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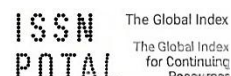
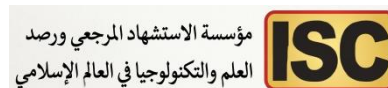
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Comparative evaluation of the efficiency of Endophytic *Aspergillus* and *Penicillium* spp. for gold nanoparticles biosynthesis

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Abstract:

Gold nanoparticles (AuNPs) are highly explored due to their interesting optical and physicochemical properties and biological synthesis is a cheaper and eco-friendly method to prepare AuNPs. In this study, the biosynthetic capacity of two fungal isolates *Penicillium* sp. (Eucalyptus leaves) and *Aspergillus* sp. (Capparis leaves) for the production of AuNP was compared. Visual color change, UV-Vis spectroscopy, particle size analysis based on microscopy and quantitative, transmission electron microscopy (TEM) and field-emission scanning electron microscopy (FESEM) were used as a means of assessing the fungal filtrates. AuNP produced by *Penicillium* sp. had a reproducible surface plasmon resonance peak of 507.00 ± 0.50 nm, with a maximum absorbance of 0.823 ± 0.012 AU, while for *Aspergillus* sp., the surface plasmon resonance peak was 376.00 ± 0.50 nm, and the maximum absorbance was 0.244 ± 0.007 AU. The differences in both cases were highly significant ($p < 0.001$), with the peak wavelength exhibiting a very large effect size ($r = 0.99998$) and the maximum absorbance having a very large effect size ($r = 0.99971$). The average particle size

of 60 microscopy derived measurements was 33.53 ± 10.21 nm with a range between 15.65 and 57.27 nm, corresponding to a polydisperse population of nanoscale particles. The shape of the particles was spherical or quasi-spherical, mostly, and dispersion and localized aggregation were variable as observed by electron microscopy. The CVs across the three independent replicates of *Penicillium* sp were 0.10%, 1.47% and 1.20% for peak wavelength, maximum absorbance and mean particle size respectively, respectively indicating good experimental reproducibility. *Penicillium* sp. has been found to be more reproducible and prolific AuNP associated response than *Aspergillus* sp. under the tested conditions making it an eco friendly biological system for gold nanoparticle biosynthesis.

Keywords: *Aspergillus* sp; Biosynthesis; Gold nanoparticles; Nanobiotechnology; *Penicillium* sp.; UV-Vis. spectroscopy;

1. Introduction

Over the past few decades, nanotechnology has been considered as one of a significant field with applications across different technological and scientific fields. It has been involved in

electronics, medicines, environmental remediation, and catalysis, making nanotechnology of the most promising areas of contemporary research (Sellami *et al.*, 2022). The physicochemical properties of gold nanoparticles (AuNPs) have attracted significant attention among the different types of nanomaterial because of their large surface area, biocompatibility, and exceptional optical properties associated with surface plasmon resonance (SPR) (Iravani, 2014). Traditional techniques for the synthesis AuNPs frequently rely on energy-intensive physical and chemical methods that involve hazardous substances, potentially leading to the aggregation of hazard residues.

Recent trends in nanoparticles synthesis have concentrated on synthesizing sustainable and environmentally friendly alternatives, particularly those based on natural approaches. The use of living organisms, such as bacteria, fungi, and algae in nanoparticles synthesis has raised as a viable and cost-effective method (Rampogu *et al.*, 2018).

The use of fungi in biosynthesis of NPs has raised owing to their ability to secrete substantial quantities of extracellular proteins and metabolites. These compounds act as stabilizing and reducing agents, facilitating the transformation of metallic ions into nanoparticles. The involvement of fungal species from genera, such as *Aspergillus* and *Penicillium* in the production of metallic nanoparticles, including silver and gold, has been thoroughly investigated (Kullai Setty *et al.*, 2011). The disparity in biosynthetic efficiency among different fungal species are remains as an interesting area of exploration, despite the large number of studies involving the use of fungi in nanoparticle production (Oyebamiji *et al.*, 2025). The capacity of fungi to synthesize nanoparticles can be significantly influenced by factors including enzyme activity, metabolic profiles, and environmental circumstances (Adeleke *et al.*, 2024).

Based on the available literature, there is an importance need to investigate efficient biological agents for the eco-friendly synthesis of nanoparticles. Fungal metabolites, such as protein, amino acid and polysaccharide, have been demonstrated to accomplish two major functions in NPs biosynthesis, reducing and stabilizing. Fungi are suitable for controlling nanoparticle production because their metabolites influence the size, shape, and stability of nanoparticles (Abd-El salam, 2024; Molnár *et al.*, 2018). System parameters, including pH, thermal condition, salt concentration and reaction time regulate mycosynthesis outcomes by driving fungal enzymatic activity and nucleation speed, thereby directly influencing the size geometry and colloidal behavior of the synthesized nanostructures (Bhatnagar & Aoyagi, 2025). The phytochemical diversity of *Eucalyptus* and *Capparis* supports endophytic fungal strain with strong enzymatic potential for metal ion reduction (Giri *et al.*, 2023; Osman *et al.*, 2024).

The present study is focused on the biosynthetic abilities of two fungal strains *Penicillium* species and *Aspergillus* species from *Eucalyptus* leaves and *Capparis* leaves respectively. The isolates were determined according to their morphological characteristics and AuNP biosynthesis was analyzed from visual evidence of synthesis, UV–Vis spectral characteristics, particle-size analysis from quantitative microscopy, TEM, FESEM and experimental reproducibility in replicate measurements.

2. Materials and Methods

2.1 . Samples collection and preparation

Eucalyptus and *Capparis* leaves were collected from a local garden at the University of Missan/Iraq, between October-November/2022. The collected leaves were transferred to the Biotechnology Lab at the University of Missan for further analysis.

2.2 . Surface Sterilization of Leaves

The collected leaves were subjected to a surface sterilization method to eliminate external contaminants, following the protocol described by (Greenfield *et al.*, 2015). This method was applied to isolate endophytic fungal species residing within the leaf tissue. The sterilized leaf fragments were sat on appropriate media for fungal isolation under aseptic condition.

2.3. Isolation of and Identification of Fungal Isolates

To allow the growth of fungi residing within the leaf, small pieces of sterilized leaves were placed on an appropriate development growth in potato dextrose agar medium PDA (consisting of 200g potato, 20g dextrose and 150 agar each one litter of distill water) and incubated for 3-7 day at 27 °C. The plates were examined daily to monitor fungal colonies growth. Subsequently, fungal isolates were sub-cultured and purified on PDA medium with chloramphenicol (0.5 g/ml) under aseptic conditions and incubated at 27 °C for (3-7) days (Molnár *et al.*, 2018).

The isolates were scrutinized microscopically and characterized based on their morphological characteristics.

2.4. Biosynthesis of Gold Nanoparticles (AuNPs)

The fungal isolates were sub-cultured in 1000 ml flask containing MGY medium (consist of 3 g yeast extract, 10 g glucose, 3 g malt extract and 5 g peptone in 1000 ml distilled water) and incubated at 27-28 °C with shaking at 120 rpm for 7-10 days. Later, the fungal mycelium was collected using Whatman filter paper and washed with sterile distilled water (DW) repeatedly to erase medium traces. Ten grams dry weight of mycelia were suspended in 100 ml deionized water and incubated in dark for 72 hours at 27 °C with shaking (Shariff *et al.*, 2026). The cells-free filtrate was collected using a vacuum filtration system. Following, 100 ml of 2 mM $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ was added as precursors to initiate the synthesis of gold nanoparticles (AuNPs) (Gupta & Chundawat, 2020).

2.5. Characterization of AuNPs

2.5.1 UV-Vis spectroscopy

UV-Vis spectroscopy was used to determine the optical properties of biosynthesized AuNPs. The absorption spectrum was measured at a wavelength range of 200-800 nm using a Double Pillar UV-1800 spectrophotometer (Shimadzu, Japan). Two ml of the prepared AuNPs suspension were transferred in a quartz cuvette, and D.W. was utilized as blank control (Xu *et al.*, 2024b).

2.5.2 Electron Microscopy and Particle-Size Assessment

characteristics of the synthesized AuNPs The morphology, dispersion, surface features and size were assessed by electron microscopy. The surface morphology after the deposition of the nanoparticle suspension on a specimen stub and drying at room temperature was observed by with respect to particle morphology and dispersion, SEM. TEM micrographs were examined with complementary high magnification information from the FESEM micrographs with regard microscopy images -to surface morphology and aggregate organization. The available electron size -scopy measurements which were used for quantitative particle were used to derive 60 mi analysis. The size of the particles was determined with help of the software ImageJ and then summarized using statistical procedures described in Section 2.6

2.6. Statistical Analysis

The microscopy-derived particle-size dataset ($n = 60$) was summarized using the mean, standard deviation (SD), median, interquartile range (IQR), minimum, maximum, coefficient of variation (CV), and 95% confidence interval (CI) for the mean. UV-Vis replicate measurements were reported as mean $\pm$ SD. Differences between *Penicillium* sp. and *Aspergillus* sp. in peak wavelength and maximum absorbance were evaluated using Welch's independent-samples t test. A p value < 0.05 was considered statistically significant. Reproducibility across three independent

Penicillium sp. replicates was assessed using SD and CV for peak wavelength, maximum absorbance, and replicate mean particle size. Effect size was quantified using the correlation-type effect size r derived from the Welch t statistic and its degrees of freedom, with values approaching 1.00 indicating a very large effect.

3. Results

3.1. Identification of Fungal Isolates

The fungal isolates were characterized on the basis of colony appearance, spore structure, and hyphal morphology. The Penicillium isolate showed the characteristic brush-like conidiophore architecture, whereas the Aspergillus isolate showed the characteristic conidial head arrangement.

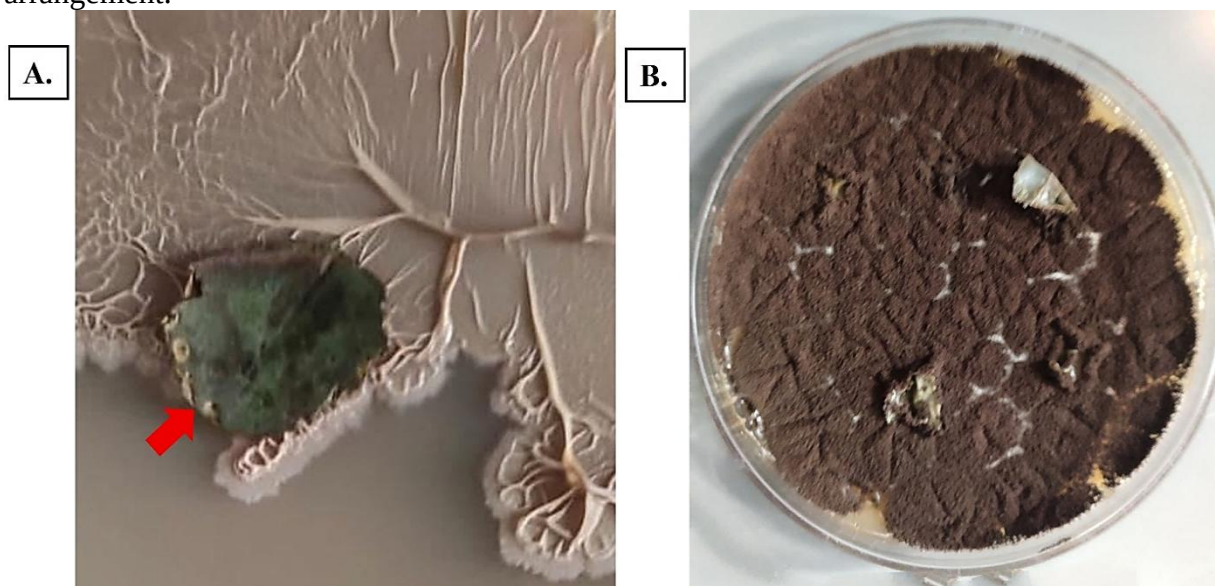


Figure 3.1. Growth of the fungal isolates on culture medium. A. Penicillium sp. isolated from Eucalyptus leaves. B. Aspergillus sp. isolated from Capparis leaves.

3.2. Biosynthesis of AuNPs

A visible color change was observed in the Penicillium sp. filtrate after addition of the gold precursor, whereas no apparent color change was observed in the Aspergillus sp. filtrate under the applied experimental conditions.

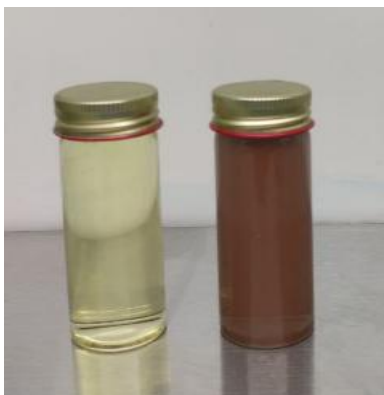


Figure 3.2. Visual indication of AuNP biosynthesis by Penicillium sp. before and after addition of $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$.



Figure 3.3. *Aspergillus* sp. filtrate with gold chloride showing no color alternation.

3.3. Characterization of Nanoparticles

3.3.1. UV–Visible Spectral Analysis

The UV–Vis replicate measurements demonstrated a marked difference between the two fungal preparations. *Penicillium* sp. exhibited a reproducible peak at 507.00 ± 0.50 nm, whereas *Aspergillus* sp. exhibited a peak at 376.00 ± 0.50 nm. Maximum absorbance was 0.823 ± 0.012 AU for *Penicillium* sp. and 0.244 ± 0.007 AU for *Aspergillus* sp.

Table 3.1. Statistical Comparison of UV–Vis Measurements between *Penicillium* sp. and *Aspergillus* sp.

Parameter	<i>Penicillium</i> sp. (Mean $\pm$ SD)	<i>Aspergillus</i> sp. (Mean $\pm$ SD)	Mean Difference	Welch t	df	p-value	Effect size / Interpretation
Peak wavelength (nm)	507.00 $\pm$ 0.50	376.00 $\pm$ 0.50	131.00	320.88	4.00	<0.001	r = 0.99998 Very large effect
Maximum absorbance (AU)	0.823 $\pm$ 0.012	0.244 $\pm$ 0.007	0.579	72.84	3.06	<0.001	r = 0.99971 Very large effect

Both UV–Vis comparisons were statistically significant ($p < 0.001$). The effect sizes were $r = 0.99998$ for peak wavelength and $r = 0.99971$ for maximum absorbance, indicating very large differences between the two fungal preparations. The 507 nm response was consistent with the AuNP-associated SPR observed for *Penicillium* sp., whereas the approximately 376 nm maximum in *Aspergillus* sp. was outside the characteristic AuNP-associated SPR response observed in the *Penicillium* preparation.

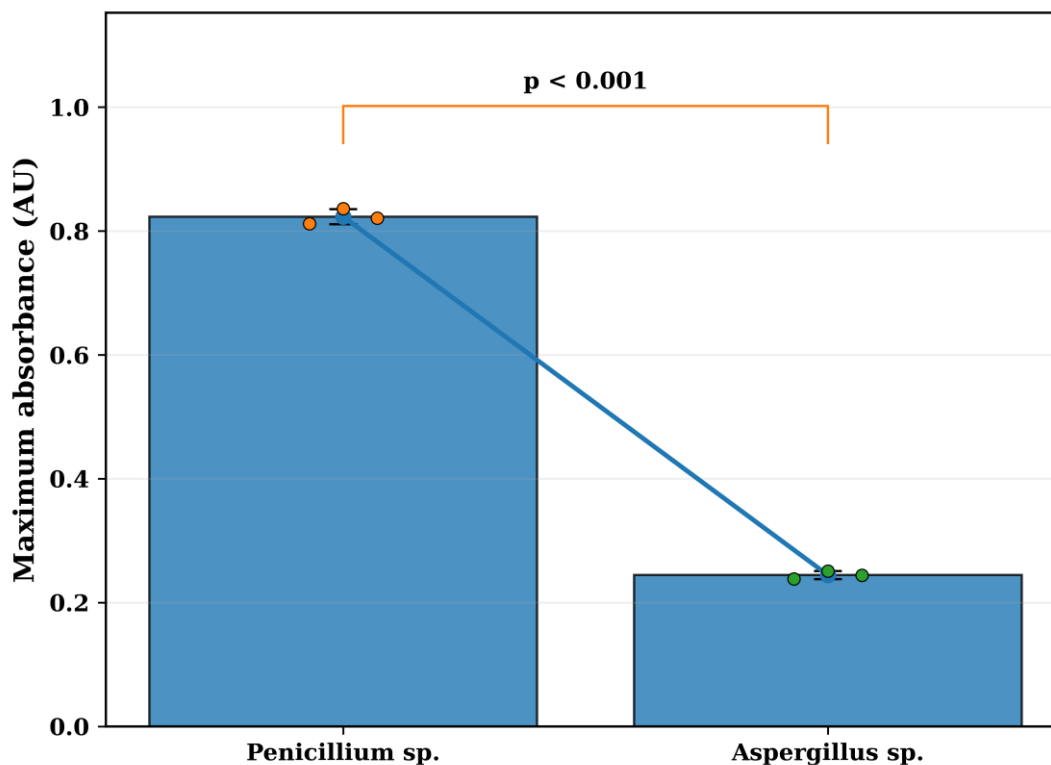


Figure 3.4. Comparison of UV-Vis Maximum Absorbance between *Penicillium* sp. and *Aspergillus* sp.

The columns represent mean maximum absorbance values, the error bars represent SD, and the overlaid line emphasizes the marked difference between the fungal preparations. The individual replicate points show close within-group agreement.

3.3.2. Quantitative Particle Size Analysis

Table 3.2. Descriptive Statistics of Microscopy-Derived AuNP Particle Size.

n	Mean (nm)	SD (nm)	Median (nm)	Q1 (nm)	Q3 (nm)	Minimum (nm)	Maximum (nm)	CV (%)	95% CI of Mean (nm)
60	33.53	10.21	31.55	27.34	41.90	15.65	57.27	30.45	30.89–36.16

Sixty microscopy-derived particle-size measurements were analyzed. The mean particle size was 33.53 ± 10.21 nm, with a median of 31.55 nm and an observed range from 15.65 to 57.27 nm. The interquartile range was 27.34–41.90 nm and the CV was 30.45%, indicating appreciable particle-size heterogeneity and a polydisperse nanoscale population.

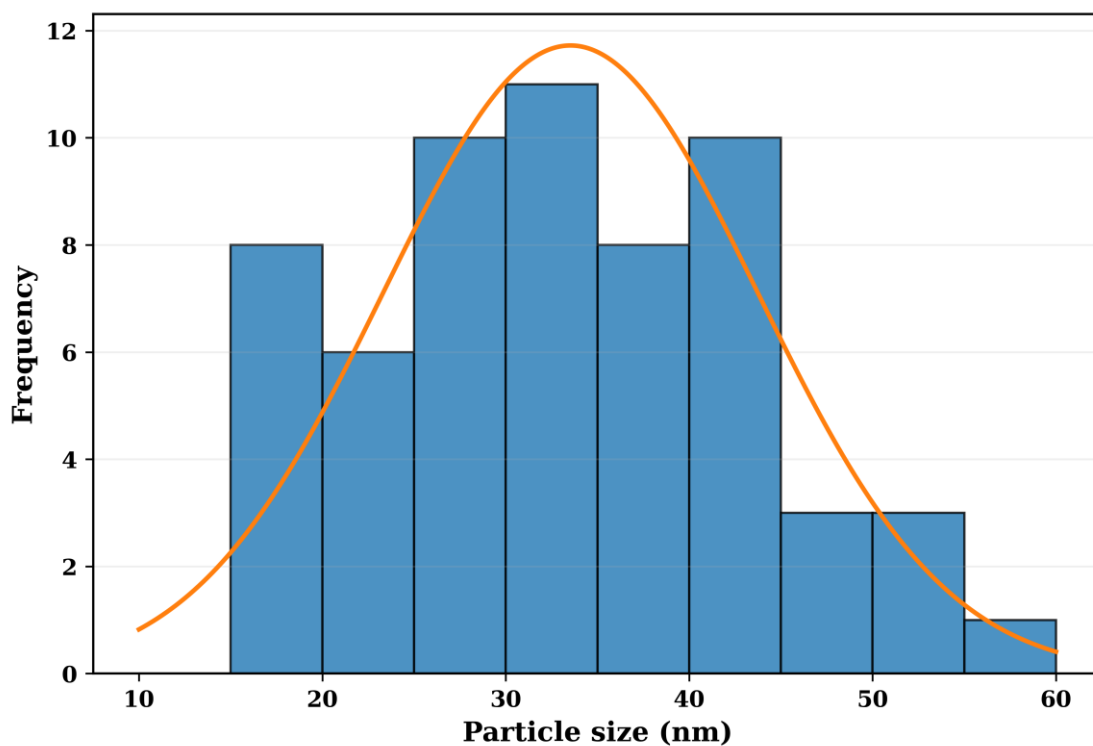


Figure 3.5. Particle Size Distribution of AuNPs.

The histogram shows that most particle sizes were concentrated approximately between 25 and 45 nm, with a smaller right-sided extension toward larger particles. The smooth overlaid curve summarizes the overall distribution pattern.

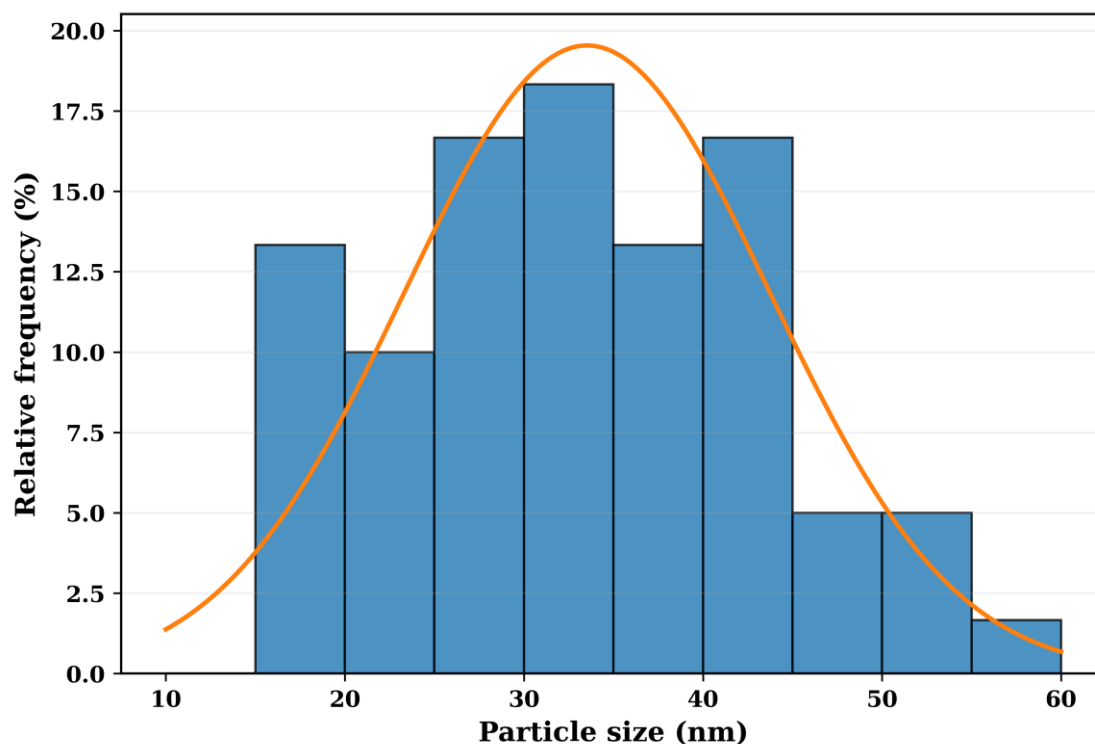


Figure 3.6. Relative Frequency Distribution of AuNP Particle Size.

The relative-frequency histogram confirms that the greatest proportion of observations occurred in the central size classes, with fewer particles at the lower and upper extremes.

3.3.3. Transmission Electron Microscopy (TEM)

TEM examination showed predominantly spherical to quasi-spherical electron-dense nanoparticles with variable dispersion and localized aggregation. In conjunction with the microscopy-derived quantitative dataset, the observed nanoscale population ranged from 15.65 to 57.27 nm with a mean size of 33.53 ± 10.21 nm.

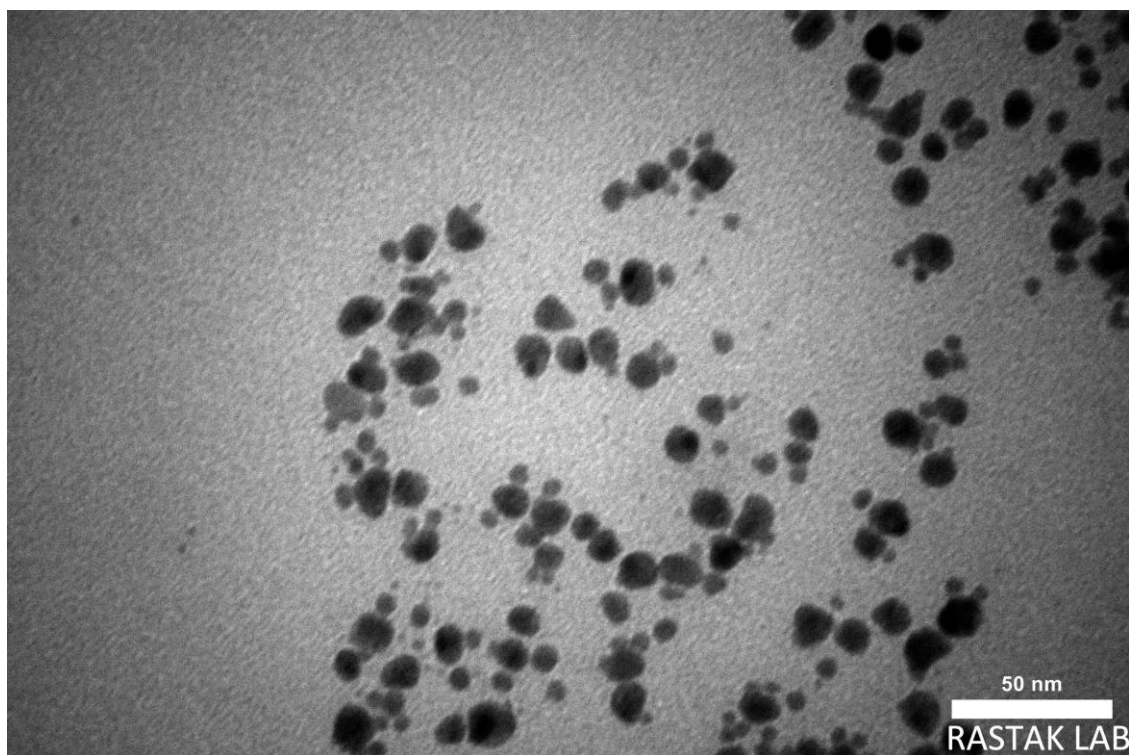


Figure 3.7. Representative TEM Micrograph of the AuNP Population at 50 nm Scale. This TEM field clearly shows numerous electron-dense nanoparticles over a broad area, allowing visualization of nanoscale dimensions, rounded morphology, and moderate particle-size variation.

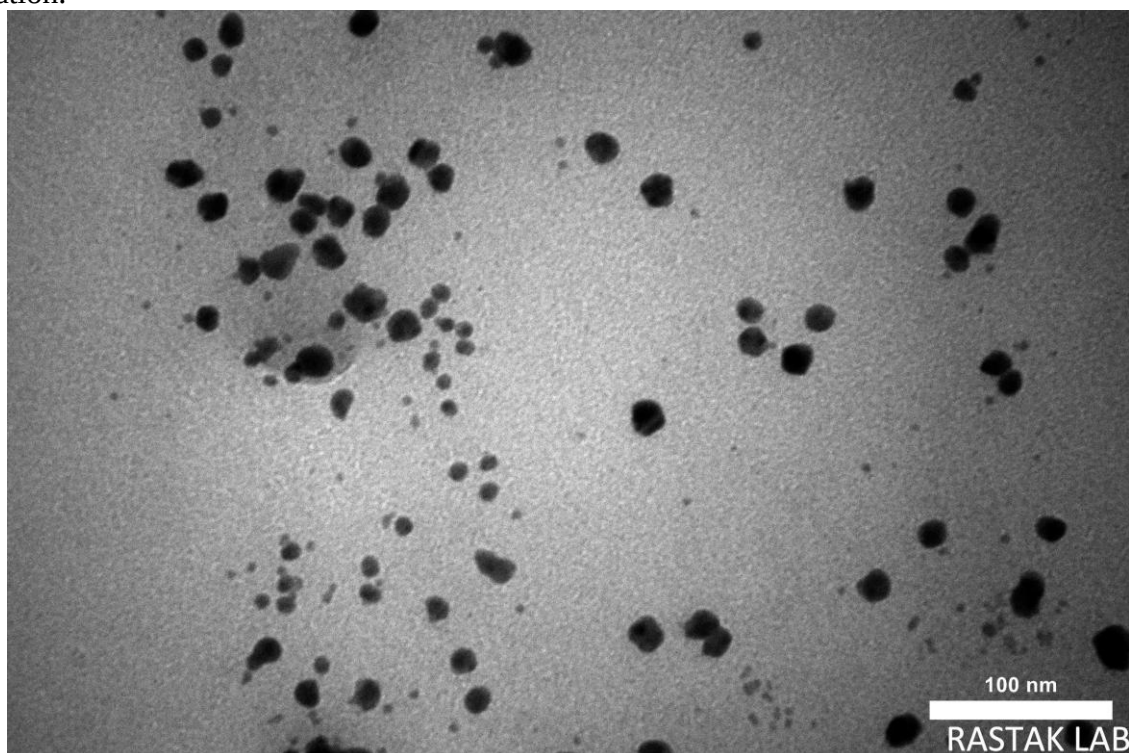


Figure 3.8. Representative TEM Micrograph of AuNP Dispersion at 100 nm Scale.

The lower-magnification TEM field demonstrates the overall dispersion pattern, with particles distributed throughout the field and localized areas of clustering.

3.3.4. Field-Emission Scanning Electron Microscopy (FESEM)

FESEM examination provided complementary information on the surface morphology and aggregate organization of the synthesized material at two magnification levels.

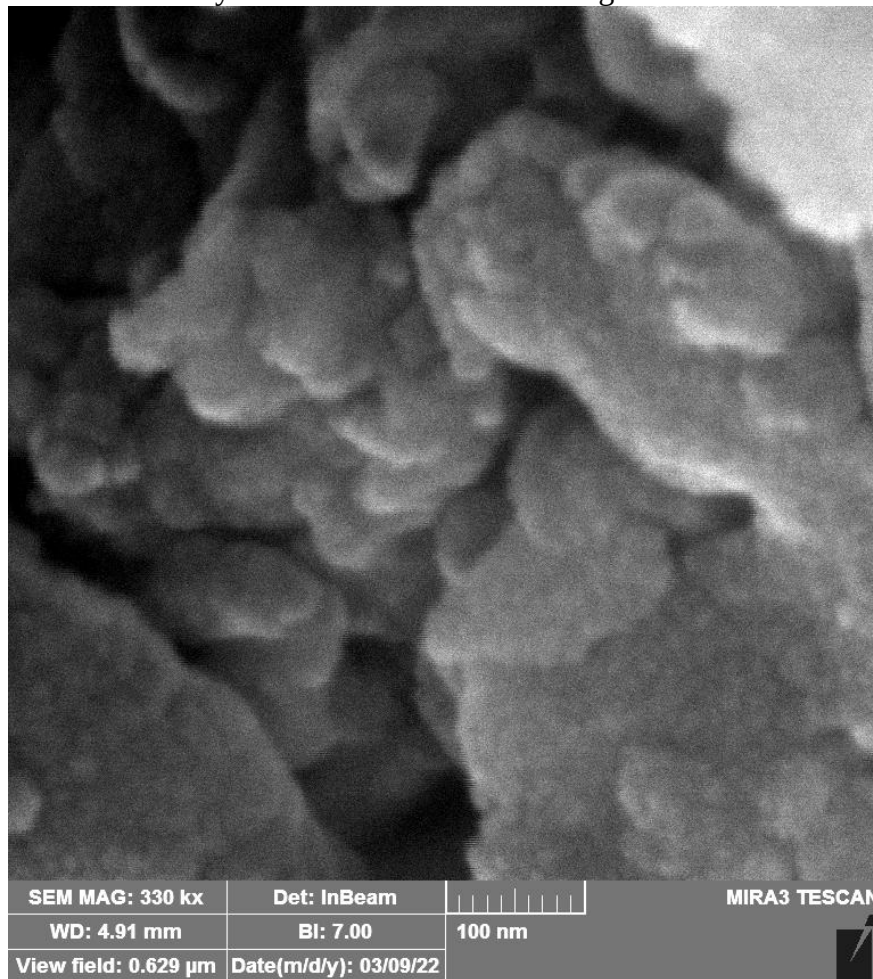


Figure 3.9. High-Magnification FESEM Micrograph (330 kx; Scale Bar = 100 nm).

The high-magnification FESEM image shows nanoscale surface morphology and compact aggregated structures at the 100 nm scale.

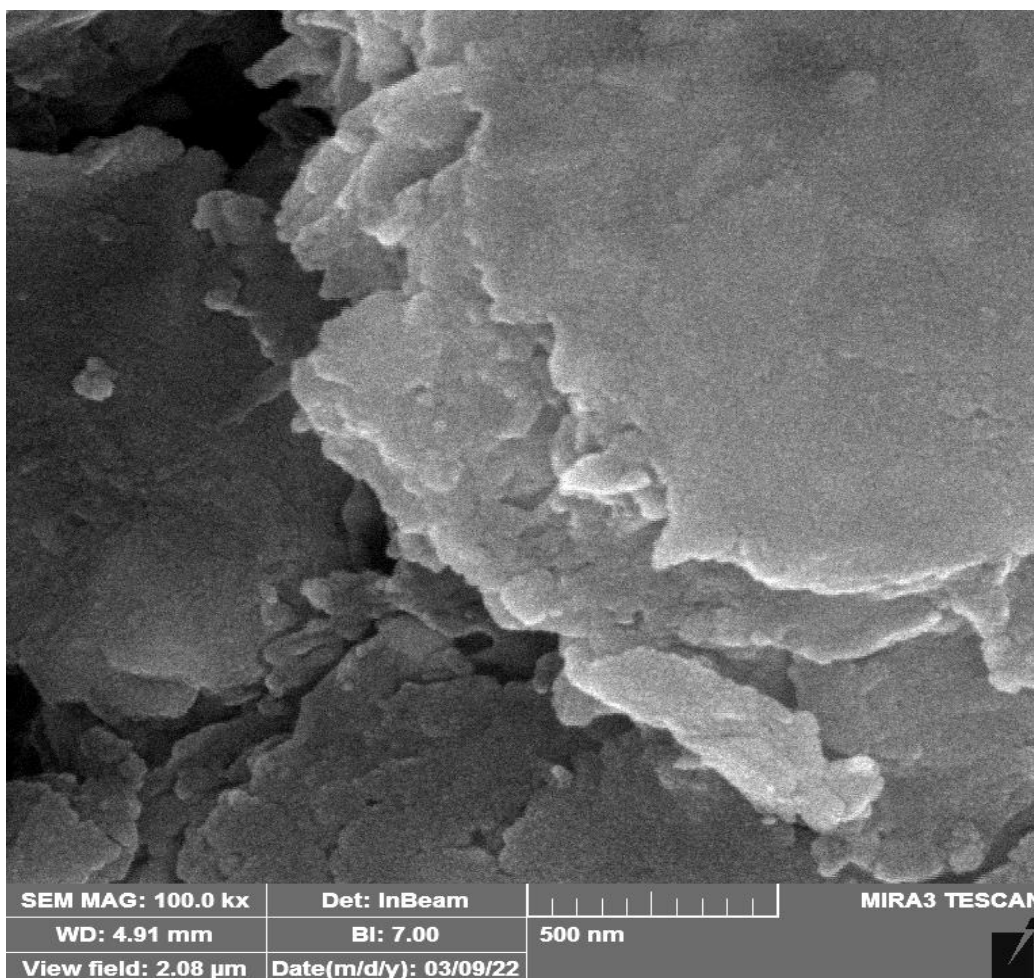


Figure 3.10. FESEM Micrograph (100 kx; Scale Bar = 500 nm).

The 100 kx FESEM field provides a broader view of the surface architecture and the larger organization of the aggregates.

3.3.5. Reproducibility of AuNP Biosynthesis

Table 3.3. Reproducibility of AuNP Biosynthesis across Experimental Replicates.

Replicate	Peak wavelength (nm)	Maximum absorbance (AU)	Mean particle size (nm)	Particle-size SD (nm)	Assessment
1	506.5	0.812	31.84	10.74	Consistent
2	507.0	0.836	32.61	11.08	Consistent
3	507.5	0.821	32.17	10.91	Consistent
Overall	507.00 ± 0.50 (CV 0.10%)	0.823 ± 0.012 (CV 1.47%)	32.21 ± 0.39 (CV 1.20%)	10.91	High reproducibility

Across the three *Penicillium* sp. experimental replicates, peak wavelength showed a CV of 0.10%, maximum absorbance a CV of 1.47%, and replicate mean particle size a CV of 1.20%. These low between-replicate CV values indicate good analytical and experimental reproducibility.

4. Discussion

The present study demonstrated a clear difference between the two endophytic fungal isolates in their response to gold nanoparticle biosynthesis under the applied experimental conditions. *Penicillium* sp. produced a reproducible AuNP-associated optical response at 507.00 ± 0.50 nm with a maximum absorbance of 0.823 ± 0.012 AU, whereas *Aspergillus* sp. showed a maximum at 376.00 ± 0.50 nm and a substantially lower absorbance of 0.244 ± 0.007 AU. The highly significant differences ($p < 0.001$) and very large effect sizes support a markedly stronger AuNP-associated response for *Penicillium* sp. in this experiment. This species-dependent behavior is biologically plausible because fungal synthesis of AuNPs is mediated by extracellular proteins, peptides, enzymes, phenolic compounds and other metabolites whose abundance and reducing capacity vary among fungi and according to culture conditions (Sawalha et al., 2025; Xu et al., 2024b). The absorption maximum near 507 nm observed for *Penicillium* sp. is consistent with the visible-region surface plasmon resonance behavior commonly reported for biosynthesized AuNPs. Recent fungal studies have shown that the position and intensity of the AuNP absorption band can vary with particle size, morphology, aggregation and the composition of the biological capping layer.

(Hammad et al., 2025), for example, reported endophytic-fungus-assisted AuNP formation with a characteristic visible-region absorption response, while recent reviews emphasize that pH, precursor concentration, fungal biomass, temperature and reaction time can shift nanoparticle optical and structural characteristics (Verma et al., 2024; Xu et al., 2024a).

The approximately 376 nm maximum recorded for *Aspergillus* sp. should therefore be interpreted cautiously. In the present study it was outside the AuNP-associated SPR response observed for the *Penicillium* preparation and was accompanied by no apparent color transformation and much lower absorbance. These findings provide weaker evidence for AuNP formation by the *Aspergillus* isolate under the tested conditions, but they should not be interpreted as evidence that *Aspergillus* species are intrinsically unable to synthesize AuNPs. Fungal nanoparticle production is strongly strain- and condition-dependent, and optimization of reaction variables can substantially influence nucleation, growth, particle morphology and final yield (Verma et al., 2024; Xu et al., 2024a).

Quantitative microscopy further supported the nanoscale nature of the particles associated with the *Penicillium* preparation. The 60 microscopy-derived measurements yielded a mean particle size of 33.53 ± 10.21 nm and a range of 15.65–57.27 nm. TEM showed predominantly spherical to quasi-spherical electron-dense particles, whereas FESEM provided complementary evidence of nanoscale surface morphology and localized aggregate organization. Comparable spherical or quasi-spherical morphologies and nanoscale dimensions have been reported in recent fungal-mediated AuNP studies, although the exact dimensions vary among isolates and synthesis protocols (Hammad et al., 2025; Olvera-Aripez et al., 2024).

The particle-size CV of 30.45% indicates appreciable heterogeneity and is consistent with a polydisperse population. Such variability is expected in biological synthesis because fungal filtrates contain complex mixtures of reducing and stabilizing biomolecules, creating multiple nucleation and growth environments. (Xu et al., 2024a) identified control of AuNP size and shape as a continuing challenge in fungus-mediated synthesis and noted that reaction temperature, pH, fungal biomass, precursor concentration and reaction time can be adjusted to improve control of nanoparticle characteristics. The localized aggregation observed by TEM and FESEM may likewise reflect interactions among particles and biomolecular capping components.

Importantly, particle-level heterogeneity did not prevent good between-replicate reproducibility. Across the three independent *Penicillium* sp. replicates, the CV was only 0.10% for peak wavelength, 1.47% for maximum absorbance and 1.20% for replicate mean particle size. These low between-replicate CV values indicate that the overall optical response and average particle dimensions were stable across repeated experiments, even though individual particles varied in size. This reproducibility strengthens the evidence that the observed *Penicillium*-associated response was not restricted to a single experimental run.

The stronger performance of *Penicillium* sp. may be related to differences in extracellular enzyme activity and secondary-metabolite secretion. Endophytic fungi can act as biological nanofactories because their enzymes, proteins, reducing sugars, phenolic compounds and other metabolites participate in metal-ion reduction and can subsequently cap and stabilize the newly formed nanoparticles (Sawalha *et al.*, 2025).

Accordingly, the

difference between the two isolates in the present study may reflect isolate-specific metabolic activity as well as the interaction between fungal physiology and the experimental environment rather than a universal difference between the two genera.

Overall, the combined evidence from visual observation, UV–Vis spectroscopy, quantitative particle-size analysis, TEM, FESEM and replicate reproducibility supports more effective AuNP biosynthesis by the *Penicillium* isolate under the conditions examined. Nevertheless, the findings remain specific to the tested isolates and experimental conditions. Additional characterization by X-ray diffraction, FTIR spectroscopy and zeta-potential analysis would strengthen confirmation of crystallinity, surface functional groups and colloidal stability. Future studies should also include multiple isolates and systematic optimization of pH, precursor concentration, biomass, temperature and reaction time to determine the extent to which the observed differences are species-, strain- or condition-dependent (Sawalha *et al.*, 2025; Verma *et al.*, 2024; Xu *et al.*, 2024b)

5. Conclusion

The present study demonstrated clear differences between the two endophytic fungal isolates in their response to gold nanoparticle biosynthesis under the tested experimental conditions. *Penicillium* sp., isolated from Eucalyptus leaves, showed a reproducible AuNP-associated surface plasmon resonance peak at 507.00 ± 0.50 nm with a maximum absorbance of 0.823 ± 0.012 AU, whereas *Aspergillus* sp., isolated from Capparis leaves, showed a peak at 376.00 ± 0.50 nm with a substantially lower maximum absorbance of 0.244 ± 0.007 AU. Both UV–Vis comparisons were statistically significant ($p < 0.001$) and showed very large effect sizes ($r = 0.99998$ for peak wavelength and $r = 0.99971$ for maximum absorbance). Quantitative analysis of 60 microscopy-derived particle-size measurements confirmed a nanoscale, polydisperse population with a mean particle size of 33.53 ± 10.21 nm and a range of 15.65–57.27 nm. TEM and FESEM observations showed predominantly spherical to quasi-spherical particles with variable dispersion and localized aggregation. Reproducibility across three independent *Penicillium* sp. replicates was high, with CVs of 0.10% for peak wavelength, 1.47% for maximum absorbance, and 1.20% for replicate mean particle size. Therefore, within the conditions examined in this study, *Penicillium* sp. exhibited stronger and more reproducible AuNP-associated biosynthetic performance than *Aspergillus* sp. Further optimization and complementary physicochemical characterization are recommended before broader conclusions are made regarding species-specific biosynthetic capacity.

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Conflict of Interest:

The author declare that there are no financial, personal, or professional conflicts of interest that could inappropriately influence, bias, or interfere with the findings, results, or interpretation presented in this manuscript.

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