



Antimicrobial Activity of Biosynthesized Silver Nanoparticles Against Bacterial and Fungal Pathogens: A Comparative Experimental Analysis

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Abstract

The biologically synthesized silver nanoparticles (AgNPs) offer a natural alternative for microbial pathogen management and offer alternative to traditional chemical synthesis. The results of this study showed that two AgNPs (S1_9 and S4_7) formulations prepared with *Pseudomonas* sp. metabolites exhibited comparable antimicrobial activities. Under various synthesis conditions, Z9.3 with various conditions. Six human-associated bacterial pathogens, three phytopathogenic bacteria and four fungal pathogens were analyzed in open experimental data at multiple levels of AgNP. Bacterial activity was measured as inhibition-zone and fungal responses as percentage growth inhibition compared to untreated controls. The effects of formulation, concentration, pathogen and formulation by concentration were evaluated by descriptive statistics and factorial analysis of variance. Both preparations showed an increase in antibacterial activity with increasing concentration and the mean inhibition zones provided by S1 were greater than S4. The species of bacteria and formulation and concentration were found to have significant effects on the antibacterial response and the formulation-by-bacterial-species interaction was not significant. There was also a concentration dependent effect on the antifungal activity, S4 being more effective than S1, on each of the fungi tested. *Alternaria* sp. was the most responsive fungal pathogen especially at 120 concentration units. These results show that the biosynthesized AgNPs have antimicrobial activity and that this effect is formulation-dependent and concentration-dependent, thus warranting further study to be used as antimicrobial materials in biomedical and agricultural applications. The analysis provides a reproducible comparative framework.

Keywords: Silver nanoparticles, Biosynthesis, Antibacterial activity, Antifungal activity, Nanobiotechnology

1. Introduction

A growing need for antimicrobial agents that can combat a wide range of bacterial and fungal pathogens persists due to antimicrobial resistance and chronic microbial diseases. The metallic nanomaterials have been paid attention to due to the fact that at the nanoscale, they present physicochemical properties which are very different from those of the bulk. Among such systems, silver nanoparticles (AgNPs) are remarkable for their wide antimicrobial spectrum and their capacity to regulate the interactions with microbial membranes, proteins, nucleic acids, and redox processes. Biologically fabricated AgNPs have exhibited antibacterial and antifungal activity, and raised key questions about selectivity and mammalian-cell compatibility (Khan et al., 2020). Biological metabolites are attractive as they serve as reducing and stabilizing agents, which could also decrease the number of hazardous substances and make it easier to produce nanoparticles. Thus, Green synthesis is a crucial novel direction in Antimicrobial Nanotechnology (Roy et al., 2019). In addition, very recently, biosynthesized AgNPs have been found to inhibit bacterial and fungal phytopathogens as well, which suggest their use as not only clinical infection controlling agents but also in other directions as well (Khan et al., 2021).

The antimicrobial activity of AgNPs is influenced by the method of synthesis, the size and shape of particles, the biological capping molecules, the concentration of the nanoparticles, and the type of microorganisms. Studies on bioactive AgNPs highlight the importance of green synthesis for obtaining particles with significant antibacterial activity, and biological substances for controlling the nucleation and stabilization process (Huq et al., 2022). Further, experimental studies have shown that the structurally characterized green AgNPs are capable of inhibiting bacterial growth, which establishes successful formation of nanoparticles and biological performance (Asif et al., 2022). Plant mediated systems also demonstrate functional AgNPs with measurable antibacterial activity in naturally reducing environments (Shanmugam et al., 2022). Additionally, biosynthesized nanoparticles have been reported to exhibit antibacterial and antioxidant activities, and biological compatibility is crucial for practical applications (Suman et al., 2022). This indicates that efficiency in the antimicrobial activity of silver nanomaterials must be considered along with synthesis parameters and the susceptibility of the individual organisms and not as an universal parameter.

Fungi pathogens pose an added problem due to the different cellular organisation from bacteria. The green-synthesized AgNPs have been tested for therapeutic activity against candidiasis, indicating that biologically synthesized AgNPs can have significant antifungal effects (Abdallah & Ali, 2022). Activity has also been observed against phytopathogenic fungi which may have applications in agriculture, where fungal growth has a direct impact on plant health and crop productivity (Dashora et al., 2022). In an analogous manner, biosynthesized AgNPs have shown inhibition of bacterial plant pathogens like *Pectobacterium carotovorum*, suggesting that nanoparticles can be used in microbial control in various microbial systems (Beltrán Pineda et al., 2022). Physicochemical characterization still plays an important role in this type of research, as the size of the nanoparticles, their surface characteristics, and the biomolecules that they carry can influence the interaction between nanoparticles and microorganisms (Wasilewska et al., 2023). Comparative antifungal analysis with *Fusarium* and *Rhizopus* also reveals that susceptibility to green AgNPs can significantly differ between fungal genera (Moreno-Vargas et al., 2023) and combination studies with *Candida* and *Aspergillus* support that green AgNPs can be used in conjunction with existing antifungal drugs (Amini et al., 2023).

The concentration is also an important factor of the antimicrobial response. As the exposure of nanoparticles increases, so can the contact to the surfaces of the microorganisms and the biologically available silver (which can differ depending on the organism). AgNPs synthesized by ultrasound-assisted green approach have shown

their antibacterial effect in a concentration-dependent manner (Fatimah et al., 2023) and against the use of multidrug resistant *Acinetobacter baumannii* (Gautam et al., 2023), highlighting their potential relevance to tackle challenging bacterial infections. But, few analyses have investigated two biosynthesized AgNP preparations in human-associated bacteria, phytopathogenic bacteria and fungi with experimental measurements at the replicate level within a single analytical framework. These comparisons can be used to separate formulation specific and pathogen specific responses from general concentration responses.

These results provide a reproducible framework for assessing antimicrobial efficacy based on different synthetic conditions. To discern dose-response patterns without merging assay scales, replicate-level observations were retained and bacterial inhibition zones were separated from standardized fungal growth inhibition. This methodology also allows to test formulation and concentration effects.

Hence, a comparative secondary analysis of the antimicrobial data generated from experiments was undertaken in the present study for two biosynthesized AgNP preparations, which were prepared under different conditions. The analysis separated bacterial and fungal assay endpoints, and quantified fungal inhibition relative to untreated controls to facilitate a biologically appropriate interpretation.

Research objectives:

1. To evaluate concentration-dependent antimicrobial activity of biosynthesized silver nanoparticles against selected bacterial and fungal pathogens.
2. To compare the antimicrobial efficacy of S1 and S4 biosynthesized silver nanoparticle preparations across pathogen groups.
3. To determine pathogen-specific susceptibility patterns and quantify formulation, concentration, and interaction effects on antimicrobial responses.

2. Methodology

2.1 Study Design and Experimental Dataset

Open experimental data was analyzed secondarily quantitatively. The data set included characterization and antimicrobial data of the AgNPs biosynthesized using the metabolites of *Pseudomonas* sp. The dataset was downloaded from Zenodo repository (Arslan et al., 2024) and was labeled as Z9.3. The repository contained antibacterial measurements for six human-associated bacterial pathogens, three phytopathogenic bacteria and antifungal measurements for four fungal pathogens. Experimental variability was maintained by retaining individual replicate observations.

2.2 Biosynthesized Silver Nanoparticle Preparations

Two AgNP preparations were tested which were biologically synthesized. S1_9 was synthesized at a pH of 9 at a ratio of 5:1 (bacterial filtrate: 5 mM AgNO₃), and S4_7 was synthesized at a pH of 7 at a ratio of 2:4 (bacterial filtrate: 5 mM AgNO₃). These preparations were referred to as S1 and S4 for statistical analysis purposes. The concentrations of bacteria used were 10, 25, 50, 75 and 100; and the concentrations of fungi used were 30, 60 and 120. There were three replicates for each treatment.

2.3 Bacterial and Fungal Antimicrobial Outcomes

The response variable was the diameter of the inhibition zone, which was used for the analysis of bacterial activity. The bacteria panel included *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus* sp., *Salmonella* sp., *Pseudomonas aeruginosa*, *Escherichia coli*, two *Xanthomonas campestris*, and *Pseudomonas syringae* DC3000. The fungi analysed were *Alternaria* sp., *Stemphylium* sp., *Rhizopus* sp. and *Fusarium* sp. Due to a higher activity of smaller growth diameters, fungal data were converted to percentage growth inhibition compared to an untreated species-

specific control.

2.4 Data Processing and Derived Measures

Imported three antimicrobial CSV files with a delimiter of ‘;’ and numbers in decimal format. Data were converted from wide to long format, treatment labels were broken down to get the formulation and concentration, and organism names were harmonized for display. In the case of replicate observations, the mean and standard deviation were calculated. The percentage of inhibition of fungal growth was calculated as (control growth - treatment growth)/control growth x 100%. Because these were different endpoints for the assays, the determination of the bacterial inhibition-zone diameters and diameters of fungal growth were not combined.

2.5 Statistical Analysis

Data was analyzed for each formulation, concentration and pathogen using descriptive statistics. Bacterial inhibition zones and fungal percentage inhibition were analyzed separately by factorial analysis of variance (ANOVA). For each model, the factors examined were the AgNP formulation, the concentration, the pathogen and the interaction between the AgNP formulation and concentration. Partial eta squared was used as effect-size measure. Results were considered statistically significant if $p < 0.05$. All analyses were run in python with pandas, SciPy, statsmodels and Matplotlib.

3. Results

3.1 Experimental Profile

The analytical data set comprised 13 pathogens, including 6 human associated bacteria, 3 phytopathogenic bacteria and 4 fungi. Three replicates were used in the evaluation of both S1 and S4. Five concentration levels were used in the bacterial experiments and three in the fungal experiments. The experimental structure is summarized in Table 1 and the distribution of the pathogen-groups is shown in Figure 1.

Table 1. Experimental Profile of Biosynthesized AgNPs and Tested Pathogens

Experimental component	Dataset profile
AgNP preparations	S1_9 and S4_7
Human-associated bacteria	6 species
Phytopathogenic bacteria	3 species
Fungal pathogens	4 species
Bacterial concentrations	10, 25, 50, 75, 100
Fungal concentrations	30, 60, 120
Replicates	3 per condition

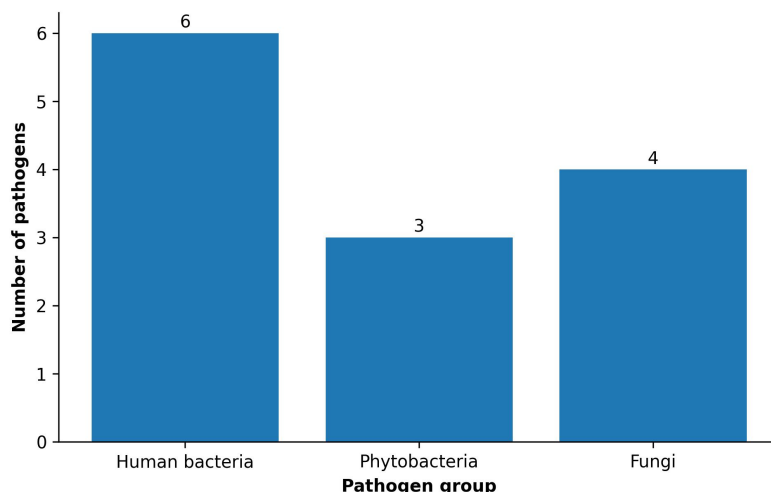


Figure 1. Distribution of Pathogen Groups Included in the Comparative Analysis

3.2 Concentration-Dependent Antibacterial Activity

For both preparations, the antibacterial activity increased gradually as the concentration of the preparations increased. The pooled mean inhibition zone of S1 was 10.02 mm at 10 and 13.03 mm at 100. As a result, S1 was greater than S4 at all matched concentrations (Table 2; Figure 2). In general, *X. campestris* var tomato was most effective in all treatments (11.59 mm) and *Enterococcus* sp. least effective (10.06 mm). The heat map in Figure 3 shows that the magnitude of the response was consistently different depending on the pathogen, formulation and concentration.

Table 2. Concentration-Dependent Antibacterial Activity of S1 and S4 AgNPs

AgNP	Concentration	Mean inhibition zone (mm)	SD
S1	10.0	10.02	1.06
S1	25.0	11.27	0.99
S1	50.0	12.15	1.11
S1	75.0	12.46	1.08
S1	100.0	13.03	1.29
S4	10.0	8.4	1.4
S4	25.0	9.14	1.28
S4	50.0	9.71	1.21
S4	75.0	10.33	1.03
S4	100.0	11.16	0.75

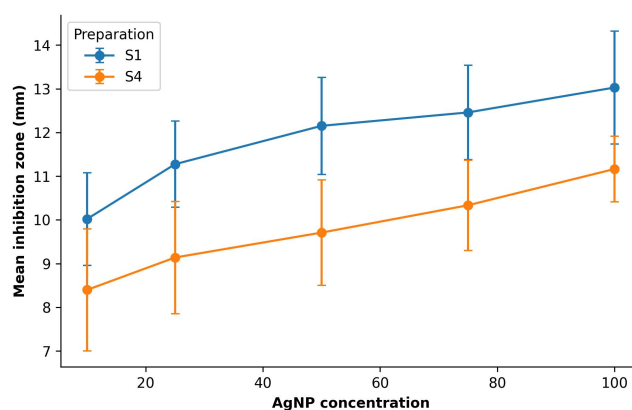


Figure 2. Concentration-Dependent Antibacterial Activity of S1 and S4 AgNPs

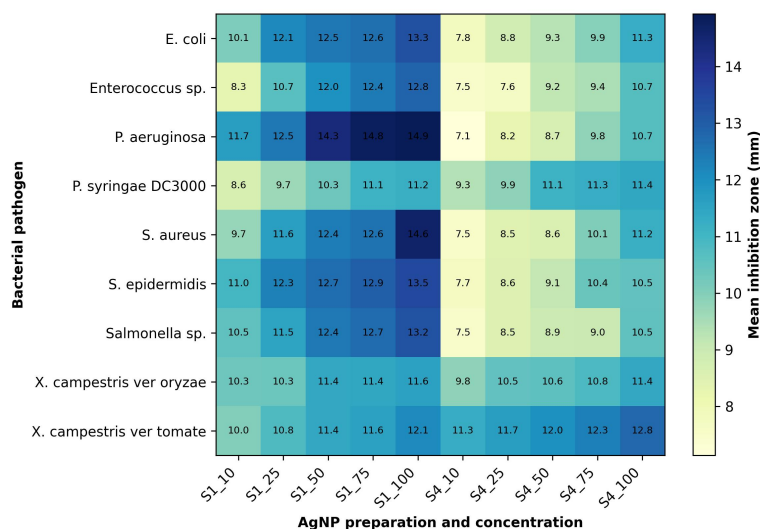


Figure 3. Antibacterial Activity Heatmap Across Pathogens, Preparations, and Concentrations

3.3 Comparative Antifungal Activity

The concentration dependence of the fungal inhibition was very strong, and at the same concentration the inhibition for S4 was always higher than for S1 (Table 3). *Alternaria* sp. was the most susceptible. The mean inhibition values obtained at concentration 120 were 65.67% and 74.98% for S1 and S4 respectively. *Stemphylium* sp. was 49.57% and 66.29%. Intermediate inhibition was observed with *Fusarium* sp. which was 38.52% in S1 and 45.60% in S4 while *Rhizopus* sp. was least responsive at 23.10% and 32.71%. As shown in Figure 4, the level of inhibition and the separation between formulations increase.

Table 3. Antifungal Activity and Growth Inhibition of Biosynthesized AgNPs

Fungal pathogen	AgNP	Concentration	Mean growth (mm)	Growth inhibition (%)	SD
<i>Alternaria</i> sp.	S1	30.0	68.94	22.31	0.26
<i>Alternaria</i> sp.	S1	60.0	37.5	57.74	1.72
<i>Alternaria</i> sp.	S1	120.0	30.46	65.67	2.33
<i>Alternaria</i> sp.	S4	30.0	34.42	61.21	3.47
<i>Alternaria</i> sp.	S4	60.0	24.82	72.03	0.76
<i>Alternaria</i> sp.	S4	120.0	22.21	74.98	1.58
<i>Fusarium</i> sp.	S1	30.0	58.34	11.0	0.84
<i>Fusarium</i> sp.	S1	60.0	46.84	28.54	6.48
<i>Fusarium</i> sp.	S1	120.0	40.3	38.52	2.47
<i>Fusarium</i> sp.	S4	30.0	47.05	28.23	2.73
<i>Fusarium</i> sp.	S4	60.0	40.11	38.81	2.74
<i>Fusarium</i> sp.	S4	120.0	35.66	45.6	2.94
<i>Rhizopus</i> sp.	S1	30.0	67.69	1.63	0.84
<i>Rhizopus</i> sp.	S1	60.0	56.86	17.37	5.26
<i>Rhizopus</i> sp.	S1	120.0	52.92	23.1	3.07
<i>Rhizopus</i> sp.	S4	30.0	55.3	19.64	0.1

Rhizopus sp.	S4	60.0	50.52	26.59	2.2
Rhizopus sp.	S4	120.0	46.31	32.71	3.32
Stemphylium sp.	S1	30.0	73.17	7.12	1.91
Stemphylium sp.	S1	60.0	53.74	31.79	6.69
Stemphylium sp.	S1	120.0	39.73	49.57	1.78
Stemphylium sp.	S4	30.0	55.27	29.85	1.44
Stemphylium sp.	S4	60.0	37.3	52.65	10.35
Stemphylium sp.	S4	120.0	26.56	66.29	1.69

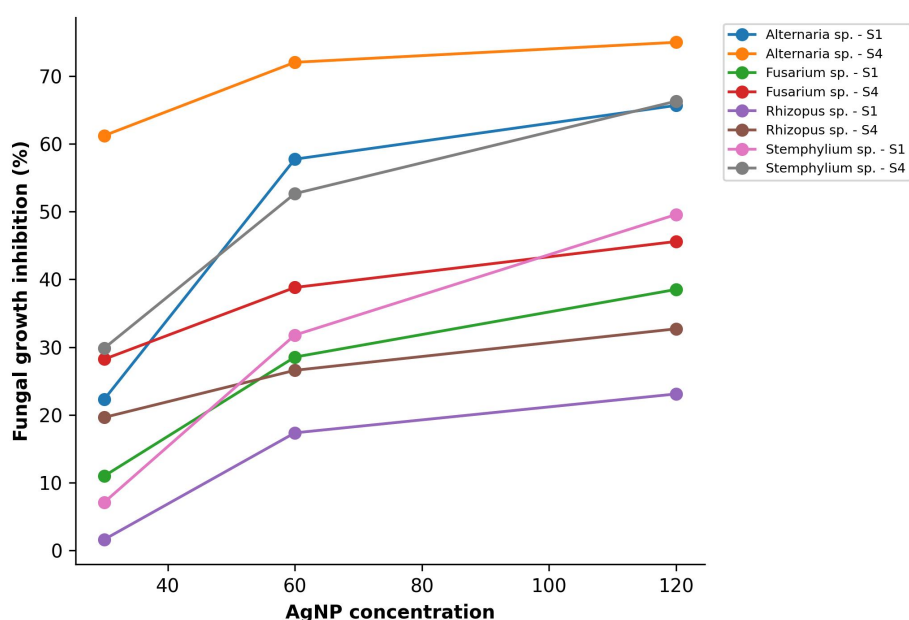


Figure 4. Concentration-Dependent Fungal Growth Inhibition by S1 and S4 AgNPs

3.4 Statistical Effects of Formulation, Concentration, and Pathogen

The results of the factorial analysis showed that the effect of the formulation ($F=248.68$, $p<0.001$, partial eta squared=0.497), the concentration ($F=59.00$, $p<0.001$, partial eta squared=0.484) and the pathogen ($F=5.58$, $p<0.001$, partial eta squared=0.150) was significant. The interaction between the formulation and concentration was not significant ($F=1.15$, $p=0.335$) suggesting broadly parallel increases as concentration increased for both S1 and S4. Fungicide formulation ($F=134.62$), concentration ($F=129.06$) and pathogen ($F=136.81$) were significant at $p<0.001$ for fungal inhibition. The formulation-by-concentration interaction also was significant ($F=8.67$, $p<0.001$), suggesting that the size of the S1-S4 difference varied with fungal concentration. The complete inferential results are given in Table 4.

Table 4. Effects of AgNP Formulation, Concentration, and Pathogen on Antimicrobial Activity

Analysis	Factor	df	F	p-value	Partial eta
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					squared
Antibacterial	AgNP formulation	1.0	248.68	<0.001	0.497
Antibacterial	Concentration	4.0	59.0	<0.001	0.484
Antibacterial	Pathogen	8.0	5.58	<0.001	0.15
Antibacterial	Formulation x concentration	4.0	1.15	0.335	0.018
Antifungal	AgNP formulation	1.0	134.62	<0.001	0.681
Antifungal	Concentration	2.0	129.06	<0.001	0.804
Antifungal	Pathogen	3.0	136.81	<0.001	0.867
Antifungal	Formulation x concentration	2.0	8.67	<0.001	0.216

4. Discussion

The comparative analysis showed that the biosynthesized AgNPs exhibited antimicrobial activity in all the groups of bacteria and fungi, however, the extent of the activity was formulation, concentration, and pathogen dependent. The trend of increasing the bacterial inhibition with the concentration is consistent with the overall findings that bio-synthesized AgNPs could impart effective antimicrobial activity if enough exposure of the nanoparticles is achieved. The antimicrobial activity of green-synthesized AgNPs against multidrug-resistant and clinically relevant bacteria has been demonstrated, highlighting their potential as alternative antimicrobial materials (Ajaykumar et al., 2023). Similar biological activity has been seen with plant mediated nanoparticles against other bacterial targets but the amount of inhibition is dependent on the chemistry of synthesis and the characteristics of the organism (Namulinda et al., 2023). The present bacterial results complement this evidence, as they demonstrate statistically significant bacteria formulation and concentration effect over the mixed panel of human associated and phytopathogenic organisms.

Formulation accounted for a significant portion of the variance in the response and S1 resulted in larger bacterial inhibition zones at all concentrations matched. This difference is significant as it shows that antimicrobial effect is not due to nanosilver alone. Comparative studies have also revealed that different green-synthesis conditions can result in differing antibacterial properties and biological activity (Lethongkam et al., 2023). The two preparations in the present dataset were prepared with different filtrate-to-silver ratio and pH, which are all factors that could influence nucleation, particle size, surface chemistry, and biological capping. Articles assessing green AgNP production methods highlight that synthesis variables and reducing agents play a crucial role in determining the dispersion, morphology, and antimicrobial activity of the nanoparticles (Gong et al., 2024). The non-significant formulation by concentration interaction implies a relative advantage of S1 throughout the concentration range tested and not just at a specific concentration.

Bacterial inhibition was also influenced by the identity of the pathogen, indicating that the susceptibilities were not the same. This heterogeneity is biologically reasonable since the composition of the microbial envelope, membrane permeability, efflux capacity, response to stress, and interactions with the surface of the nanoparticles could affect the susceptibility to silver. Biosynthesized AgNPs have been found to inhibit resistant pathogenic bacteria, which can suggest that nanosilver can also be active against the bacteria of various antimicrobial-resistance backgrounds (Swain et

al., 2024). In parallel, the antibacterial activity of the Banana-extract mediated AgNPs has been demonstrated, highlighting the common link between green synthesis of nanoparticles and antimicrobial properties (Ohiduzzaman et al., 2024). However, the present results should not be viewed as proof that one taxonomic group of bacteria is invariably more sensitive since the number of species included in the data set was limited and no attempt was made to provide matched measurements of cell-envelope responses.

The pattern in the fungal analysis was different: S4 was always more effective in inhibiting fungal growth than S1 and the interaction between formulation and concentration was significant. This means that between the synthesis-dependent difference grew or the difference in magnitude changed with an increase in fungicide exposure. Fungi susceptibility to green AgNPs has been shown in various systems such as the fungal source AgNPs which possesses measurable antifungal activities (Ahmad et al., 2024). Considering fungal infections, green AgNPs have shown effectiveness against drug-resistant *Candida auris*, which could be important for fungal infections (Verma et al., 2024). *Alternaria* sp. appeared the most sensitive and *Rhizopus* sp. appeared the least sensitive to the present dataset with *Stemphylium* and *Fusarium* being intermediate. These differences might be explained by differences in cell wall structure, growth, tolerance to oxidative stress and interactions with the silver species unique to each organism.

The concentration had a particularly strong effect in the case of fungi. However, greater inhibition of *Alternaria* ($\neq 75\%$) and *Stemphylium* ($\neq 66\%$) was observed at the highest concentration tested as compared to inhibition of *Fusarium* and *Rhizopus*. These patterns coincided with the related experimental study on AgNPs synthesized from *Pseudomonas* Z9.3, which revealed their high antibacterial and antifungal activity with *Alternaria* exhibiting a maximum sensitivity to S4-7 (Plokhovska et al., 2025). Moreover, the research on macrofungal mediated biosynthesis has revealed potential links to the antibacterial and wound healing applications of AgNPs, demonstrating the vast translational potential of biosynthesis of nanosilver (Vijayakumar et al., 2024). The present data analysis provides quantification of replicate variability and standardized fungal inhibition and factorial effects to aid interpretation of the underlying data beyond descriptive comparisons.

There are some restrictions in interpretation. This was secondary analysis and the organisms, concentrations, and assay designs were those of the original design. Because these two biological endpoints were different (bacterial inhibition zones and fungal growth diameters), they were analyzed independently with no attempt to directly rank bacteria against fungi. Resistant clinical isolates, standardized particle-dose metrics, nanoparticle physicochemical characteristics, and mammalian-cell toxicity should be included in future experiments.

5. Conclusion

This comparative experimental analysis revealed that silver nanoparticles biosynthesised using *Pseudomonas* sp. The Z9.3 metabolites showed detectable antimicrobial activity against a wide range of pathogens of humans and plants. The zone of inhibitory activity for both the nanoparticles preparations gradually increased with concentration and the S1 at the same concentration showed larger zone of inhibition than S4. The results of statistical analysis clearly showed that the formulation of AgNP, the concentration (AgNP dose), and the bacterial pathogen were significant, whereas the formulation-by-concentration interaction was not significant, which reflects broadly parallel dose-related antibacterial responses. Conversely, fungal growth inhibition was consistently higher in S4 and there were significant formulation, concentration, pathogen and interaction effects showing a more formulation-dependent antifungal response. *Alternaria* sp. exhibited the highest level of fungal susceptibility followed by *Stemphylium* sp. while the *Rhizopus* sp. was

comparatively less susceptible. The results highlight that apart from the concentration of nanoparticles, biosynthesis conditions and biological characteristics of pathogens play a key role in determining the antimicrobial efficacy. The differential activity of S1 and S4 towards the bacterial and fungal targets also underscores the need to optimise the production of nanoparticles for the desired antimicrobial activity. Biosynthesized AgNPs can thus be a potential candidate for antimicrobial development for biomedical and agriculture applications. The results, however, need to be expanded with standardized MIC, MBC, MFC assays, detailed physicochemical-response relationships, mechanistic studies, resistant clinical isolates and cytotoxicity testing prior to translation. This validation would help to understand efficacy, safety and formulation specific benefits under clinically and environmentally relevant conditions. Further studies should also correlate antimicrobial activity with the size, morphology, surface chemistry, silver ion release, and biological capping of the nanoparticles to determine the physicochemical parameters that enhance antimicrobial activity and negate potential toxicity.

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