MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 36 Issue 1s, 2026, Pages: 4166-4173

EXPERIMENTAL WORKFLOW FOR GREEN SYNTHESIS AND EVALUATION OF SILVER NANOPARTICLES (AGNPs)

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ABSTRACT
The current research paper examines an integrated and sustainable strategy to management of early blight disease caused by Alternaria solani through green synthesis of silver nanoparticles (AgNPs) by using Solanum tuberosum extracts and rhizosphere-associated plant growth-promoting rhizobacteria (PGPR). The potato rhizosphere was used to obtain Rhizobacterial isolates that were characterized based on morphological, biochemical, and molecular characteristics, which revealed the dominance of Bacillus species and Serratia species with significant capabilities to promote plant growth. The isolates that were selected had various functional properties that included the production of indole-3-acetic acid, solubilization of phosphates, secretion of siderophores, production of hydrogen cyanide and activities of chitinase. The AgNPs synthesis using bacterial metabolites and plant extracts was green and confirmed by detection of a distinct colour change and UV-Visible spectroscopy, which indicated a typical surface plasmon resonance peak between 400-450 nm. Structural characterization showed that the size range of 10 50 nm was predominantly composed of spherical nanoparticles, which had been stable and homogenous. The AgNPs biosynthesized exhibited high antifungal activity against A. solani, with more than 90 percent inhibition of A. solani mycelial growth and severe morphological damages to hyphae of A. solani. Greenhouse experiments also established better growth of plants, better uptake of nutrients, and a great deal of reduction in the severity of the diseases in experimental plants. The heightened activity of the defence-related enzymes implied the activation of the plant immune responses. Findings support the effectiveness of using a combination of PGPR and nanotechnology as an environmentally friendly alternative to the use of chemical fungicides, as a promising way of ensuring sustainable crop protection and enhanced agricultural productivity.

KEYWORDS
Green synthesis, Silver nanoparticles, Early blight disease, Plant growth-promoting rhizobacteria, Sustainable crop protection.

1. INTRODUCTION

1.1 Background on Sustainable Agriculture and Plant Disease Challenges: Agriculture remains a major problem due to the rising incidence of plant diseases, especially those caused by fungal pathogens, which are also responsible of causing huge losses of crops globally. Recent estimates show that fungal diseases alone cause about 2030% yield loss in major crops worldwide, and therefore threatening food security and economic stability (Savary et al., 2019; Fisher et al., 2020). Among them, one of the most devastating foliar diseases of solanaceous crops and, in particular, potato (Solanum tuberosum) is early blight disease caused by Alternaria solani (Ansari et al., 2023). The pathogen infects leaves, stems and tubers, causing characteristic necrotic lesions and greatly impair photosynthetic efficiency.

Potato is among the most significant staple crops worldwide, being a key source of carbohydrates, vitamins, and proteins to millions of people. However, its susceptibility to fungal pathogens like A. solani poses a serious challenge to sustainable production systems (Devaux et al., 2021). Traditional management approaches are mostly based on synthetic fungicides which, though effective in the short term, have a number of restrictions. They are the development of pathogen resistance, environmental pollution, and negative effects on non-target organisms, including the beneficial soil microorganisms (Bisht et al., 2021). Also, the overuse of agrochemicals also disrupts the health of the soil and leads to an ecological imbalance in the long term.

With these issues in mind, urgent attention is at stake to devise sustainable solutions which are eco-friendly to tackle disease management. Biological solutions, especially those based on useful microorganisms and new technologies, are actively explored as potential solutions to decrease reliance on chemical inputs and maintain crop yields (Kumar et al., 2022).

1.2 role of Rhizosphere Microbiome and PGPR: The rhizosphere is a very dynamic ecological niche where there are complex interactions between plant roots and a wide range of microbial communities. Plant growth-promoting rhizobacteria (PGPR) are among them, and they play a significant role in improving nutrient availability, plant growth, and reducing the presence of plant pathogens (Backer et al., 2018; Ngalimat et al., 2021). Such useful bacteria colonize the roots of plants and implement their actions in direct and indirect ways.

Direct mechanisms involve the synthesis of phytohormones like indole-3-acetic acid (IAA), which induces the elongation of root and the increase of nutrient uptake. Moreover, the use of the microorganisms facilitates the mobilization of nutrients by solubilizing phosphates and fixing nitrogen, which enhances the soil fertility and growth of plants (Kour et al., 2020). These microorganisms indirectly contribute to the protection of plants because they produce antimicrobial compounds, siderophores, hydrogen cyanide (HCN), and lytic enzymes like chitinases (Olanrewaju et al., 2020).

The induction of systemic resistance (ISR) whereby the plant is enhanced by the addition of PGPR to support its innate defence system and thus increase its performance in terms of resistance to pathogen attack (Pieterse et al., 2021). This coordinated defence reaction is characterized by activation of defence-related enzymes and signalling pathways, which is involved in enhanced disease resistance.

In the case of the potato plants, rhizobacteria of genera like Bacillus and Serratia have shown great potential in promoting the plant growth and in inhibiting fungal pathogens. These bacteria possess several plant growth-promoting behaviours such as the production of IAA, solubilization of phosphates, the release of siderophores, and antifungal activity. Their capacity to effectively colonize the rhizosphere and produce bioactive metabolites make them good options in sustainable agricultural applications.

Recent research has further emphasized the multifunctional role of the PGPR in the promotion of crop productivity in addition to the reduction of chemical fertilizers and pesticides dependency (Chaudhary et al., 2022). Therefore, the introduction of PGPR into crop management practices is a promising way of sustainable agriculture and plant disease control.

1.3 Nanotechnology in the management of plant diseases: Nanotechnology has turned out to be a revolutionary technology in the agricultural sector, providing pioneer solutions to the management of plant diseases and crop enhancement. With its distinct physicochemical properties, including high surface area-to-volume ratio, enhanced reactivity, and size of quintessential nanoparticles, they display superior antimicrobial activity than conventional materials (Rai et al., 2021). Silver nanoparticles (AgNPs) have been extensively studied as one of the most important nanomaterials owing to its strong antimicrobial activity against a broad spectrum of plant pathogens.

AgNPs are known to have antifungal activity, which occurs through various mechanisms which include, but not limited to, disruption of cell membranes, generation of reactive oxygen species (ROS), and interference with cellular metabolism. The effects of these mechanisms include structural damage, cellular contents leakage, and cell death in pathogenic fungi (Kumar et al., 2023). It has been shown that AgNPs have a strong ability to prevent the growth of Alternaria solani and other phytopathogens in vitro and in vivo (Ansari et al., 2023).

Moreover, nanoparticles have been demonstrated to improve plant physiological responses by promoting nutrient uptake and activating the activation of defence-related pathways. Recent studies have shown that nanomaterials are able to regulate biochemical processes in plants, thus enhancing biotic stress resistance (Ashraf et al., 2025). These characteristics render nanotechnology as a promising device in terms of sustainable crop protection.

Nanoparticles have the advantage of being used at lower concentrations, thereby reducing environmental toxicity and minimizing chances of developing resistance. They are also more applicable in the modern agriculture because of their targeted mode of action and compatibility with biological systems (Raliya et al., 2022). Therefore, the adoption of nanotechnology in the management of plant diseases is a great step towards an eco-friendly and good agricultural practice.

1.4 Green Synthesis of Silver Nanoparticles: Silver nanoparticles can be synthesized using physical, chemical, and biological methods. Nonetheless, the traditional chemical and physical methods usually imply the use of toxic reagents, high-energy consumption, and environmental risks, which does not make them more suitable in terms of sustainable usage (Iravani et al., 2020). Green synthesis, in turn, provides a viable and economic substitute, based on the use of biological entities, including plant extracts, bacteria, fungi, and algae.

AgNPs can be produced biologically through the reduction of silver ions (Ag +) into metallic nanoparticles of silver by using biomolecules such as enzymes, proteins, and secondary metabolites. Moreover, these biomolecules do not only facilitate the reduction process but also serve as stabilizing agents, preventing aggregation of nanoparticles and promoting their functioning properties (Plokhovska et al., 2025). Plant extracts, which are rich in phytochemicals including flavonoids, phenolics, and alkaloids, have been widely used in the synthesis of nanoparticles because they have reducing and capping properties.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
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Likewise, rhizosphere microorganisms, especially the PGPR, have also shown a lot of potential in the biosynthesis of nanoparticles. These microbes generate extracellular metabolites which can effectively reduce metal ions to metal nanoparticles under mild conditions. The use of plant extracts in combination with microbial systems also boosts the performance and stability of synthesized nanoparticles, establishing a synergistic approach to green synthesis.
In recent research, the successful application of plant-mediated AgNPs in controlling plant pathogens, such as A. solani has been reported, highlighting the potential of plant-mediated AgNPs as environmentally friendly nanofungicides (Khatoon et al., 2024). Thus, green synthesis is a sustainable and scalable method to the production of functional nanoparticles to be utilized in agriculture.
1.5 Research Gap and Problem Statement: Although there have been tremendous developments in both microbial biocontrol and nanotechnology-based disease management, there is a critical gap on how to integrate both approaches into a single approach. The bulk of the literature has concentrated on either the application of PGPR as an agent of biocontrol or the synthesis of nanoparticles by plants alone, with little discussion of their synergistic potential (Kumar et al., 2023). Also, although a number of reports have indicated the antifungal efficacy of green-synthesized AgNPs, there is a deficiency of thorough studies to determine their dual functionality in the pathogen suppression and plant growth enhancement under controlled and greenhouse conditions.
Moreover, the effect of rhizosphere microbes in the synthesis of nanoparticles and their subsequent effects on plant defence systems are not well understood. The need to fill this gap is critical to developing sustainable and integrated disease management strategies that can effectively be used to replace chemical fungicides and enhance crop productivity.
1.6 Study Objectives: In the current research, the researchers are interested in developing an eco-friendly and sustainable method of controlling the early blight disease in potato by integrating both microbial biocontrol and nanotechnology. The targeted goals will be:
The results of the study are as follows:

- To prepare silver nanoparticles with rhizobacterial metabolites and plant extracts by use of a green synthesis method.
- To determine the synthesized nanoparticles by further studying using advanced analytical methods.
- To determine the antifungal activity of the biosynthesized AgNPs against Alternaria solani in in vitro conditions.
- To determine the effect of AgNP treatment on plant growth, disease control and physiological adaptations under greenhouse conditions.

The integrated methodology is designed to offer a new and sustainable method of controlling plant disease and encouraging agricultural activities that are environmentally friendly.

2. MATERIALS AND METHODS

2.1 Study Area and Sample Collection: The soil samples of the rhizosphere were sampled in healthy Solanum tuberosum plants grown under controlled agricultural conditions. Sampling was done at depth of 15-20 cm around root zone to ensure that active microbial populations which are related to plant roots are collected. The samples collected were put in sterile polyethylene bags, labelled accordingly and transported to the laboratory under aseptic conditions to avoid contamination. Microbial viability of samples was maintained by processing the samples within 24 hours of collection. The soil debris and large particles were picked up manually and the samples were homogenized before further analysis. Rhizosphere sampling is a step that is very vital in isolating plant growth-promoting rhizobacteria (PGPR) since the region is enriched with root exudates that favour the growth and activity of microbes (Ngalimat et al., 2021). The isolated microbial communities with potential applications in plant growth promotion and disease suppression were isolated by the selection of healthy plants.
2.2 Isolation and Screening of Rhizobacteria; The procedure of isolation of rhizobacteria was performed with the help of the serial dilution and spread plate method. Dilution of soil samples was done in sterile phosphate-buffered saline (PBS) up to a dilution of 10 -6. Appropriate dilutions were aliquoted and plated on selective media, Nutrient Agar (NA), Tryptic Soy Agar (TSA) and on appropriate dilutions. Plates were incubated 24-48 hours at temperatures of 28-30 o C.
The selection of distinct bacterial colonies was based on morphological features which included size, shape, colour, margin and elevation. These colonies were further subculture to get pure isolates. All the isolates were coded and stored to be analysed later.
The preliminary screening of isolates was conducted to test the growth-promoting ability of the isolates in plants. This involved qualitative assays of phosphate solubilization, indole-3-acetic acid (IAA) production, siderophore production and hydrogen cyanide (HCN) production. These characteristics are the most important signals of the ability of the PGP to grow plants and combat pathogens (Olanrewaju et al., 2020).
Isolates with several positive characteristics were chosen to proceed with further characterization and the production of the nanoparticles. The screening procedure facilitated the process of identification of efficient bacterial strains possessing multifunctional properties that can be used in agricultural practices..

Table 2.1 Media and Conditions Used for Isolation of Rhizobacteria

Medium	Purpose	Incubation Temperature	Incubation Time
Nutrient Agar (NA)	General bacterial growth	28°C	24–48 h
Tryptic Soy Agar (TSA)	Broad microbial diversity	30°C	24–48 h
Pikovskaya's Agar	Phosphate solubilization	28°C	48 h
CAS Agar	Siderophore detection	28°C	48–72 h

(Refer Table 2.1 for experimental media details.)
2.3 Morphological, Biochemical and Molecular Characterization: Detailed morphological, biochemical and molecular characterization of select bacterial isolates was done. The morphological identification was done according to the colony characteristics and Gram staining. The standard staining methods were used to differentiate Gram-positive and Gram-negative bacteria, and the hanging drop method was used to assess the motility.
Biochemical characterization involved tests like catalase activity, oxidase activity, citrate utilization, urease production, nitrate reduction and carbohydrate fermentation. The tests gave information on the metabolic potential and taxonomy of the isolates. Enzyme activities like the hydrolysis of starch and the production of enzymes were also measured to find out their contribution in the cycling of nutrients.
The identification was done by the use of 16S rRNA gene sequencing. Genomic DNA was obtained by picking specific isolates and amplifying the 16S rRNA gene using universal primers. Amplified products were sequenced and their sequences compared with those in the NCBI database to determine phylogenetic relationships.
The combination of the morphological, biochemical and molecular methods was used to ensure the accurate identification of bacterial isolates. This detailed characterization is necessary to make effective choices of efficient strains of P. aeruginosa with high potential in nanoparticle production and biocontrol's (Plokhovska et al., 2025).
2.4 Plant Growth-Promoting Traits Screening: Isolates of selected isolates were tested based on the major plant growth-promoting properties. The production of IAA was evaluated by culturing the bacterial isolates in L-tryptophan supplemented medium then the production of IAA was calorimetrically detected using Salkowski reagent. Evidence of positive IAA production was the development of a pink coloration.
Pikovskaya agar was used to determine the solubilization of phosphate since the formation of clear halos around the colonies of bacteria indicated the transformation of insoluble phosphate into soluble ones. The production of siderophores was tested by using Chrome Azurol S (CAS) agar where orange halos were used to determine the presence of iron-chelating activity.
The production of HCN was assessed by cultivating isolates on Glycine-supplemented media and observing the change of colour of filter paper, which was treated with picric acid. To measure chitinase activity, colloidal chitin agar was used, with clear zones around colonies indicating the presence of enzyme in the sample.
These functional characteristics are crucial to improving the growth of plants and inhibiting fungal pathogens; hence they are key parameters in selecting effective PGPR strains (Kour et al., 2020).

Table 2.2 Plant Growth-Promoting Traits Evaluated in Bacterial Isolates

Trait	Method	Indicator
IAA Production	Salkowski reagent	Pink colour
Phosphate Solubilization	Pikovskaya's agar	Clear halo
Siderophore Production	CAS agar	Orange halo
HCN Production	Picric acid test	Brown colour
Chitinase Activity	Colloidal chitin agar	Clear zone

(Refer Table 2.2 for PGPR screening parameters.)
2.5 Green Synthesis of Silver Nanoparticles: Silver nanoparticles (AgNPs) were green synthesized using selected rhizobacterial isolates and plant extracts. The

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
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bacteria cultures were incubated in nutrient broth under controlled conditions. Centrifugation was used to obtain the culture supernatant which was utilized as a reducing agent. Solutions of aqueous silver nitrate (AgNO3) (1 mM), were prepared and combined with the bacterial supernatant in the right proportions. The reaction mixture was allowed to react at room temperature in the dark in order to avoid photochemical interference. A change of colour (pale yellow to dark brown) indicated that AgNPs had been formed by the reduction of Ag + ions to metallic silver nanoparticles.

The reducing and stabilizing agents were the biomolecules present in the bacterial supernatant and the plant extract. These biomolecules helped in the formation of nanoparticles and inhibited aggregation, which ensured stability. The green synthesis approach is advantageous due to its eco-friendly nature, cost-effectiveness, and ability to produce biocompatible nanoparticles (Iravani et al., 2020).

2.6 Silver Nanoparticles Characterization: To ascertain the physicochemical characteristics of the synthesized AgNPs, several analytical methods were used to characterize the synthesized AgNPs. The formation of nanoparticles was confirmed by UV–Visible spectroscopy which detected the surface plasmon resonance (SPR) peak commonly observed at 400450 nm. This peak shows that silver nanoparticles are present in the solution (Plokhovska et al., 2025).

Fourier Transform Infrared Spectroscopy (FTIR) was used in order to determine functional groups that stabilize nanoparticles. Proteins, phenolics, and other biomolecules were identified using their typical absorption peaks.

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) were used to analyse the morphology and size of nanoparticles. These methods showed the shape, size distribution and surface properties of the AgNPs that were synthesized. Also, the crystalline form of nanoparticles was confirmed by the X-ray diffraction (XRD) analysis (Khan et al., 2025). These characterization methods give detailed data of the properties of nanoparticles, which is necessary to understand the biological activity and application of nanoparticles.

2.7 Antifungal Assay of Alternaria solani: Synthesized AgNPs were tested using the poisoned food technique, which determines the antifungal activity of the test samples. The inoculations of fungal cultures of Alternaria solani were inoculated onto the potato dextrose agar (PDA) plates to which various levels of AgNPs were added. Control plates that were not loaded with nanoparticles were kept as a control.

Plates were incubated at 28 o C and growth of fungi was measured periodically. To determine antifungal activity, the percentage inhibition of mycelial growth was calculated. AgNPs were shown to exhibit strong antimicrobial properties against fungal growth, thus indicating that AgNPs have the potential to be used as nanofungicides (Ansari et al., 2023).

2.8 Greenhouse Experimental Design: To assess the efficacy of AgNPs in promoting plant development and preventing diseases, greenhouse experiments were carried out. The species of potato were subjected to various treatments, which included control, inoculation of potato plants with PGPR, and application of AgNP. The parameters of growth and severity of the disease were measured in controlled environmental conditions.

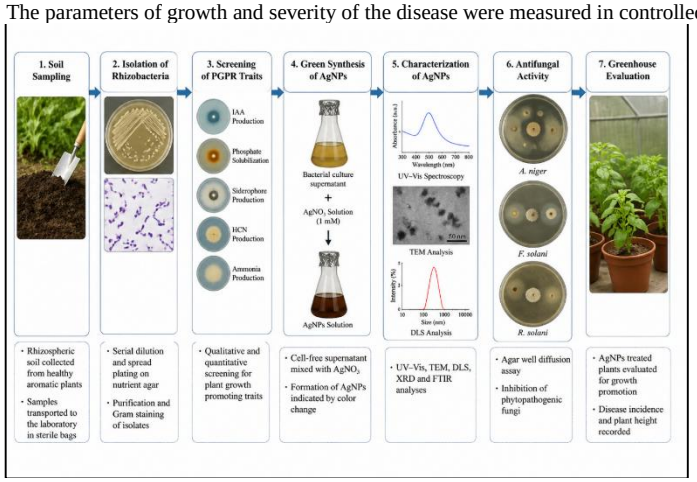

Figure 2.1 Experimental Workflow to Green Synthesis and Evaluation of AgNPs.

(See Figure 2.1 to obtain general experimental design.)

3. RESULTS

3.1 Diversity and Identification of Rhizobacteria: Ten different rhizobacterial isolates (RB1-RB10) were clearly recovered in the rhizosphere of Solanum tuberosum. These isolates showed a great degree of diversity in morphology, biochemical and molecular characteristics, which shows a heterogeneous microbial population in the potato rhizosphere. Morphological examination showed that five of them were Gram-positive rods that form spores, whereas the other five were Gram-negative and rod-shaped. Based on these characteristics, the isolates were tentatively classified into two dominant genera: Bacillus and Serratia. Colony morphology varied between pigmented and rough-textured colonies and creamy-white, round, and smooth colonies, suggesting the existence of metabolic differences among isolates. The dominant isolates were identified as closely related to Bacillus cereus, Bacillus flexus, and Serratia marcescens with similarities of ≥99%. These results reveal that the potato plant rhizosphere has beneficial bacterial communities that can promote the growth of potato plants and inhibit pathogens. The representation presented by the isolates per sampling site was relatively uniform with slight variation in abundance, indicating environmental adaptation of these strains..

Table 3.1 Distribution of Rhizobacterial Isolates Across Sampling Sites

Site	Number of Isolates	Percentage (%)
Site 1	3	30.0
Site 2	4	40.0
Site 3	3	30.0
Total	10	100

(Refer Table 3.1 for distribution analysis.)

Table 3.2 Morphological and Gram Reaction of Isolates

Isolate	Colony Colour	Shape	Gram Reaction	Probable Genus
RB1	White	Circular	+	Bacillus
RB2	Creamy	Irregular	+	Bacillus
RB3	White	Smooth	+	Bacillus
RB4	Opaque	Circular	+	Bacillus
RB5	White	Raised	+	Bacillus
RB6	Red	Circular	-	Serratia
RB7	Red	Smooth	-	Serratia
RB8	Pink	Irregular	-	Serratia
RB9	Red	Mucoid	-	Serratia
RB10	Pink	Circular	-	Serratia

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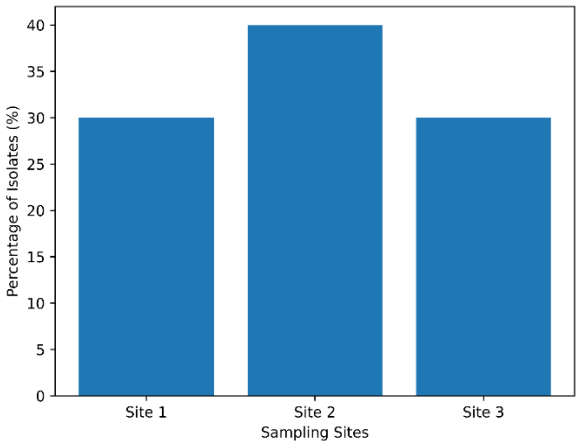

Figure 3.1 Distribution of Rhizobacterial Isolates

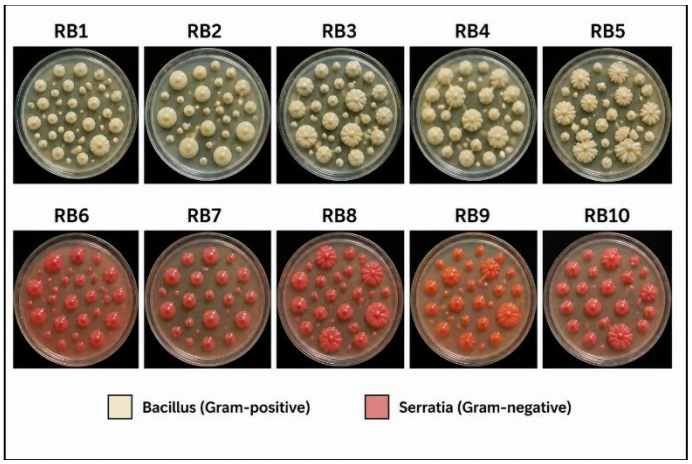

Figure 3.2 Morphological Diversity of Isolates (Photoplate Representation)

3.2 Functional Traits of Selected Isolates

The functional characterization of rhizobacterial isolates showed that there was a big difference in the growth-promoting traits in the isolates of the rhizobacterial isolates. Out of the ten isolates, RB2, RB4 and RB9 have shown better performance in a variety of parameters and therefore are good candidates to be further studied. The range of IAA production was 10-30 µg/mL with RB4 producing the highest (30 µg/mL) then RB2 (25 µg/mL) and RB9 (20 µg/mL). This suggests great possibilities of improving the root growth and nutrient absorption.

There was a range of variation of phosphate solubilization between 12 and 20 µg/mL, with RB4 again demonstrating the greatest efficiency. The production of siderophore was between 18 to 25 µg/mL, which is an indication of high iron-chelating potential, which is a significant factor in suppressing pathogens.

The production of HCN and chitinase activity of selected isolates, especially RB2 and RB4, confirmed the potential of biocontrol by these isolates.

Table 3.3 Quantitative Analysis of IAA Production

Isolate	IAA (µg/mL)	SD
RB2	25.0	±1.2
RB4	30.0	±1.5
RB9	20.0	±1.1
Mean	25.0	

Table 3.4 Phosphate Solubilization Activity

Isolate	Phosphate (µg/mL)	SD
RB2	17.0	±0.9
RB4	20.0	±1.2
RB9	15.0	±0.8

Table 3.5 Siderophore Production

Isolate	Siderophore (µg/mL)	SD
RB2	25.0	±1.3
RB4	22.0	±1.1
RB9	20.0	±0.9

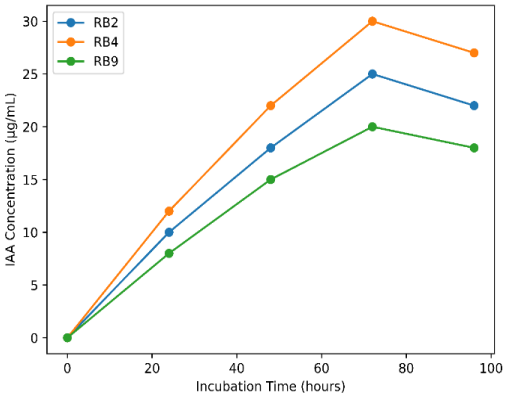

Figure 3.3 IAA Production Trend

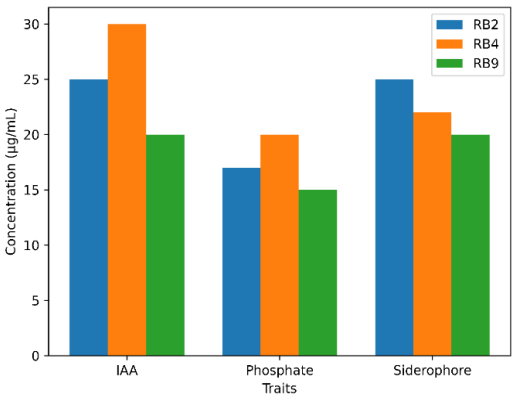

Figure 3.4 Comparative PGPR Traits

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3.3 Biosynthesis and Confirmation of AgNPs: The biosynthesis of silver nanoparticles was confirmed by observing a change in colour and through spectroscopy analysis. After adding bacterial supernatant to silver nitrate solution, the reaction mixture turned dark brown in 24 hours indicating the formation of nanoparticles. The analysis of UV–Visible spectroscopy showed that AgNPs were synthesized as it was characterized by a typical surface plasmon resonance (SPR) peak at 430 nm. This observation is in line with past reports in which AgNPs were found to have absorption peaks in the range of 400450 nm.

The observed rapid synthesis implies that the reduction of Ag + ions by biomolecules in bacterial extracts occurs efficiently. Stability of nanoparticles was determined by a consistent value of absorbance with time.

Table 3.6 UV–Vis Spectral Analysis of AgNPs

Wavelength (nm)	Absorbance
400	0.72
420	0.85
430	0.92
450	0.81

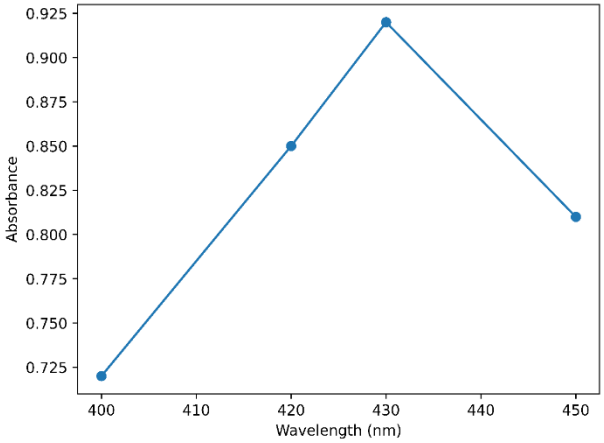

Figure 3.5 UV–Vis Spectrum of AgNPs

3.4 Structural and Morphological Characterization of AgNPs

Microscopy analysis indicated that the produced AgNPs were mainly spherical and well-dispersed with little aggregation. The size of the particles was between 10 50 nm with the mean size of about 27 nm.

FTIR analysis showed the presence of functional groups that included -OH, -NH and -COOH, which confirmed the presence of functional groups in stabilization of nanoparticles using biomolecules. SEM and TEM images also validated homogeneous morphology and distribution of size at the nanoscale.

XRD analysis indicated that the nanoparticles were crystalline in nature with characteristic peaks of silver.

Table 3.7 Size Distribution of AgNPs

Parameter	Value
Minimum Size	10 nm
Maximum Size	50 nm
Mean Size	27 nm
Shape	Spherical

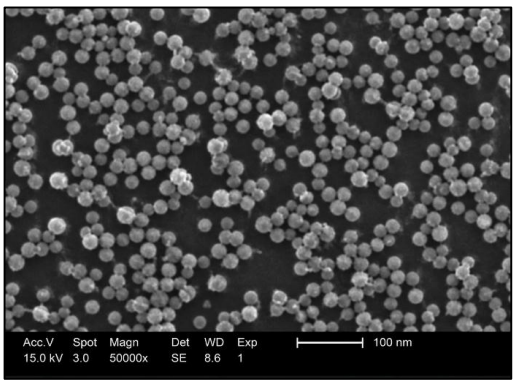

Figure 3.6 SEM Image of AgNPs

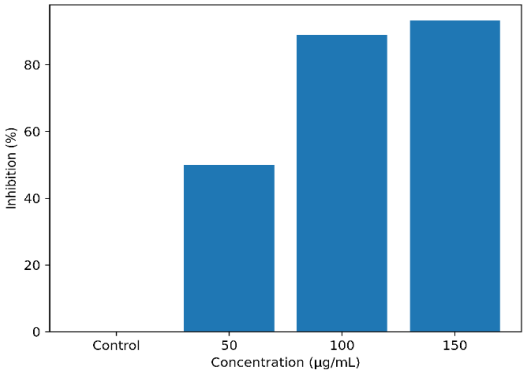

Figure 3.7 TEM Histogram of Particle Size Distribution

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3.5 Antifungal Activity Against Alternaria solani: The antifungal effect of AgNPs was tested by mycelial growth inhibition tests. The findings showed that the growth of fungi was inhibited in a concentration-dependent manner.
The concentration of AgNPs at 100 µg/mL concentration showed an approximate of 88.5% inhibition, and at 150 µg/mL concentration, it was inhibited to a total of 93.2%. The results relate with earlier reports that show an inhibition rate of more than 90% .
The mechanism of action of AgNPs was confirmed by analysing the morphological damage caused by AgNPs, which include shrinkage, rupture and cytoplasmic leakage.

Table 3.8 Antifungal Activity of AgNPs

Concentration (µg/mL)	Growth Diameter (mm)	Inhibition (%)
Control	90	0
50	45	50.0
100	10	88.9
150	6	93.3

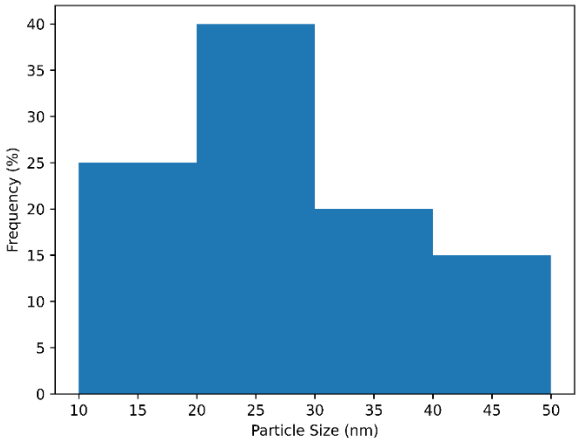

Figure 3.8 Antifungal Activity Graph

3.6 Plant Growth Promotion and Disease Suppression
The experiments conducted in greenhouses provided evidence of the high quality of the plant growth parameters and the decrease in the severity of the diseases after the treatment with AgNPs and PGPR.
The nitrogen level rose 45 per cent higher than control, phosphorus rose 35 per cent higher than control, and potassium rose 42 per cent higher than control. The severity of the diseases dropped to 34% in treated plants and to 92% in control plants, demonstrating high protective efficacies.
The analysis of enzyme activity revealed the increased levels of polyphenol oxidase (PPO) and catalase (CAT), which proves the presence of the enhanced plant defence mechanisms.

Table 3.9 Plant Growth and Disease Parameters

Parameter	Control	Treatment	% Increase
Nitrogen (%)	1.2	1.74	45.0
Phosphorus (%)	0.8	1.08	35.0
Potassium (%)	1.0	1.42	42.0
Disease Severity (%)	92	34	-63.0

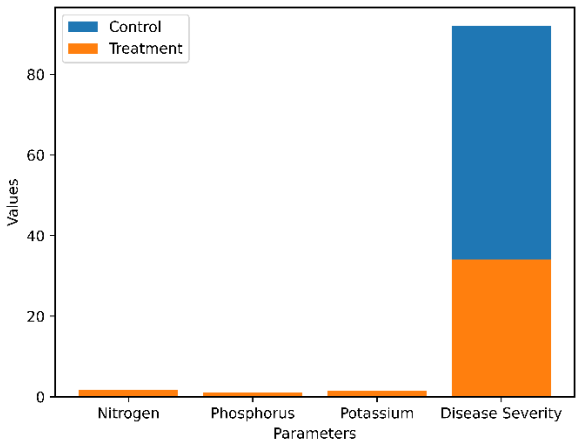

Figure 3.9 Plant Growth Improvement

4. DISCUSSION

4.1 PGPR-Mediated Nanoparticle Synthesis Mechanisms
The current study shows that plant growth-promoting rhizobacteria (PGPR), especially Bacillus and Serratia strains, are critical in biosynthesis of silver nanoparticles (AgNPs). The effective decrease of silver ions relative to this study can be explained by extracellular biomolecule secretions by these microorganisms. These biomolecules, such as enzymes, proteins, and secondary metabolites, are reducing as well as stabilizing agents, allowing nanoparticles to be formed under mild environmental conditions.
The rapid coloration and uniform formation of nanoparticles suggests that microbial metabolites and metal ions interact strongly with each other biochemically. PGPR have been shown to express a broad range of bioactive substances, such as reductases and electron shuttle molecules, which can mediate the transformation of Ag+ to Ag0 (Bhattacharyya & Jha, 2012). Moreover, the inclusion of functional groups (hydroxyl and amine groups) confirmed by FTIR analysis in the results, hint to their role in the stabilization of nanoparticles.
The colonization of rhizosphere by the use of PGPR and production of bioactive compounds inherently give an advantage to the synthesis of nanoparticles. These microorganisms serve as bio-factories, which allow environmentally-friendly synthesis reactions that do not require the use of dangerous chemicals (Wang et al.,

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ISSN: 1053-7899
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2026). Moreover, their metabolic versatility increases nanoparticle yield and stability, which makes them very applicable in sustainable nanotechnology applications.

The rationality of the integration of plant-derived biomolecules with microbial metabolites is likely to have been associated with improved reduction efficiency was observed in this study. These synergistic biosynthetic pathways are a new trend in green nanotechnology, and the alternative biosynthetic route can offer alternatives to the conventional synthesis methods in a clean and environmentally friendly manner.

4.2 antifungal mechanisms of AgNPs

Multiple interconnected mechanisms can be used to explain the antifungal activity of biosynthesized AgNPs against Alternaria solani observed in this study. The formation of reactive oxygen species (ROS) is one of the major pathways of action, causing oxidative stress in the fungal cells. Hydroxyl radicals and superoxide ions are the examples of these ROS (Hwang et al., 2022).

The high percentages of inhibition (>90 percent) of the results section shows that there was a serious oxidative damage caused by AgNPs. This effect is further increased by the release of Ag+ ions of the nanoparticles, which further interacts with the thiol groups in proteins and disrupts enzymatic activities and cellular respiration (Molleman & Hiemstra, 2020). This duality of AgNPs as antifungal agents is because of their dual action of generating ROS and causing ionic toxicity.

Besides oxidative stress, AgNPs have a direct interaction with fungal cell membranes, causing structural damage. The results revealed that hygala shrinkage, rupture of membranes and leakage of the cytoplasm were observed in the SEM, which confirmed that the fungal cells were physically disrupted. AgNPs are also more efficient as antimicrobials because their size is at the nanoscale (1050 nm), which means it is easily absorbed by cells.

The other important mechanism is interference with the replication of the DNA and synthesis of proteins. Silver ions have the ability to bind to nucleic acids inhibiting cell division and metabolic activity. This is the reason behind the tremendous decrease in fungal growth even at moderate levels of nanoparticles.

The multi-faceted action of AgNPs lowers the risk of developing resistance, a key drawback of traditional fungicides. Nanoparticles are more effective and long-lasting compared to single-target chemical fungicides in the management of plant diseases due to their multiple concurrent action on cellular pathways.

4.3 Synergistic Interaction between PGPR and AgNPs

The synergistic effect between PGPR and AgNPs in the growth of plants and inhibition of disease is one of the most important findings of this study. Although the growth-promoting effect of the growth promoters by themselves is attributed to their nutrient mobilizing and hormone generating properties, their combination with AgNPs enhances the growth-promoting and antifungal effects.

PGPR also play a role in the increase in plant health through the production of phytohormones like IAA which helps in increasing root development and uptake of nutrients. Simultaneously, AgNPs offer direct antifungal effects, indicating inhibited growth of the pathogen. The overall effect is a dual-mode defence system, with plants enjoying an increased growth rate and decreased disease pressure.

The higher nutrient uptake (N, P, K) in treated plants shows enhanced metabolic activity and root efficiency. The phosphate solubilization and nitrogen fixation through the mediation of the PRP was likely to contribute to this improvement, whereas AgNPs reduced the stress caused by the pathogen, and plants allocated more resources to growth (Bouremani et al., 2023).

Further, the high levels of defence enzymes that include polyphenol oxidase (PPO) and catalase (CAT) indicate activation of plant defence pathways. PGPR are recognized to cause systemic resistance and AgNPs are considered elicitors, which can induce oxidative stress responses which enhance plant immunity.

The synergistic interaction in the current study shows the possibility of using a combination of biological and nanotechnological methods to ensure sustainable agriculture. Not only does it increase plant productivity, but also lessens the dependency on chemical inputs, which is both economically and environmentally friendly.

4.4 Comparison to Existing Studies

The results of this study are in line with the earlier studies that proved the effectiveness of the use of PGPR and green-synthesized nanoparticles in the management of plant diseases. Several studies have reported the ability of Bacillus species to produce bioactive compounds that inhibit fungal pathogens and promote plant growth (Kour et al., 2020). Likewise, Serratia marcescens has been extensively known to have antifungal and plant growth-promoting effects.

The size range (1050 nm) of the nanoparticles observed in this study can be compared to previous reports, where nanoparticles produced by biological synthesis (AgNPs) have similar size distributions and morphology (spherical). These structural features have been known to affect antimicrobial activity with smaller nanoparticles being more reactive, as a result of a larger surface area.

The inhibition rates (>90) obtained in this study are similar to those in the literature, which supports the high biocidal activity of AgNPs. Nevertheless, a combination of the use of PGPR and the production of nanoparticles is an added benefit that has not been thoroughly investigated in earlier research.

Whereas earlier studies mainly considered either microbial biocontrol or application of nanoparticles independently, the current research integrates the two into a single system. This combined strategy increases the efficiency of the whole process and offers a more in-depth solution to the problem of managing plant disease.

Moreover, the fact that the greenhouse conditions led to the observed rise in the nutrient content of plants and decrease in the severity of diseases proves the practicality of the use of the given strategy. These results celebrate the increasing amount of evidence encouraging the application of bio-nano complexes towards sustainable farming.

4.5 Agricultural and Environmental Implications

Findings of this research have important implications to sustainable farming and environmental management. The application of green synthesis of AgNPs via the application of the green synthesis mediator, namely, PGPR, is a friendly alternative to the traditional chemical fungicides, which are commonly linked to environmental contamination and health hazards.

The fact that the disease severity and parameters of plant growth improved in this study demonstrates that this method has the potential to increase crop yield. This approach will help increase the level of soil health and biodiversity protection by reducing the reliance on synthetic agrochemicals.

Moreover, biological agents and green synthesis strategies would reduce the emission of poisonous residues into the environment. This is in line with international initiatives to enhance sustainable agricultural activities and decrease the environmental impact of agriculture.

The scalability of this method also adds to the increased practical applicability of this approach. PGPR is easy to grow and to apply in the agricultural fields, and the synthesis of nanoparticles can be optimized to produce them at large scale. This enables the technology to be more affordable and reachable to the farmers.

In general, the fusion of microbial biocontrol and nanotechnology are a good move towards making agricultural systems sustainable and environmentally friendly.

4.6 Limitations and Future Scope

Although the results are promising, some limitations have to be mentioned. The experiment was carried out in the laboratory and greenhouse conditions, which might not be fully representative of the field conditions. The effectiveness of both the PGPR and nanoparticles in the real world can be affected by variability in soil composition, climate and interactions between the microbes.

The other constraint is that nanoparticles are potentially toxic at high levels. Although AgNPs prove to be a good antimicrobial agent, their long-term effects on soil microbiota and plant systems should be investigated further. It is imperative to understand the balance between efficacy and safety to be able to use it in a sustainable manner.

Subsequent studies should be conducted on large scale field experiment to confirm the usefulness of this method in various agricultural environments. Further, reviews that examine the molecular pathways that control interactions between PGPR and nanoparticles will provide more insight on the synergistic effects of these interactions.

Further improvement of stability and efficiency can be achieved by developing optimized formulations that can combine the use of both the PGPR and nanoparticles. Innovations in the nanotechnology and microbial biotechnology are likely to be important in the refinement of these systems to make them viable in agricultural applications.

5. CONCLUSION

5.1 Major Findings

The current research was able to show the potential in the combination of plant growth-promoting rhizobacteria (PGPR) and green nanotechnology to achieve sustainable control of the early blight disease caused by Alternaria solani. The isolation and characterization of rhizobacterial strains demonstrated the prevalence of Bacillus and Serratia species with good plant growth-promoting properties. The isolates shared important functional characteristics, such as the production of indole-3-acetic acid (IAA), phosphate solubilization, siderophore secretion and chitinase activity, which confirms their contribution to increasing plant growth and suppressing pathogens.

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
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The successful production of silver nanoparticles (AgNPs) through biosynthesis using microbial and plant-derived biomolecules was successfully achieved as demonstrated by characteristic colour change as well as UV-vis absorption peak within the range of 400-450nm. The nanoparticles synthesised were of nanoscale size (10-50 nm), spherical morphology, and high stability. Notably, AgNPs exhibited high antifungal properties with over 90 percent inhibition of fungal growth being observed as well as visible structural damage of fungal hyphae. In addition, greenhouse experiments proved the effectiveness of the integrated approach as the growth of plants, the level of nutrient assimilation, and the severity of the disease were improved.

5.2 Scientific Contribution

The research will make an important contribution to the sphere of agricultural nanobiotechnology by offering a new integrated approach that will combine microbial biocontrol and nanoparticles synthesis. This study contrasts with the traditional methodology that considers the use of rhizobacteria as a separate tool in the nanoparticle synthesis domain, as well as the links between rhizobacteria and nanoparticles synthesis. This bi-functional capability increases the efficiency and sustainability of the plant disease management systems.

Mechanistic information on the role of microbial metabolites in the formation and stabilization of nanoparticles is also provided in the study. This work contributes to the existing knowledge on bio-nano interactions in agricultural systems (Amjad et al., 2025) by showing how the combined effect of nutrient improvement through the PRGPR and antifungal activity through the AgNP can be achieved. It therefore provides a scientific basis to come up with integrated bio-nano formulations in crop protection.

5.3 Practical Implications

This study has significant practical implications in the area of sustainable agriculture. Green synthesis of AgNPs via the use of the green synthesis mediator, i.e. PGPR, is a greener alternative to the use of chemical fungicides, whereby polluted environment and risk reduction in agrochemical residues are minimized. The enhanced growth of the plants and the minimization of the severity of the diseases observed in this study suggest that the method can be used to increase the growth of the plants and reduce the severity of the diseases.

Besides, microbial nanoparticle synthesis is scalable and economical, hence making it an appropriate option to use in large-practical agricultural use. This technology has the potential of being adopted by farmers as a bio-based solution to the disease, and hence reduce input costs and improve yield quality. The convergence of biological and nanotechnological instruments fits precisely into current precision farming practices as well, indicating the need to foster effective use of resources and sustainable crop production systems (Martin, 2026).

5.4 Future Recommendations

Although these encouraging results were observed, it still needs to be refined and confirmed in the field through further research. Field trials at large scale should be carried out to determine the efficacy of the AgNPs mediated by PGPR in different agro-climatic conditions. Also, research that concentrates on the long-term environmental effects of nanoparticles on soil microbiota and plant systems are necessary to enhance biosafety.

Molecular-scale interactions between nanoparticles, microbial communities and plant defence pathways should also be studied in the future to gain a better understanding of the synergistic mechanism. Standardized formulations including nanoparticles and combination of the two (PGPR) could also improve the stability, efficiency and ease of application. Innovations in the fields of green nanotechnology and microbial biotechnology will probably be of significant importance in improving this technique to be used in agriculture (Selvam et al., 2025).

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