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EXPLORING THE POTENTIAL OF GREEN SILVER NANOPARTICLES FROM *BERBERIS VULGARIS* AGAINST BACTERIAL SPOT OF TOMATO AND ITS SURVEILLANCE IN POONCH DISTRICT

[a]Basharat Mahmood, [a]Muhammad Abbas, [b]Sajjad Hussain, [c]Nasir Rahim, [d]Saima Shafique, [e]Rizwan T. Khan, [c]Tahmina Zafar, [b]Rafia M. Bhatti, [a]Abu Bakar, [a]Muhammad T. Younas, [d]Syeda O. Javed, [f]Shameen Arif

[a] *Department of Plant Pathology, University of Poonch Rawalakot, AJK, Pakistan.*
[b] *Department of Botany, University of Poonch Rawalakot, AJK, Pakistan.*
[c] *Department of Soil and Environmental Sciences, University of Poonch Rawalakot, AJK, Pakistan.*
[d] *Department of Plant Breeding and Molecular Genetics, University of Poonch Rawalakot, AJK, Pakistan.*
[e] *Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad, AJK, Pakistan.*
[f] *Department of Botany, Mohi-ud-Din Islamic University Nerian Sharif, AJK, Pakistan.*

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ABSTRACT

Bacterial spot of tomato is a major constraint to tomato production in tropical, subtropical, and temperate climates, leading to significant crop losses. The current study aimed to manage the highly devastating disease bacterial spot of tomato, caused by *Xanthomonas perforans*, using green silver nanoparticles based on *Berberis vulgaris* plant extract. Disease parameters, namely disease prevalence and disease incidence, were calculated from tomato growing areas of district Poonch, AJK, to document the current status of bacterial spot disease on local tomato cultivars. The associated pathogenic strains were purified, and virulence study was conducted on healthy tomato seedlings followed by characterization using morphological, biochemical, and molecular analysis. *B. vulgaris* plant extract was used for the preparation of green silver nanoparticles (AgNPs), and three different concentrations were prepared (0.2%, 0.4%, and 0.6%). For texture and molecular composition study, characterization of green AgNPs was done using Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). Green silver nanoparticles were then evaluated using the inhibition zone technique in the lab, and it was found that the maximum inhibition zone of 24.32 mm was observed at a 0.6% concentration. Similarly, in the greenhouse experiment, the minimum disease incidence was recorded in the treatment with a 0.6% concentration of green AgNPs. The results of the current study showed a significant reduction in disease incidence while using green silver nanoparticles against bacterial spot of tomato.

Corresponding Author: Basharat Mahmood
Email: rajabasharat@upr.edu.pk

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important dietary food globally and plays a crucial role in the vegetable industry, both on an industrial and agricultural level (Babalola and Agbola, 2008). It is commonly used as a fresh vegetable in salads, as a culinary spice for long-term use in ketchup and juices, and as an ingredient in numerous dishes. Tomatoes are a source of a well-balanced and healthy diet, and every 100g of dry weight contains approximately 23 kilo calories. Nutritionally, tomatoes are

rich in minerals and vitamins, including vitamin C, potassium, folic acid, and carotenoids (Perveen *et al.*, 2015; Fernández-Bedmar *et al.*, 2018; Ahmad *et al.*, 2023).

Over the last four decades, the production of tomatoes, both in processed and fresh forms, has increased by 300%. In Pakistan, tomatoes are cultivated on 64,254 hectares, with an average yield of 9.40 tons per hectare (FAO, 2021). Tomatoes are a common and popular plant to grow in Pakistan, and they are grown in every major part of the country. They are available for consumers in public places all year round. However, the average yield of tomatoes in Pakistan is relatively low compared to other countries, such as China (57.96 t. ha^{-1}) and India (25.25 t. ha^{-1}) (FAO, 2021).

Tomato production is often affected by several biotic and abiotic factors, as the crop is typically grown in climates with relatively high temperature and humidity. Among the biotic factors, tomato is vulnerable to several diseases, especially bacterial spot of tomato, which is a devastating disease that not only deteriorates the quality of the produce but also reduces the overall production of tomato crops. The causal organism of this disease includes four species of the genus *Xanthomonas*, including *X. perforans*, *X. gardneri*, *X. euvesicatoria*, and *X. vesicatoria* (Jones and Miller, 2014; Stall *et al.*, 2009). Among these four species, *X. perforans* is widely associated with the disease and is reported worldwide as a gram-negative bacteria that may survive at temperatures of 25-30 ℃ and in relatively humid environments (Horvath *et al.*, 2012). The symptoms of bacterial spot of tomato include leaf and fruit lesions, as well as defoliation, which reduce overall yield and quality, making the produce less suitable for sale in the market. Due to the severe nature of bacterial spot, the yield can be reduced by up to 50%, which is a significant loss for both industrial and agricultural crops.

Efficient control of plant diseases is crucial for crop growers, environmentalists, farmers, implementers, and policy-makers since plant diseases can cause significant reductions in plant growth and yield, resulting in considerable losses (Anil Kumar *et al.*, 2007; Bashir *et al.*, 2020). The management strategies for plant diseases depend heavily on agrochemicals, particularly pesticides, which are applied using well-timed pesticide applications and sanitary practices (Klaus-Joerger *et al.*, 2001).

Khan and Rizvi (2014) suggested that the physical and chemical properties of nanomaterials can be utilized for various applications that benefit society, including plant disease management. Nanoparticles are commonly used on seeds, soil, and leaves to control disease infections caused by plant pathogens. Nanotechnology involves technology implemented at the nanoscale, and it has real-world applications. New methods of controlling plant pathogens are based on nanotechnology (Bhattacharya and Gupta, 2005).

According to a study by Nuruzzaman *et al.* (2016), nanomaterials comprising silver nanoparticles, gold, and FeONPs are used as pesticides. Various methods and applications for the characterization of nanoparticles and their formulation have been reported by different researchers, as well as the effects of nanoparticles on plant disease management (Al-Samarrai, 2012).

The use of nanotechnology can reduce environmental pollution caused by the excessive use of pesticides and chemical fertilizers, as it is considered environmentally friendly. It supports agriculture and minimizes environmental pollution by producing pesticides and chemical fertilizers, and controlling plant diseases using nanoparticles that are eco-friendly, resulting in increased pesticide efficiency with lower doses to manage phytopathogenic diseases (Bhattacharyya *et al.*, 2016; Fraceto *et al.*, 2016; Prasad *et al.*, 2017).

This study aims to control *Xanthomonas perforans* to maintain the quality of food, feed and dietary fiber produced by tomato producers. To achieve this goal, the biosynthesis of silver nanoparticles for controlling *Xanthomonas perforans* was evaluated.

MATERIALS AND METHODS

Surveillance of Bacterial Spot

A survey was conducted in tomato growing areas of the Poonch district in AJK, Pakistan to investigate the current status of bacterial spot disease of tomato during the summer season 2022. Based on typical symptoms in the literature of bacterial spot disease including necrotic and water soaked lesions, small circular and dark brown spots on all the aerial parts of the tomato plant were observed and data was recorded. The plant parts and tomato fruits with symptoms were collected and properly labelled and transported to the laboratory for further processing for the confirmation pathogen. From the recorded surveillance data in the field disease incidence and prevalence were calculated by the following formulas;

$$Disease\ Incidence\% = \frac{No.\ of diseased\ plants}{Total\ No.\ of\ plants\ observed} \times 100$$

$$Disease\ Prevalance\% = \frac{N0.of\ Infected\ fields}{Total\ No.of\ field\ selected} \times 100$$

Pathogen Isolation

The symptomatic plant samples from the surveyed locations were collected for the isolation of pathogen. Samples were washed thrice with water followed by washing with sodium hypochlorite then cut into small pieces for direct plating on the nutrient agar media followed by incubation at 25±2 ℃ for 24-48 hours. Purification was done from the obtained bacterial colonies separately by picking a single bacterial colony and was streaked on the media plates individually followed by incubation again at 25±2 ℃ for another 24-48 hours. Further purification of associated bacterial culture was done using a streaking method and the obtained pure cultures were preserved in 10% glycerol. All the virulent isolates were further subjected to identification on the basis of morphological characters and biochemical characteristics including micro and macroscopic features. Furthermore, molecular identification of selected bacterial isolates was done using 16sDNA primers followed by sequencing and sequence analysis using the maximum likelihood method (Tamura *et al.*, 2013).

Virulence study

To confirm Koch's postulates pathogenicity test was done on healthy tomato fruits by inoculating 24h old fresh bacterial suspension with a measured concentration of 10^5 cfu/ml on healthy tomato fruits. Symptoms were observed after 2-4 days. From the observed symptoms bacterial isolates were re-isolated for the confirmation of Koch's postulates.

Preparation of Plant Extract

For the preparation of plant extract stock 30g of *Berberis vulgaris* plants were cut into small pieces and were boiled using 100 ml sterile distilled water at 100 °C for 5-10min followed by filtration of crude extract through the Whatman filter paper. The filtrate was then preserved at 4 °C as a stock solution for the synthesis of silver nanoparticles (AgNPs) (Manik *et al.*, 2020).

Biosynthesis and Characterization of Green Silver Nanoparticles

Prepared stock solution of *B. vulgaris* extract was added to the silver nitrate solution prepared using 0.05 M silver nitrate (AgNO3) for the synthesis of green AgNPs. The mixture was then heated continuously until the change of color from dark brown to black followed by centrifugation of solution at 10,000 rpm for 10 minutes for the collection of silver nanoparticles after removing the supernatant. The collected Ag-nanoparticles were then placed in covered glass vials for characterization and further studies (Manik *et al.*, 2020).

Then characterization of green AgNPs was done using scanning electron microscopy (SEM) and FTIR to check the structure of the dry mixture and the composition of active functional group respectively (Vivek *et al.*, 2011).

***In-vitro* Evaluation of Green Silver Nanoparticles against Pathogen**

B. vulgaris based AgNPs were then evaluated against *Xanthomonas perforans* using the inhibition zone technique. Three different concentrations (0.2%, 0.4% and 0.6%) were prepared using 0.2g, 0.4g and 0.6g of prepared green silver nanoparticles in 100 ml of sterilized double distilled water. Nutrient agar media plates were used for the streaking of *X. perforans*. Then sterilized blotter paper was placed on the inoculated plates after dipping in the prepared concentrations of green AgNPs. For control treatment distilled water was used for dipping of blotter paper. Treated plates were then incubated at 28 ℃ for 24 hours. Growth inhibition was observed after 24 hours and the zone of inhibition was measured for consecutive three days (Elbeshehy *et al.*, 2015).

Greenhouse Evaluation of Green Silver Nanoparticles against Pathogen

Seedling of susceptible tomato varieties were transplanted in pots. One week after transplanting the tomato seedlings were inoculated with a virulent *X. perforans* isolate. One week after inoculation of pathogenic isolate with 100 ml different concentrations of green AgNPs solution were applied. Each treatment was repeated five times in a fully randomized design and the pots were placed in a greenhouse at 25 ± 2°C for 8 weeks. After the specified period, the plants were carefully examined to determine disease parameters. Sterile distilled water served as a negative control in all the experiments. No mineral fertilizer or pesticides were used (Vicente and Roberts, 2003).

Data Analysis

The recorded data were analyzed using analysis of variance with a completely randomized design (CRD) for the pot study conducted in a greenhouse. CRD was chosen as the meteorological conditions in the greenhouse remained constant, with no variations.

RESULTS AND DISCUSSION

Extensive visits to tomato growing areas of the Poonch

district of Azad Jammu and Kashmir were done in the year 2021-22. Based on symptoms, 139 infected leaf, stem and fruit samples were collected from tomato fields. On the basis of symptomlogical parameters disease surveillance data was calculated. In district Poonch tomato fields were visited in three tehsils including Rawalakot, Hajira and Abbaspur and it was found that the bacterial spot has prevailed in all three tehsils of district Poonch and it was recorded 100% disease prevalence in district Poonch Figure 1.

For the calculation of disease incidence during 2021, in tehsil Rawalakot six locations including Rawalakot, Drake, Trar, Khrick, Check, and Chare were surveyed and was observed that the maximum disease incidence of bacterial spot on tomato was recorded at 40% in Trar while minimum disease incidence on tomato was recorded in Khrick that was 10% (Figure 2).

Similarly, in tehsil Hajira 10 locations were surveyed and was found that the maximum disease incidence of the bacterial spot of tomato was recorded in Kahuta which was 55% while the lowest disease incidence was recorded in Davigali which was 30% (Figure 3).

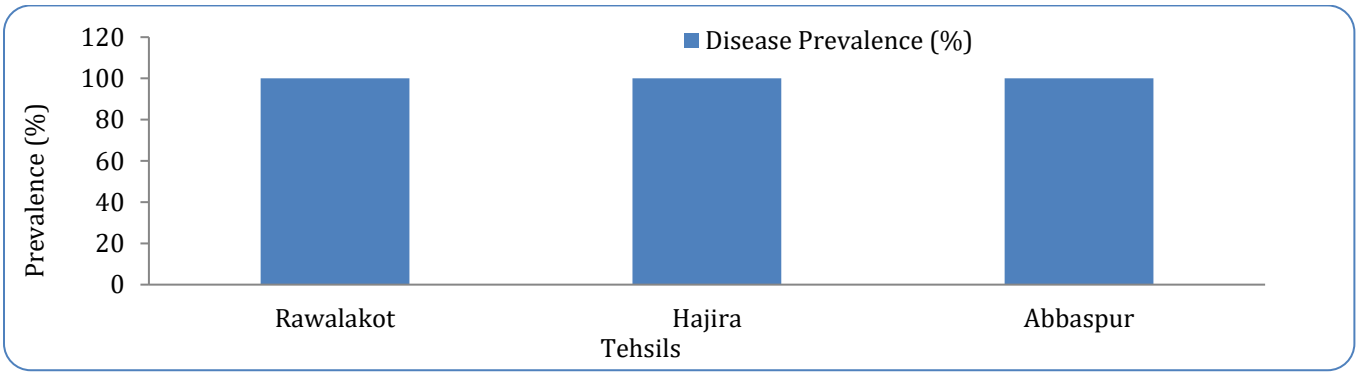

Figure 1. Percent disease prevalence in district Poonch.

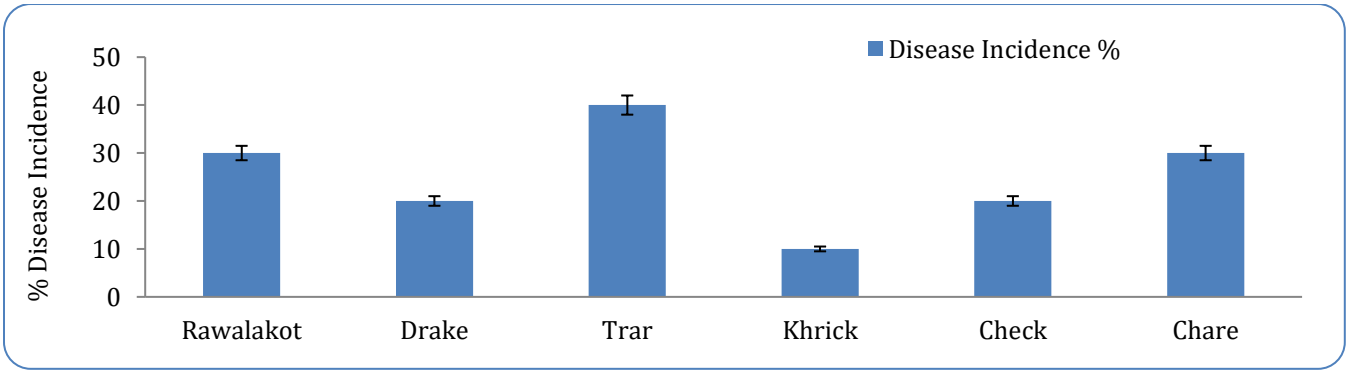

Figure 2. Disease incidence of bacterial spot on tomato in Rawalakot.

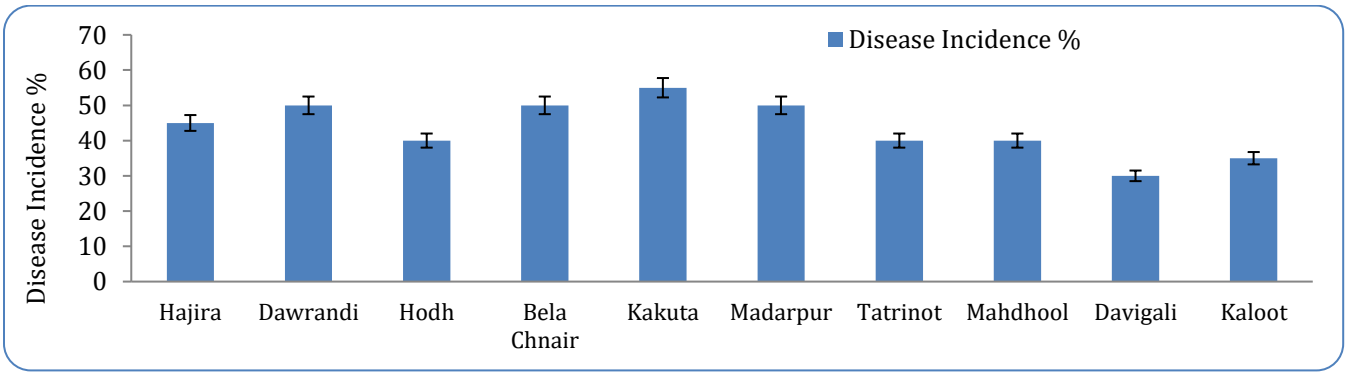

Figure 3. Disease incidence of bacterial spot on tomato in Hajira.

Similarly, in Abbaspur 10 locations were selected the highest percent disease incidence of bacterial spot of tomato was recorded in Manghar which was 60% while the minimum disease incidence was recorded in Chuffar which was 40% (Figure 4).

According to Kavitha and Umesha (2007), two consecutive field surveys in the major tomato growing district of Karnataka revealed a high incidence of bacterial spot disease in tomato fields. The disease was particularly severe, with an average incidence rate of 29% in the Mysore region, 50% in the Mandya region when the PKM-1 cultivar was used, 31% in the Bangalore region, and 22% in the Kolar region when using the Avinash and Allrounder tomato cultivars. These findings suggest that bacterial spot disease poses a significant threat to tomato crops in the region and that effective measures need to be taken to control its spread.

Similarly, according to Bashan *et al.* (1985) severe bacterial spot infections can lead to significant reductions in fruit yield, with direct losses ranging from 23% to 44%. Indirect losses can also occur when infected plants shed leaves, leaving fruits exposed to sunlight, which can cause sunscald. In North America, the two most significant bacterial pathogens affecting tomato crops are *X. gardneri* and *X. perforans*. *X. perforans*, specifically race T4, are prevalent on the east coast and are responsible for causing significant yield losses of up to 66%. On the other hand, *X. gardneri* dominates in the Midwest and can also result in substantial yield losses for tomato growers (Adhikari *et al.*, 2020).

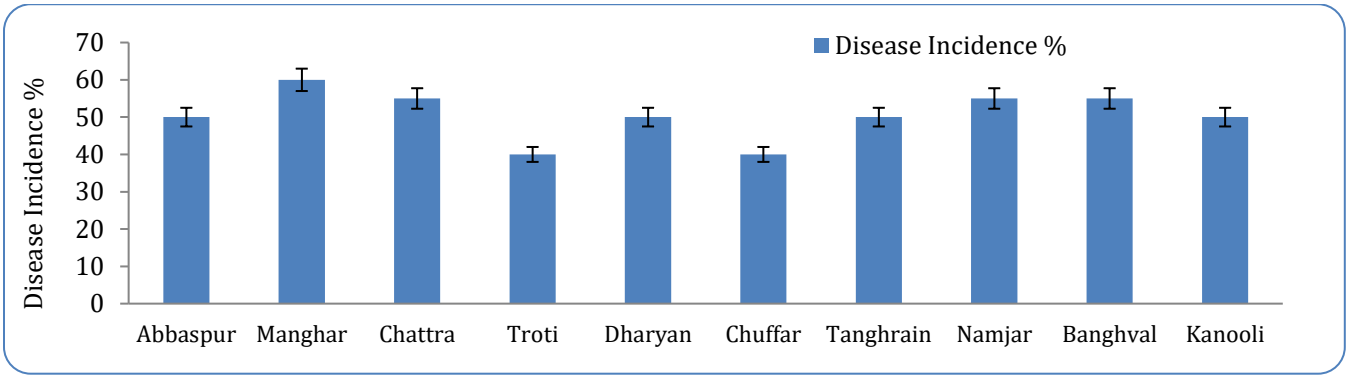

Figure 4. Disease incidence of bacterial spot on tomato in Abbaspur.

The present study's findings align with those of Ahmad and Ahmad (2022), who reported that the highest calculated percent disease incidence (%DI) of bacterial spot of tomato in 2017 was recorded in the Swat district, reaching 64.42% by the end of August, followed by the Mansehra district. In contrast, the lowest %DI was recorded in the Lower Dir district at 46.42%. Moreover, the Swat district had the highest percent disease severity (%DS) at the end of August, with a value of 49.17%, followed by the Mansehra district with 45.57%, while the lowest %DS was observed in the Lower Dir district (41.94%).

Pathogen and their Virulence

Of the total 139 samples 26 bacterial isolates were purified on the basis of their colony morphology, color and texture. After incubation of pure cultures at 25±2 ℃ on nutrient agar media the expected translucent growth of *X. Perforans* was found in 20 isolates screened initially as the description of bacteria was reported by Aiello *et al.* (2013).

The results of the study were parallel with the previous findings of Abrahamian *et al.* (2021) that *X. perforans* was identified as an aerobic, rod shaped mono flagellated gram-negative bacteria is a devastating pathogen causing bacterial spot of tomato flourishes in relatively high humidity as well as high temperature. Similarly, Roach *et al.* (2018) reported the gram staining test and loop test the *X. Perforans* showed negative results for gram staining and positive for the loop test.

All the 20 isolates after initial screening were further tested for their virulence studies on fresh tomato fruits. After incubation of 3-4 days, it was found that 13 isolates were virulent showing clear symptoms of bacterial spot on tomato fruits (Table 1) and were subjected to further characterization. The results were similar to the findings of Jones and Miller (2014) that reported the symptoms of bacterial spot of tomato on all the aerial parts of the tomato plant including the fruits. Symptoms include the lesions as water soaked irregular, circular spots up to 3-4 mm in diameter (Aiello *et al.*, 2013).

Table 1. Virulence study of bacterial isolates on fresh tomato fruits.

S. No.	Isolates	Response	S. No.	Isolates	Response
1	XP-1	-	11	XP-14	+
2	XP-2	+	12	XP-15	-
3	XP-3	+	13	XP-16	+
4	XP-6	-	14	XP-18	+
5	XP-7	-	15	XP-19	+
6	XP-8	+	16	XP-20	-
7	XP-9	+	17	XP-21	+
8	XP-11	+	18	XP-23	+
9	XP-12	+	19	XP-25	+
10	XP-13	-	20	XP-26	-

Identification of Pathogenic Isolates

The recovered isolates underwent biochemical tests, including oxidase, levan formation, pigment production, and hypersensitivity response. It was observed that all the isolates produced mucoid and dome-shaped colonies, indicating a positive result for levan formation on glucose-rich nutrient agar media (Table 2). Additionally, 10 bacterial isolates tested negative for oxidase activity, while three bacterial isolates (XP-16, XP-21, and XP-25) showed positive results. All isolates subjected to hypersensitivity reaction on healthy potato slices demonstrated positive results, except for XP-8 and XP-25 (Table 2).

These findings are consistent with the results of Kebede *et al.* (2014), who reported levan-positive isolates and performed hypersensitive reactions on tobacco, while negative oxidase reactions were indicative of *X. perforans*. The presence of the bacterium was confirmed by examining the inoculated Nutrient Agar (NA) media plates, which exhibited yellow-colored, shiny, and dome-shaped colonies, tentatively identifying the bacterium as *Xanthomonas* spp. Ahmad and Ahmad (2022).

Table 2. Biochemical response of virulent bacterial isolates.

Sr. No	Isolates	Oxidase Activity	Levan Production	Pigment Production	HR
1	XP-2	_	+	+	+
2	XP-3	_	+	+	+
3	XP-8	_	+	+	_
4	XP-9	_	+	+	+
5	XP-11	_	+	+	+
6	XP-12	_	+	+	+
7	XP-14	_	+	+	+
8	XP-16	+	+	_	+
9	XP-18	_	+	+	+
10	XP-19	_	+	+	+
11	XP-21	+	+	_	+
12	XP-23	_	+	+	+
13	XP-25	+	+	_	_

Similarly according to Burlakoti *et al.* (2018) two strains of *Xanthomonas vesicatoria* were tested and found to have positive reactions for starch hydrolysis and the oxidative-fermentativ (OF) test, but negative for lipase activity. In contrast, all 21 tested strains of *Xanthomonas perforans* showed positive reactions for all three biochemical tests, which include starch hydrolysis, the OF test, and lipase activity (Scortichini *et al.*, 2013; Roach *et al.*, 2018; Timilsina *et al.*, 2015).

The DNA was extracted from all 10 isolates and was used as template DNA. PCR product was amplified by troubleshooting the primer temperature and a reaction

mixture of 50 µL was used. All the 10 isolates were approximately segregated at 1500bp band size while using 1kb DNA ladder on 1% agarose gel. After purification of the PCR product using a GeneJet PCR purification kit, the product was sequenced from Macrogen Korea. A study by Adhikari *et al.* (2019) used a qPCR assay targeting the highly conserved *hrcN* gene to identify *Xanthomonas* strains from tomato in North Carolina as a single species, *X. perforans*. The detection of only green fluorescence confirmed that all of the strains were *X. perforans*.

The obtained sequences from Macrogen Korea were then manipulated and cleaned followed by BLAST showed that the sequences were 99-100% similar to the sequences of already submitted *X. Perforans* strains on the NCBI database. For phylogenetic analysis of all the 10 sequences and the sequences of *X. Perforans* downloaded from the NCBI database was done using MEGA 7.0. Phylogenetic tree of all the sequences constructed using MEGA 7.0 adopting the maximum likelihood method (Figure 5). The molecular findings of the current study were similar to the previous findings and recommendations that molecular identification was important for *Xanthomonas* species using species-specific primers in PCR and MLSA. The specific primers are already developed and reported in the literature can be used to detect all four species of *Xanthomonas* species causing bacterial spots of tomato (Jones *et al.*, 2004; Koenraadt *et al.*, 2007).

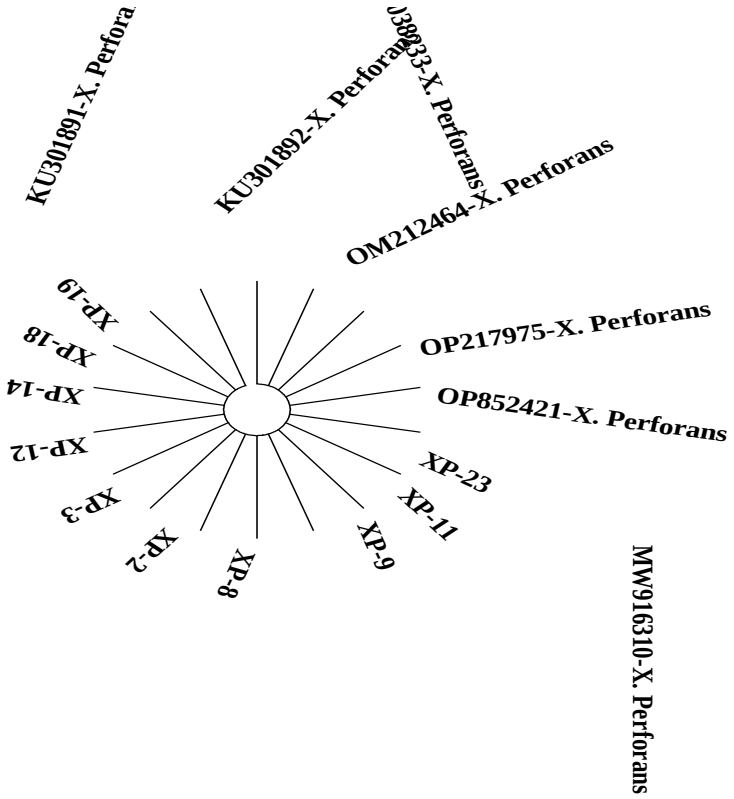

Figure 5. Phylogenetic analysis of ten sequences using 16s rRNA primer with seven already known sequences using the maximum likelihood method.

Characterization of Green silver Nanoparticles (AgNPs-Bv)

The micrograph of *B. vulgaris* AgNPs showed that they had minute-size in range, globose ball-shaped and regular dissemination (Figure 6). As it was noticed when utilizing silver nitrate as a follower, the AgO particles develop gradually and appear minute globular framework. The results were similar to the previous SEM studies of AgNPs reported the shape and morphology of green synthesized AgNPs (Daphedar and Taranath, 2018; Jamdagni *et al.*, 2018). Similarly, the findings supported by the previous findings of Javed *et al.* (2020) that Initially, to confirm the synthesis of silver nanoparticles (AgNPs), a change in the color of the

reaction mixture was observed, which is believed to be caused by the reaction of AgNPs to light. The use of a plant extract and silver salt in a ratio of 1 to 9 was found to be effective in reducing and stabilizing AgNPs while consuming only a minimal amount of secondary metabolites, ensuring reproducibility. By adjusting the molar ratios of dsDNA, reducing agent, and silver precursor, it is possible to control the morphology and size of silver nanoparticles (AgNPs) synthesized on double-stranded DNA (dsDNA). According to Beddoes *et al.* (2015), the process of synthesizing silver nanoparticles (AgNPs) can be influenced by various physicochemical factors, such as temperature, pH, and reaction time. These factors play a critical role in determining the size, shape, and biological corona of the AgNPs, which in turn can affect their biomedical properties. Therefore, it is important to carefully control the physicochemical parameters during the synthesis of AgNPs in order to steer their properties towards the desired biomedical application.

Similarly, the results of FTIR showed the surface contents of prepared green AgNPs to observe the functional group capped on the surface of green AgNPs (Sambalova *et al.*, 2018; Ssekatawa *et al.*, 2021). The results showed the peaks were prominent in the higher concentration of plant extract used with silver nitrate solution. The bands at 2156cm^{-1} confirm the presence of C – C stretching bound of the alkynes molecule (Figure 7). The results also showed small peaks at 1200-1000 cm^{-1} confirming the presence of polyphenols reported the presence of phenolic and carbonyl compounds on the silver nanoparticle surface. The results are similar to the previous findings of Gebremedhn *et al.* (2019) and Awwad and Salem (2012) that the peak at 1021.14 cm^{-1} and 800.58 cm^{-1} can be attributed to C–O and C–H bending, respectively. The observed peaks ranging from 1412.3 to 2236.4 cm^{-1} correspond to O–H bending and C=C stretching. The peak at 3440.2 cm^{-1} is assigned to N–H stretching, which may be attributed to the presence of amino acids that also act as capping agents.

The functional groups present on the surface of AgNPs were analyzed using FTIR. According to the literature, the peak at 800.58 cm^{-1} can be attributed to C-O and C-H bending, respectively. The observed peaks ranging from 1412.3 to 2236.4 cm^{-1} correspond to O-H bending and C=C stretching. The peak at 3440.2 cm^{-1} is assigned to N-H stretching, which may be attributed to the presence of amino acids that also act as capping agents (Yusof *et al.*, 2018; Sambalova *et al.*, 2018).

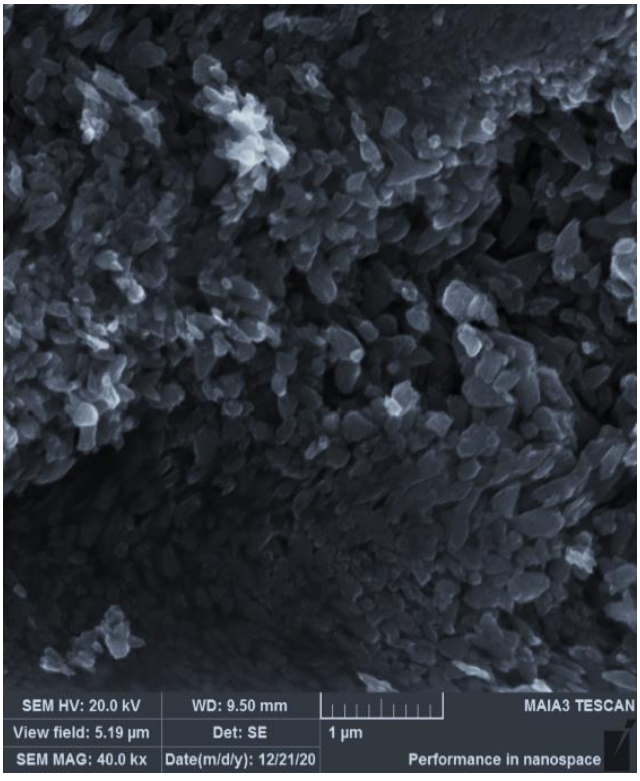

Figure 6. Morphological studies of prepared green AgNPs using scanning electron microscopy.

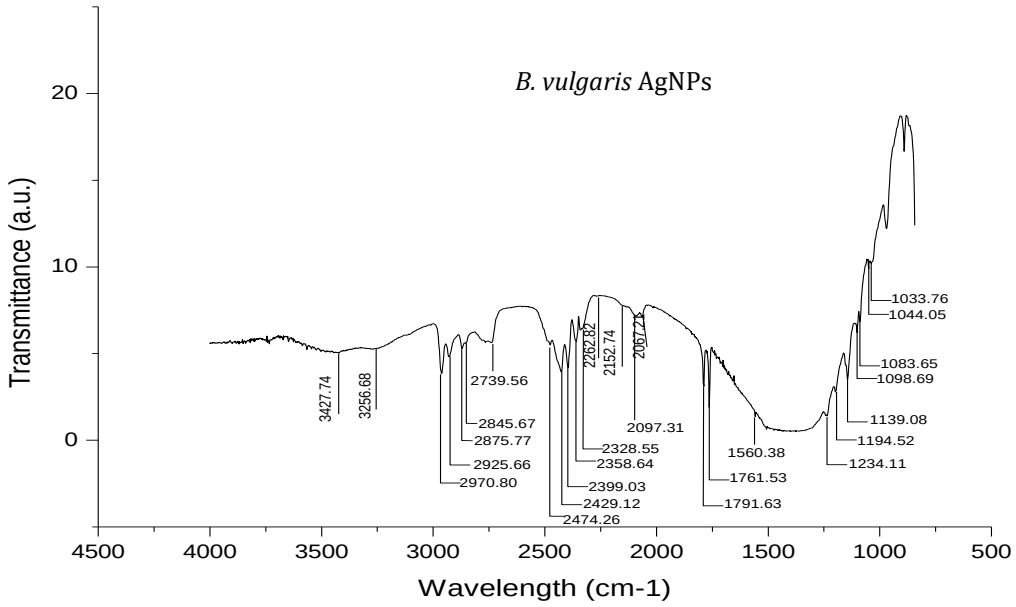

Figure 7. FTIR spectra of green silver nanoparticle prepared using *B. vulgaris*.

***In-vitro* evaluation of green silver nanoparticles**

All the concentrations of green silver nanoparticles used for the inhibition zone technique showed significant results but the maximum inhibition zone (28.12 mm) was recorded for the concentration 0.6% followed by 0.4% that was 16.93 mm and 12.25 mm was recorded for the concentration 0.2% comparing the control treatment (Figure 8).

Ocsoy *et al.* (2013) synthesized two composites of DNA-directed silver nanoparticles (AgNPs) on graphene oxide (GO), with sizes of 18 nm and 5 nm, and confirmed that they exhibit antibacterial activity against *Xanthomonas perforans*. The optimal antibacterial effect was observed with a concentration of 20 ppm for the 18 nm AgNPs composite and 16 ppm for the 5 nm AgNPs composite. These results suggest that these composites have potential as antibacterial agents against *X. perforans*. The antibacterial activity of DNA-directed silver nanoparticles grown on graphene oxide composites towards *X. perforans*, a model plant pathogenic bacterium, is enhanced by the synergistic effect of the AgNPs and GO (Nicosia *et al.*, 2020).

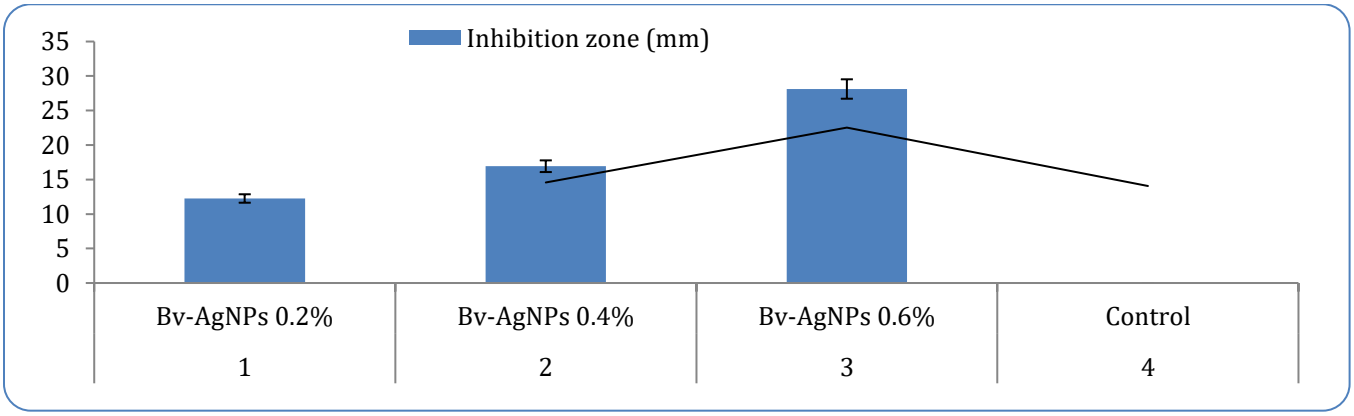

Figure 8. *In vitro* evaluation of different concentrations of green AgNPs on the growth of *X. Perforans*.

Greenhouse Evaluation of Green AgNPs

All the concentrations of green silver nanoparticles showed positive results against *X. Perforans*. The current study results showed that green silver nanoparticles having a concentration 0.6% showed a minimum disease incidence that was 28.1% followed by 34.9% using 0.4%

concentration of green silver nanoparticles while maximum disease incidence was recorded in the treatment using AgNPs concentration of 0.2% that was 37.3% as compared to control that was 71% (Figure 9).

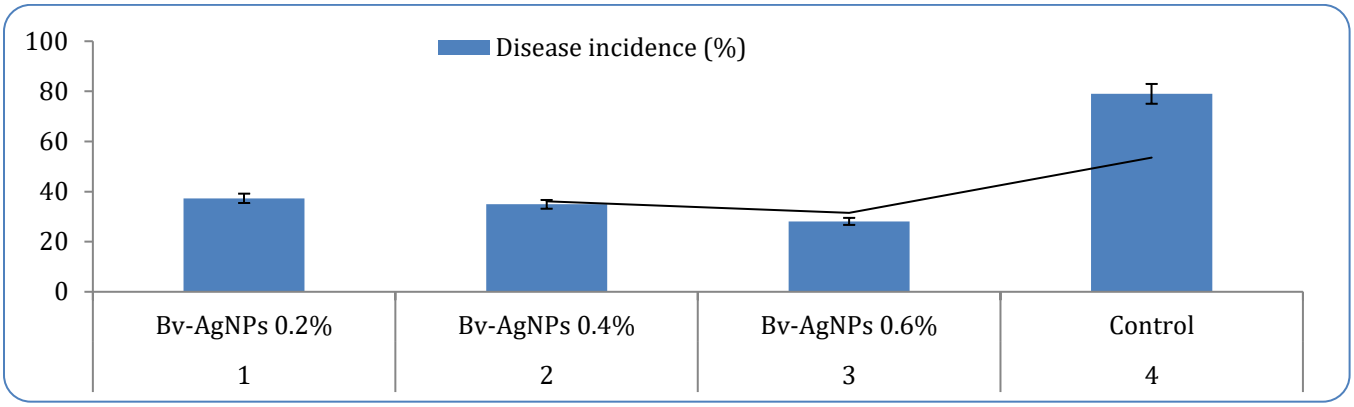

Figure 9. Effect of different concentrations of green AgNPs on % disease incidence of *X. Perforans* on tomato.

The results of the current research were supported by the previous findings of Varympopi *et al.* (2022) which observed the growth of *Xanthomonas campestris* pv. *vesicatoria* was significantly suppressed by green based nanoparticles. The study outcomes were also related to the findings of Abel *et al.* (2021) who investigated the technique of biosynthesis of nanoparticles from leaf extract of moringa. The previous related findings also supported that the use of the green culinary technique is better due to its cost-effective and environment friendly impact. Moradian *et al.* (2018) revealed the green nano-particles (NPs) synthesized using plant extracts were effective against *plant diseases by* expressing the *hrpE* gene response in plants. Also, a study was conducted to investigate the bactericidal activity of chitosans (CSs) and chitosan nanoparticles (CsNPs) against two bacterial strains, *Pseudomonas fluorescens* and *P. carotovorum*. The results of the study showed that both CSs and CsNPs exhibited significant inhibitory effects on bacterial growth, with concentrations ranging from 0.009% to 0.15% (w/v) proving effective in reducing bacterial growth as compared to the control. These findings suggest that both CSs and CsNPs may have potential applications as antibacterial agents in the control of *Pseudomonas*-associated plant diseases (Mohammadi *et al.*, 2016).

CONCLUSION

The study successfully demonstrated the potential of green silver nanoparticles derived from *Berberis vulgaris* plant extract for managing the highly devastating disease bacterial spot of tomato caused by *Xanthomonas perforans*. Disease parameters were calculated to document the current status of bacterial spot disease in the tomato-growing areas of district Poonch, AJK. The associated pathogenic strains were characterized using morphological, biochemical, and molecular analysis. Characterization of green AgNPs was done using FTIR and SEM, and the maximum inhibition zone of 24.32 mm was observed at a 0.6% concentration. The minimum disease incidence was recorded in the treatment with a 0.6% concentration of green AgNPs in the greenhouse experiment. Overall, the study results showed a significant reduction in disease incidence while using green silver nanoparticles, highlighting their potential for effective disease management in tomato production.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally to the research and compiling the data as well as editing the manuscript.

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