavy metals. From the findings, Fusarium proliferatum shown resistance to a several heavy metals at variant concentrations, such as copper (Cu) , nickel (Ni), zinc (Zn), silver (Ag), and mercury (Hg). Range of tolerance was shown depending on the metal, the colony formation of Fusarium proliferatum showed with certain strains being extremely resistant, moderately tolerant, or susceptible**.**

By removing heavy metals from contaminated soils, this strain of Fusarium proliferatum has the ability to be used in bioremediation. Also, encouraging antibacterial qualities was shown by using the creation of Ag nanoparticles, That made them useful against microbes which are resistant to many drugs**.**

1. Introduction:

Another special area of research that has uses in many facets of human life is nanotechnology. Nanotechnology, in opposite to other scientific disciplines, combines a several disciplines, including mathematics, physics, engineering, , and technology (1). Nanoparticles (NPs), one of the several

types of nanotechnology, are manufactured for a wide range of uses, the most crucial is in medical systems (2). Due to the creation and manufacturing of nanoparticles Therapeutic agents have great advanced, improving medical therapies (3). The distribution, size and morphology of nanoparticles, set them apart from bulk materials because they are ultra-small particles having a nano-sized structure, (4).
In contrast to chemical approaches and conventional physical, there has been an increasing interest in using biological systems to synthesis and stabilize nanoparticles lately (5). Because of their reductive abilities, biological systems such as, fungus, bacteria, yeasts, plants, and algae have shown the ability to convert inorganic metal ions into nanoparticles (6). Because of their low energy uses and environmental friendliness, these biological synthesis procedures have been popular as an alternative to more

high-pressure, hazardous, and high-temperature chemical ways (7). Many products, such as sanitary coatings, toothpaste, sunscreens, and even food products, include nanomaterials (8).
To be more specific, consumer goods that touch the human body frequently contain metal nanoparticles in the form of silver, gold, and several inorganic forms (9). This led to scientists investigations in the manufacturing of nanoparticles using biocatalysts like yeasts and fungi (10). Due to their rapid growth and increasing rate in extracellular enzyme secretion, on a wide scale these organisms are especially well-suited for producing nanoparticles (11). Because of reductase enzymes, fungi can create metal nanoparticles both intracellularly and extracellularly, and they are renowned for their ability to withstand and accumulate heavy metals (12).
Because of their capacity to produce nanoparticles fungi are useful in bioremediation, which can help in the elimination of heavy metals from contaminated settings (13). *Fusarium proliferatum* In particular, has demonstrated abilities in the synthesis of silver nanoparticles (AgNPs) and is agents a number of heavy metals, such as copper, zinc, nickel, and mercury (14). One type of metallic nanoparticle that has drawn a lot of interest are silver nanoparticles (AgNPs) because of its potent antibacterial qualities, low toxicity, and wide range of uses in both in in vivo and in vitro settings (15). Variety of research have focused on them because of their abilities in medical uses, especially as antibacterial agents (16).

1.1. Biological Systems for Metal Nanoparticle Synthesis

There are two types of nanoparticles: inorganic and organic. Inorganic nanoparticles include magnetic and metal nanoparticles, including silver and gold, whereas organic nanoparticles are usually made of carbon-based compounds. Researchers use the living biomass of microbes to create metallic nanoparticles because it makes it easier to reduce metal ions into nanoparticle form using simple procedures.
Inorganic nanoparticles, such as those of silver, nickel, zinc, mercury, and copper, can be produced intracellularly or extracellularly by microorganisms such as bacteria, actinomycetes, yeast, and fungi. The culture conditions and level of metal tolerance of these microorganisms determine their capacity to withstand heavy metals and generate nanoparticles. For the manufacture of nanoparticles on a wide scale, these requirements must be standardized. In particular, fungi are effective at creating metal nanoparticles and show traits that are comparable to those of artificial nanomaterials.

1.2.Biosynthesis of Nanoparticles by Fungi

Eukaryotic creatures that are frequently found in damp habitats are fungi. By releasing enzymes that degrade complex organic compounds into smaller molecules, fungus act as decomposers and absorb nutrients. Fungi are important in biotechnological applications, such as the creation of nanoparticles, because of their capacity to release enzymes. In example, filamentous fungi are efficient biocatalysts for the production of nanoparticles (17).

Additionally, to their capacity to tolerate heavy metals and bioaccumulate, fungi such as *Fusarium proliferatum* also produce large amounts of proteins, which increases the synthesis of nanoparticles (18). Fungi are perfect for laboratory applications and large-scale nanoparticle manufacture due to their effectiveness as extracellular enzyme secretors and their fast development (19). Several fungi, such as *Fusarium proliferatum*, have powerful metal-binding potentials on the cell wall and within the cell. According to (20), this makes them good candidates for the synthesis of silver nanoparticles and several metallic nanoparticles.
In this work, to create silver nanoparticles using the reductase enzymes of s *Fusarium proliferatum* silver ions are reduced. The fungus's ability to withstand heavy metals like copper, zinc, nickel, and mercury as well as silver underscores its promise for environmental bioremediation and nanoparticle creation. In addition to that, the produced nanoparticles have antibacterial properties, particularly against bacteria that are resistant to drugs. This makes *Fusarium proliferatum* a useful organism for environmental and medical uses.

2. Materials and Methods

Metal nanoparticles can be biosynthesized using a variety of methods and microorganisms. In this work, metal nanoparticles were mycosynthesised by a local isolate of *Fusarium proliferatum*. In an Erlenmeyer flask, For three days a fungal culture maintained in glycerol was cultivated in 250 milliliters of potato dextrose broth., the flask was continuously shaken at 150 rpm while being incubated at 28°C. Mycelial pads were aseptically extracted from the conidia using filtration Using sterile cheesecloth. To examine the conidial suspension under a microscope lactophenol cotton blue (LPCB) stain was then used. In an Eppendorf tube, the suspension was diluted 1:1 with peptone water to measure the conidia concentration. The diluted suspension was counted using a hemocytometer chamber to calculate the total number of cells per milliliter.
Fusarium proliferatum was identified macroscopically using four general-purpose mediums. The spotting method was used to inoculate Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Sabouraud Dextrose Agar (SDA) with a 100 mL fungal conidial suspension. For four days, the inoculum was incubated at 28°C.In addition to that , following LPCB staining, the mycelial pads of *Fusarium proliferatum* were examined under a light microscope. Based on the diameter, color, and form of the mycelial colonies, Malt Extract Agar was chosen for the mycosynthesis of metal nanoparticles.

2.1. Heavy Metal Tolerance of Fusarium proliferatum

On Malt Extract Agar (MEA) a 50 mL conidial suspension was cultivated with four distinct doses of silver nitrate in order to evaluate *Fusarium proliferatum*'s heavy metal resistance to silver nitrate ($AgNO_3$). After preparing and autoclaving a 10 mM stock solution of $AgNO_3$ (0.08 g $AgNO_3$ in 50 mL distilled water), it was diluted to concentrations of 0.25 mM, 0.50 mM, 0.75 mM, and 1 mM., The plates were incubated at 28°C for seven days.
Fusarium proliferatum was examinate for its ability to withstand several heavy metals, such as nickel nitrate ($Ni(NO_3)_2.6H_2O$), copper sulfate ($CuSO_4$), zinc chloride ($ZnCl_2$), and mercury chloride ($HgCl_2$), in addition to $AgNO_3$. Autoclaving was used to sterilize stock solutions containing 10 mM of each element (0.06 g $ZnCl_2$, 0.13 g $HgCl_2$, 0.08 g $CuSO_4$, and 0.14 g

$Ni(NO_3)_2.6H_2O$ in 50 mL distilled water). $ZnCl_2$ (1 mM, 2 mM, 3 mM, and 4 mM), $HgCl_2$ (0.1 mM, 0.15 mM, 0.2 mM, and 0.25 mM), $CuSO_4$ (1 mM, 1.5 mM, 2 mM, and 2.5 mM), and $Ni(NO_3)_2$ (1 mM, 1.5 mM, 2 mM, and 2.5 mM) based on their toxicity levels were subsequently diluted to the following amounts., The plates were incubated at 28°C. for seven days

3.Results

3.1. Growth of *Fusarium proliferatum* on Different Media

After Fusarium proliferatum was isolated and cultured, the mycelial pads were removed, and a conidial suspension was made for both macroscopic and microscopic analysis. A hemocytometer was used to count the conidia, and the average concentration in potato dextrose broth was 6.6×10^5 conidia/mL. Lactophenol cotton blue (LPCB) staining under a microscope demonstrated well-developed mycelium, microconidia, and macroconidia, as seen in Figure 1.
On several fungal media, macroscopic identification revealed varying growth patterns. Across Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Sabouraud Dextrose Agar (SDA), the mycelial colonies displayed unique morphological characteristics (Figure 2). Colonies on SDA developed a cottony edge with a white-reddish center, while colonies on the MEA looked white and cottony with an orange reverse. Colonies remained tiny and white on PDA, and growth was restricted.
Because it supports vigorous fungal growth, Malt Extract Agar was chosen as the best medium for assessing *Fusarium proliferatum*'s heavy metal tolerance based on the total colony diameter and morphological appearance.

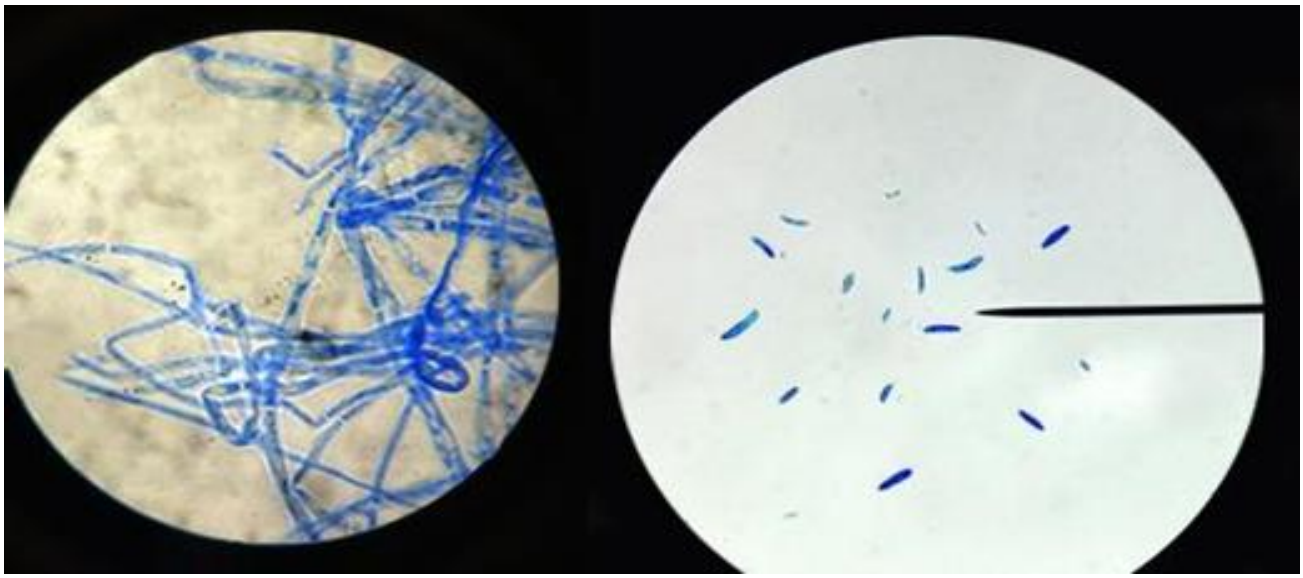

Figure 1. Microscopic examination of *Fusarium proliferatum* stained with LPCB:

A) Mycelium, B) Microconidia and macroconidia.

The conidial solution was inoculated onto four general-purpose media (PDA, MEA, and SDA) in order to evaluate macroscopic growth patterns. Across the media, different colony morphologies were noted (Figure 2):
Colonies showed up as cottony white with an orange reversal on MEA, reddish in the center with a white cottony border on SDA, and compact and white on PDA due to restricted colony expansion.
Malt Extract Agar (MEA) was chosen as the best medium for additional research, including heavy metal tolerance tests, based on colony size, margin, and reverse coloring.

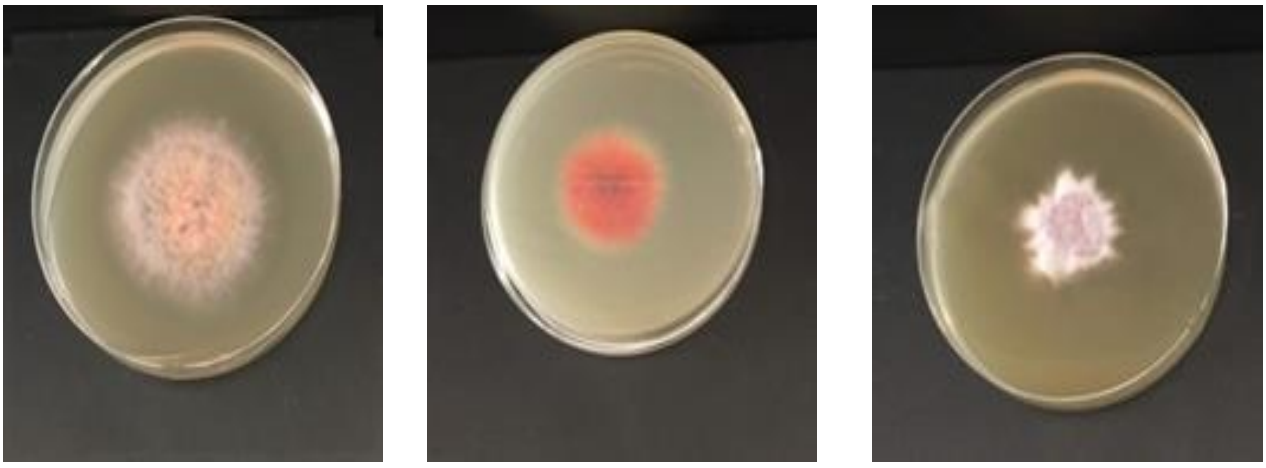

Figure2. Colony morphology of *Fusarium proliferatum* on different media:

A) MEA, B) SDA, C) PDA.

1.1. Heavy Metal Tolerance of *Fusarium proliferatum*

In this study, by monitoring its growth on malt extract agar (MEA) plates supplemented with varying concentrations of specific metal salts—silver nitrate ($AgNO_3$), zinc chloride ($ZnCl_2$), mercury chloride ($HgCl_2$), copper sulfate ($CuSO_4$), and nickel nitrate [$Ni(NO_3)_2 \cdot 6H_2O$]—over a 170-hour period, the heavy metal tolerance of *Fusarium proliferatum* was evaluated.

Only when silver nitrate ($AgNO_3$) was present did *F. proliferatum* show discernible and prolonged growth among all tested metals. The fungus retained distinct morphological traits, including dry, cottony white colonies with a peculiar yellow-brown pigmentation on the reverse side, even at concentrations as high as 1 mM (Figure 3). Complete suppression was not seen, despite the fact that increasing $AgNO_3$ concentrations caused a progressive decrease in colony diameter, suggesting a high degree of tolerance to silver ions.

The other heavy metals examined, $ZnCl_2$, $HgCl_2$, $CuSO_4$, and $Ni(NO_3)_2 \cdot 6H_2O$, on the other hand, did not cause any detectable or persistent fungal growth at any concentration. Either total colony formation suppression or incredibly sparse and irregular patches of fungal biomass that failed to mature into colonies were observed on plates treated with these metals. This implies that *F. proliferatum* is highly sensitive to these metal ions and that, in the experimental setup, there are no efficient detoxifying or resistance mechanisms against them.
These results demonstrate that *F. proliferatum* has a selective tolerance to silver, but not to zinc, mercury, copper, or nickel. This selectivity raises the possibility that the fungus has structural adaptations or metal-specific resistance pathways that allow it to survive in environments with higher concentrations of silver. In addition to making, it a viable organism for silver bioremediation, *F. proliferatum*'s resistance to silver toxicity supports its possible use in the mycosynthesis of silver nanoparticles (AgNPs), a process that is gaining attention in the field of green nanotechnology.
In addition to helping to develop sustainable and environmentally friendly methods for managing silver contamination in industrial and environmental settings, more research into the physiological and molecular reactions of *F. proliferatum* to silver ions may improve our knowledge of fungal metal interactions.

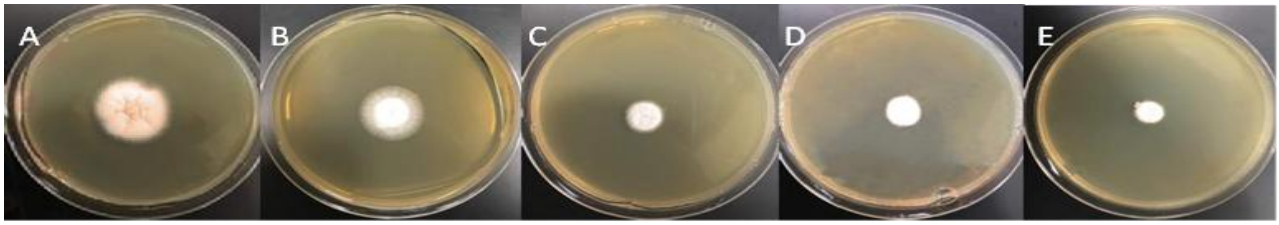

Figure3. Growth of F. proliferatum on MEA with varying $AgNO_3$ concentrations: A) Control, B) 0.25 mM, C) 0.50 mM, D) 0.75 mM, E) 1.00 mM.

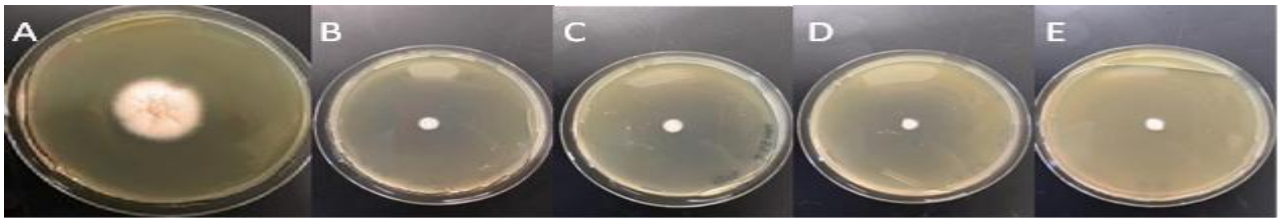

Figure4. Growth on MEA with $ZnCl_2$:
A) Control, B) 1 mM, C) 2 mM, D) 3 mM, E) 4 mM.

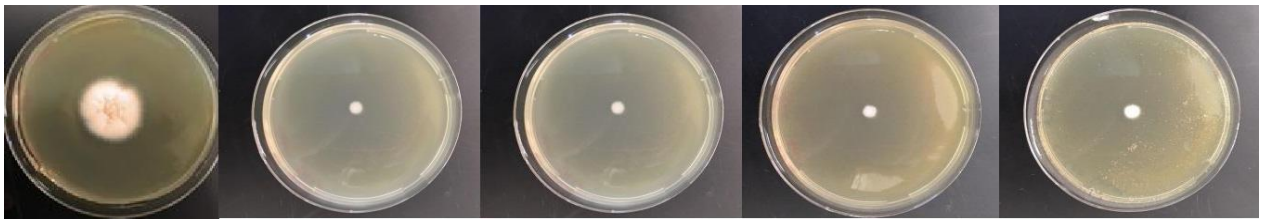

Figure5. Growth on MEA with $HgCl_2$:
A) Control, B) 0.10 mM, C) 0.15 mM, D) 0.20 mM, E) 0.25 mM.

Figure6. Growthon MEA with $CuSO_4$:
A) Control, B) 1.0 mM, C) 1.5 mM, D) 2.0 mM, E) 2.5 mM.

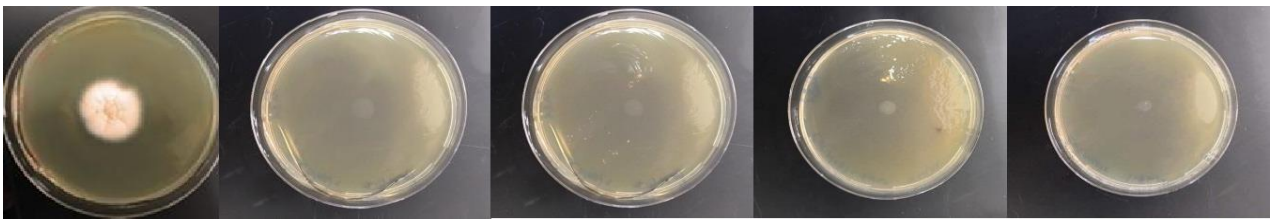

Figure7. Growth on MEA with $Ni(NO_3)_2.6H_2O$:

A) Control, B) 1.0 mM, C) 1.5 mM, D) 2.0 mM, E) 2.5 mM.

4.Discussion

Due to the contaminants' extreme toxicity, persistence, and non-degradability, heavy metal contamination stays as a serious environmental concern. Mining operations, agricultural runoff, industrial discharge, and incorrect waste disposal are some of the reasons of contamination. Over time, these metals—which include copper, nickel, zinc, silver, and mercury—can pile up in ecosystems, changing soil microbiomes, decreasing biodiversity, and compromising vital ecological functions. The effects of heavy metal poisoning on microbial populations, which are important for the maintaining of ecosystems and the cycling of nutrients, are among its most remarkable consequences. Biological components can be harmed by These metals' harmful effects by causing oxidative stress, suppressing enzyme activity, and preventing microbial growth.
In this regard, fungi have shown great promise as heavy metal bioremediation and environmental detoxifying agents. They are useful instruments for ecological restoration because of their strong cell walls, adaptability as enzymatic organisms, and capacity to generate a variety of metal- binding compounds. Using malt extract agar (MEA) as a growth medium, we assessed the tolerance of a native fungal isolate, *Fusarium proliferatum*, against a panel of heavy metals in a lab setting. Surprisingly, *F. proliferatum* only showed discernible and prolonged growth when silver nitrate ($AgNO_3$) was present, up to a concentration of 1.0 mM. Although the colony width gradually decreased as the quantity of silver increased, the colonies maintained their characteristic cottony shape and white coloration with a yellow-brown backside. Given that silver is known to have antibacterial qualities and frequently prevents microbial growth at even low concentrations, this observation is especially noteworthy. *F. proliferatum*'s observed tolerance to silver raises the possibility of specialized resistance mechanisms, such as the production of metal-chelating proteins and stress-related enzymes, extracellular sequestration of silver ions, or cell wall alterations that restrict ion penetration.
On MEA plates treated with zinc chloride, mercury chloride, copper sulfate, or nickel nitrate at any tested dose, however, no discernible fungal growth was seen. These findings suggest that either the concentration ranges examined were higher than the threshold for fungal viability, or that *F. proliferatum* lacks efficient resistance mechanisms against these specific metals. The uniqueness of fungal-metal interactions is highlighted by this stark difference with the silver response, which also raises the possibility that tolerance mechanisms vary depending on the metal type.
The recognized high toxicity of nickel ($Ni(NO_3)_2 \cdot 6H_2O$) and mercury ($HgCl_2$) is consistent with the fungus's inability to develop in their presence. While nickel blocks the absorption of vital nutrients and causes oxidative stress, which can damage DNA and proteins, mercury interacts with the function of proteins by attaching itself to thiol (-SH) groups. Likewise, copper ($CuSO_4$) and zinc ($ZnCl_2$), while necessary trace minerals at low quantities, become extremely toxic when present in excess, resulting in metabolic dysfunctions and decreased enzyme activity. These metals' total suppression of fungal growth emphasizes the thin line separating essentiality from toxicity and underscores the necessity for more thorough research on tolerance thresholds.
The selective tolerance of *F. proliferatum* to silver suggests its potential application in biotechnological and environmental remediation efforts, especially in contexts where silver contamination is prevalent. Moreover, this selective resistance presents an opportunity to harness the fungus for the green synthesis of silver nanoparticles (AgNPs), which are valuable in medical, industrial, and environmental applications due to their antimicrobial and catalytic properties.
the fungus for the green synthesis of silver nanoparticles (AgNPs), which are valuable in medical, industrial, and environmental applications due to their antimicrobial and catalytic properties.

This work aligns closely with several United Nations Sustainable Development Goals (SDGs):
• SDG 6 (Clean Water and Sanitation): By contributing to the development of biological tools for heavy metal removal, this study supports efforts to ensure access to clean water by reducing contamination from industrial pollutants.
• SDG 12 (Responsible Consumption and Production): The application of *F. proliferatum* for eco-friendly remediation and biosynthesis of nanoparticles promotes sustainable industrial practices that minimize environmental harm.
• SDG 13 (Climate Action): Addressing soil and water pollution contributes indirectly to climate resilience by enhancing the health and productivity of natural ecosystems.
• SDG 15 (Life on Land): Mitigating heavy metal pollution in soils protects terrestrial biodiversity and fosters ecosystem recovery, which is essential for sustainable land use and conservation.

5.Conclusion

For traditional chemical and physical processes A viable substitute, biological production of nanoparticles allows a less dangerous and more ecologically friendly method. The non-human-threatening but plant-pathogenic fungus *Fusarium proliferatum* showed great promise in this work as a biocatalyst for the production of silver nanoparticles. Its inherent resistance to different heavy metals emphasizes its dual potential for environmental bioremediation and nanomaterial synthesis.
Beside promoting sustainable nanotechnology, the successful production of silver nanoparticles utilizing *Fusarium proliferatum* creates opportunities for medical uses, especially in antibacterial therapy. The produced nanoparticles' antibacterial qualities point to possible future use in the fight against bacteria that are resistant to drugs and perhaps even in cancer treatment plans.
This work emphasizes the valuable role of *Fusarium proliferatum* in green nanotechnology and sets the stage for further exploration into its clinical and environmental applications.

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