International Science and Technology Journal
المجلة الدولية للعلوم والتقنية
Volume 39 العدد
Part 2 المجلد
ISTJ

Clove-Mediated Green Synthesis of Silver Nanoparticles and Their In Vitro Antimicrobial Activity Against Plant Pathogens

http://www.doi.org/10.62341/istj-vol39-2-NAS28

Received	**2026/10/14**	**تم استلام الورقة العلمية في**
Accepted	**2026/10/31**	**تم قبول الورقة العلمية في**
Published	**2026/11/01**	**تم نشر الورقة العلمية في**

Clove-Mediated Green Synthesis of Silver Nanoparticles and Their In Vitro Antimicrobial Activity Against Plant Pathogens

Nagia Mohamed Jadallah, Aboubakr Mansour Faraj

Department of Plant Protection, Omar Al-Mukhtar University
Al Bayda, Libya
nagia.mohamed@omu.edu.ly
[ORCID iD:0009-0008-2851-187X]

Abstract

Plant pathogenic bacteria and fungi cause major crop losses, while repeated reliance on conventional bactericides and fungicides raises concerns regarding resistance and environmental impacts. This study evaluated silver nanoparticles (AgNPs) prepared using an aqueous clove (Syzygium aromaticum) bud extract against Agrobacterium tumefaciens (MG706145), Pectobacterium carotovorum subsp. carotovorum (ON248562.1), and Alternaria alternata. AgNPs were prepared by mixing 90 mL of 1 mM $AgNO_3$ with 10 mL of crude clove extract, followed by heating at 60 °C for 45 min and incubation in the dark for 24 h. SEM of the clove-mediated AgNPs showed predominantly spherical particles, with particle sizes ranging from 22.95 to 32.95 nm and a mean size of 27.54 ± 3.82 nm and a silver content of 43.87 ± 0.62 wt% by EDX. Antibacterial activity was evaluated by inhibition-zone diameter using three replicates per treatment. AgNPs produced larger inhibition zones than the clove extract against Agrobacterium tumefaciens (MG706145) (14.67 ± 1.15 vs. 7.67 ± 1.15 mm) and P. carotovorum subsp. carotovorum (13.67 ± 0.58 vs. 10.67 ± 1.15 mm). Two-way ANOVA showed a significant treatment effect ($F_{1,8}$ = 69.23, $p < 0.001$) and treatment × bacterium interaction ($F_{1,8}$ = 11.08, $p = 0.010$). For A. alternata, AgNPs reduced colony diameter from 15.67 ± 0.58 mm at 5% to 3.67 ± 1.15 mm at 20% (one-way

Clove-Mediated Green Synthesis of Silver Nanoparticles and Their In Vitro Antimicrobial Activity Against Plant Pathogens
http://www.doi.org/10.62341/istj-vol39-2-NAS28

ANOVA, $F_{3,8} = 43.63$, $p < 0.001$), corresponding to 82.6–95.9% mycelial growth inhibition when calculated against a 90-mm untreated control. Overall, the results indicate enhanced antibacterial activity of clove-mediated AgNPs relative to the extract alone and a concentration-dependent antifungal effect against A. alternata.

Keywords: Silver nanoparticles; Green synthesis; Syzygium aromaticum; Clove extract; Plant pathogens; Antibacterial activity; Antifungal activity

التخليق الأخضر لجسيمات الفضة النانوية بوساطة القرنفل ونشاطها المضاد للميكروبات مختبريًا ضد مسببات أمراض النبات

نجية محمد جادالله، ابوبكر منصور فرج

قسم وقاية النبات جامعه عمر المختار/ البيضاء_ ليبيا
nagia.mohamed@omu.edu.ly

الملخص

تهدف هذه الدراسة إلى تقييم النشاط المضاد للميكروبات لجسيمات الفضة النانوية (AgNPs) المُحضَّرة بالتخليق الأخضر باستخدام المستخلص المائي لبراعم القرنفل (Syzygium aromaticum) ضد بعض مسببات أمراض النبات. حُضِّرت الجسيمات النانوية بخلط 90 مل من نترات الفضة ($AgNO_3$) بتركيز 1 ملي مولار مع 10 مل من مستخلص القرنفل الخام، ثم التسخين عند 60 °م لمدة 45 دقيقة مع التحريك المستمر، تلاه التحضين في الظلام لمدة 24 ساعة. أظهرت صور المجهر الإلكتروني الماسح (SEM) جسيمات غالبًا كروية، تراوحت أحجامها بين 22.95 و32.95 نانومتر، بمتوسط قدره 27.54 ± 3.82 نانومتر، بينما أظهر تحليل طيف الأشعة السينية المشتتة للطاقة (EDX) محتوى من الفضة قدره 43.87 ± 0.62% بالوزن. تم تقييم النشاط المضاد للبكتيريا من خلال قياس أقطار مناطق التثبيط باستخدام ثلاث مكررات لكل معاملة. حققت جسيمات الفضة النانوية مناطق تثبيط أكبر من مستخلص القرنفل ضد

Clove-Mediated Green Synthesis of Silver Nanoparticles and Their In Vitro Antimicrobial Activity Against Plant Pathogens
http://www.doi.org/10.62341/istj-vol39-2-NAS28

Agrobacterium tumefaciens (MG706145)، حيث بلغت 14.67 ± 1.15 مم مقارنةً بـ7.67 ± 1.15 مم للمستخلص، وضد Pectobacterium carotovorum subsp. carotovorum، حيث بلغت 13.67 ± 0.58 مم مقارنةً بـ10.67 ± 1.15 مم للمستخلص. أظهر تحليل التباين ثنائي الاتجاه تأثيرًا معنويًا للمعاملة ($F_{1,8}$ = 69.23، $p < 0.001$)، وكذلك تفاعلًا معنويًا بين المعاملة والبكتيريا ($F_{1,8}$ = 11.08، $p = 0.010$). وبالنسبة للفطر Alternaria alternata، أدت جسيمات الفضة النانوية إلى خفض قطر المستعمرة من 15.67 ± 0.58 مم عند تركيز 5% إلى 3.67 ± 1.15 مم عند تركيز 20%، وفقًا لتحليل التباين أحادي الاتجاه ($F_{3,8}$ = 43.63، $p < 0.001$)، وهو ما يعادل تثبيطًا للنمو الميسيلي بنسبة تراوحت بين 82.6 و95.9% مقارنةً بالكنترول غير المعامل البالغ قطره 90 مم. تشير النتائج إلى أن جسيمات الفضة النانوية المُحضَّرة باستخدام القرنفل عززت النشاط المضاد للبكتيريا مقارنةً بالمستخلص المائي وحده، كما أظهرت تأثيرًا مضادًا للفطر يعتمد على التركيز. وتبرز هذه النتائج أهمية إجراء دراسات لاحقة أكثر تفصيلًا تشمل تحديد الحد الأدنى للتركيز المثبط (MIC)، والحد الأدنى للتركيز القاتل للبكتيريا (MBC)، والحد الأدنى للتركيز القاتل للفطريات (MFC)، إلى جانب تقييم تأثيرها على النباتات وإجراء تجارب تحت ظروف حيوية.

الكلمات المفتاحية: جسيمات الفضة النانوية؛ التخليق الأخضر؛ Syzygium aromaticum؛ مستخلص القرنفل؛ مسببات أمراض النبات؛ النشاط المضاد للبكتيريا؛ النشاط المضاد للفطريات.

1. Introduction

Bacterial and fungal diseases are among the major constraints on crop production. Agrobacterium tumefaciens causes crown gall disease, Pectobacterium carotovorum subsp. carotovorum (ON248562.1) is a widespread soft-rot pathogen affecting cultivated plants, and Alternaria alternata includes pathotypes responsible for diseases in important crops (Gohlke and Deeken, 2014; Perfileva et al., 2025; Tsuge et al., 2013). Conventional disease-management programs commonly rely on copper compounds, antibiotics, and synthetic fungicides, while repeated use can select for resistant

pathogen populations and raise environmental concerns (Fan et al., 2022; Sundin and Wang, 2018; Yang et al., 2019).

Plant-derived products represent a potential complementary strategy because their chemically diverse constituents can exhibit multiple biological activities. Clove (Syzygium aromaticum) buds are rich in phenolic constituents, particularly eugenol and related compounds, and have documented antibacterial, antifungal, and antioxidant properties (Chaieb et al., 2007; Cortés-Rojas et al., 2014; Rahim et al., 2025).

Green nanotechnology can combine the biological properties of plant extracts with the physicochemical properties of metallic nanoparticles. Phytochemicals can participate in the reduction of Ag^{+} to Ag^{0} and in stabilization of the resulting nanoparticles, while silver nanoparticles can interact with microbial cell envelopes and promote oxidative stress (Singh et al., 2010; Bansal et al., 2018; Sati et al., 2025). Studies specifically using Syzygium aromaticum have confirmed the feasibility of clove-mediated AgNP synthesis and have reported antimicrobial activity, with particle size and optical properties varying substantially according to the plant material and synthesis conditions (Vijayaraghavan et al., 2012; Ajitha et al., 2019; Kasim et al., 2021; Wattimena et al., 2021; Madiabu et al., 2024; Mohammed et al., 2026).

Reported clove-mediated AgNPs have shown surface plasmon resonance bands around 415–440 nm and particle sizes ranging from a few nanometres to several tens of nanometres, illustrating the strong influence of extract source and synthesis conditions on nanoparticle characteristics (Ajitha et al., 2019; Vijayaraghavan et al., 2012; Wattimena et al., 2021; Madiabu et al., 2024). Antibacterial activity has also been demonstrated against several bacterial species, while antifungal effects have been reported in some clove-AgNP systems (Ajitha et al., 2019; Kasim et al., 2021; Muruganandham et al., 2023). However, comparatively little information is available on the direct activity of a clove-mediated AgNP formulation against plant-associated bacterial pathogens together with a concentration-response assessment against a plant-pathogenic fungus. This study was designed to address that gap using the available experimental formulation and measured antimicrobial responses.

http://www.doi.org/10.62341/istj-vol39-2-NAS28

Previous work with clove-mediated AgNPs demonstrated enhanced biological activity relative to clove extract in an insect model (Hmed et al., 2026). The present study extends this approach to plant-associated microbial pathogens by comparing the antibacterial activity of the AgNP formulation with the aqueous clove extract against Agrobacterium tumefaciens (MG706145) and P. carotovorum subsp. carotovorum and by evaluating concentration-dependent effects of the AgNP formulation against A. alternata.

2. Materials and Methods

2.1 Plant material and aqueous extract preparation

Clove buds were obtained from a local market, identified by a botanist, and ground to powder. An aqueous extract was prepared by macerating 50 g of powdered clove in 250 mL of distilled water for 24 h with regular shaking. The mixture was filtered through Whatman No. 1 paper, centrifuged, and the supernatant was stored at 4 °C (Hmed et al., 2026).

2.2 Green synthesis of AgNPs

AgNPs were prepared using the clove-mediated biogenic reduction procedure reported by Hmed et al. (2026). Briefly, 90 mL of 1 mM $AgNO_3$ was mixed with 10 mL of crude aqueous clove extract and heated at 60 °C for 45 min under continuous magnetic stirring while protected from direct light. The reaction mixture was then maintained in darkness for 24 h. Formation of AgNPs was indicated by the change in solution colour from pale to dark brown.

2.3 Characterization of AgNPs

Particle morphology and elemental composition were assessed by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). The SEM micrographs and EDX spectrum used in this manuscript correspond to the same clove-mediated AgNP batch characterized in Hmed et al. (2026). For characterization of that batch, the AgNP suspension was centrifuged at 12,000 rpm for 20 min, washed three times with distilled water, dried at 80 °C, gently ground with a few drops of ethanol to obtain a fine powder, and stored at 7 °C until analysis. SEM/EDX measurements were performed using a JSM-IT200 scanning electron microscope (JEOL, Japan) at 20 kV, 10 mm working distance, ×170 magnification, under high vacuum, with a 30-s live

time; quantitative elemental analysis used the standardless ZAF correction method (Hmed et al., 2026). The reported EDX analysis showed a prominent Ag signal with a silver content of 43.87 ± 0.62 wt% (10.53 ± 0.15 atomic%).

Six representative particle diameters were re-measured from the available SEM micrographs, ranging from 22.95 to 32.95 nm (mean 27.54 ± 3.82 nm), for descriptive reporting only. The FTIR procedure reported for the same clove-derived material used a Perkin-Elmer SPECTRUM 10 spectrometer, with spectra recorded from 450–4000 cm^{-1} at a resolution of 8 cm^{-1}; the FTIR bands summarized in this manuscript are reference data from Hmed et al. (2026) and are not presented as a newly acquired spectrum for the current nanoparticle sample. No UV–Vis spectrum is reported for the present formulation.

2.4 Plant pathogens

The tested microorganisms were Agrobacterium tumefaciens (MG706145), Pectobacterium carotovorum subsp. carotovorum (ON248562.1), and Alternaria alternata.

2.5 Antibacterial assay

Antibacterial activity of the clove extract and clove-mediated AgNPs was evaluated against Agrobacterium tumefaciens (MG706145) and Pectobacterium carotovorum subsp. carotovorum (ON248562.1) using the paper-disc diffusion method described previously (Jadalla, 2024). Bacterial suspensions adjusted to 10^8 CFU/mL were spread on nutrient agar with a sterile cotton swab, and sterile blank discs loaded with 20 µL of the clove extract or the AgNP suspension were placed on the inoculated plates. The plates were incubated at 28 ± 2 °C for 24 h, and inhibition-zone diameter was then recorded in millimetres. Three replicates were used for each treatment. The aqueous clove extract served as the comparator for the AgNP formulation. No standard (reference) antibiotic, $AgNO_3$ treatment, or negative control was included in the bacterial assay. The disc diameter and the concentration of the test materials loaded on the discs were not available in the experimental records and are therefore not reported.

2.6 Antifungal assay

The effect of clove-mediated AgNPs on Alternaria alternata was evaluated at 5, 10, 15, and 20% (v/v) using the poisoned-food

http://www.doi.org/10.62341/istj-vol39-2-NAS28

technique in 9-cm Petri dishes containing potato dextrose agar (PDA), with three replicates per concentration and an untreated control. The dishes were incubated at 25 °C until the untreated control colony completed its growth, which took approximately one week. Colony diameter (mm) refers to the measured diameter of the fungal colony, not the radial growth (radius) from the inoculation point. Because the radius is half the diameter, MGI is unchanged whichever measure is used, provided the same convention is applied to control and treated dishes. The untreated control colony reached the edge of the 9-cm Petri dish, corresponding to 90 mm. Mycelial growth inhibition (MGI) was calculated as: MGI (%) = [(DC − DT) / DC] × 100, where DC is the untreated control colony diameter and DT is the treated colony diameter. The fungal inoculum size was not available in the experimental records and is therefore not reported. A parallel clove-extract treatment was not included in the antifungal assay; the antifungal results therefore describe the AgNP formulation alone and cannot be compared directly with the extract.

2.7 Statistical analysis

Bacterial inhibition-zone data were analysed using two-way analysis of variance (ANOVA) with treatment and bacterial species/isolate as factors, followed by Tukey's HSD test. Fungal colony diameter was analysed by one-way ANOVA followed by Tukey's HSD test. Data are presented as mean ± standard deviation (SD), with three replicates per treatment. Statistical significance was considered at $p < 0.05$. With three replicates per group, the assumptions of ANOVA (normality and homogeneity of variance) could not be tested reliably.

3. Results

3.1 Characterization of the clove-mediated AgNPs

The SEM micrographs correspond to the same clove-mediated AgNP batch previously characterized by Hmed et al. (2026). Six representative particle diameters visible in those micrographs (23.87, 32.95, 22.95, 26.43, 29.51, and 29.51 nm) were re-measured for descriptive reporting, giving a mean of 27.54 ± 3.82 nm and an observed range of 22.95–32.95 nm (Fig. 1; Table 1). The previously reported EDX spectrum from the same batch showed a prominent Ag signal at approximately 3 keV, with Ag accounting for 43.87 ±

Clove-Mediated Green Synthesis of Silver Nanoparticles and Their In Vitro Antimicrobial Activity Against Plant Pathogens

http://www.doi.org/10.62341/istj-vol39-2-NAS28

0.62 wt% and 10.53 ± 0.15 atomic % (Fig. 2; Table 1). Carbon and oxygen were also prominent, while Na, Mg, Si, S, Cl, K, and Ca occurred at lower levels (Table 1). The mean obtained here is based on six measurements and differs slightly from the 28.34 ± 1.203 nm reported by Hmed et al. (2026) from a size-distribution analysis of multiple images; both values fall within the same size range.

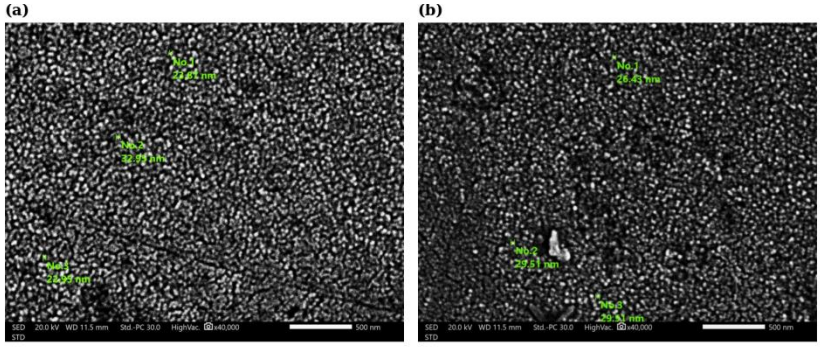


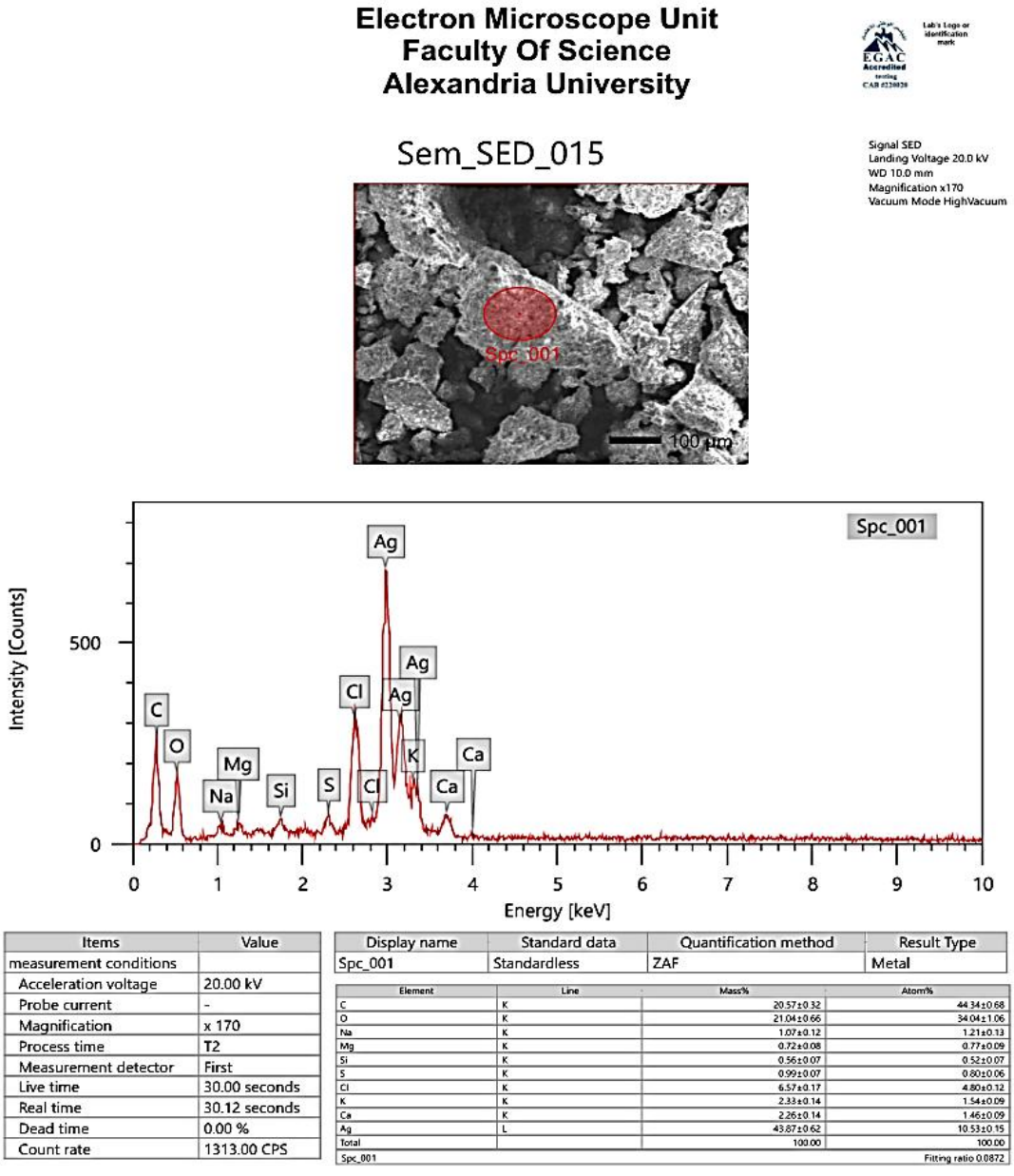
Fig.1. Previously reported SEM micrographs of the same clove-mediated AgNP batch characterized by Hmed et al. (2026), showing predominantly spherical particles. The marked particle diameters were re-measured in the present analysis: (a) 23.87, 32.95, 22.95 nm; (b) 26.43, 29.51, 29.51 nm. Scale bar: 500 nm.

Table 1. Previously reported characterization data for the same clove-mediated AgNP batch (Hmed et al., 2026).

Parameter	Value
Shape	Predominantly spherical
Mean particle size (SEM)	27.54 ± 3.82 nm (range: 22.95–32.95 nm)
Ag (EDX)	43.87 ± 0.62 wt%; 10.53 ± 0.15 atomic %
C / O (EDX)	20.57 / 21.04 wt%
Other elements (EDX, wt%)	Cl 6.57; K 2.33; Ca 2.26; Na 1.07; S 0.99; Mg 0.72; Si 0.56

Clove-Mediated Green Synthesis of Silver Nanoparticles and Their In Vitro Antimicrobial Activity Against Plant Pathogens

http://www.doi.org/10.62341/istj-vol39-2-NAS28



Items	Value
measurement conditions	
Acceleration voltage	20.00 kV
Probe current	-
Magnification	x 170
Process time	T2
Measurement detector	First
Live time	30.00 seconds
Real time	30.12 seconds
Dead time	0.00 %
Count rate	1313.00 CPS

Display name	Standard data	Quantification method	Result Type
Spc_001	Standardless	ZAF	Metal

Element	Line	Mass%	Atom%
C	K	20.57±0.32	44.34±0.68
O	K	21.04±0.66	34.04±1.06
Na	K	1.07±0.12	1.21±0.13
Mg	K	0.72±0.08	0.77±0.09
Si	K	0.56±0.07	0.52±0.07
S	K	0.99±0.07	0.80±0.06
Cl	K	6.57±0.17	4.80±0.12
K	K	2.33±0.14	1.54±0.09
Ca	K	2.26±0.14	1.46±0.09
Ag	L	43.87±0.62	10.53±0.15
Total		100.00	100.00
Spc_001			Fitting ratio 0.0872

Fig.2. Previously reported EDX elemental spectrum and quantitative composition of the same clove-mediated AgNP batch characterized by Hmed et al. (2026). The figure is reproduced from the previously reported characterization record and is included here with citation.

Clove-Mediated Green Synthesis of Silver Nanoparticles and Their In Vitro Antimicrobial Activity Against Plant Pathogens
http://www.doi.org/10.62341/istj-vol39-2-NAS28

3.2 Antibacterial activity

Clove-mediated AgNPs produced larger inhibition zones than the clove extract against both bacterial targets (Table 2; Fig. 3). Against Agrobacterium tumefaciens (MG706145), the inhibition zone increased from 7.67 ± 1.15 mm with the extract to 14.67 ± 1.15 mm with AgNPs. Against P. carotovorum subsp. carotovorum, the corresponding values were 10.67 ± 1.15 and 13.67 ± 0.58 mm. Two-way ANOVA showed a significant treatment effect ($F_{1,8} = 69.23$, $p < 0.001$) and a significant treatment × bacterium interaction ($F_{1,8} = 11.08$, $p = 0.010$; Fig. 4), whereas the main effect of bacterium was not significant ($F_{1,8} = 2.77$, $p = 0.135$).

Table 2. Inhibition-zone diameter (mm, mean ± SD, n = 3).

Treatment	Agrobacterium tumefaciens	P. carotovorum subsp. carotovorum
Clove-mediated AgNPs	14.67 ± 1.15 a	13.67 ± 0.58 a
Clove extract	7.67 ± 1.15 c	10.67 ± 1.15 b

Different letters indicate significant differences among the four means (Tukey's HSD, $p < 0.05$).

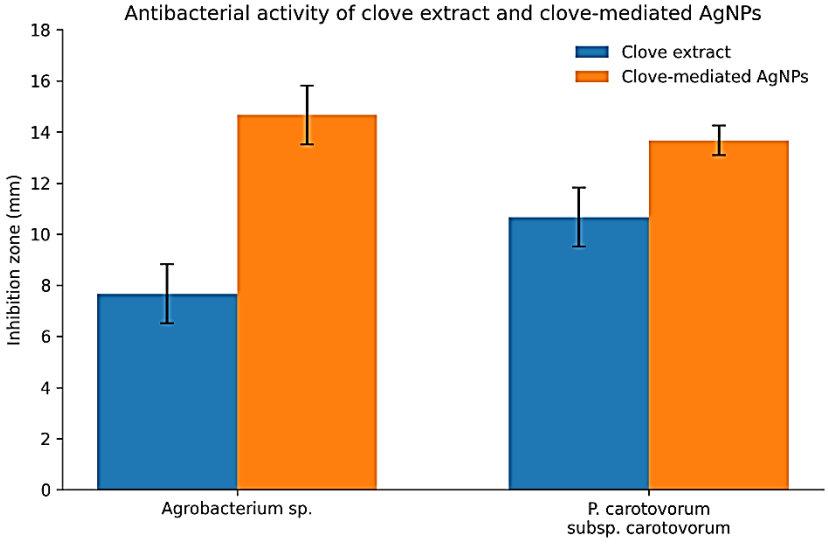


Fig.3. Antibacterial inhibition zones produced by clove extract and clove-mediated AgNPs.

Clove-Mediated Green Synthesis of Silver Nanoparticles and Their In Vitro Antimicrobial Activity Against Plant Pathogens

http://www.doi.org/10.62341/istj-vol39-2-NAS28

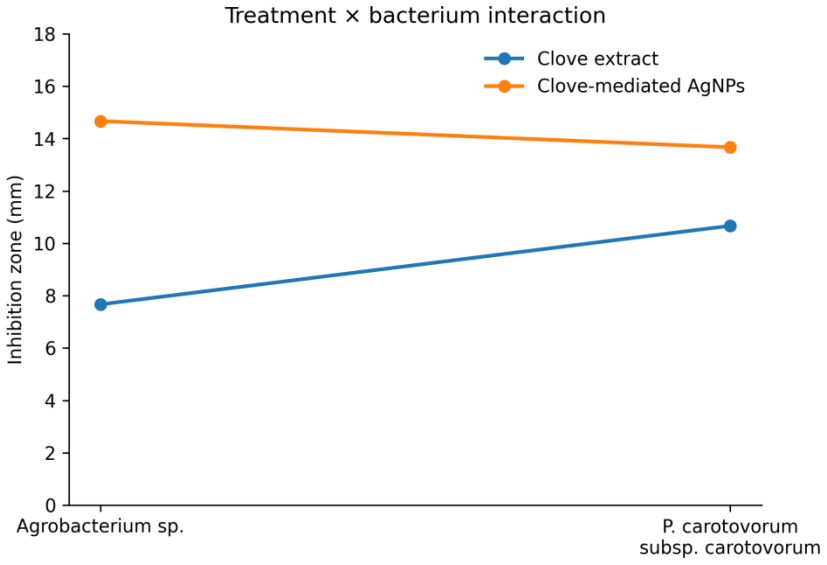


Fig.4. Treatment × bacterium interaction for inhibition-zone diameter.

3.3 Antifungal activity against Alternaria alternata

Colony diameter decreased progressively as the AgNP concentration increased (Table 3; Fig. 5). Mean colony diameter was 15.67 ± 0.58 mm at 5%, 9.33 ± 1.53 mm at 10%, 6.50 ± 1.80 mm at 15%, and 3.67 ± 1.15 mm at 20%. One-way ANOVA indicated a highly significant concentration effect ($F_{3,8} = 43.63$, $p < 0.001$). Using a 90-mm untreated control, the corresponding MGI values were 82.6, 89.6, 92.8, and 95.9%, respectively (Table 3; Fig. 6).

Table 3. Effect of clove-mediated AgNP concentration on A. alternata colony diameter.

Concentration (%)	Colony diameter (mm, mean ± SD)	MGI (%)
Control	90	0
5	15.67 ± 0.58 a	82.6
10	9.33 ± 1.53 b	89.6
15	6.50 ± 1.80 bc	92.8
20	3.67 ± 1.15 c	95.9

Clove-Mediated Green Synthesis of Silver Nanoparticles and Their In Vitro Antimicrobial Activity Against Plant Pathogens
http://www.doi.org/10.62341/istj-vol39-2-NAS28

Different letters indicate significant differences among AgNP concentrations (Tukey's HSD, $p < 0.05$); the control was not included in the test. The untreated control colony reached the edge of the 9-cm dish, corresponding to 90 mm.

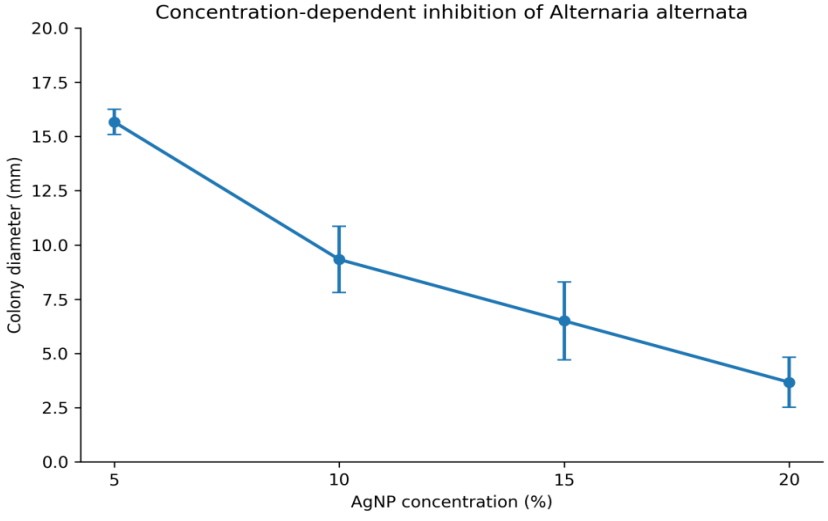


Fig.5. Concentration-dependent reduction in A. alternata colony diameter.

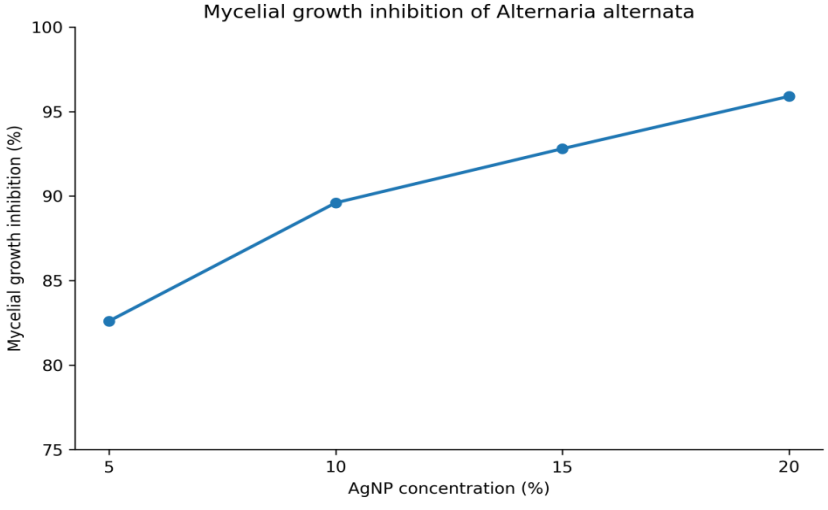


Fig.6. Mycelial growth inhibition (%) of A. alternata relative to the 90-mm untreated control.

4. Discussion

The present results support the potential of clove-mediated AgNPs as a plant-derived antimicrobial formulation. The antibacterial response was consistently greater for AgNPs than for the aqueous clove extract, with the strongest relative enhancement observed against Agrobacterium tumefaciens (MG706145). This pattern agrees with previous studies showing antibacterial activity of clove-mediated AgNPs and, in some systems, greater activity of the nanoparticle preparation than the corresponding plant extract (Ajitha et al., 2019; Madiabu et al., 2024; Al-Khafaji, 2025; Muruganandham et al., 2023). The agreement should nevertheless be interpreted cautiously because the earlier studies used different clove materials, nanoparticle preparation conditions, bacterial species, and assay formats.

The antifungal results showed a clear concentration-dependent reduction in A. alternata colony diameter. At 20% AgNPs, the calculated MGI reached 95.9% relative to the 90-mm control. The progressive response is compatible with reported antifungal activity of clove-mediated AgNPs (Ajitha et al., 2019; Muruganandham et al., 2023) and with the broader antimicrobial activity reported for green-synthesized silver nanoparticles. However, direct quantitative comparison with previous studies is limited because fungal species, nanoparticle concentrations, exposure periods, and response metrics differed among experiments. At the cellular level, silver nanoparticles can interact with microbial membranes and induce oxidative stress (Sati et al., 2025; Gwada et al., 2025).

The re-measured particle size range from the previously reported SEM micrographs (22.95–32.95 nm; mean 27.54 ± 3.82 nm) falls within the range reported for several clove-derived AgNP systems. Madiabu et al. (2024) reported particles of 14–32 nm from clove leaf extract, while Wattimena et al. (2021) reported a broader 2.9–33.6 nm range with a mean of 20.5 ± 4.9 nm. Other studies have reported smaller or larger particles depending on the synthesis system, including approximately 9.5 nm in a recent clove-mediated preparation and 38–79 nm in a study using clove extract against antibiotic-resistant bacteria (Mohammed et al., 2026; Al-Khafaji, 2025). These comparisons illustrate the variability of particle size across clove-mediated synthesis systems and should be interpreted

in relation to extraction method, $AgNO_3$ concentration, reaction conditions, and characterization technique.

The previously reported characterization data provide context for the biological activity. The predominantly spherical morphology and nanoscale size provide a surface for interaction with microbial cells, while the EDX profile confirms a strong silver signal. The carbon and oxygen content may reflect plant-derived organic residues associated with the nanoparticle preparation. FTIR bands previously reported for the clove extract, including O–H, C=O, aromatic C=C, and C–O/C–O–C signals, support the possible participation of phenolic and other oxygen-containing constituents in the reduction and stabilization process (Hmed et al., 2026).

Eugenol is the principal phenolic constituent of clove buds and is widely regarded as a major contributor to the bioactivity of clove preparations (Chaieb et al., 2007; Cortés-Rojas et al., 2014; Rahim et al., 2025). In the green synthesis of AgNPs, phenolic compounds such as eugenol are generally proposed to act as both reducing and stabilizing agents: the phenolic hydroxyl group can donate electrons (and hydrogen) to reduce Ag^+ to Ag^0, while the oxidized phenolics and other oxygen-containing molecules can adsorb onto the particle surface and limit aggregation through steric and electrostatic stabilization (Singh et al., 2010; Bansal et al., 2018; Sati et al., 2025). The colour change to dark brown observed during synthesis is consistent with AgNP formation, although it is only a visual indicator because no UV–Vis spectrum was recorded, and the FTIR bands previously reported for the clove extract (O–H, C=O, aromatic C=C, and C–O) are compatible with the participation of such constituents (Hmed et al., 2026). Because eugenol was not quantified and the capping molecules on the particle surface were not identified in the present work, the involvement of eugenol should be regarded as a plausible mechanism supported by the literature rather than a finding of this study.

The greater antibacterial activity of the AgNPs than of the extract alone may reflect the combined action of silver and extract-derived surface molecules. Both bacterial targets are Gram-negative, and AgNPs are generally thought to adhere to the outer membrane, increase membrane permeability, and release Ag^+ ions that interact with membrane proteins, thiol-containing enzymes, and nucleic

acids; these events can disturb respiratory activity and promote the generation of reactive oxygen species (ROS), leading to oxidative stress and cell damage (Sati et al., 2025; Gwada et al., 2025). Eugenol associated with the nanoparticle surface could add to this effect, since eugenol itself is known to affect membrane integrity (Chaieb et al., 2007; Rahim et al., 2025), which would be consistent with an additive or synergistic response. A comparable sequence of interaction with the cell wall and plasma membrane, ion release, and oxidative stress may underlie the inhibition of A. alternata hyphal growth, although fungal cell-wall composition differs from that of bacteria. These mechanisms are inferred from the literature; membrane integrity, ROS generation, and Ag^{+} release were not measured here, so the mechanistic interpretation remains hypothetical.

An important limitation is that $AgNO_3$ alone was not tested as a separate treatment. Therefore, the contribution of residual silver ions cannot be completely separated from the activity attributable to the nanoparticle formulation. In addition, the bacterial assay did not include a negative control or a standard antibiotic, and only three replicates were available, so the statistically significant treatment × bacterium interaction should be interpreted with appropriate caution. The fungal experiment evaluated the nanoparticle formulation but did not include a parallel clove-extract treatment; consequently, the contribution of the extract's own antifungal constituents (for example, eugenol) to the observed inhibition could not be separated from that of the nanoparticles. Furthermore, the fungal inoculum size, disc diameter, and the concentration of material loaded per disc were not documented, which limits the exact reproducibility of both assays. Future work should therefore include $AgNO_3$, appropriate negative and positive controls (a reference antibiotic and a reference fungicide), a parallel extract treatment in the antifungal assay, fully documented assay conditions, MIC/MBC/MFC determinations, and host-plant assays.

5. Conclusion

Clove-mediated AgNPs showed enhanced antibacterial activity compared with the aqueous clove extract against Agrobacterium tumefaciens (MG706145) and P. carotovorum subsp. carotovorum,

with the greater relative enhancement observed against Agrobacterium. The same formulation produced concentration-dependent inhibition of A. alternata growth, reaching 95.9% calculated MGI at 20% relative to the 90-mm untreated control. Together, these findings support further investigation of clove-mediated AgNPs as a plant-derived antimicrobial formulation, particularly through controlled dose-response studies, MIC/MBC/MFC testing, phytotoxicity assessment, and in vivo plant-pathogen experiments.

References

Ajitha, B., Reddy, Y. A. K., Lee, Y., Kim, M. J., and Ahn, C. W., 2019, "Biomimetic synthesis of silver nanoparticles using Syzygium aromaticum (clove) extract: Catalytic and antimicrobial effects," Applied Organometallic Chemistry, 33(5), e4867. DOI: 10.1002/aoc.4867.

Al-Khafaji, A. A. K., 2025, "Biological activity of biosynthesized silver nanoparticles from Syzygium aromaticum extract using green technology against pathogenic and antibiotic-resistant bacteria isolated from burn infections," Technium BioChemMed, 13, 126–140. DOI: 10.47577/biochemmed.v13i.13133.

Bansal, H., Kaushal, J., Chahar, V., Shukla, G., and Bhatnagar, A., 2018, "Green synthesis of silver nanoparticles using Syzygium aromaticum (clove) bud extract: Reaction optimization, characterization and antimicrobial activity," Journal of Bionanoscience, 12(3), 378–389.

Chaieb, K., Hajlaoui, H., Zmantar, T., Kahla-Nakbi, A. B., Rouabhia, M., Mahdouani, K., and Bakhrouf, A., 2007, "The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (Syzygium aromaticum L. Myrtaceae): A short review," Phytotherapy Research, 21(6), 501–506. DOI: 10.1002/ptr.2124.

Cortés-Rojas, D. F., de Souza, C. R. F., and Oliveira, W. P., 2014, "Clove (Syzygium aromaticum): A precious spice," Asian Pacific Journal of Tropical Biomedicine, 4(2), 90–96. DOI: 10.1016/S2221-1691(14)60215-X.

Fan, X., Saleem, T., and Zou, H., 2022, "Copper resistance mechanisms in plant pathogenic bacteria," Phytopathologia Mediterranea, 61(1), 129–138. DOI: 10.36253/phyto-13282.

Gohlke, J., and Deeken, R., 2014, "Plant responses to Agrobacterium tumefaciens and crown gall development," Frontiers in Plant Science, 5, 155. DOI: 10.3389/fpls.2014.00155.

Gwada, C. A., Ndivhuwo, P. S., Matshetshe, K., Aradi, E., Mdluli, P., Moloto, N., Otieno, F., and Airo, M., 2025, "Phytochemical-assisted synthesis, optimization, and characterization of silver nanoparticles for antimicrobial activity," RSC Advances, 15(18), 14170–14181. DOI: 10.1039/D4RA08900F.

Hmed, F. S., Albrany, F. M., and Jadalla, N. M., 2026, "Enhancing the biological efficacy of clove extract via silver nano-loading to combat the stored drug beetle (Coleoptera: Anobiidae)," Bani Waleed University Journal of Humanities and Applied Sciences, 11(1), 101–115.

Jadalla, N. M., 2024, "Green synthesis, characterization and antimicrobial activities of silver nanoparticles against some phytopathogenic bacteria," Journal of Nano Research, 85, 21–35. DOI: 10.4028/p-o81zRe.

Kasim, S., Dali, S., and Rahmah, M., 2021, "Synthesis of silver nanoparticles using bioreductors from clove leaf extract (Syzygium aromaticum) and test of its antibacterial activity," Journal of Physics: Conference Series, 1763, 012051. DOI: 10.1088/1742-6596/1763/1/012051.

Madiabu, M. J., Abdul Aziz, I. T., Supriyono, Putra, A. P., Cahyotomo, A., and Panglipur, H. S., 2024, "Synthesis of silver nanoparticles with Syzygium aromaticum leaves extract as antioxidant and antimicrobial materials," Key Engineering Materials, 989, 109–120. DOI: 10.4028/p-0HqqKo.

Mohammed, E. J., Laftah, A. R., Salem, S. S., Seyam, E. A., Al-Bakarey, A. G., Modafer, Y., Sayed Youssef, F., Abdelaziz, A. E. M., Al-Habibi, M. M., Khedr, M., Alfaifi, M., Shati, A. A., Hussein, A. M., and Mekky, A. E., 2026, "Clove-mediated green synthesis of silver nanoparticles and in vitro investigation of their antimicrobial, antioxidant, and anticancer potential,"

Frontiers in Microbiology, 17, 1860045. DOI: 10.3389/fmicb.2026.1860045.

Muruganandham, M., Al-Otibi, F. O., Alharbi, R. I., Sivasubramanian, K., Raja, R. K., Velmurugan, P., and Basavegowda, N., 2023, "Antibacterial, antifungal, antioxidant, and cytotoxicity activities of the aqueous extract of Syzygium aromaticum-mediated synthesized novel silver nanoparticles," Green Processing and Synthesis, 12(1), 20230188. DOI: 10.1515/gps-2023-0188.

Perfileva, A. I., Strekalovskaya, E. I., Klushina, N. V., Gorbenko, I. V., and Krutovsky, K. V., 2025, "The causative agent of soft rot in plants, the phytopathogenic bacterium Pectobacterium carotovorum subsp. carotovorum: A brief description and an overview of methods to control it," Agronomy, 15(7), 1578. DOI: 10.3390/agronomy15071578.

Rahim, N., Derabli, C., Bramki, A., Mahdjoub, S., Rup-Jacques, S., Barboucha, G., Hesse, S., and Boulebd, H., 2025, "Evaluating the multifaceted bioactivity of Syzygium aromaticum essential oil: The central role of eugenol," Turkish Journal of Biology, 49(1), 102–117.

Sati, A., Ranade, T. N., Mali, S. N., Yasin, H. K. A., and Pratap, A., 2025, "Silver nanoparticles (AgNPs): Comprehensive insights into bio/synthesis, key influencing factors, multifaceted applications, and toxicity—a 2024 update," ACS Omega, 10(8), 7549–7582.

Singh, A. K., Talat, M., Singh, D. P., and Srivastava, O. N., 2010, "Biosynthesis of gold and silver nanoparticles by natural precursor clove and their functionalization with amine group," Journal of Nanoparticle Research, 12(5), 1667–1675. DOI: 10.1007/s11051-009-9835-3.

Sundin, G. W., and Wang, N., 2018, "Antibiotic resistance in plant-pathogenic bacteria," Annual Review of Phytopathology, 56, 161–180. DOI: 10.1146/annurev-phyto-080417-045946.

Tsuge, T., Harimoto, Y., Akimitsu, K., Ohtani, K., Kodama, M., Akagi, Y., Egusa, M., Yamamoto, M., and Otani, H., 2013, "Host-selective toxins produced by the plant pathogenic fungus Alternaria alternata," FEMS Microbiology Reviews, 37(1), 44–66. DOI: 10.1111/j.1574-6976.2012.00350.x.

Vijayaraghavan, K., Nalini, S. P. K., Prakash, N. U., and Madhankumar, D., 2012, "Biomimetic synthesis of silver nanoparticles by aqueous extract of Syzygium aromaticum," Materials Letters, 75, 33–35. DOI: 10.1016/j.matlet.2012.01.083.

Wattimena, S. C., Reniwuryaan, A. A., and Patty, P. J., 2021, "Physical-chemical and antibacterial properties of green-synthesized silver nanoparticles mediated by leaf extract of Syzygium aromaticum L," Journal of Physics: Conference Series, 1825(1), 012090. DOI: 10.1088/1742-6596/1825/1/012090.

Yang, L.-N., He, M.-H., Ouyang, H.-B., Zhu, W., Pan, Z.-C., Sui, Q.-J., Shang, L.-P., and Zhan, J., 2019, "Cross-resistance of the pathogenic fungus Alternaria alternata to fungicides with different modes of action," BMC Microbiology, 19, 205. DOI: 10.1186/s12866-019-1574-8.