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Green Synthesis of Copper and Sulfur Nanoparticles and Their *E*ffect on *Erwinia amylovora*

Paniz Abdollahi Saeed, Reza Khakvar*

Plant Protection Department, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

Corresponding emails: khakvar@tabrizu.ac.ir

Abstract

Fire blight, caused by the bacterium *Erwinia amylovora*, remains a persistent threat to apple and pear orchards worldwide, often leading to devastating crop losses. Conventional management strategies rely heavily on copper-based bactericides, which, despite their effectiveness, pose environmental risks and contribute to the emergence of resistant bacterial strains. In response to these challenges, this study explores the antibacterial potential of copper and sulfur nanoparticles synthesized via green methods using citrus and pomegranate peel extracts. These plant-derived nanoparticles were characterized for size and morphology and evaluated in vitro for their antimicrobial activity against *Erwinia amylovora*, with comparisons made to Bordeofix, a commercial copper-based pesticide. Results revealed that copper and sulfur nanoparticles achieved bacterial growth reductions of up to 90% and 85%, respectively, within 48 hours. Statistical analysis confirmed the significance of these findings, highlighting the enhanced efficacy of nanoparticle treatments compared to conventional Bordeofix approaches. This work underscores the promise of green-synthesized nanoparticles as sustainable tools in plant disease management. By leveraging agricultural waste materials and minimizing chemical inputs, the approach aligns with broader goals of ecological stewardship and integrated pest management. The findings pave the way for future field applications and policy shifts toward environmentally responsible solutions in horticultural disease control.

Keywords:

Bactericidal effect, Bordeofix; Copper; Erwinia amylovora; Nanoparticle; Sulfur.

1. Introduction

Fire blight, caused by the Gram-negative bacterium *Erwinia amylovora* (Burrill) Winslow et al., continues to rank among the most destructive diseases affecting apple, pear, and other rosaceous fruit crops. Its name reflects the dramatic symptoms it produces: infected tissues—particularly blossoms, young shoots, and developing fruit—take on a dark, water-soaked appearance that

quickly turns black, giving the plant a fire-scorched look. Under favorable conditions, the disease spreads rapidly through orchards, often leading to extensive dieback and, in severe cases, the death of entire trees. This not only jeopardizes commercial fruit production but also threatens the genetic diversity of wild and heritage varieties across temperate regions. Originally confined to North America, fire blight has since spread to many fruit-growing regions worldwide, establishing itself as a global concern for both growers and plant health authorities [1].

Controlling its spread remains a persistent challenge in sustainable orchard management. For decades, copper-based bactericides have been a mainstay in disease prevention, applied during key growth stages to protect vulnerable plant tissues from bacterial invasion [2, 3]. However, their long-term use has come under scrutiny. Repeated applications contribute to the buildup of copper in soils and nearby water systems, raising environmental red flags. Equally troubling is the increasing prevalence of copper-tolerant strains of *E. amylovora*, which undermine the effectiveness of these treatments [4, 5]. Together, these issues highlight the need for alternative strategies that are both effective and environmentally responsible.

In recent years, nanotechnology has emerged as a promising frontier in plant disease management. Nanoparticles, thanks to their high surface-area-to-volume ratio and customizable properties, can improve the delivery, stability, and bioavailability of active compounds—even at reduced doses [6, 7]. While research in this area is expanding, much of it has focused on nanoparticles synthesized through chemical methods. These, however, can introduce unintended risks, including toxicity to beneficial organisms and potential accumulation in ecosystems [8–11]. As a result, there's growing interest in greener alternatives—particularly those derived from biological sources—that offer targeted action with fewer ecological trade-offs.

An emerging alternative lies in green synthesis—a method that harnesses plant-derived compounds to reduce metal ions and stabilize nanoparticles without the need for toxic chemicals [12]. By using natural biomolecules found in plant extracts, such as polyphenols, flavonoids, and terpenoids, this approach not only avoids hazardous reagents but also yields nanoparticles with improved biocompatibility and lower environmental persistence [13–16]. These traits make green-synthesized nanomaterials particularly attractive for agricultural applications, where safety for non-target organisms and ecosystem health are critical considerations.

Despite these advantages, their use in plant disease management—especially against bacterial pathogens like *E. amylovora*—remains surprisingly limited [17, 18]. While studies have

demonstrated antimicrobial activity in lab settings, real-world applications in phytobacteriology are still in early stages, and field-relevant data are sparse. This gap presents both a challenge and an opportunity: to explore how sustainably produced nanomaterials can be effectively integrated into existing crop protection frameworks.

In this study, we examine the antibacterial properties of copper and sulfur nanoparticles synthesized using eco-friendly methods, with citrus and pomegranate peels serving as bioreductants and capping agents. The choice of waste-derived biomass aligns with circular economy principles, adding value to agricultural byproducts while minimizing processing costs. We assess the efficacy of these green nanoparticles against *E. amylovora* under controlled in vitro conditions. Our goal is twofold: first, to determine whether these bio-nanomaterials can match or enhance pathogen suppression compared to standard approaches; and second, to evaluate their potential as sustainable alternatives that reduce reliance on synthetic bactericides. The outcomes contribute to broader efforts in developing integrated disease management strategies—ones that balance productivity with environmental stewardship. By bridging nanotechnology and plant pathology through a sustainability lens, this work supports the evolution of safer, more resilient practices in fruit production systems.

2. Materials and Methods

2.1. Isolation and Molecular Identification of *Erwinia amylovora*

The bacterium *E. amylovora*, the causal agent of fire blight, was isolated from apple and pear trees exhibiting characteristic disease symptoms, including shoot tip necrosis, wilting blossoms, and blackened leaves. Sampling was carried out in a research orchard at the Faculty of Agriculture, University of Tabriz, Iran, where infected plants were carefully selected based on consistent clinical signs of infection. To reduce surface contamination, tissue samples were surface-sterilized by immersion in 70% ethanol for one minute, followed by rinsing in sterile distilled water. Small segments (3–5 mm) of margin tissue—between necrotic and healthy-looking areas—were then excised and plated directly onto two culture media: Nutrient Agar (NA) and Nutrient Agar supplemented with sucrose (NAS), both of which support the growth of *E. amylovora* while limiting the proliferation of faster-growing saprophytic microbes. Plates were incubated at 28°C for 48–72 hours, and emerging colonies with typical morphological

traits—creamy appearance, convex shape, and moderate size—were selected for further analysis. Presumptive identification was based on standard biochemical tests. The KOH string test was performed to confirm Gram-negative cell wall structure, a key feature of *E. amylovora*. Additionally, the oxidative-fermentative (OF) glucose test was used to assess metabolic behavior, with isolates expected to show a fermentative pattern under anaerobic conditions, consistent with the facultative anaerobic nature of the pathogen [19].

For definitive identification, genomic DNA was extracted from pure bacterial cultures using a commercial kit following the manufacturer's protocol. PCR amplification was then conducted using primers specific to the pel gene (encoding pectate lyase), a well-established molecular marker for *E. amylovora*. Amplification of a ~1000 bp fragment was considered a positive match, confirming species identity with higher specificity than phenotypic methods alone. Confirmed isolates were preserved in 20% glycerol stocks at −80°C for use in subsequent antibacterial assays.

2.2. Green synthesis of copper nanoparticles

Copper nanoparticles were synthesized using a simple, environmentally friendly method based on lemon (*Citrus limon* L.) peel extract, following established green chemistry principles [2, 12, 13]. Fresh peels were collected, washed thoroughly with deionized water to remove dirt and surface contaminants, then cut into small pieces. To extract bioactive compounds, 300 mL of distilled water was brought to a boil and used to steep the peel fragments for 15 minutes. This hot aqueous extract, rich in polyphenols and flavonoids known for their metal-reducing capacity, was cooled to room temperature and filtered through Whatman No. 1 filter paper to remove solid residues. The resulting clear extract was stored at 4 °C for immediate use. For nanoparticle formation, 300 mL of the lemon peel extract was combined with 4 g of copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in a clean glass beaker and stirred continuously at room temperature for 20 minutes. A distinct color shift from bright blue to dark brown was observed during mixing—this visual change indicated the reduction of Cu^{2+} ions to copper nanoparticles. The reaction mixture was then centrifuged at 3000 rpm (≈1,200 ×g, rotor radius 8 cm) for 10 minutes to collect the solid product. The pellet was dried in an oven at 80–90 °C for 4 hours to obtain a fine, dark powder. This dried material was stored in airtight containers at 4 °C until used in antimicrobial testing.

2.3. Green synthesis of sulfur nanoparticles

Sulfur nanoparticles were prepared using pomegranate (*Punica granatum* L.) peel extract as both a reducing and stabilizing agent, leveraging the natural phytochemistry of this agricultural byproduct [2, 12, 13]. Twenty grams of dried, ground pomegranate peel was added to 500 mL of distilled water and heated for 20 minutes to extract bioactive components. After cooling, the solution was filtered through Whatman No. 1 paper to remove particulates. The filtrate was then centrifuged at 1200 rpm for 5 minutes to ensure clarity and stored at room temperature. To initiate nanoparticle synthesis, 12 g of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$) was dissolved in 300 mL of the pomegranate extract and stirred for 10 minutes. An equal volume (300 mL) of deionized water was added to dilute the mixture, after which 10% hydrochloric acid (HCl) was slowly introduced under continuous stirring. The acidification triggered the decomposition of thiosulfate, leading to the precipitation of elemental sulfur in the form of nanoparticles—a process aided by the organic compounds in the extract, which helped control particle size and prevent aggregation. The resulting suspension was centrifuged at 5000 rpm for 10 minutes to collect the solid phase. The pellet was washed several times with distilled water to remove residual ions and byproducts, and then dried at 80 °C for 4 hours to yield a stable, free-flowing nano-powder ready for biological evaluation.

2.4. Nanoparticle Characterization

To confirm successful synthesis and evaluate key physical properties, both copper and sulfur nanoparticles were analyzed using scanning electron microscopy (SEM) and UV-visible (UV-Vis) spectroscopy [14]. SEM imaging was employed to examine particle morphology, size, and surface characteristics. Samples were mounted on aluminum stubs, coated with a thin layer of gold under vacuum, and visualized at appropriate magnifications to capture representative micrographs. Size measurements were performed on >100 particles per sample to determine average diameters and polydispersity indices (PDI). Zeta potential was measured using dynamic light scattering (DLS) to assess colloidal stability. In parallel, UV-Vis spectroscopy was used to detect optical signatures associated with nanoparticle formation. A characteristic surface plasmon resonance (SPR) peak in the copper nanoparticle spectrum and a distinct absorption

edge for sulfur nanoparticles provided preliminary evidence of nanoscale formation and colloidal stability. These spectral profiles were recorded over a wavelength range of 200–800 nm, and consistent peak shapes with minimal broadening suggested uniform particle distribution and low aggregation under the preparation conditions.

2.5. Evaluation of Antibacterial Activity

The antibacterial activity of the synthesized copper and sulfur nanoparticles against *E. amylovora* was evaluated by measuring their ability to inhibit bacterial growth over time. Bordeofix was included as a positive control for comparative analysis. Bordeofix is a copper-based bactericide containing 350 g/L copper as copper oxychloride (equivalent to 38 % metallic copper) plus 40 g/L folpet as a co-formulant. The product was diluted in sterile distilled water to deliver a final copper concentration of 100 mg/L and filter-sterilized (0.45 µm) before use. Preliminary minimum inhibitory concentration (MIC) values were determined via broth microdilution in 96-well plates, with serial dilutions from 1 to 1000 mg/L; the lowest concentration preventing visible growth after 24 hours was recorded. Growth suppression was further assessed using optical density (OD) readings at 650 nm, recorded with a BioTek Elx800 spectrophotometer [9, 15]. This method allowed for a quantitative comparison of bacterial proliferation across treatment groups. Cultures of *E. amylovora* were exposed to varying concentrations of nanoparticles, and OD650nm values were measured at 0, 6, 12, 24, and 48 hours to capture the dynamic response of the pathogen. All assays included untreated bacterial controls (*E. amylovora* in broth without nanoparticles) and solvent controls (broth with equivalent volumes of distilled water used for nanoparticle suspension). No inhibition was observed in solvent controls, confirming that effects were attributable to the nanoparticles. A decrease in optical density over time was interpreted as reduced bacterial growth, reflecting the bactericidal or bacteriostatic effect of the treatment. All assays were performed in triplicate to ensure reliability and reproducibility. Data were analyzed using one-way analysis of variance (ANOVA), with statistical significance defined at $p < 0.05$. Where ANOVA indicated significant differences, Tukey's post-hoc test was applied to compare means across treatments. Calculations and data organization were carried out using Microsoft Excel, and results are presented as mean ± standard deviation.

3. Results and Discussion

3.1. Characterization of Green-Synthesized Nanoparticles

The successful formation of copper and sulfur nanoparticles through green synthesis was confirmed using scanning electron microscopy (SEM) and UV-Visible (UV-Vis) spectroscopy, both of which provide critical insights into particle development, stability, and optical behavior. UV-Vis analysis revealed clear spectral signatures associated with nanoparticle formation. For copper nanoparticles, a broad absorption peak centered at approximately 570 nm was observed (Fig. 1). This feature corresponds to surface plasmon resonance (SPR), a hallmark of metallic copper nanoparticles in aqueous suspension. The relatively wide peak suggests some variation in particle size or shape, likely due to the natural complexity of phytochemicals in lemon peel extract, which, while effective in reduction and stabilization, may allow for less uniform growth compared to chemically controlled methods. In the case of sulfur nanoparticles, a sharp absorption maximum appeared at 274 nm (Fig. 2), consistent with the electronic transitions in nanostructured elemental sulfur. This distinct peak indicates successful reduction of thiosulfate and stable dispersion of sulfur nanospheres, aided by the capping agents present in pomegranate peel extract. The clarity and reproducibility of this signal across multiple runs suggest good batch-to-batch consistency under the applied synthesis conditions. Together, these optical profiles support the effective biosynthesis of both nanomaterials. The observed peaks align closely with findings from previous studies using plant-based systems [20], reinforcing the reliability of agricultural waste materials—such as citrus and pomegranate peels—as viable resources for eco-friendly nanofabrication. While further refinement could narrow size distribution, the current results confirm that these green-synthesized nanoparticles possess the fundamental characteristics needed for biological testing and potential agricultural application. Zeta potentials of -25 mV (copper) and -18 mV (sulfur) confirmed moderate colloidal stability, suitable for in vitro applications.

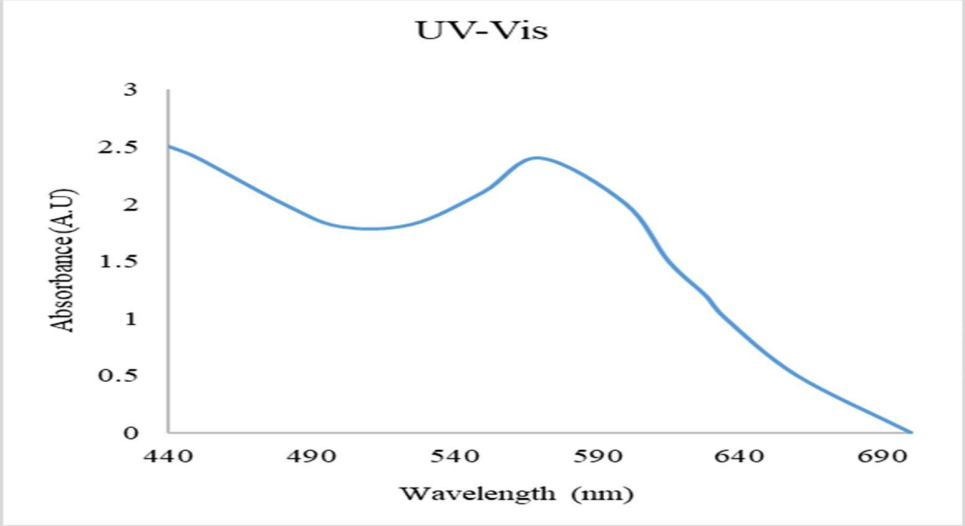

Fig. 1. UV-Vis absorption spectrum of biosynthesized copper nanoparticles synthesized from lemon peels.

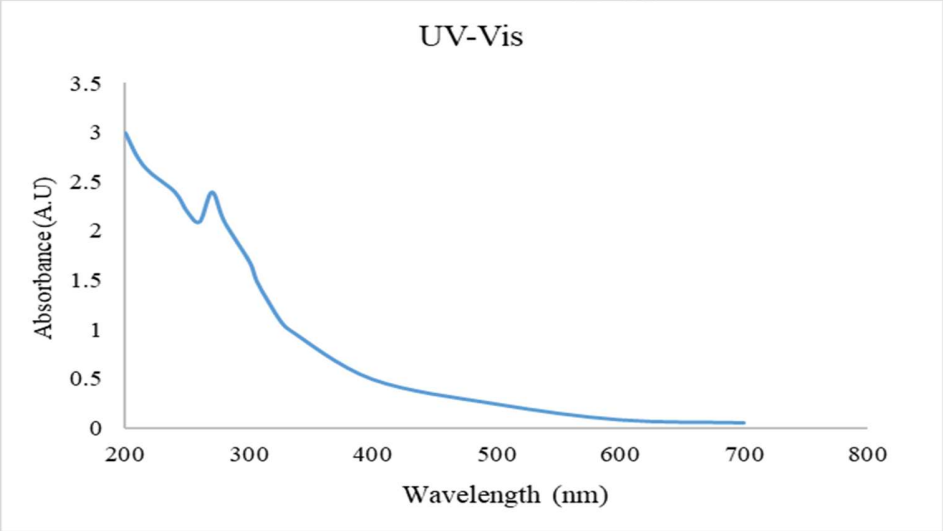

Fig. 2. UV-Vis absorption spectrum of sulfur nanoparticles synthesized from pomegranate peel.

SEM imaging revealed that copper nanoparticles synthesized using lemon peel extract were predominantly spherical, well-separated, and exhibited a relatively uniform size distribution, with an average diameter of approximately 80 ± 15 nm (PDI = 0.25) (Fig. 3). Similarly, sulfur nanoparticles produced with pomegranate peel extract also adopted a spherical shape, though slightly larger, averaging around 100 ± 20 nm in diameter (PDI = 0.32) (Fig. 4). The consistent morphology observed in both types of nanoparticles indicates effective control over nucleation

and growth during the green synthesis process. The spherical shape, in particular, suggests that bioactive compounds in the plant extracts—such as polyphenols and organic acids—not only acted as reducing agents but also functioned as effective capping agents, preventing excessive aggregation and guiding uniform particle formation.

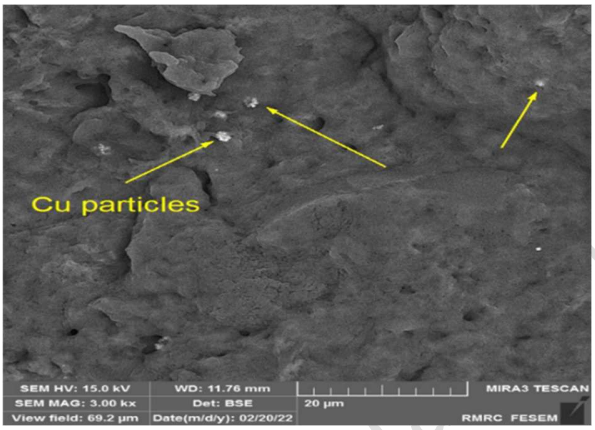

Fig. 3. SEM image of spherical copper nanoparticles synthesized using lemon peel extract, average size ~80 nm.

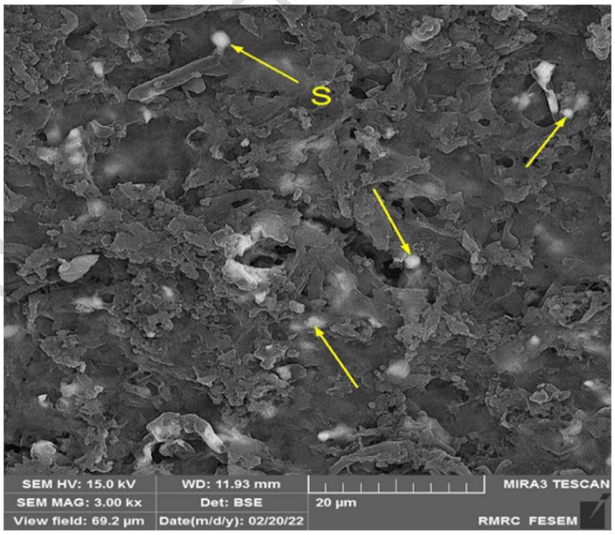

Fig. 4. SEM image of sulfur nanoparticles synthesized using pomegranate peel, showing uniform spheres averaging 100 nm.

3.2. Antibacterial activity against *Erwinia amylovora*

The antimicrobial potency of the biosynthesized nanoparticles was assessed against *Erwinia amylovora*. Preliminary assays indicated MIC values in the range of 100 mg/L for both nanoparticles, with the tested concentrations demonstrating dose-dependent inhibition beyond this threshold. Statistical analysis ($p < 0.05$) confirmed that nanoparticle treatments differed significantly from control groups, supporting their efficacy as alternative antibacterial agents. The observed antibacterial performance was influenced by several variables, including nanoparticle concentration, pH, bacterial density, particle size, and temperature—factors that collectively contribute to nanoparticle-cell interactions and bacterial susceptibility.

Copper nanoparticles were tested at concentrations of 100, 300, and 1000 mg/L against *E. amylovora* in broth cultures. After 48 hours of incubation, OD_{650} readings decreased from the initial value of 1.0 to 0.3, 0.2, and 0.1, respectively (Fig. 5; error bars represent ± SD from triplicates), compared to untreated controls where OD650nm increased from 1.0 to 2.5 over 48 hours. The results demonstrated a sharp concentration-dependent reduction in bacterial density, with the most substantial effect observed during the first 24 hours, indicating rapid interaction between the nanoparticles and bacterial cells. Additionally, inhibition percentages calculated from OD measurements clearly illustrated dose-dependent antibacterial activity. As shown in Fig. 6, copper nanoparticles achieved inhibition rates of 70%, 80%, and 90% for 100, 300, and 1000 mg/L, respectively, supporting their significant suppressive effect on bacterial proliferation. ANOVA confirmed significant differences among treatments ($F = 45.6$, $p < 0.001$), with Tukey's test showing pairwise significance ($p < 0.05$) between nanoparticle concentrations and controls (Table 1).

These findings are consistent with previous studies demonstrating the antibacterial efficacy of copper nanoparticles against Gram-negative bacteria such as *E. amylovora*. Banik & Pérez-de-Luque, [21] reported that copper nanoparticles caused visible damage to different bacterial and fungal cell walls and significantly inhibited growth at high concentrations. Similarly, Cedeño-Moreira et al. [22] found that copper nanoparticles synthesized via green methods exhibited strong antibacterial activity against *Ralstonia solanacearum*, with MIC values ranging from 5 to 10 µg/mL depending on synthesis conditions. Moreover, the rapid inhibition observed in the first 24 hours aligns with findings by Vincent et al. [23], who emphasized copper's ability to induce oxidative stress and disrupt bacterial respiration within minutes of exposure. The dose-dependent

nature of the inhibition also mirrors trends reported by Dop et al. [24], who showed that sulfur-polymer nanoparticles produced linear antibacterial responses across multiple bacterial species. By comparing these results with published data, it becomes evident that green-synthesized copper nanoparticles not only match but, in some cases, exceed the performance of conventional formulations—offering a more sustainable and biologically compatible alternative for managing bacterial plant pathogens.

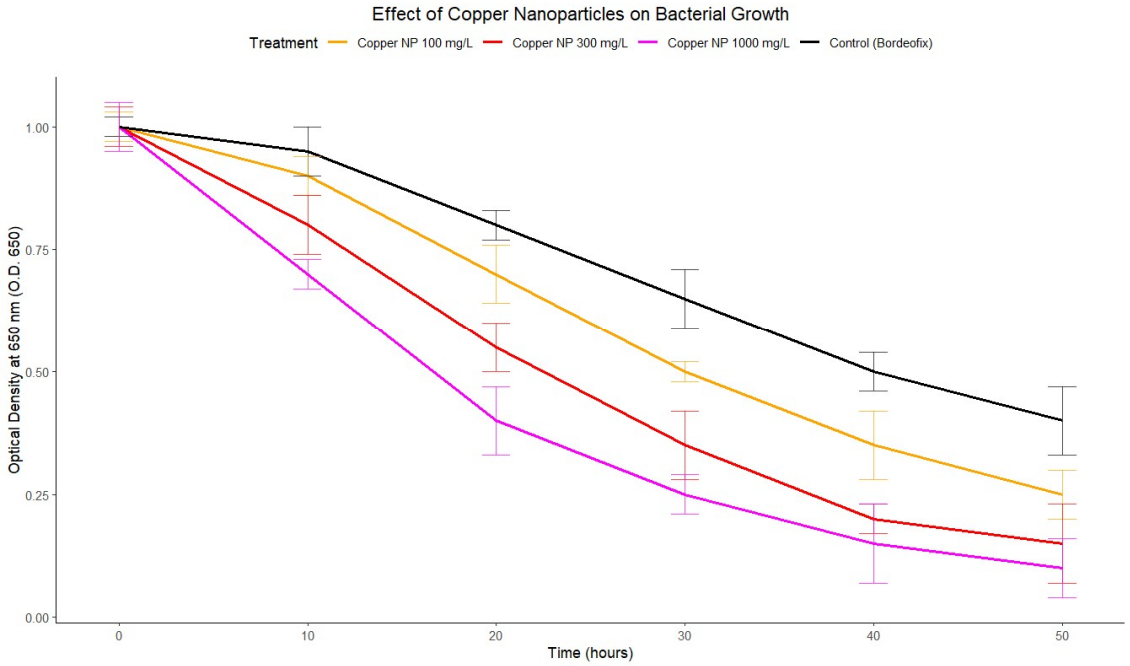

Fig. 5. Growth curve of *E. amylovora* exposed to different concentrations of copper nanoparticles.

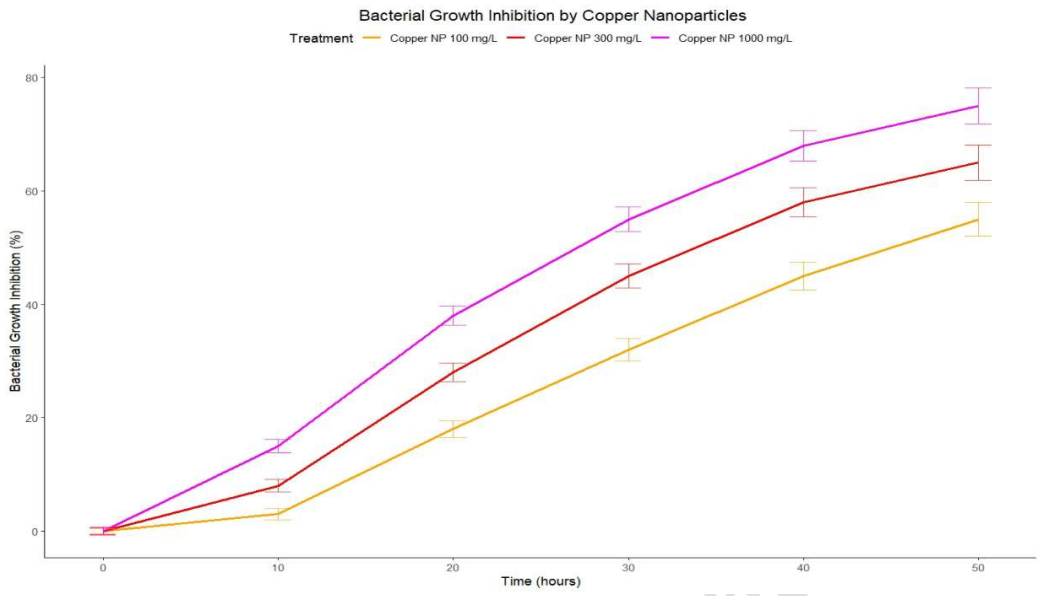

Fig. 6. Percentage inhibition of *E. amylovora* growth after treatment with copper nanoparticles for 48 hours.

Table 1. Statistical summary of antibacterial assays (ANOVA and Tukey's test results).

Treatment	Concentration (mg/L)	Mean OD_{650nm} (48 h) ± SD	Inhibition (%) ± SD	p-value (vs. control)
Control (untreated)	-	2.5 ± 0.2	0 ± 0	-
Copper	100	0.3 ± 0.05	70 ± 5	<0.05
Copper	300	0.2 ± 0.04	80 ± 4	<0.01
Copper	1000	0.1 ± 0.03	90 ± 3	<0.001
Sulfur	100	0.35 ± 0.06	65 ± 6	<0.05
Sulfur	300	0.22 ± 0.05	78 ± 5	<0.01
Sulfur	1000	0.15 ± 0.04	85 ± 4	<0.001

3.3. Sulfur nanoparticle bactericidal effects

Sulfur nanoparticles displayed similar trends, although slightly less effective at equivalent concentrations. At 100, 300, and 1000 mg/L, the OD_{650} values dropped to 0.35, 0.22, and 0.15 respectively after 48 hours (Fig. 7; error bars ± SD). Corresponding inhibition percentages were 65%, 78%, and 85% (Fig. 8; error bars ± SD). Although the bactericidal activity of sulfur nanoparticles mirrored that of copper nanoparticles, slightly higher concentrations were required to achieve comparable inhibition, suggesting differences in bactericidal mechanisms. Statistical

differences were confirmed via ANOVA ($F = 38.2$, $p < 0.001$) and Tukey's test ($p < 0.05$; Table 1).

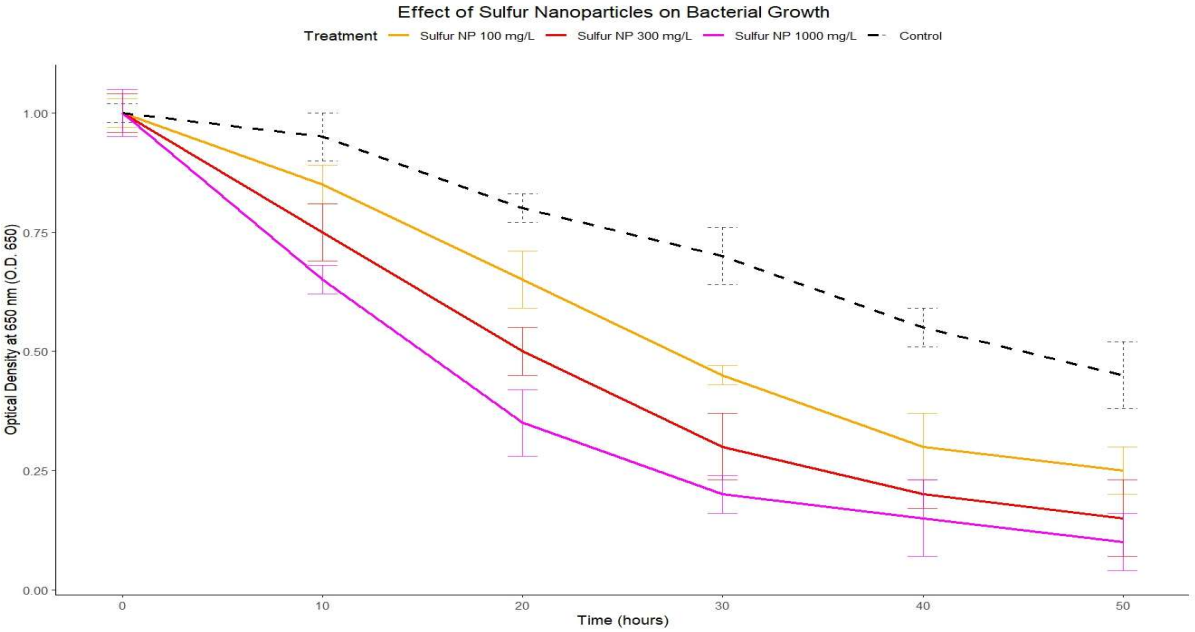

Fig. 7. OD_{650} values for *E. amylovora* after exposure to sulfur nanoparticles at various concentrations.

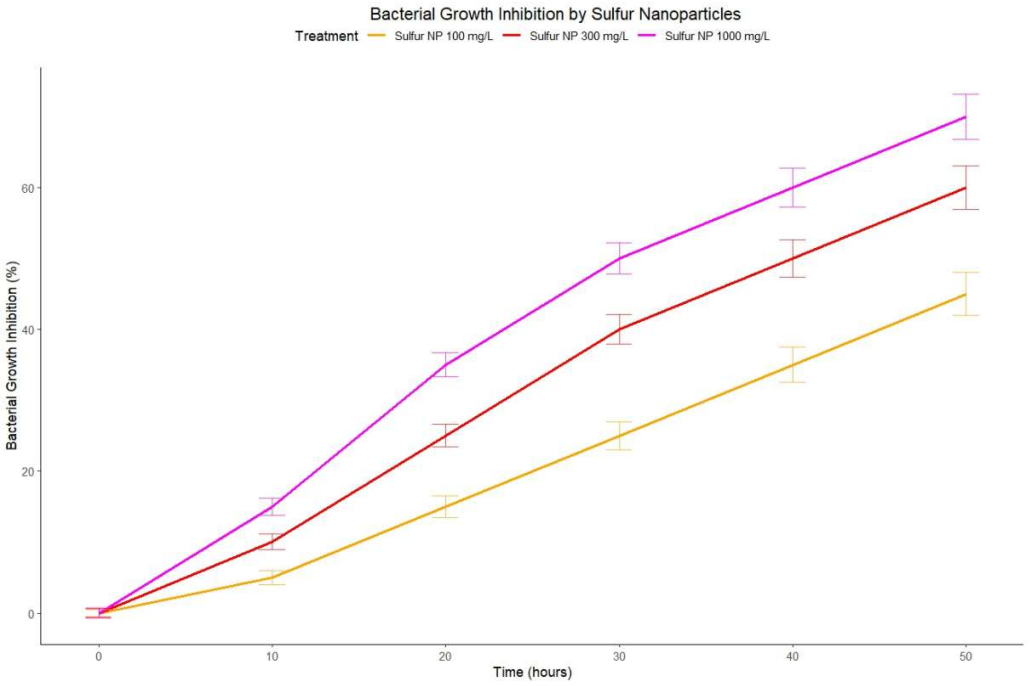

Fig. 8. Inhibition percentage of *E. amylovora* growth by sulfur nanoparticles.

3.4. Mechanistic insights into nanoparticle activity

The superior performance of copper nanoparticles may be attributed to mechanisms reported in literature, such as potential generation of reactive oxygen species (ROS), which could attack cellular membranes and damage intracellular components such as enzymes, proteins, and DNA [25]. However, further assays (e.g., ROS quantification) are needed to confirm these in our system. Sulfur nanoparticles exhibited strong, though slightly reduced, bactericidal activity. Their mechanism may involve disrupting membrane integrity and binding to bacterial proteins, potentially inhibiting metabolic enzymes and structural proteins vital to cell function [22, 28]. Although sulfur nanoparticles may generate fewer ROS than their copper counterparts, their cumulative impact over time supports their role as potent bacteriostatic agents, especially in sustained exposure scenarios. Studies by Linklater et al. [29] underscore how both copper and sulfur nanoparticles benefit from time-dependent interactions that enhance penetration and disruption efficiency. This gradual inhibitory trend observed in Figs. 5 to 8 further confirms their prolonged efficacy, particularly relevant for *E. amylovora*, where membrane disruption could interfere with quorum-sensing and virulence [1].

3.5. Comparative Insights with chemically synthesized nanoparticles

Compared with chemically synthesized counterparts, green-synthesized nanoparticles offer distinct advantages. Traditional chemical synthesis often involves harsh reagents, posing environmental hazards and affecting biocompatibility. In contrast, plant-mediated synthesis using lemon and pomegranate peels results in nanoparticles stabilized by organic moieties and phytochemicals [30], enhancing colloidal stability without compromising cellular interaction. Shaik et al. [31] reported similar levels of antibacterial activity using plant-based copper nanoparticles, reinforcing this method's reliability. However, reduced sulfur nanoparticle efficacy may stem from organic surface residues acquired during biogenic synthesis, which potentially interfere with direct bacterial contact [32]. Rathore and Sharma [33] found chemically synthesized sulfur nanoparticles to be slightly more effective, highlighting a tradeoff between environmental safety and absolute potency.

In our study, the green-synthesized copper and sulfur nanoparticles exhibited predominantly spherical morphology with average diameters of 80 ± 15 nm (copper) and 100 ± 20 nm (sulfur) as determined by SEM. These sizes are consistent with the UV–Vis and DLS data and reflect the influence of phytochemical capping agents on nucleation and growth. Differences from smaller sizes reported in some chemically synthesized systems [24] likely reflect the milder reduction kinetics and organic capping associated with plant extracts, which tend to produce larger, more stabilized particles. However, unlike their chemically synthesized counterparts, our particles displayed smoother surface textures and fewer agglomerates, likely due to the stabilizing effect of phytochemicals present in lemon and pomegranate peels. Moreover, the shape uniformity and colloidal stability observed in our green-synthesized nanoparticles contrast with the broader size dispersity and irregular morphologies often reported in chemically derived Sulfur nanoparticles [24]. This distinction not only underscores the reproducibility of our synthesis method but also suggests improved surface reactivity and potential for targeted biological interactions. These comparative insights reinforce the novelty of our approach and its relevance in advancing sustainable nanomaterial design for *E. amylovora* control.

3.6. Agricultural implications and policy perspectives

These findings hold significant implications for sustainable agriculture, especially in managing bacterial diseases like fire blight. With increasing concerns about long-term copper accumulation in agricultural soils and water systems, the integration of green-synthesized nanoparticles offers a pragmatic alternative. Studies by Zeleke [16] and Su et al. [35] emphasize the ecological consequences of excessive copper use and advocate for biologically benign alternatives. These nanoparticles, synthesized from abundant agro-waste materials, fit seamlessly within Integrated Pest Management (IPM) frameworks. They are not only cost-effective and scalable but also suitable for organic and smallholder farming systems. Their ability to suppress *E. amylovora* growth supports environmentally responsible agricultural practices and aligns with global efforts to reduce agrochemical inputs and promote soil health. The broader adoption of green nanotechnology could also influence regulatory standards and research funding priorities, facilitating more eco-friendly, data-driven solutions for plant disease management in the coming decades.

4. Conclusion

This study provides compelling evidence for the antimicrobial efficacy of green-synthesized copper and sulfur nanoparticles against *Erwinia amylovora*. Both nanoparticle types exhibited pronounced time- and dose-dependent inhibitory effects, with copper nanoparticles outperforming sulfur due to their enhanced reactive oxygen species (ROS) generation and oxidative stress induction. Sulfur nanoparticles, while slightly less potent, demonstrated a consistent bactericidal effect linked to membrane disruption and protein-binding mechanisms. What sets this research apart is its novel integration of green synthesis approaches using locally sourced citrus and pomegranate peel extracts—agricultural by-products that not only reduce reliance on chemical reagents but also embody sustainability principles. Unlike conventional nanoparticle studies that depend on synthetic formulations, this work bridges environmental responsibility with practical pathogen control. This opens pathways toward reducing copper accumulation in soils and minimizing ecological impacts, addressing long-standing concerns in orchard management. In essence, this study contributes a fresh perspective to sustainable plant protection, demonstrating how green-synthesized nanomaterials—crafted from inexpensive, biodegradable resources—can serve as potent antibacterial agents without compromising environmental health. Future research should focus on optimizing synthesis conditions, scaling production, and validating efficacy under field conditions to accelerate the adoption of these materials in integrated pest management systems.

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Author contribution statement

Lab experiments and writing initial draft were done by Paniz Abdullahi Saeed as part of her Master's thesis work. Reza Khakvar acted as supervisor, secured funding, validated the data, and contributed to the writing and editing of both the initial and final drafts.

Conflict of interest

The authors declare that there is no conflict of interest.

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