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Biosynthesis and Characterization of Silver-Selenium Nanoparticles from Endophytic Fungi and Their Biological Activity

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Abstract

Background: Silver (Ag) and selenium (Se) nanoparticles are known for their unique antimicrobial properties. This study aims to synthesize Silver-Selenium (Ag-Se) nanoparticles using endophytic fungi and evaluate their antibacterial and anti-biofilm activities against clinically relevant bacterial strains. **Methods**: Endophytic fungi were isolated from various plant samples, and Ag-Se nanoparticles were synthesized through a green synthesis method involving the reduction of silver nitrate ($AgNO_3$) and sodium selenite (Na_2SeO_3) using fungal extracts. The synthesized nanoparticles were characterized using techniques such as SEM, TEM, DLS, FTIR, and XRD. Antibacterial activity was assessed via the agar well diffusion method and Minimum Inhibitory Concentration (MIC) determination. Anti-biofilm activity was evaluated using a microtiter plate assay to quantify biofilm inhibition. **Results**: The Ag-Se nanoparticles were characterized as spherical with an average size of 20-50 nm and exhibited a zeta potential of -27.3 mV, indicating good stability. The nanoparticles demonstrated significant antibacterial activity, with zones of inhibition of 16.1 mm against *Escherichia coli*, 21.0 mm against *Staphylococcus aureus*, and 14.8 mm against

Pseudomonas aeruginosa. MIC values for Ag-Se nanoparticles were 25 µg/mL for *E. coli*, 30 µg/mL for *S. aureus*, and 40 µg/mL for *P. aeruginosa*, significantly lower than those for individual Ag (50, 60, and 70 µg/mL, respectively) and Se nanoparticles. Biofilm inhibition percentages were 65% for *E. coli*, 70% for *S. aureus*, and 58% for *P. aeruginosa*, with statistical significance confirmed by ANOVA and Tukey's post hoc test ($p < 0.01$). **Conclusion**: The study demonstrates that Ag-Se nanoparticles synthesized from endophytic fungi exhibit superior antibacterial and anti-biofilm properties compared to their counterparts. These findings suggest the potential of Ag-Se nanoparticles as effective agents in combating bacterial infections and biofilm-related challenges, paving the way for their application in medical and industrial settings.

Keywords: Silver-Selenium nanoparticles, Endophytic fungi, Antibacterial activity, Anti-biofilm activity.

1. Introduction

Nanotechnology has emerged as a transformative field in medicine, offering innovative solutions for diagnostics, drug delivery, and therapeutic applications(1). Among the various nanomaterials, metallic nanoparticles have gained significant attention due to their unique physical, chemical, and biological properties. Silver (Ag) nanoparticles, in particular, are well known for their potent antimicrobial activity against a wide range of pathogens, including antibiotic-resistant bacteria. Their ability to disrupt bacterial membranes and generate Reactive Oxygen Species (ROS) makes them effective in combating infections. Selenium (Se) nanoparticles, on the other hand, possess notable antioxidant, anti-inflammatory, and anticancer properties. Selenium is an essential trace element in humans, playing a crucial role in immune function and cellular defense mechanisms. However, at the nanoscale, selenium exhibits enhanced bioavailability and biological activity, making it a promising candidate for biomedical applications.

The synthesis of nanoparticles has traditionally relied on chemical and physical methods, which often require toxic reagents, high energy consumption, and generate hazardous by-products(3). These limitations have led to a growing interest in green synthesis approaches, which utilize biological entities such as plants, bacteria, fungi, and algae for nanoparticle production. Among these, endophytic fungi have shown great promise as bio-factories for the synthesis of nanoparticles. Endophytic fungi,

residing symbiotically within plant tissues, produce a wide range of secondary metabolites, including enzymes, phenolic compounds, and proteins, which can facilitate the reduction and stabilization of metal ions into nanoparticles. This method offers a sustainable and eco-friendly alternative to conventional synthesis methods, reducing environmental impact and enhancing the biocompatibility of the synthesized nanoparticles.

Combining silver and selenium into a single nanoparticle formulation can potentially leverage the unique properties of both elements, creating a synergistic effect that enhances their antibacterial, anti-biofilm, and anticancer activities(5). The idea behind synthesizing Ag-Se nanoparticles lies in their ability to combine the broad-spectrum antibacterial action of silver with the selective cytotoxicity and antioxidant properties of selenium. Previous studies have highlighted the individual benefits of silver and selenium nanoparticles, but limited research has explored their combined potential, predominantly when synthesized using biological methods. This gap in research presents an opportunity to explore novel applications of Ag-Se nanoparticles in combating infections and treating cancers.

The current study aims to address this gap by isolating endophytic fungi from various localities in Egypt and screening their potential for synthesizing Ag-Se nanoparticles(7). The most potent fungal isolates will be used to synthesize and characterize Ag-Se nanoparticles, followed by a comprehensive evaluation of their antibacterial, anti-biofilm, and anticancer properties. A particular focus is placed on liver cancer, a significant health concern worldwide, with high morbidity and mortality rates. The study aims to investigate whether Ag-Se nanoparticles can selectively target cancer cells while minimizing cytotoxic effects on normal cells, thereby providing a potential new avenue for cancer therapy.

Moreover, bacterial biofilm formation poses a significant challenge in clinical settings, particularly in hospital-acquired infections and the contamination of medical devices(9). Biofilms confer protection to bacteria against antibiotics and immune responses, making infections difficult to eradicate. The ability of Ag-Se nanoparticles to inhibit biofilm formation could make them valuable in preventing such persistent infections. Understanding the mechanisms through which Ag-Se nanoparticles interact with bacterial cells and biofilms could open new strategies for managing resistant infections.

The novelty of this study lies in the use of endophytic fungi as a green synthesis platform for Ag-Se nanoparticles and the exploration of their combined antimicrobial and antibiofilm properties. By integrating biological synthesis with advanced characterization techniques and biological assays, this research aims to contribute to the development of safe and effective nanomaterials for biomedical applications. The findings from this study could pave the way for future research into the therapeutic potential of biogenic Ag-Se nanoparticles, offering a sustainable and practical approach to addressing global health challenges.

2. Materials and methods

2.1. Sample Collection and Fungal Isolation:

Samples were collected from various ecological niches to obtain a diverse range of endophytic fungi(10). Sampling sites were selected based on their varied environmental conditions to ensure a broad spectrum of fungal diversity. Plant samples, including roots, leaves, and stems, as well as soil samples, were collected using sterilized tools to minimize contamination. Each sample was placed in sterile polyethylene bags and transported to the laboratory under cool conditions to preserve the integrity of the microbial communities.

Upon arrival at the laboratory, plant samples were processed for surface sterilization to eliminate any epiphytic microorganisms. This process involved a sequential immersion of the plant material in 70% ethanol for 1 minute, followed by sodium hypochlorite (2% v/v) for 3 minutes, and a final rinse in sterile distilled water three times to remove any residual sterilizing agent. Soil samples were subjected to serial dilution and plated directly on culture media. The sterilized plant tissues were aseptically cut into small segments (approximately 1 cm) and placed onto Potato Dextrose Agar (PDA) plates supplemented with 100 µg/mL chloramphenicol to inhibit bacterial growth. The plates were incubated at 28°C for 5-7 days, during which endophytic fungi emerging from the plant tissues were observed and isolated. The growing fungal colonies were then sub-cultured onto fresh PDA plates to obtain pure cultures. For further purification, hyphal tip isolation was performed to ensure that each fungal isolate originated from a single colony. The isolates were cultured at room temperature until sufficient mycelial growth was observed. Each pure isolate was then transferred to PDA slants and stored at 4°C for short-term preservation, with glycerol stocks prepared for long-term storage at -80°C.

2.2. Green Synthesis of Silver-Selenium Nanoparticles Using Endophytic Fungi

The green synthesis of Ag-Se NPs using endophytic fungi was conducted as an eco-friendly alternative to conventional chemical methods. This approach leverages the metabolic capabilities of fungi to reduce metal ions and stabilize the synthesized nanoparticles, ensuring minimal use of hazardous chemicals. The steps involved in this process are outlined below:

2.3. Preparation of Fungal Extracts

Selected endophytic fungal isolates, identified as potent nanoparticle synthesizers based on preliminary screening, were cultured in liquid Potato Dextrose Broth (PDB)(11). A loopful of each fungal isolate was inoculated into 250 mL Erlenmeyer flasks containing 100 mL of sterilized PDB. The flasks were incubated at 28°C for 7-10 days under shaking conditions (120 rpm) to promote fungal growth and metabolite production. After the incubation period, the fungal biomass was separated from the culture medium by filtration through Whatman No. 1 filter paper under sterile conditions.

2.4. Preparation of Fungal Culture Filtrate

The collected fungal biomass was rinsed thoroughly with sterile distilled water to remove any residual medium components(12,13). Approximately 10 grams of fresh fungal biomass was then transferred to a fresh 250 mL Erlenmeyer flask containing 100 mL of sterile distilled water. The mixture was incubated for 48 hours at 28°C with continuous shaking (120 rpm) to allow the release of extracellular enzymes and metabolites into the aqueous medium. After incubation, the fungal culture filtrate was obtained by filtering the mixture through Whatman No. 1 filter paper, followed by centrifugation at 10,000 rpm for 10 minutes to remove any remaining fungal debris. The resulting supernatant served as the fungal extract, which contained the bioactive compounds required for nanoparticle synthesis.

2.5. Synthesis of Ag-Se Nanoparticles

The biosynthesis of Ag-Se nanoparticles was carried out by mixing the fungal culture filtrate with aqueous solutions of silver nitrate ($AgNO_3$) and sodium selenite (Na_2SeO_3). The synthesis process involved the following steps: A 1 mM solution of $AgNO_3$ and a 1 mM solution of Na_2SeO_3 were prepared using sterile distilled water. 50 mL of the fungal culture filtrate was mixed with 25 mL of 1 mM $AgNO_3$ solution and 25 mL of 1 mM Na_2SeO_3 solution in a 250 mL Erlenmeyer flask, resulting in a final concentration of 0.5 mM for each metal ion. The reaction mixture was maintained at room

temperature (25°C) under dark conditions to prevent photodegradation of the metal ions and stirred continuously at 120 rpm.

2.6. Observation of Nanoparticle Synthesis

The synthesis of Ag-Se nanoparticles was monitored through a change in the color of the reaction mixture, which served as a preliminary indication of nanoparticle formation. The color change from pale yellow to reddish-brown, observed within 24 hours, indicated the reduction of silver and selenium ions by the bioactive compounds present in the fungal filtrate. This color change suggested the formation of Ag-Se nanoparticles as the metal ions were reduced and stabilized in the reaction medium.

2.7. Characterization of Ag-Se Nanoparticles

The synthesized nanoparticles were characterized using various analytical techniques to determine their size, shape, surface charge, and crystalline structure(14). Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) were employed to visualize the morphology and size distribution of the nanoparticles. Dynamic Light Scattering (DLS) was used to measure the hydrodynamic diameter and zeta potential, providing insights into the stability of the nanoparticles in suspension. Fourier-Transform Infrared Spectroscopy (FTIR) was performed to identify functional groups involved in the capping and stabilization of the nanoparticles. Additionally, X-ray Diffraction (XRD) analysis was conducted to determine the crystalline nature of the synthesized Ag-Se nanoparticles.

2.8. Antimicrobial Activity Assay

The antibacterial activity of the synthesized Ag-Se NPs was evaluated against a range of Gram-positive and Gram-negative bacterial strains(15). The selected bacterial strains included *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive), and *Pseudomonas aeruginosa* (Gram-negative). These strains were chosen due to their clinical relevance and their joint association with antibiotic-resistant infections. The antibacterial potential of the Ag-Se nanoparticles was assessed using both the agar well diffusion method and the determination of Minimum Inhibitory Concentration (MIC).

2.9. Anti-Biofilm Activity of Silver-Selenium Nanoparticles

The ability of the synthesized Ag-Se nanoparticles to inhibit biofilm formation by bacterial strains was assessed using a microtiter plate assay(16). This assay measures the ability of the nanoparticles to

prevent biofilm formation on a solid surface and is quantified by staining the biofilm with crystal violet.

a) **Preparation of Bacterial Cultures:** Fresh cultures of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were grown in Luria-Bertani (LB) broth at 37°C overnight. The bacterial cultures were adjusted to an optical density (OD) of 0.5 at 600 nm, which corresponds to approximately 1×10^8 CFU/mL, using sterile LB broth.

b) **Preparation of Nanoparticle Solutions:** The Ag-Se nanoparticle stock solution was prepared in sterile distilled water, and serial dilutions were made to obtain concentrations ranging from 5 µg/mL to 100 µg/mL. As controls, separate solutions of Ag nanoparticles (Ag NPs) and Se nanoparticles (Se NPs) were prepared at similar concentrations.

c) Biofilm Formation Assay:

- **Inoculation in Microtiter Plates:** 100 µL of the bacterial suspension was added to each well of a sterile 96-well flat-bottom microtiter plate. 100 µL of the diluted nanoparticle solution was then added to each well, resulting in final concentrations of 5 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL of the nanoparticles. Control wells containing only bacterial suspension (positive control) and LB broth (negative control) were also included.
- **Incubation:** The microtiter plates were covered and incubated at 37°C for 24 hours under static conditions to allow biofilm formation. The assays were performed in triplicate to ensure reproducibility.

2.10. Statistical Analysis

Each experiment was performed in triplicate, and the findings were presented as Mean ± standard deviation (SD)(17). The statistical significance of the results was evaluated using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value of less than 0.05 was considered to indicate statistical significance.

3. Results

3.1. Characterization of Nanoparticles:

SEM analysis revealed that the synthesized Ag-Se nanoparticles had an average size ranging from 20 to 50 nm. The nanoparticles appeared well-dispersed, with a Mean ± SD size of 35 ± 5 nm. The size range indicates a relatively uniform synthesis process with reasonable control over particle dimensions, which is vital for consistent biological activity. TEM images showed that the Ag-Se nanoparticles were predominantly

spherical in shape. The morphology of the nanoparticles was consistent across different samples, indicating that the synthesis method produced uniform particles with a consistent spherical structure. The spherical shape is advantageous for maximizing surface area, which may enhance the interaction of nanoparticles with biological membranes. The Polydispersity Index (PDI) of the Ag-Se nanoparticles was 0.182 ± 0.03. PDI values range from 0 to 1, with values closer to 0 indicating uniform particle size distribution.

The low PDI value observed here suggests that the synthesized nanoparticles were monodisperse, with a uniform size distribution, which is desirable for consistent performance in biological applications. FTIR analysis identified several functional groups on the surface of the Ag-Se nanoparticles, indicating their role in capping and stabilization. Critical peaks were observed at 3400 cm^{-1}, corresponding to hydroxyl (OH) groups, at 1600 cm^{-1}, corresponding to amine (NH) groups, and at 1230 cm^{-1}, corresponding to C-O stretching vibrations. These functional groups likely originate from the metabolites present in the fungal culture filtrate, which act as reducing and stabilizing agents during the nanoparticle synthesis process.

The presence of these groups suggests that the nanoparticles are capped by bio-organic molecules, enhancing their biocompatibility. XRD analysis confirmed the crystalline nature of the synthesized Ag-Se nanoparticles. Characteristic diffraction peaks were observed at 2θ angles of 27°, 32°, and 38°, which correspond to the crystalline phases of silver and selenium. These peaks match well with standard diffraction patterns for Ag-Se phases, indicating the successful incorporation of both elements into the nanoparticle structure. The crystallinity of the nanoparticles contributes to their stability and potentially enhances their biological activity by providing well-defined surface properties, as revealed in **Table 1**.

Table 1: Physicochemical Characterization of Silver-Selenium Nanoparticles

Characterization Technique	Parameter Analyzed	Observed Results	Statistical Analysis
SEM	Average Size	20-50 nm	Mean ± SD: 35 ± 5 nm
TEM	Morphology	Spherical	Consistent across replicates
Dynamic Light Scattering (DLS)	Zeta Potential (mV)	-27.3 ± 1.2 mV	Indicates good stability
DLS	Polydispersity Index (PDI)	0.182 ± 0.03	Low, indicating uniformity
FTIR	Functional Groups	Peaks at 3400 cm^{-1} (OH), 1600 cm^{-1} (NH), 1230 cm^{-1} (C-O)	Consistent with stabilization molecules
XRD	Crystallinity	Crystalline nature with peaks at 2θ of 27°, 32°, 38°	Indexed to Ag-Se phases

3.2. Antibacterial Activity

The antibacterial activity of Ag-Se NPs synthesized using endophytic fungi was evaluated against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* using the agar well diffusion method, with results indicating significant efficacy. The zone of inhibition (ZOI) for *E. coli* was 16.1 ± 0.3 mm; for *S. aureus* it was 21.0 ± 0.4 mm, and for *P. aeruginosa* it was 14.8 ± 0.5 mm, with the Ag-Se nanoparticles demonstrating superior antibacterial activity compared to individual Ag (10.4 ± 0.6 mm for *E. coli*, 12.3 ± 0.5 mm for *S. aureus*, and 9.8 ± 0.5 mm for *P. aeruginosa*) and Se nanoparticles (8.7 ± 0.4 mm for *E. coli*, 9.2 ± 0.3 mm for *S. aureus*, and 7.5 ± 0.3 mm for *P. aeruginosa*). Statistical analysis via one-way ANOVA revealed significant differences ($p < 0.01$ for *E. coli* and *S. aureus*; $p < 0.05$ for *P. aeruginosa*), with Tukey's post hoc test confirming that Ag-Se nanoparticles were significantly more effective than both Ag and Se nanoparticles (*Ag-Se NPs > Ag NPs > Se NPs*). These findings suggest that Ag-Se nanoparticles possess antibacterial solid properties, likely due to the synergistic effects of silver and selenium, making them promising candidates for addressing antibiotic-resistant bacterial infections **(Table 2)**.

Table 2: Antibacterial Activity of Ag-Se Nanoparticles Against Various Strains

Bacterial Strain	Zone of Inhibition (mm)	Ag NPs	Se NPs	Ag-Se NPs	p-value (ANOVA)
E. coli	Mean ± SD	10.4 ± 0.6	8.7 ± 0.4	**16.1 ± 0.3**	$p < 0.01$
S. aureus	Mean ± SD	12.3 ± 0.5	9.2 ± 0.3	**21.0 ± 0.4**	$p < 0.01$
P. aeruginosa	Mean ± SD	9.8 ± 0.5	7.5 ± 0.3	**14.8 ± 0.5**	$p < 0.05$

3.3. The Minimum Inhibitory Concentration (MIC) of Ag-Se nanoparticles assessment

The Minimum Inhibitory Concentration (MIC) of Ag-Se NPs synthesized using endophytic fungi was determined against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The results indicated that Ag NPs had MIC values of 50 µg/mL for *E. coli*, 60 µg/mL for *S. aureus*, and 70 µg/mL for *P. aeruginosa*. In contrast, Se NPs exhibited higher MIC values of 75 µg/mL, 80 µg/mL, and 90 µg/mL for the respective bacterial strains. Notably, Ag-Se NPs demonstrated significantly lower MIC values of 25 µg/mL for *E. coli*, 30 µg/mL for *S. aureus*, and 40 µg/mL for *P. aeruginosa*. Statistical analysis confirmed that the Ag-Se NPs had significantly lower MIC values compared to both Ag and Se nanoparticles, with p-values of less than 0.01 for *E. coli* and *S. aureus* and less than 0.05 for *P. aeruginosa*, indicating their superior antibacterial efficacy **(Table 3)**.

Table 3: Minimum Inhibitory Concentration (MIC) of Nanoparticles (µg/mL)

Bacterial Strain	Ag NPs	Se NPs	Ag-Se NPs	Statistical Significance
E. coli	50	75	**25**	Ag-Se NPs significantly lower MIC ($p < 0.01$)
S. aureus	60	80	**30**	Ag-Se NPs significantly lower MIC ($p < 0.01$)
P. aeruginosa	70	90	**40**	Ag-Se NPs significantly lower MIC ($p < 0.05$)

3.4. Anti-biofilm Activity

The anti-biofilm activity of Ag-Se NPs synthesized from endophytic fungi was evaluated against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* by measuring the percentage of biofilm inhibition. The results showed that Ag NPs inhibited biofilm formation by 45 ± 3% for *E. coli*, 52 ± 4% for *S. aureus*, and 40 ± 3% for *P. aeruginosa*, while Se NPs exhibited slightly lower inhibition rates of 38 ± 2%, 40 ± 3%, and 35 ± 2%, respectively. In contrast, Ag-Se NPs significantly enhanced biofilm inhibition, achieving rates of 65 ± 3% for *E. coli*, 70 ± 4% for *S. aureus*, and 58 ± 3% for *P. aeruginosa*. Statistical analysis using ANOVA revealed significant differences, with p-values less than 0.01 for *E. coli* and *S. aureus* and less than 0.05 for *P. aeruginosa*. Tukey's post hoc test confirmed that the Ag-Se NPs exhibited greater anti-biofilm efficacy compared to both Ag and Se nanoparticles, indicating their potential as effective agents in preventing biofilm-related infections **(Table 4)**.

Table 4: Biofilm Inhibition by Nanoparticles

Bacterial Strain	Biofilm Inhibition (%)	Ag NPs	Se NPs	Ag-Se NPs	p-value (ANOVA)
E. coli	Mean ± SD	45 ± 3	38 ± 2	**65 ± 3**	$p < 0.01$
S. aureus	Mean ± SD	52 ± 4	40 ± 3	**70 ± 4**	$p < 0.01$
P. aeruginosa	Mean ± SD	40 ± 3	35 ± 2	**58 ± 3**	$p < 0.05$

4. Discussion

The results from this study underscore the potential of Ag-Se NPs synthesized using endophytic fungi as effective antimicrobial agents. The characterization of these nanoparticles revealed an average size of 20-50 nm, predominantly in a spherical morphology, which is critical for maximizing surface area and enhancing interaction with microbial cells. This small size facilitates improved penetration into bacterial cells, contributing to their higher antibacterial efficacy compared to larger particles. The negative zeta potential of -27.3 mV indicates good stability in suspension, which is essential for maintaining their bioactivity in biological environments.

Tests showed that Ag-Se nanoparticles were much better at stopping the growth of *E. coli*, *S. aureus*, and *P. aeruginosa* bacteria than just Ag or Se nanoparticles alone. The Ag-Se nanoparticles were particularly good at stopping *E. coli* (with a stopping zone of 16.1 mm) and *S. aureus* (with a stopping zone of 21.0 mm), showing they work well against different types of bacteria. The observed antibacterial activity may be attributed to the synergistic effects of silver and selenium ions. Silver ions are known to disrupt bacterial cell membranes and generate reactive oxygen species (ROS), leading to oxidative stress and cell death. Meanwhile, selenium contributes to this mechanism by enhancing antioxidant activity and potentially increasing the permeability of bacterial membranes to silver ions. The combination of these mechanisms makes Ag-Se nanoparticles a promising candidate for tackling multi-drug-resistant bacterial infections.

Furthermore, the determination of Minimum Inhibitory Concentration (MIC) values showed that Ag-Se nanoparticles had significantly lower MICs (25 µg/mL for *E. coli*, 30 µg/mL for *S. aureus*, and 40 µg/mL for *P. aeruginosa*) compared to Ag and Se nanoparticles alone. This indicates that lower concentrations of Ag-Se nanoparticles are sufficient to inhibit bacterial growth, which is particularly advantageous in clinical applications where minimizing the concentration of antimicrobial agents can reduce potential side effects and toxicity.

The ability of Ag-Se nanoparticles to inhibit biofilm formation was also assessed, yielding promising results. Biofilms are structured communities of bacteria that adhere to surfaces and are encased in a protective extracellular matrix, making them

highly resistant to conventional antibiotics and immune responses. The substantial biofilm inhibition percentages observed (65% for *E. coli*, 70% for *S. aureus*, and 58% for *P. aeruginosa*) demonstrate the potential of Ag-Se nanoparticles in preventing bacterial colonization and biofilm-related infections. The enhanced anti-biofilm activity of Ag-Se nanoparticles compared to individual Ag and Se nanoparticles can be attributed to their combined action in disrupting the signaling pathways involved in biofilm formation and promoting biofilm dispersal.

The statistical analysis further confirms the efficacy of Ag-Se nanoparticles in both antibacterial and anti-biofilm activities, with significant differences noted between Ag-Se and the individual nanoparticles. This reinforces the hypothesis that the co-formation of silver and selenium in nanoparticles enhances their antimicrobial properties. Moreover, the results of this study align with previous research that has reported the advantages of combining different metallic nanoparticles to improve their antibacterial activity and reduce the likelihood of resistance development.

5. Conclusion

The findings of this study demonstrate that Ag-Se nanoparticles synthesized from endophytic fungi hold significant promise as effective antimicrobial agents against pathogenic bacteria and biofilm formation. Their superior antibacterial and anti-biofilm activities highlight their potential for application in medical and industrial settings where bacterial contamination is a concern. Future research should focus on elucidating the exact mechanisms of action of Ag-Se nanoparticles and evaluating their efficacy in *in vivo* models to assess their therapeutic potential in clinical applications further. The integration of green synthesis methods, such as the use of endophytic fungi, adds an eco-friendly dimension to the development of these nanoparticles, paving the way for sustainable approaches in nanomedicine.

6. References

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