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Biologically Synthesized Silver Nanoparticles Efficiently Control Plant Pathogenic Bacteria-*Erwinia carotovora* and *Ralstonia solanacearum*

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Abstract: Biogenic nanoparticles are considered effective alternatives to chemical pesticides for the management of pathogenic plant diseases. This study was focused on the synthesis of stable silver nanoparticles (AgNPs) to control challenging plant pathogenic bacteria in vitro and in planta. We synthesized AgNPs by reacting different proportions of silver nitrate and aqueous extract of *Hedera nepalensis*. The physicochemical properties of the synthesized AgNPs were determined by using various physical techniques. The TEM analysis revealed the AgNPs less than 50 nm in size and spherical shaped. For antibacterial assays, different concentrations (1000–15.62 µg/mL, 2-fold dilutions) of the extract-free AgNPs (Ef-AgNPs) or extract-mixed AgNPs (Em-AgNPs), and fruit extracts (FE) were used against plant pathogenic bacteria *Erwinia carotovora* subsp. *carotovora*, *Erwinia carotovora* subsp. *atroseptica*, and *Ralstonia solanacearum*. In the in vitro assays, we found significant inhibition of both bacterial species in response to maximum concentrations of AgNPs. Overall, Ef-AgNPs exhibited a higher percent inhibition of bacterial pathogens. In potato tubers assay, complete inhibition of *Erwinia carotovora* was observed, except for the lowest AgNPs concentration of 15.62 µg/mL. Similarly, exposure of tomato plants to *Ralstonia solanacearum* suspensions (OD600 = 0.2) in the soil-drenching experiment and post-exposure treatment with 1000 µg/mL and 125 µg/mL of AgNPs resulted in disease inhibition. This study provides the basis that biogenic nanoparticles prepared from *Hedera nepalensis* are one of the best substitutes to synthetic pesticide, having displayed better results to control the growth of phytopathogenic microbes. However, field studies need to be conducted in a controlled environment to scale up the current work and find out the efficacy of nanoparticles on a larger scale.

Keywords: silver nanoparticles; phytopathogenic; *Erwinia carotovora*; *Ralstonia solanacearum*; in planta

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1. Introduction

Plant diseases lead to huge economic losses due to compromised agricultural productivity. Pathogenic microorganisms deteriorate the quantity and quality of plants worldwide, which leads to billion-dollar losses [1]. The current agricultural production system is going through the challenge of lower productivity due to multiple factors such as pathogens attack, stress condition, and crop loss, which ultimately minimize the whole productivity by up to 25% [2].

Conventional approaches to control plant pathogens include biological control agents, the release of new varieties showing resistance to pathogens, chemical pesticides, and the stimulation of plant defense system [3]. Contrary to the beneficial effects of biopesticides on the environment [4], synthetic pesticides are hazardous for humans and the environment. As a result, pest management has been challenged with economic and ecological constraints globally [5]. However, the sustainable applications of biopesticides are hindered by various operational and developmental constraints.

The emergence of nanomaterials and their applications in physical, chemical, and biosciences is gaining tremendous interest [6,7]. Multiple applications of nano-based materials led to the emergence of nano-revolution based on the particulate size, which plays a key role in various sectors of biological sciences [8]. In agriculture, nanofertilizers, nanopesticides, and nanosensors are under extensive research [9]. The nano-based materials have the potential to directly affect the target sites rather than effecting other non-target tissues and can be easily degraded by various microorganisms. This shows the dominancy of nano-based materials over the synthetic compounds [10–12]. They have persistent biological effects which may reduce the extensive use of conventional pesticides.

The plant-based synthesis of nanoparticles is superior in the context of the availability of plants as a biological substrate in bulk, their operational ease, and the presence of a variety of reducing metabolites in their tissues [13,14]. Thorley and Tetley [15] explained that nanoparticles can be used in numerous applications in various sectors, such as cosmetics, food products/processing, diagnostics, and in medicine. Several reports are available on the plant-based nanoparticle-induced inhibition of phytopathogens, including fungi and bacteria [16–19]. The emergence of nanoparticles associated with silver as ideal candidates for antimicrobial activities has been excellently reviewed [20]. Various studies have reported that nanoparticles of silver and its compounds have significant bactericidal, fungicidal, and antiviral activities [21–23]. The biogenic silver nanoparticles have shown efficient inhibition of Phytophthora species in planta experiments without negatively affecting the normal morphological characteristics of the host plant Nicotiana [24].

As a novel study, we report the synthesis of stable AgNPs by using *Hedera nepalensis* aqueous fruit extract as a reducing and capping substrate, and the synthesized AgNPs either alone or in combination with plant extract were tested against the plant pathogens *Erwinia carotovora* and *Ralstonia solanacearum* in vitro and in planta.

2. Results

2.1. Visual Observation of the Synthesized AgNPs

After mixing the same volumes of silver nitrate (6, 4, 2 Mm) and plant extract (10, 5, 2.5 mg/mL), the change in the color of reaction mixture to yellowish brown or dark brown indicated biosynthesis of AgNPs. After 24 h of mixing, brown colored colloidal solutions of AgNPs were obtained. No color change was observed with the plant extract or $AgNO_3$ alone under the same conditions.

2.2. Characterization of AgNPs by UV-Vis Spectroscopy

UV-visible spectroscopy analysis showed (Figure 1) an increase in UV-vis spectrum above 350 nm, with the most pronounced peak in the 400 to 500 nm range. The most appropriate peak of the mixture of fruit extract and silver nitrate (5 mg/mL + 6 mM) resulted in a narrower peak at 442 nm.

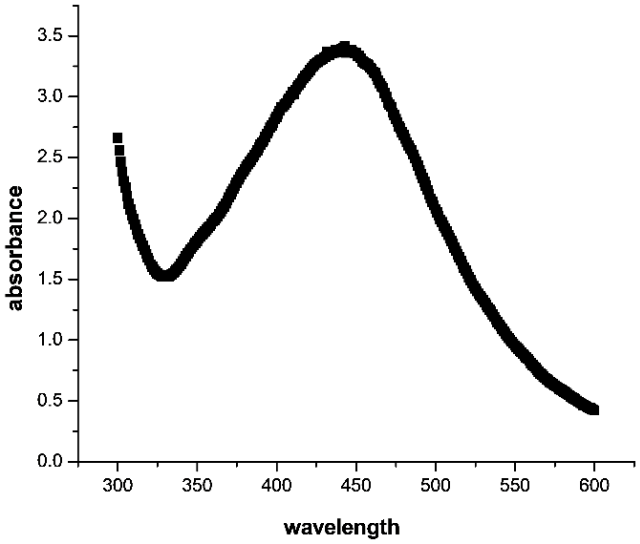


Figure 1. UV-vis absorbance spectrum of 6 mM $AgNO_3$ and 5 mg/mL of *Hedera nepalensis* fruit extract mixed in 1:1 (*v*/*v*) ratio.

2.3. Physical Characterization

2.3.1. Fourier Transform Infrared Spectroscopy

The Figure 2 showed that synthesized nanoparticles exhibited functional groups with seven peaks at diverse sites ranging from 500 to 4500 cm^{-1}. The functional groups identified with their corresponding peaks were OH group (3356 cm^{-1}), CH and CH2 stretching bond of aliphatic group (2625 cm^{-1}), C=O functional group of quinone compound (2625 cm^{-1}), C=O bond of inorganic carbonate (1453 cm^{-1}), CH aliphatic bending group (1371 cm^{-1}), the amide C-N (1259 cm^{-1}), and the alkyl amine (1037 cm^{-1}).

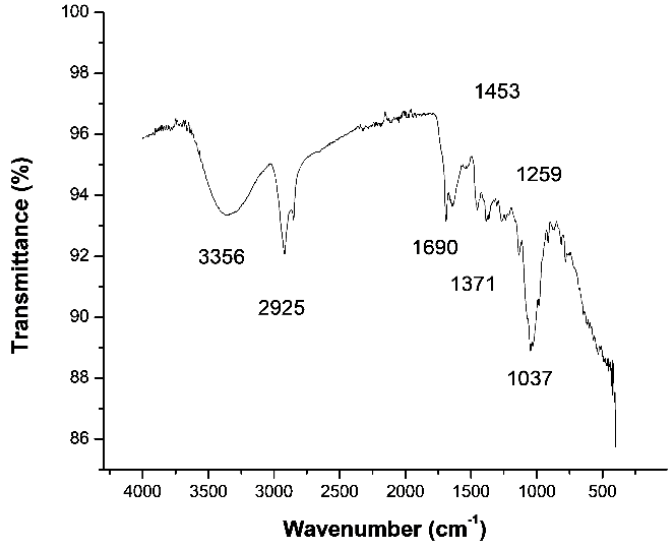


Figure 2. FTIR spectrum of AgNPs synthesized by using the fruit extract of *Hedera nepalensis*.

2.3.2. Transmission Electron Microscopy Analysis

In this study, the TEM analysis (Figure 3) was focused to evaluate the morphological features of the synthesized nanoparticles. The results showed maximum nanoparticle ovals that were spherical in shape and less than 50 nm in diameter. Moreover, agglomeration of nanoparticles into clusters was also observed.

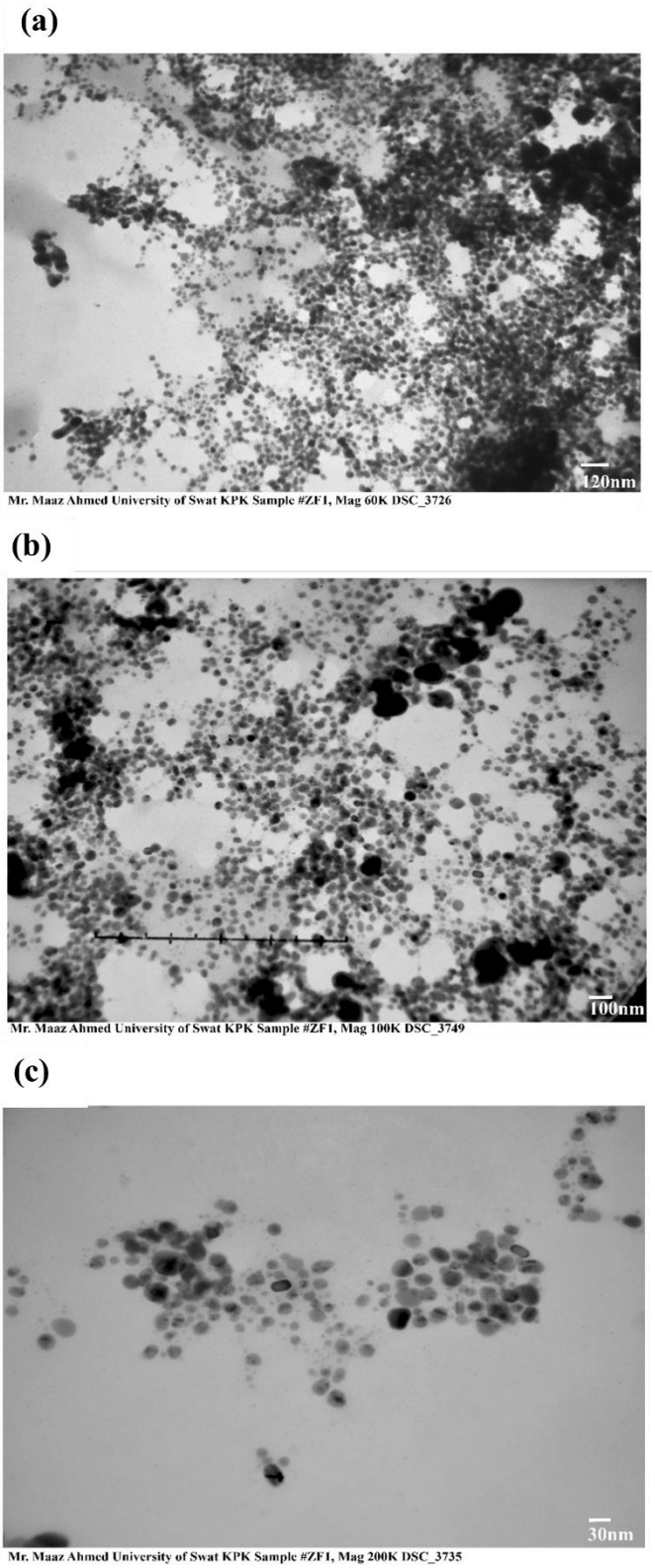


Figure 3. Magnification wise distribution of the TEM micrograph ((**a**) 120 nm, (**b**) 100 nm, and (**c**) 30 nm) of the synthesized AgNPs.

2.3.3. EDX Analysis

EDX spectra (Figure 4) revealed major energy peaks corresponding to Ag, Cl, C, and O. Several peaks were observed around the Ag main peak, which indicated the different valency states of Ag in the particles. Other peaks may be attributed to C-coated grids.

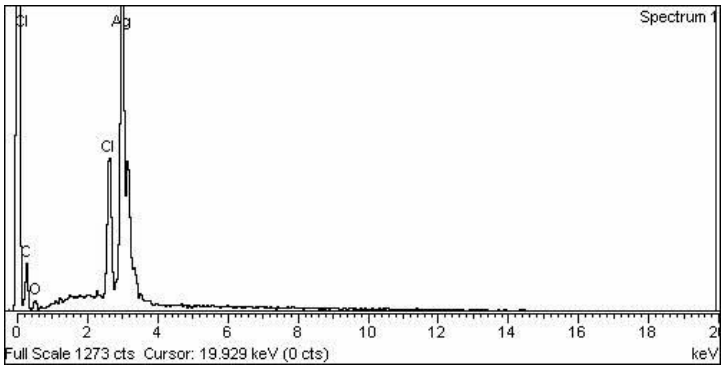


Figure 4. EDX spectrum of AgNPs synthesized by using the fruit extract of *Hedera nepalensis*.

2.4. In Vitro Antimicrobial Analysis

2.4.1. Growth Inhibition of *Erwinia carotovora* subsp. *atroseptica* (ECA)

According to Figure 5 The highest concentrations, T1 (1000 µg/mL) and T2 (500 µg/mL), of Ef-AgNPs exhibited 100% growth inhibition of the tested bacteria, T3 (250 µg/mL) inhibited about 90% growth, and T4 (125 µg/mL) and T5 (62.5 µg/mL) showed more than 80% activity against *Erwinia carotovora* subsp. *atroseptica*. The lowest concentration, T7 (15.62 µg/mL), inhibited the bacterium by more than 50%. Em-AgNPs exhibited 100% activity at T1, T2, and T3. However, T4 inhibited more than 90% growth of ECA. Further, only 5% of growth inhibition was observed at T7 concentration. Less than 30% of antibacterial activity was observed in response to most of the fruit extract concentrations.

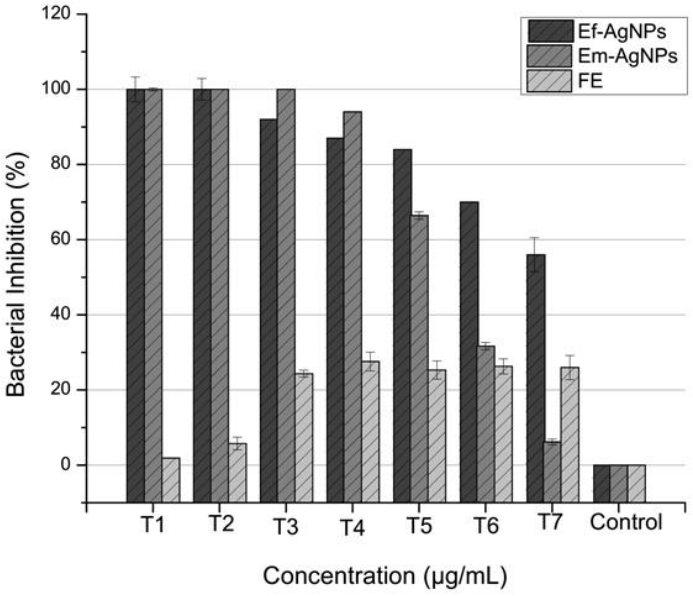


Figure 5. Inhibition of *Erwina carortovora* subsp. *atroseptica* by extract-free silver nanoparticles (Ef-AgNPs), extract-mixed silver nanoparticles (Em-AgNPs), and fruit extract (FE) of *Hedera nepalensis*. Concentrations (µg/mL) (T1:1000, T2: 500, T3: 250, T4: 125, T5: 62, T6: 31.25, T7: 15.62). The error bars indicate standard error of mean, and values are mean ± standard error of three replicates.

2.4.2. Growth Inhibition of *Erwinia carotovora* subsp. *carotovora* (ECC)

In different concentrations (Figure 6) of Ef-AgNPs, the highest antibacterial activity of 100% was shown by T3 (250 µg/mL), followed by a gradual decrease in activity with decreasing nanoparticles concentration. T5 inhibited bacterial growth by more than 80%. However, T7 showed minimum inhibition activity above 30%. In the case of Em-AgNPs, lower concentration such as 125 µg/mL exhibited 100% inhibition of ECC. About 40% of activity was shown by most of the plant extract concentrations.

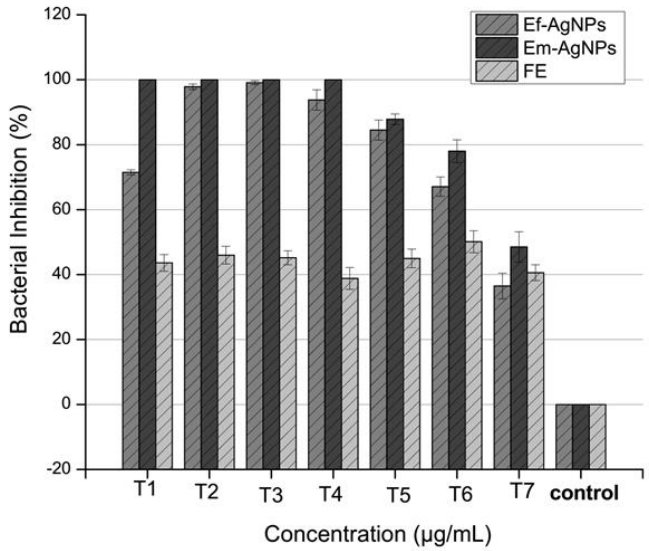


Figure 6. Inhibition of *Erwinia carotovora* subsp. *carotovora* by extract-free silver nanoparticles (Ef-AgNPs), extract-mixed silver nanoparticles (Em-AgNPs), and fruit extract (FE) of *Hedera nepalensis*. Concentrations (µg/mL) (T1:1000, T2: 500, T3: 250, T4: 125, T5: 62, T6: 31.25, T7: 15.62). The error bars indicate standard error of mean, and values are mean ± standard error of three replicates.

2.4.3. Growth Inhibition of *Ralstonia solanacearum*

In the in vitro experiment (Figure 7), more than 90% inhibition of *R. solanacearum* was observed in response to 1000 µg/mL of Ef-AgNPs. The same concentration of Em-AgNPs caused reduced activity. Unlike the dose-dependent bacterial inhibition of Ef-AgNPs, 250 µg/mL of Em-AgNPs resulted in 100% antibacterial activity. However, the IC-50 for both types of nanoparticles was found at 62.5 µg/mL. The highest concentration (1000 µg/mL) fruit extract of *Hedera nepalensis* exhibited almost less than 30% bacterial inhibition, followed by reduced activity in response to diluted concentrations.

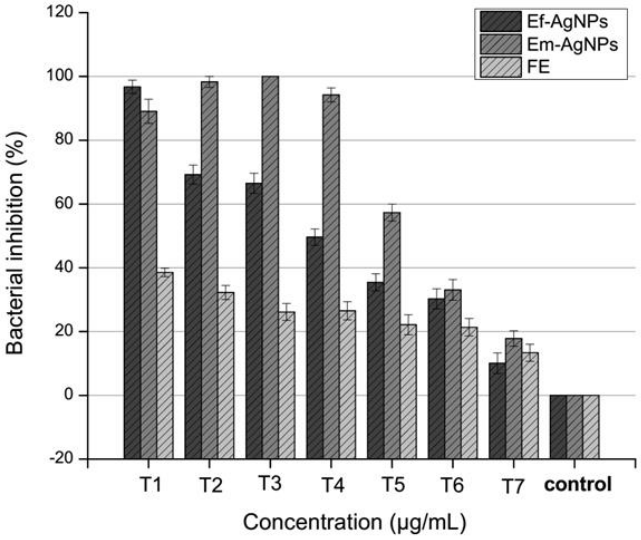
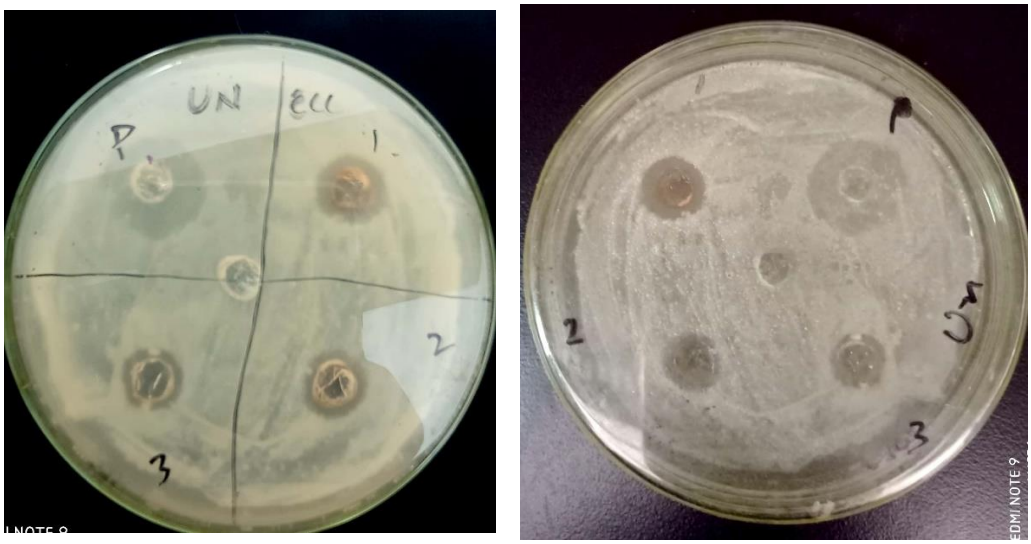


Figure 7. Inhibition of *Ralstonia solanacearum* by extract-free silver nanoparticles (Ef-AgNPs), extract-mixed silver nanoparticles (Em-AgNPs), and fruit extract (FE) of *Hedera nepalensis*. Concentrations (µg/mL) (T1:1000, T2: 500, T3: 250, T4: 125, T5: 62, T6: 31.25, T7: 15.62). The error bars indicate standard error of mean, and values are mean ± standard error of three replicates.

2.4.4. Well Diffusion Assay

Against *Erwinia carotovora* subsp. *carotovora*, the Em-AgNPs showed higher activity than the Ef-AgNPs. The zone of inhibition in response to 1000 µgm/mL, 250 µgm/mL, and 62.5 µgm/mL was found to be 15 mm, 13 mm, and 12 mm, respectively (Figure 8). Similarly, 1000 µgm/mL, 250 µgm/mL, and 62.5 µgm/mL of the Ef-AgNPs exhibited 12 mm, 11 mm, and 9 mm zone of inhibition. Against *Ralstonia solanacearum*, the zone of inhibition was found to be 15 mm, 12 mm, and 7 mm in response to Em-AgNPs of 1000 µgm/mL, 250 µgm/mL, and 62.5 µgm/mL, respectively. The Ef-AgNPs resulted in 12 mm, 7 mm, and 4 mm zones of inhibition in response to the three tested concentrations. No zone of inhibition was detected in response to plant extracts against either bacterium. Ultimately, 100 ppm of streptomycin used as positive control, and displayed 22 mm zone of inhibition.



(**a**) (**b**)

Figure 8. Inhibition of *Erwinia carotovora* (**a**) and *Ralstonia solanacearum* (**b**) by 1000 µgm/mL, 250 µgm/mL, and 62.5 µgm/mL of Em-AgNPs.

2.5. Potato Tubers Infectivity Assay

The results of treating potato tubers with mixtures of bacterial suspension and each individual concentration of Em-AgNPs showed (Figure 9) that the lack of disease appearance was observed until the concentration T5 (62.5 µg/mL). The proceeding concentrations resulted in the appearance of the characteristic lesion of *Erwinia* infection, and the controlled treatment without nanoparticles appeared to be completely infected by the bacterium.

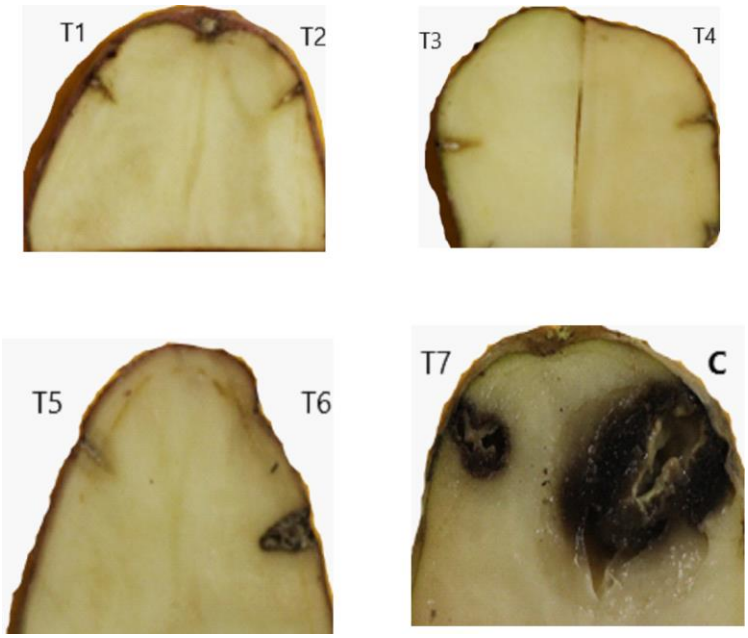


Figure 9. Potato tuber infectivity assay with various concentrations (T1: 1000 µg/mL through 125 µg/mL) of Em-AgNPs against *ECC*.

2.6. Prevention of Bacterial Wilt Disease in Tomato Plants

For this assay, the pot-grown tomato plants were raised in a controlled environment. Drenching Em-AgNPs with lower concentrations such as 125 µg/mL resulted in disease-free plants (Figure 10). Moreover, the plants were healthy and no visible phytotoxicity was observed.

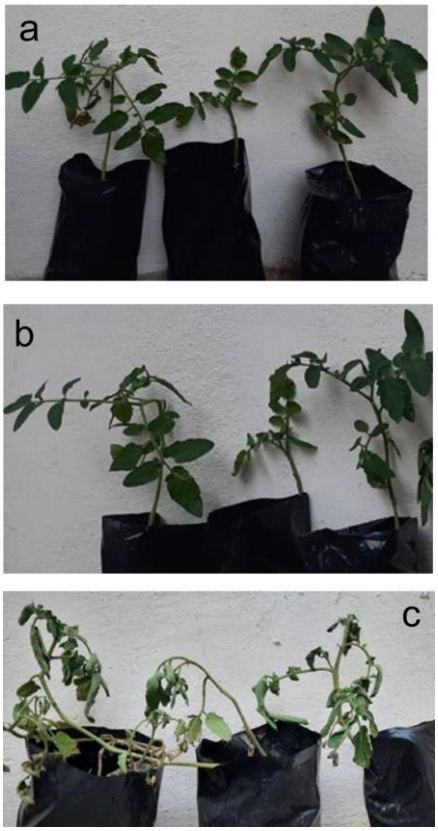


Figure 10. Prevention of tomato wilt by Em-AgNPs. (**a**) 1000 µg/mL. (**b**) 125 µg/mL. (**c**) Control.

3. Discussion

Generally, the main strategy for pest management comprises of using pesticides such as herbicides, fungicides, and insecticides. However, their nonspecific inhibition of non-target organisms and the developing resistance in microorganisms against chemical pesticides are among some of the limiting factors in their extensive use [25]. Agricultural nanotechnology has attracted immense attention, especially for the crop protection of crops and disease management [11]. Recently, the effective use of nanoparticles against various plant pathogens has been excellently reviewed [26–28]. Nanoparticles possess high surface to volume ratios, and therefore can target the pathogenic organisms in a shorter time. The biological synthesis of nanoparticles needs nontoxic chemicals, eco-friendly solvents, and renewable materials [14].

In this study, we have synthesized silver nanoparticles with desirable physical characteristics to control the challenging plant pathogens *Erwinia* and *Ralstonia*. For the synthesis, we mixed aqueous solutions of silver nitrate and *Hedera nepalensis* aqueous extract in different ratios. The first indication of the synthesis of AgNPs was the appearance of dark brown color of the reaction mixtures, as has been previously reported by Ali et al. [29]. Thereafter, UV-visible spectroscopic analysis revealed a narrower peak at 442 nm of the reaction mixture, consisting of silver nitrate (6 mM) and fruit extract (5 mg/mL) (Figure 1). The results were consistent with various previous studies, where the characteristic peaks for silver were observed at 433 nm [30], 436 nm [31], and 420 nm [32]. The FTIR spectrum of the silver nanoparticle-synthesized AgNPs specified several functional groups existing at diverse sites ranging from 500 to 4500 cm^{-1} (Figure 2), which indicated the presence of capping biomolecules of the plant extract adsorbed to the nanoparticles. The overall spectrum indicated the presence of stretching OH group, CH and CH2 stretching bond of aliphatic group, C=O functional group of quinone compound, C=O bond of inorganic carbonate, CH aliphatic bending group, the functional group amide C-N, and the bond present in alkyl amine functional group. Previously, iron oxide nanoparticles with capping biomolecules have been reported by Alam, Khan, Ali, Sher, Ullah, and Ali [19]. The capping molecules may also have a potential role in the reduction and stability of the synthesized nanoparticles [33]. The capping ligands may also be involved in preventing agglomeration of the synthesized nanoparticles [34].

Direct imaging by transmission electron microscopy showed most of the synthesized AgNPs to be smaller than 30 nm in size and spherical in shape. However, larger particles, probably due to agglomeration of the smaller nanoparticles, were also visible. Agglomeration has been found to be a common phenomenon in green synthesis of nanoparticles which has been linked to increase in initial plant extract concentration [29,35]. The shape and size of nanoparticles has also been reported to be affected by the plant extract concentration [36]. However, our optimized sample of AgNPs showed that most of the nanoparticles were smaller than 50 nm and exhibited minimal agglomeration.

The EDX spectral analysis of AgNPs showed major energy peaks for Ag, Cl, C, and O (Figure 4). The presence of carbon spectrum showed that organic moiety plays a crucial role as capping agent, as reported previously [19]. Other peaks were observed around the Ag main peak, which indicated the variable valency states of Ag in the particles.

In the in vitro antibacterial assays, the Em-AgNPs were superior to Ef-AgNPs in inhibiting the tested bacteria which indicates the potential synergistic role of plant extract and AgNPs. In the case of ECA, 100% inhibition was recorded in response to 125 µg/mL of Em-AgNPs, while the same concentration of Ef-AgNPs resulted in more than 90% inhibition. Similarly, the antibacterial activity against ECC was 100% in response to Em-AgNPs (125 µg/mL); however, the same concentration of Ef-AgNPs caused less than 100% inhibition of the same bacterium. On the other hand, most of the concentrations of the aqueous fruit extract of *Hedera nepalensis* showed less than 40% inhibition of both bacteria. The synergistic effects of biogenic AgNPs and plant extracts against various microbes are already documented [37,38]. Recently, Dilbar et al. [39] reported the high efficacy of the synergistic treatment of AgNPs and *Stachys emodi* against *Erwinia carotovora*. Similarly, Arif,

Ullah, Ahmad, Ali, Ullah, Ali, Al-Joufi, Zahoor, and Sher [18] reported plant extract-coated AgNPs as highly effective against *Xanthomonas axonopodis*, as compared to extract-free AgNPs. Although the exact mechanism of AgNPs-induced microbial inhibition is yet to be researched, it may be attributed to membrane leakage, protein denaturation, DNA damage, and the disassembly of bacterial ribosomes [33].

Based on the in vitro inhibition of *Ralstonia solanacearum*, we used Em-AgNPs (1000 µg/mL and 125 µg/mL) to assess the control of bacterial wilt of tomato in the soil drenching-experiment. We found that both the concentrations of AgNPs effectively controlled the diseases and healthy tomato plants were observed in the post-treated data collection. Previously, [19] reported the efficient control of bacterial wilt of tomato by the applications of biologically synthesized iron oxide nanoparticles in the in planta assay.

4. Materials and Methods

4.1. Collection and Processing of Plants

Fresh plants of *Hedera nepalensis* were collected from Kanju Township, District Swat, Pakistan. The plants were taxonomically confirmed by plant taxonomists at the Centre for Plant Sciences and Biodiversity, University of Swat, Pakistan. The plants were rinsed with tap water and then with deionized water. After separating the leaves and flowers from the plants, they were allowed to dry for three to five days at room temperature. In order to be used later, the dried fruit tissues were ground into a fine powder and kept at room temperature.

4.2. Preparation of Plant Aqueous Extract and Silver Nitrate Solutions

The aqueous extract of the fruit of *Hedera nepalensis* (10 mg/mL) was prepared by adding 1.0 g dried ground powder tissue to 100 mL of distilled water. The solution mixture was heated at about 80 °C until the plant materials surfaced the water. The mixture was cooled at room temperature and then filtered three times using Grade-1 Whatman filter paper. The final volume of the aqueous extract was adjusted by adding distilled water to replace the vaporized water. The 10 mg/mL stock aqueous extract was diluted two times to make 5 mg/mL and 2.5 mg/mL solutions. The prepared aqueous extract was stored in the refrigerator until further use.

To prepare silver nitrate stock solution (50 mM), 1.679 g of $AgNO_3$ (Sigma Aldrich, US) was dissolved in 200 mL distilled water in Erlenmeyer flask (250 mL). For protection of the solution from light, the flask was covered with aluminum foil. Different dilutions (6, 4, and 2 mM) were prepared by using the following formula: $C1V1 = C2V2$.

4.3. Biosynthesis of Silver Nanoparticles (AgNPs)

To synthesize AgNPs, plant extracts (10, 5, and 2.5 mg/mL) and $AgNO_3$ (6, 4, and 2 mM) were mixed in equal volumetric ratios (v/v). Briefly, 700 µL of plant extract (10 mg/mL) and 700 µL of each $AgNO_3$ concentration (6, 4, and 2 mM) were mixed as independent mixtures, each in a 1.5 mL Eppendorf tube. A similar pattern of mixing was followed by mixing the same volume of 5 mg/mL and 2.5 mg/mL with each concentration of $AgNO_3$. All Eppendorf tubes were exposed to sunlight for 15 min and were then placed under the room light for 24 h for the reaction to complete.

To obtain extract-free AgNPs (Ef-AgNPs), all reaction mixtures were centrifuged (13,000 rpm for 15 min), pelleted, and then washed with deionized water. The resultant supernatants were replaced by deionized water, followed by another round of washing. The process was repeated at least three times and each extract-free pellet of AgNPs was homogenized in 1.0 mL deionized water for characterization. To prepare the extract mixed AgNPs (Em-AgNPs), the synthesized nanoparticles were dried without prior centrifugation.

4.4. UV-Visible Spectroscopy

After the reaction completion, all samples were analyzed by UV-Vis spectroscopy in the range of 300–600 nm to evaluate their surface plasmon resonance (SPR) peaks. Appropriate samples in terms of spectroscopic analysis were selected for further characterization.

4.5. Physical Characterization

The morphological features of the synthesized AgNPs were determined by using different physical techniques. The biomolecules that are particularly attached to the silver surface and the local molecular environment of the capping agent on the nanoparticles were identified using Fourier Transform Infrared Spectroscopy (FTIR) spectroscopy. The size and morphology of the synthesized AgNPs were examined using Transmission Electron Microscopy (TEM). The chemical composition of the synthesized AgNPs was determined using EDX microanalysis.

4.6. Antibacterial Assays

Antibacterial activity of AgNPs was performed against certain plant pathogenic bacteria—*Erwinia carotovora* subsp. *carotovora* (ECC), *Erwinia carotovora* subsp. *atroseptica* (ECA), and *Ralstonia solenacearum*.

4.6.1. Microtiter Plate Reader Assay

In vitro antibacterial activity was performed in a high throughput microtiter plate assay, as described previously by Ali and Reddy [40]. Briefly, bacterial cultures were initiated in liquid LB media, and the assay mixtures were assembled in a 96-well flat-bottom microtiter plate, with each well containing 100 µL of bacterial suspension (OD_{600} = 0.1) and 100 µL of AgNPs (1000–15.62 µg/mL, 2-fold dilutions) as extract-free AgNPs (Ef-AgNPs) orextract-mixed AgNPs (Em-AgNPs), or plant tissue extract (1000 to 7.8 µg/mL, 2-fold dilutions) in a 300 µL total reaction volume. Controls were without AgNPs or plant tissue extract. Each treatment was replicated three times, and experiments were repeated at least three times. Microtiter plates were wrapped with parafilm and incubated at 25 °C in a humid chamber. Optical density (OD_{600} nm) of microtiter plates was recorded immediately (0 h) and 24 h after incubation with microtiter plate reader. Inhibitions of bacterial species were determined as percent antibacterial activity by the following formula:

$$Activity\ (\%) = \frac{Control - Treatment}{Control} \times 100$$

4.6.2. Well Diffusion Assay

The autoclaved nutrient agar medium was prepared via standard protocol and was poured in petri plates, and all plates were inoculated with bacterial culture. Each well was loaded with 1000 µg, 250, and 62.5 µg of the tested samples (Ef-AgNPs, Em-AgNPs, or plant extract). Antibiotic and distilled water were taken as positive and negative controls, respectively. Plates were covered with paraffin or tape and were placed in the incubator at 28 °C for 24 h. The activity was conducted in triplicates in sterile conditions. After 24 h of incubation, the plates were evaluated for the zone of inhibition around the wells. The zones were measured with a measuring scale and the readings were recorded.

4.7. In Planta Phytopathogens Inhibition Assay

4.7.1. Potato Tubers Assay

The antibacterial efficacy of Em-AgNPs against *E. carotovora* subsp. *carotovora* was assessed using potato tubers infectivity according to Ali and Reddy [40], with minor modifications. Commercially available potatoes were carefully washed to eliminate soil particles, and then dried in the shade. Briefly, 20 µL of each treatment mixture, consisting of bacterial suspension (OD600: 0.2) and Em-AgNPs (1000–15.62 µg/mL: 2-fold dilutions), was injected into the wells made in the potato tubers from either side with the help of

micropipette tips. Control treatment consisted of only bacterial suspension. Each treatment consisted of three replicates. The tubers were placed in porous plastic zipper bags, were lined with wet filter papers for humidity, and were incubated at 26 °C. After 48 h, each tuber was sliced at the site of treatment so that both the infection sites were exposed. By assessing the infection site, data regarding infection severity were gathered.

4.7.2. Soil-Drenching Experiment for Bacterial Wilt Disease

To evaluate the antibacterial activity of nanoparticles in terms of reduction of the bacterial wilt disease of tomatoes, the already-reported methodology [19] was followed with minor modifications. Briefly, 10 mL suspension of *R. solanacearum* (OD600: 0.2) was added to sterilized soil around the tomato seedlings in pots. After 3 days, each pot was drenched with appropriate volumes of 1000 µg/mL and 125 µg/mL of Em-AgNPs. Each treatment was performed in triplicate. Control was without nanoparticle treatment.

5. Conclusions

In conclusion, we found that the plant species *Hedera nepalensis* is an excellent biological substrate to reduce silver salts into particulate form, with highly stable and desirable physico-chemical properties of the synthesized AgNPs. Moreover, the synthesized AgNPs were highly effective against the challenging plant pathogens *Erwinia* and *Ralstonia*. The extract-mixed AgNPs showed significant inhibition of the tested bacteria, possibly due to the higher surface to volume ratios coupled with secondary metabolites.

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