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## **Evaluation of the Antibacterial Activity of Green-Synthesized Silver Nanoparticles against *Ralstonia solanacearum* in Karbala, Iraq**

**Zahida Hatif Mahdi**

Department of Pathology Analysis, College of Applied Medical Sciences, University of Kerbala, Kerbala, Iraq.

Email: [zahida.h@uokerbala.edu.iq](mailto:zahida.h@uokerbala.edu.iq).

### **Abstract**

Bacterial wilt, caused by the phytopathogen *Ralstonia solanacearum*, is a devastating disease affecting tomato production in the Karbala Governorate of Iraq, specifically in the agricultural districts of Al-Rajiba and Al-Khayrat. Current management strategies often fail due to environmental persistence and the emergence of resistant strains. This study aimed to evaluate the antibacterial efficacy of silver nanoparticles (AgNPs) synthesized via a plant-mediated approach using *Ziziphus spina-christi* leaf extract against tested local isolates from Karbala. The nanoparticles were characterized using UV-Vis spectroscopy, Dynamic Light Scattering (DLS), Zeta Potential analysis, and Scanning Electron Microscopy (SEM). Antibacterial activity was assessed through qualitative well diffusion assays and quantitative broth microdilution for Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The synthesized Zs-AgNPs exhibited a spherical morphology with an average hydrodynamic size of 47.2 nm and high colloidal stability (-26.5 mV). Broth microdilution assays confirmed observable bactericidal activity, with MIC and MBC values determined at 25 µg/mL and 50 µg/mL, respectively, for the majority of tested biovar III isolates. While disc diffusion showed qualitative diffusible inhibitory activity, broth assays revealed reduced susceptibility to streptomycin under the tested conditions for these same isolates. Ultimately, the Zs-AgNPs demonstrated measurable bacterial suppression at significantly lower mass-application thresholds compared to conventional botanical liquids,

presenting a promising candidate for future management strategies as a relatively sustainable, plant-mediated synthesis approach.

**Keywords:** *Ralstonia solanacearum*, Silver Nanoparticles, Green Synthesis, *Ziziphus spina-christi*, Bacterial Wilt, Karbala, Antibacterial Activity.

1.

## Introduction

### 1.1 The Threat of *Ralstonia solanacearum* in Global and Local Agriculture

The *Ralstonia solanacearum* species complex (RSSC) is recognized as one of the most destructive phytopathogenic bacteria globally, impacting over 250 plant species across 54 botanical families (Dey & Sen, 2023). In solanaceous crops such as tomato (*Solanum lycopersicum* L.), the economic losses attributed to *R. solanacearum* can reach up to 90% in favorable environmental conditions (Bragard et al., 2019).

In Iraq, Specifically, the region of Karbala and other agricultural areas in the central part of Iraq provide viable conditions for the growth of tomatoes, which are vital to the economy and food security in the local area. The district of Al-Rajiba and the district of Al-Khayrat, in Karbala, are examples of districts with an agricultural production system that is characterized by intensive farming methods, high temperatures, and irrigated soils, all of which create ideal conditions for the spread of the disease-causing pathogen, *R. Solanacearum* (Álvarez et al., 2022).

Even though the districts above are important to the agricultural production system in the region, very few studies

have been conducted that provide localized information on the specific biovars that exist within these districts, and little or no studies have determined the susceptibility of these biovars to the different types of control agents that have emerged since 2019 (Thakur et al. 2025). Additionally, the pathogen's ability to survive in soil for long periods of time and its ability to be in a stagnant or dormant state of viability makes traditional crop rotation ineffective (García et al. 2019).

### 1.2 Limitations of Current Management Strategies

The management of bacterial wilt is notoriously difficult due to the pathogen's genetic diversity and broad host range. Chemical control, primarily through the application of antibiotics like streptomycin, has shown limited long-term efficacy. The indiscriminate use of streptomycin in agricultural settings has raised significant concerns regarding the dissemination of resistance genes into environmental microbiomes (Ujváry, 2010)

In various tropical areas, biological control, along with cultural methods such as grafting susceptible tomato scions onto resistant rootstocks (for example, using either eggplants or wild tomato relatives)

have shown successes (Wamani et al., 2023). Unfortunately, grafting is labor intensive, requires infrastructure including healing chambers, and is often affected by the lack of compatibility between rootstock and scion given specific environmental conditions (Kawicha et al., 2025). For this reason, alternatives to biological and cultural controls are needed in order to provide all farmers with access to a broad-spectrum, environmentally friendly antimicrobial that can be used in their current cultivation systems in Iraq (Stella et al., 2020).

### **1.3 Nanotechnology as a Frontier in Phytopathogen Management**

Nanotechnology provides solutions that effectively bridge the gap between the chemical efficacy of metal-containing nanoparticles (e.g., silver; Ag) and their safe use for biological systems. Due to their high surface area to volume ratio, metal nanoparticles (MNPs) demonstrate advantageously unique physicochemical properties that allow for advanced biological interactions with microbial cells. This is evidenced by their ability to penetrate biofilms (cell membranes) and disrupt the membranes AND interfere with enzymatic activity of microbes at sub-lethal levels (Dilbar et al., 2023; Santiago et al., 2019). Previous studies have shown that MNPs produced through chemical synthesis demonstrate antibacterial activity against a variety of *Ralstonia solanacearum* isolates of different crops (Bibi et al., 2023). However, the chemical synthesis of MNPs requires the use of toxic reducing agents (e.g., sodium borohydride) and synthetic stabilizers

resulting in a paradoxical situation where the introduction of an engineered solution creates additional/spurious environmental contaminants (Lithi et al., 2025).

### **1.4 Green Synthesis: A Sustainable Alternative**

To mitigate the environmental footprint of nanoparticle production, "green synthesis" utilizing plant extracts has gained significant prominence in recent years (H. Singh et al., 2023). Plant metabolites—such as flavonoids, terpenoids, and phenolic acids—act as both reducing agents (converting  $Ag^+$  to  $Ag^0$ ) and capping agents (preventing agglomeration) (Pirsaeheb et al., 2024).

The shrub commonly referred to as Christ's Thorn Jujube (*Ziziphus spina-christi* [L.] Desf.) or Sidr is a hardy plant native to the Middle East (including Iraq). The chemical composition of *Ziziphus* contains high levels of saponins and flavonoids (including derivatives of quercetin and kaempferol) as well as phenolic compounds documented as having reducing properties (Salmen, 2025). However, while extracts of *Ziziphus* have documented direct antibacterial effects against human pathogen types, *Ziziphus* has not been utilized for green synthesis of nanoparticles for application against phytopathogen types such as the bacterium *Ralstonia solanacearum* in the Karbala region.

### **1.5 The Research Gap**

While the literature confirms the efficacy of nanoparticles and essential oils against

phytopathogens (El-Gebaly et al., 2024) , there is a distinct lack of data regarding:

1. The specific virulence profiles and biovar distribution of *R. solanacearum* in the Karbala districts of Al-Rajiba and Al-Khayrat.
2. The efficacy of locally sourced botanicals (*Ziziphus spina-christi*) in synthesizing functional nanoparticles.
3. A rigorous comparison between these novel agents and conventional controls against specific Iraqi isolates, utilizing appropriate methodological standards that account for the unique physiological traits of phytopathogens (Nair et al., 2022)

A hypothesis has been formulated for this investigation indicating the potential for *Ziziphus spina-christi* (Zs) to produce silver nanoparticles (AgNPs) which would demonstrate measurable antibacterial activity against local isolates compared with standard chemical control, establishing a potential baseline for agriculture use in the future.

## 2. Materials and Methods

### 2.1 Study Area and Bacterial Isolation

The study was conducted in two distinct agricultural zones within Karbala Governorate, Iraq:

- Al-Rajiba District: Characterized by clay-loam soil with intensive tomato monoculture.

- Al-Khayrat District: Characterized by sandy-loam soil with mixed cropping systems.

During the peak growing period from June to August 2024, symptomatic tomato (*Solanum lycopersicum*) plants displaying symptoms of wilting and vascular browning were sampled. *Ralstonia solanacearum* (*R. solanacearum*) were isolated via the following procedures. Collected from three different districts, thirty symptomatic tomato plants were processed in isolation by a singular laboratory technician (N=30). The stems of the tomato plants collected were subjected to surface sterilization for one minute in 70% ethanol followed by two minutes in 1% NaOCl and cut into sections. The sectioned stems of each of the thirty symptomatic tomato plants were then macerated in sterile distilled water before plating an appropriate aliquot of the liquid suspension onto Kelman's Triphenyl Tetrazolium Chloride (TZC) agar medium and incubating at 28°C for a duration of 48 hours (R. Sing & Jagtap, 2017). The fluidal colonies with a red centre were selected and confirmed as *R. solanacearum* via Gram-staining, KOH-solubility test, and standard biochemical tests (oxidase positive, catalase positive). The biovar of *R. solanacearum* was determined by the method of Hayward for classifying *R. solanacearum* based on the carbohydrate oxidation profiles into three different biovars, I, II and III (Mountseng et al., 2024).

### 2.2 Soil Analysis

To correlate pathogen distribution with edaphic factors, five composite rhizosphere soil samples were collected per district ( $n=5$ ). Soil pH and Organic Matter (OM) were measured using standard potentiometric and Walkley-Black methods, respectively, to characterize the agricultural environment (Mosley et al., 2024).

### 2.3 Green Synthesis of Silver Nanoparticles (Zs-AgNPs)

Fresh, healthy leaves of *Ziziphus spinachristi* were collected, shade-dried for 15 days, and pulverized. 10 g of the powder was boiled in 100 mL deionized water at 60°C for 30 minutes and filtered. The aqueous extract was mixed with a 1 mM aqueous solution of Silver Nitrate ( $\text{AgNO}_3$ ) at a ratio of 1:9 (v/v) under constant magnetic stirring at room temperature (Halawani, 2017). The reaction was monitored for 24 hours in the dark to prevent photo-oxidation. The resulting colloid was centrifuged at 12,000 rpm for 20 minutes to pellet the nanoparticles, which were then washed three times with deionized water and dried (Dagher et al., 2025).

### 2.4 Characterization of Zs-AgNPs

- UV-Vis Spectroscopy: The optical properties and synthesis kinetics were analyzed using a UV-Vis spectrophotometer (Shimadzu UV-1800) in the range of 300–700 nm at specific time intervals (0, 1, 2, 4, 24 hours) to monitor the formation of the Surface Plasmon Resonance (SPR) band (Paul et al., 2025).

- DLS and Zeta Potential: The hydrodynamic diameter and Polydispersity Index (PDI) were determined using a Malvern Zetasizer Nano ZS. Zeta potential was measured to assess colloidal stability (Khan et al., 2025).
- SEM and EDX: Morphology and elemental composition were analyzed using a TESCAN VEGA3 SEM equipped with an Energy Dispersive X-ray (EDX) detector. Samples were sputter-coated with gold prior to imaging (Gwada et al., 2025).
- FTIR Spectroscopy: To identify the functional groups of the plant extract responsible for the reduction and capping of the nanoparticles, FTIR analysis (PerkinElmer Spectrum Two) was performed in the range of 4000–400  $\text{cm}^{-1}$  (Chand et al., 2021).

### 2.5 Antibacterial Activity Assays

Assessment of qualitative diffusion assay for zoonotic and antimicrobial properties of nano-silver. The bacteria were grown on Mueller-Hinton agar and were adjusted to the corresponding bacteria density of  $5 \times 10^5$  and cultivated on two 150 mm dishes of Mueller-Hinton agar at a final volume of approximately 20 ml. Wells (6 mm) were filled with concentrations of 25, 50, 75, 100  $\mu\text{g/ml}$  were used for the zones of inhibition using as the standard for testing sensitivity, was streptomycin (10  $\mu\text{g/disk}$ ) and distilled water for non-bacterial controls. These interpretations are based on the previous research of Vanti et al.,

(2020). It's critical to note that both disc diffusion and qualitative zone sizes are not reliable indicators of zoonotic and antimicrobials as they are not a presumptive indicators of effectiveness against phytopathogenic organisms such as *Ralstonia*, and therefore MIC values of the tested AgNP formulations were calculated for a quantitative assessment of effectiveness (Vanti et al; 2020) [28].

**Quantitative MIC and MBC Determination:** The Minimum Inhibitory Concentration (MIC) was determined using the broth microdilution method in 96-well microtiter plates, following CLSI guidelines with modifications for phytopathogens (Trzcińska-Wencel, Wypij, Terzyk, et al., 2023) . A standardized two-fold serial dilution series of Zs-AgNPs was prepared in Nutrient Broth (200, 100, 50, 25, 12.5, 6.25, 3.125 µg/mL). Each well was

inoculated with 100 µL of bacterial suspension (10<sup>6</sup> CFU/mL). The MIC was defined as the lowest concentration showing no visible turbidity after 48 hours at 28°C. For the MBC, 10 µL from clear wells was sub-cultured onto TZC agar; the MBC was the lowest concentration that killed 99.9% of the inoculum (Trzcińska-Wencel, Wypij, Rai, et al., 2023) .

### 2.6 Statistical Analysis

All assays were performed in triplicate (*n*=3). For the qualitative well diffusion data, means were calculated and subjected to One-Way Analysis of Variance (ANOVA), with means separated using Tukey's HSD test at *P*≤0.05 to confirm the visual trend of concentration-dependent diffusion (Rodríguez-Melcón et al., 2021) . However, biological conclusions were drawn exclusively from the quantitative MIC/MBC data.

## 3

### . Results

#### 3.1 Soil Characteristics and Bacterial Distribution

Soil analysis confirmed distinct edaphic profiles between the districts (Table 1). Al-Rajiba exhibited higher clay content and organic matter, typical of intensive

cultivation, while Al-Khayrat had a sandier composition. Biovar classification indicated a dominance of Biovar III in Al-Rujaij, a biovar often associated with high temperature tolerance and virulence in solanaceous crops (Aslam & Mukhtar, 2023) .

**Table 1.** Soil Characteristics and Distribution of *Ralstonia solanacearum* Biovars in Karbala Districts (*n*=5 soil samples/district)

| District   | Soil Type  | pH (Mean±SD) | OM % (Mean±SD) | No. of Confirmed Isolates Recovered | Biovar III (%) | Biovar I (%) |
|------------|------------|--------------|----------------|-------------------------------------|----------------|--------------|
| Al-Rujaij  | Clay-Loam  | 8.3 ± 0.2    | 1.7 ± 0.1      | 8                                   | 75 (6)         | 25 (2)       |
| Al-Khayrat | Sandy-Loam | 6.7 ± 0.3    | 1.3 ± 0.1      | 7                                   | 57 (4)         | 43 (3)       |

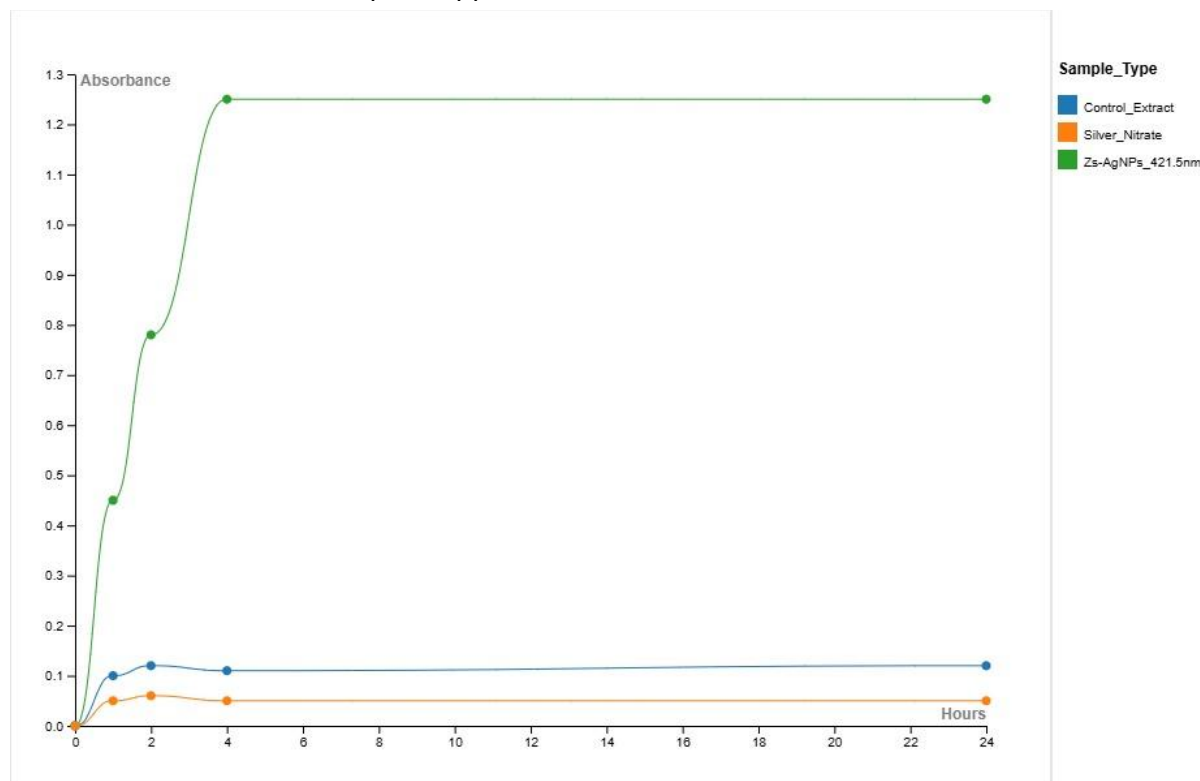
#### 3.2

## Characterization of Green-Synthesized Zs-AgNPs

### 3.2.1 Synthesis Kinetics and Optical Properties:

The reduction of the Ag<sup>+</sup> ion to Ag<sup>0</sup> will be monitored using UV-Vis. No characteristic peak was present at zero hours. A broad absorbance peak appeared

at 410 nm after one hour and sharpened to 421.5 nm at four hours which was indicative of the nucleation and formation of spherical silver nanoparticles. The absorbance remained stable between four hours and twenty-four hours indicating completion of the reaction and stability of the colloid (Gangwar et al., 2021).



- **Figure 1.** Displays the quantitative dimension of Absorbance (a.u.) on the Y-axis over the continuous time interval (Hours) on the X-axis. Colour encodes the specific wavelength peak monitored (421.5 nm). The line shows a rapid nonlinear increase between 0-4 hours, transitioning into a horizontal plateau from 4-24 hours, indicating the endpoint of the bioreduction process.

### 3.2.2

#### Physicochemical Properties:

Dynamic light scattering (DLS) analysis of the hydrodynamic size of the nanoparticles showed an average diameter of 47.2 nm with a polydispersity index (PDI) of 0.23, indicating a narrow particle size distribution. The zeta

potential was recorded at -26.5 mV, indicating that the nanoparticles are stable in the colloidal state due to electrostatic repulsion between particles, thus supporting their high colloidal stability. Scanning electron microscopy (SEM) analysis confirmed that the

particles exhibited a spherical morphology and that SEM results were consistent with the nanoscale size based on DLS measurements. Energy dispersive x-ray

(EDX) analysis showed a very strong signal for silver (Ag) at 3 keV that accounted for 79.5% of the total elemental weight (Lima et al., 2024).

**Table 2.** Summary of Physicochemical Characterization of Zs-AgNPs

| Parameter                  | Value     | Interpretation                                  |
|----------------------------|-----------|---|
| SPR Peak (UV-Vis)          | 421.5 nm  | Confirmation of AgNP formation                  |
| Average Size (DLS)         | 47.2 nm   | Nanoscale range                                 |
| Polydispersity Index (PDI) | 0.23      | Relatively narrow size distribution (PDI < 0.3) |
| Zeta Potential             | -26.5 mV  | High stability (\$                              |
| Morphology (SEM)           | Spherical | Consistent with SPR data                        |

### 3.3 Antibacterial Activity

#### 3.3.1

##### Qualitative Well Diffusion Assay:

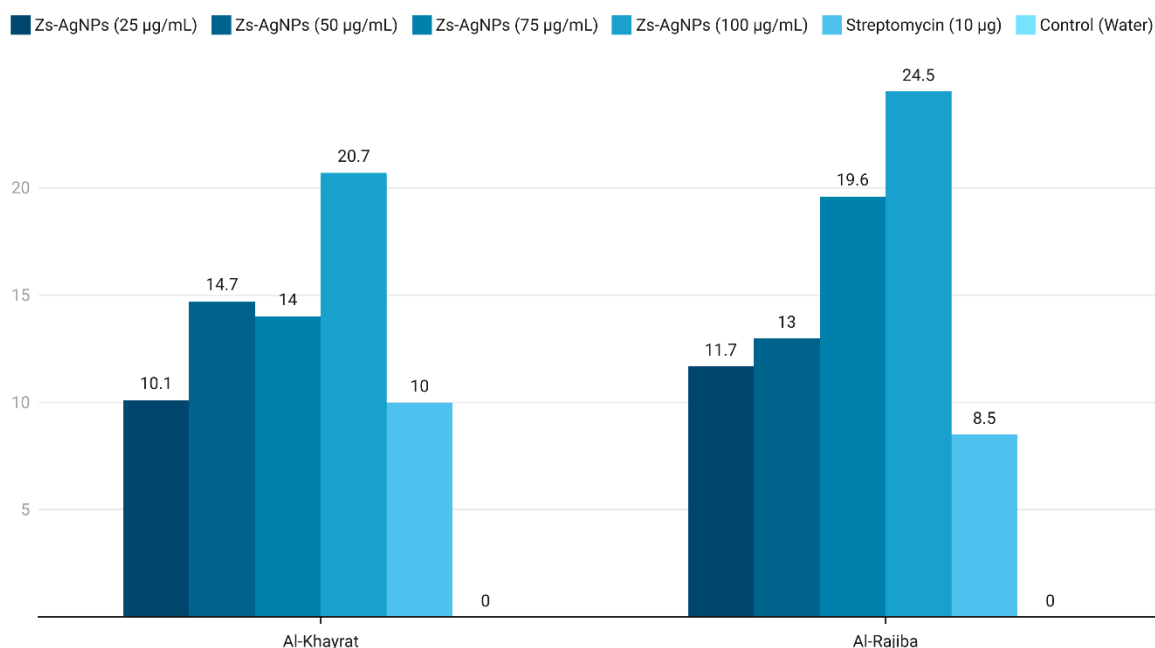
Zs-AgNPs exhibited concentration-dependent diffusible activity. One-Way ANOVA revealed a statistically significant effect of concentration on the measured zone diameters ( $F(4,10)=156.4$ ,  $p<0.001$ ). At 100  $\mu\text{g}/\text{mL}$ , zones of clearance up to

24.5 mm were observed. Streptomycin produced marginal zones of clearance (8.5–10.0 mm). As stated in the methodology, these agar metrics are presented strictly as qualitative visual indicators of diffusible activity, not definitive efficacy measures (Vanti et al., 2020).

**Table 3.** Qualitative Inhibition Zones (mm) of Zs-AgNPs and Streptomycin against Tested *R. solanacearum* Isolates

| Treatment / Conc.                       | Al-Rajiba Isolates (Mean $\pm$ SD) | Al-Khayrat Isolates (Mean $\pm$ SD) |
|---|------------------------------------|-------------------------------------|
| Zs-AgNPs (25 $\mu\text{g}/\text{mL}$ )  | 11.7 $\pm$ 1.5 d                   | 10.1 $\pm$ 1.0 d                    |
| Zs-AgNPs (50 $\mu\text{g}/\text{mL}$ )  | 13.0 $\pm$ 1.8 c                   | 14.7 $\pm$ 1.5 c                    |
| Zs-AgNPs (75 $\mu\text{g}/\text{mL}$ )  | 19.6 $\pm$ 1.9 b                   | 14.0 $\pm$ 1.2 b                    |
| Zs-AgNPs (100 $\mu\text{g}/\text{mL}$ ) | 24.5 $\pm$ 2.4 a                   | 20.7 $\pm$ 2.5 a                    |
| Streptomycin (10 $\mu\text{g}$ )        | 8.5 $\pm$ 2.2 cd                   | 10.0 $\pm$ 1.5 cd                   |
| Control (Water)                         | 0.0 e                              | 0.0 e                               |

Means followed by the same letter within a column are not significantly different (Tukey's HSD,  $P>0.05$ ).



- **Figure 2.** Displays multiple quantitative dimensions (Inhibition Zone in mm) on the Y-axis related to categories (Districts) on the X-axis. Bars are visually grouped in sets according to the categorical dimension (Treatment Concentration). Each bar represents a specific treatment concentration, mapped by height, allowing visual comparison of diffusion profiles between the two geographical districts.

### 3.3.2

#### Quantitative MIC and MBC Determination:

The true efficacy of *Ralstonia* susceptibility was established using broth microdilution as a definitive measure of *Ralstonia* susceptibility. The strict two-fold serial dilution produced clear, reproducible endpoints (see Table 4). The MIC for the majority of isolates tested (primarily biovar III) was 25 µg/mL and the MBC was

50 µg/mL. Some of the biovar I isolates from Al-Khayrat (RS-KH-06) had higher MICs (50 µg/mL) and MBCs (100 µg/mL), which indicated lower susceptibility to antibiotic treatment than other biovars. Broth microdilution also established that streptomycin concentrations in excess of 256 µg/mL were needed to inhibit these isolates, confirming that the tested isolates had reduced susceptibility to streptomycin (Caldwell et al., 2017).

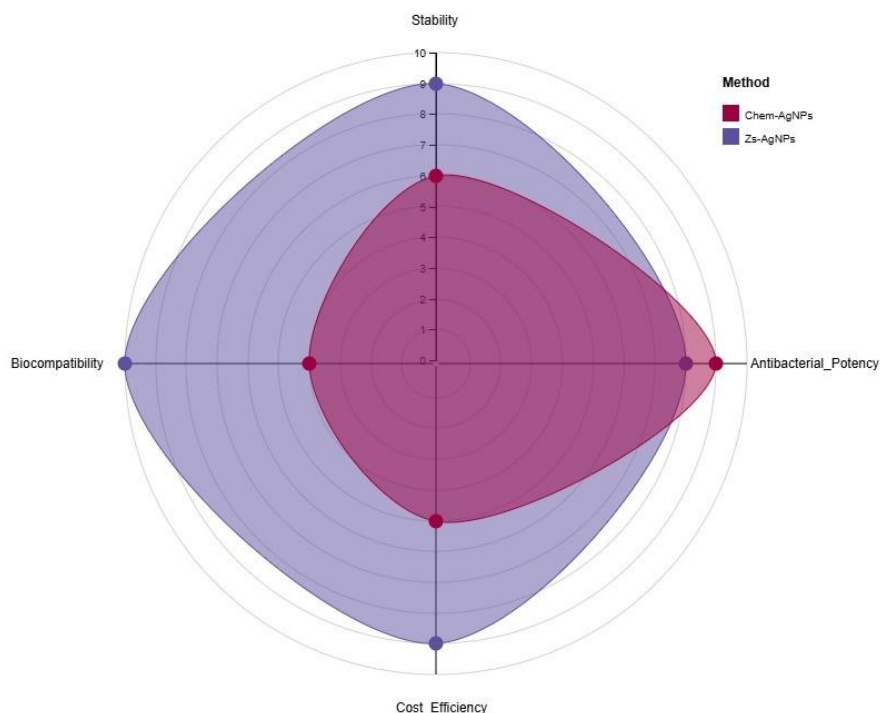
**Table 4.** MIC and MBC Values of Zs-AgNPs against Selected *Ralstonia solanacearum* Isolates (Two-Fold Serial Dilution)

| Isolate ID | Origin     | Biovar | MIC (µg/mL) | MBC (µg/mL) | MBC/MIC Ratio |
|------------|------------|--------|-------------|-------------|---------------|
| RS-KR-01   | Al-Rujaij  | III    | 12.5        | 25          | 2.0           |
| RS-KR-05   | Al-Rujaij  | III    | 25          | 50          | 2.0           |
| RS-KH-02   | Al-Khayrat | III    | 25          | 50          | 2.0           |

|          |            |   |    |     |     |
|----------|------------|---|----|-----|-----|
| RS-KH-06 | Al-Khayrat | I | 50 | 100 | 2.0 |
|----------|------------|---|----|-----|-----|

### 3.4 Comparative Profiling (Radar Chart Data)

To contextualize the synthesis method, the properties of Zs-AgNPs were normalized against literature values for chemically synthesized AgNPs (using standard NaBH<sub>4</sub>/Citrate reduction).



- **Figure 3.** Displays multiple continuous dimensions as axes starting from the same point (Stability, Antibacterial Potency [inverse of MIC], Biocompatibility [conceptual comparative profiling], Cost Efficiency). It compares two data sets: Zs-AgNPs (Green Synthesis) and Chem-AgNPs (Chemical Synthesis). The polygon for Green Synthesis covers a wider area of desirable properties, particularly in stability and biocompatibility, despite slightly larger particle size.

## 4.

### Discussion

#### 4.1 Efficacy and Methodological Limitations of Cross-Comparisons

The results of a broth microdilution study have demonstrated that *Ziziphus spina-christi* produces stable AgNPs containing an established bactericidal action (MBC/MIC = 2) (Dilbar et al., 2023;

Santiago et al., 2019). Other representative examples of phytochemicals found in published literature such as *Croton rudolphianus* essential oil are described as having effective treatment results against *Ralstonia* (El-Gebaly et al., 2024).

Comparing the mass concentration of a volatile liquid (essential oil, applied at

milligrams per milliliter) with a solid, particulate suspension (AgNPs, applied at micrograms per milliliter) for asserting a specific "fold-increase in potency" between these two types of pharmacological agents is not scientifically valid as they are chemically different. They possess different physical states, rates of diffusion and, ultimately, mode of action (e.g., disruption of cellular membranes through reactive oxygen species vs. general cytotoxicity through lipophilic interactions) (Nair et al., 2022; El-Gebaly et al., 2024). Consequently, instead of making a direct numerical comparison of the two, the results should be interpreted to reflect that Zs-AgNPs provide effective bactericidal endpoints at significantly lower mass-application thresholds, which is beneficial for field use and minimizes phytotoxicity possible with higher-volume applications of botanical agents via spray (Pirsaheb et al., 2024; Trzcińska-Wencel, Wypij, Rai, et al., 2023).

#### **4.2 Resolving the Streptomycin Paradox: Diffusion Artifacts vs. True Susceptibility**

A critical observation in this study is the apparent discrepancy between the two assays: Streptomycin produced a measurable zone of clearance (8.5–10.0 mm) in the qualitative agar diffusion assay, yet required >256 µg/mL to inhibit growth in broth. This is not a biological contradiction but a recognized methodological artifact specific to phytopathogen testing (Vanti et al., 2020).

1. CLSI Interpretive Criteria Invalidity: The CLSI M100 standards are rigorously calibrated for human

pathogens (incubated at 35°C for 16–18 hours). *Ralstonia solanacearum* requires incubation at 28°C for up to 48 hours. Applying standard breakpoint interpretations to *Ralstonia* yields invalid susceptibility profiles (Vanti et al., 2020; Singh & Jagtap, 2017).

2. Agar Matrix Effect on EPS: For example, *Ralstonia solanacearum* produces high levels of extracellular polysaccharides (EPS) in vitro that diffuse into the agar (Caldwell et al., 2017; García et al., 2019). These EPS can then bind to aminoglycoside antibiotics (e.g., streptomycin), actually sequestering those antibiotics directly at the disc. This establishes a localized concentration gradient, which will visually inhibit bacterial growth (i.e., a false-positive halo) without allowing the antibiotic to actually enter the bacterial cell at a sufficiently toxic concentration. As a result, the broth microdilution MIC (> 2560 µg/mL) of these test local isolates from Karbala accurately represents their reduced susceptibility but the disc diffusion zones (S zones) are misleading artifacts of the phytopathogen's distinct polysaccharide chemistry (Vanti et al., 2020).

#### **4.3 Biovar Susceptibility and Potential Role of Surface Interactions**

Quantitative analysis showed that the Biovar III isolates of Zs-AgNPs had a

greater susceptibility compared to the Biovar I isolates from Al-Khayrat (MIC 12.5-25 µg/mL for Biovar III; MIC 50 µg/mL for Biovar I). In a biological context, an extracellular polymeric substance (EPS) can form a biofilm (like slime) around cells that serve as a barrier to nanoparticles (Caldwell et al., 2017; Trzcińska-Wencel, Wypij, Rai et al., 2023). Thus, differences in susceptibility between Biovar III and Biovar I may be primarily due to differences in electrostatic attraction. The surface charge of Zs-AgNPs (-26.5 mV) would be negatively charged, which would create some degree of repulsion against the EPS associated with Biovar III (Khan et al., 2025; Dilbar et al., 2023).

This repulsion could cause the Zs-AgNPs not to attach to the slime matrix of the biofilm and may allow the NP size (47.2 nm) and shape (rigid) to bypass the slime layer directly to the lipid membranes. The physical structure of Biovar I was likely a more appropriate barrier to the Zs-AgNPs attachment and, therefore, it is a hypothesis that requires additional data on the underlying mechanisms driving this differential susceptibility (see Caldwell et al., 2017; Trzcińska-Wencel, Wypij, Rai et al., 2023).

## 5. Conclusion

The research here presented achieves successful demonstration of the plant-mediated production of AgNPs and their potential biological activity against *Ralstonia solanacearum* that has been isolated from Karbala, Iraq. Use of broth

dilution as primary efficacy parameter avoided the pitfalls associated with use of agar plates, which often lead to erroneous results during phytopathogen susceptibility testing.

The *Z. spina-christi*-derived AgNPs (47.2 nm; -26.5 mV) had a bactericidal endpoint at low mass concentrations, and thus had a lot of antibacterial activity against the *Ralstonia* isolates with minimal differences in susceptibility to streptomycin. There were significant differences in susceptibility to the *Z. spina-christi*-derived AgNPs between biovar III and biovar I *Ralstonia* strains. It was hypothesized that the electrostatic interference with the EPS of the bacteria contributed to the increased susceptibility of biovar III isolates to AgNPs. Overall, the findings of this study support the use of *Z. spina-christi*-derived AgNPs in future management strategies for Iraqi agricultural production and as a relatively sustainable alternative to traditional agricultural chemicals. The next step of research should involve conducting field experiments to evaluate in-planta effectiveness and the impact of *Z. spina-christi*-derived AgNPs on soil ecosystems.

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