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RESEARCH ARTICLE

Silver nanoparticles green synthesized using leaf extract of *Piper* spp. reduce *Phytophthora capsici* infection in black pepper (*Piper nigrum* L.)

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Abstract

Black pepper (*Piper nigrum* L.) is vulnerable to the devastating disease foot rot caused by the oomycete, *Phytophthora capsici*. Green synthesis of nanoparticles using plant extracts provides an eco-friendly, and economical method for plant disease management. Leaf extracts from two genotypes of pepper plants - one susceptible to foot rot disease (*P. capsici*) - *Piper nigrum*, and the other, resistant - *Piper colubrinum*, were used for synthesizing silver nanoparticles (AgNPs). These green synthesized AgNPs were characterized and evaluated for their anti-oomycete effects against *P. capsici* and ability to suppress foot rot disease in black pepper. UV Spectroscopic analysis confirmed typical absorption characteristics of AgNPs, while field emission scanning electron microscopy revealed sizes ranging from 39 to 69 nm for both samples. *In vitro* poisoned food assay demonstrated that the green synthesized AgNPs at 500 and 750 ppm showed highest inhibition on the mycelial growth of the pathogen. Among the treatments,71 % and 77 % inhibition in mycelial growth of pathogen was observed with 500 ppm and 750 ppm of Pn-AgNPs respectively, and 65 % and 73 % inhibition in growth of mycelia of pathogen with 500 ppm and 750 ppm of Pc-AgNPs respectively. Copper Oxychloride at 0.2 % completely inhibited mycelial growth. In a detached leaf assay with black pepper variety Panniyur-1, significant reduction in lesion development was observed on leaves treated with the green synthesized AgNPs compared to those treated with commercial AgNPs and chitosan nanoparticles. No lesion developed on leaves treated with 750 ppm concentration of green synthesized AgNPs. Leaf extracts were ineffective in disease suppression and pathogen growth. Mild symptoms were observed on leaves treated with the biocontrol agent (*Pseudomonas fluorescens*, 2 %), while no symptoms were found on leaves treated with the fungicide. Spraying of AgNPs green synthesized using leaf extracts of pepper genotypes reduced foot rot disease incidence in susceptible pepper genotype *P. nigrum,* efficiently. Green nanotechnology for foot rot management can benefit farmers by providing an eco-friendly, cost-effective solution that reduces dependency on chemical fungicides and minimizes environmental impact.

Keywords

black pepper; disease resistance; foot rot; green synthesis; silver nanoparticles;

Introduction

Nanotechnology is a rapidly advancing field that has effectively addressed

issues across diverse industries (1). Nanoparticles exhibit unique properties, such as surface plasmon resonance (SPR), a high surface area-to-volume ratio, and distinctive optical, biological, and electrical characteristics, which enhance their applicability. In agriculture, nanotechnology enables the use of nanoscale fertilizers, pesticides, herbicides, and supports plant disease management (2).

Nanoparticle synthesis methods include physical, chemical, and biological (green) approaches. Green synthesis, which utilizes plant extracts to form nanoparticle formation through reduction/oxidation reactions, is considered safer, more economical, and environmentally friendly compared to chemical methods (3). This method produces nanoparticles with lower toxicity due to the absence of hazardous reagents and has shown efficacy in managing various plant diseases (4). Among metals used for nanoparticle synthesis, silver is preferred for its high electrical and thermal conductivity, with aqueous plant extracts efficiently facilitating green synthesis of AgNPs. These AgNPs display potent antimicrobial activity, making them effective in agricultural applications.

Black pepper (*Piper nigrum* L.), commonly known as the King of Spices, is the most widely used spice all over the world. The plant is vulnerable to foot rot, a devastating disease caused by the oomycete, *Phytophthora capsici* Leonian (5, 6). Symptoms appear as fast advancing dark brown lesions on leaves with a fimbriate margin and rotting of aerial portions of stems and spikes leads to varying degrees of defoliation and spike shedding, ultimately reducing bush size. Although cultural, biological, and chemical approaches are recommended for disease management (7), on a global scale, an annual crop loss of $ 4.5- 8 million has been reported due to this disease. Because of this higher crop loss percentage, there is an increasing demand for an alternative method for disease management. Though all the cultivars of black pepper are susceptible to the disease, is resistant to the foot rot disease (8).

Effective management of foot rot disease is urgent and significant as it prevents crop losses that impact farmers' incomes and food security, while reducing the need for chemical treatments that can harm soil health and the surrounding ecosystem. Therefore, the study aims at green synthesize AgNPs using leaf extracts from the foot rot resistant genotype *P. colubrinum* and the susceptible genotype, *P. nigrum,* and to evaluate the disease-suppressing efficiency of these AgNPs in a susceptible black pepper cultivar.

Materials and Methods

Green synthesis of silver nanoparticles

Using aqueous leaf extracts of *Piper nigrum* and *P. colubrinum,* AgNPs were green synthesized (9). Fresh and healthy leaves (5 g) were collected, extensively cleaned using tap water, and washed with Tween 20, and subsequently rinsed three times with sterile distilled water. They were finely cut, boiled in 100 mL of sterile double-distilled water, and then filtered using Whatman No. 2 filter paper to obtain the leaf extract. The leaf extract was added to the aqueous solution of 1 mM silver nitrate (Sigma-Aldrich, India) prepared with double-distilled water in 9:1 (V/V) ratio. The mixture was microwaved for a duration of 40 s and then left at room temperature (28±2°C) in darkness for 1 hr to facilitate AgNPs formation. A colour change from light brown to dark brown after incubation indicated that the Ag^{+} reduced to AgNPs. The silver nanoparticle suspension was centrifuged for 15 min at 10,000 rpm. The resulting pellet of AgNPs was re-suspended in germ-free deionized water, again centrifuged for 15 min at 10,000 rpm, dried for 20-25 min at 40 ºC in a hot air oven, and weighed to record its dry weight. The pellet was then re-suspended in 1 mL germ free deionized water and stored for later use. Silver ion bioreduction was confirmed by adding 0.1 M aqueous sodium chloride to the supernatant obtained after centrifuging the colloidal solution of silver nitrate and leaf extracts. The absence of any precipitate indicates the complete reduction of silver ions (10). The AgNPs were named based on the plant genotypes utilized for the green synthesis. AgNPs synthesized using *P. nigrum* was named as Pn-AgNPs, while those from *P. colubrinum* was named as Pc-AgNPs.

Silver nanoparticle-Physical characterisation

UV-visible spectroscopy

The transformation of Ag+ to AgNPs was observed by analyzing the absorption peaks of the silver nanoparticle solution using a UV-Visible spectrometer (Shimadzu, 1900, Japan) The suspension was scanned at wavelengths ranging from 300 to 600 nm. Germ- free deionized water served as blank solution.

Field emission scanning electron microscopy

The aqueous AgNPs suspension sample was applied onto FE-SEM sample stub using carbon tape and allowed to air-dry. Analysis using Field Emission Scanning Electron Microscope was conducted using MAIA TC software.

In vitro bioassay of green synthesized silver nanoparticles against the pathogen

Black pepper leaf samples with symptoms of foot rot disease were collected and pathogen *P. capsici* was isolated. Its pathogenicity was confirmed as per Koch's postulates in Panniyur-1 (susceptible cultivar of black pepper).

Poisoned food technique

Equal volume of green synthesized silver nanoparticles (GSAgNPs) and sterilized double strength PDA was mixed to obtain a final concentration of 250, 500, 750 and 1000 ppm of the amendment. The mixture was poured into 5 cm diameter Petri plates. Each plate was inoculated at its centre with a 5 mm diameter mycelial disc of *P. capsici* and then placed in an incubator for five days at 26 °C. It was examined for inhibition of radial growth of mycelia of the pathogen. The two most effective concentrations of GSAgNPs were selected for subsequent experiments based on the mycelial growth inhibition. Commercially available silver nanoparticle suspension and chitosan nano powder (ChNPs) at the same concentrations mentioned above were also tested. Additionally, filter-sterilized (0.2 µm) leaf

extracts from *P. nigrum* and *P. colubrinum*, used previously for green synthesis of AgNPs, were added to PDA medium at 50 % concentration and the pathogen grown on it. As a chemical control, the medium was amended with 0.2 % Copper Oxychloride, while plates with no amendments served as absolute controls. Assessment of Percentage Inhibition (PI) was done as per the given formula:

$$PI = \frac{\text{Radial growth of mycelia in control plates - radial growth of mycelia in treatment plates}}{\text{Radial growth of mycelia in control plates}} \times 100$$

(Eqn. 1)

Detached leaf test

Healthy black pepper leaves (var Panniyur-1) were collected, cleaned, and washed with germ- free distilled water. Green-synthesized Pn-AgNPs, Pc-AgNPs, commercially available AgNPs, and ChNPs were prepared at the two most effective concentrations obtained from the poisoned food assay, and applied to detached leaves, then air-dried. Additionally, filter-sterilized (0.2 µm) leaf extracts from *P. nigrum* and *P. colubrinum* and recommended biocontrol agent *P. fluorescens* (2 %) were sprayed to detached leaves as separate treatments and also allowed to air dry. For challenge inoculation, after making pinpricks on the lower side of the treated leaves, a 4 mm mycelial disc of the pathogen was placed on each leaf. Adequate humidity was maintained by keeping a thin layer of moist cotton above the mycelial disc. Petri dishes with treated leaves were incubated for three days at 26 ºC. Pathogen-inoculated leaves treated with 0.2 % Copper Oxychloride solution served as chemical control, while those treated with sterile water, served as inoculated control. Two sets of experiments were conducted separately each with three replications.

Cost analysis

A financial analysis was conducted to evaluate the cost-effectiveness of using green nanotechnology for foot rot disease management. This involved calculating the treatment cost per plant, including materials/ reactants, energy, equipment depreciation, labour, and application costs. The cost analysis will provide insights into the economic feasibility of this method for farmers.

Statistical analysis

Statistical analysis was performed using one-way Analysis of Variance (ANOVA) and the treatment means were compared by Duncan's Multiple Range Test (DMRT) at 5 % level of significance. The analysis was conducted using the statistical package *GrapesAgri1*, (version 1.1.0.) in R (version 4.2.3).

Results

Green synthesis of silver nanoparticles

The colour of solution containing $AgNO_3$ and leaf extract changed from light brown to dark brown (Fig. 1, 2). This colour change confirmed the formation of AgNPs. In the precipitation test, sodium chloride was added to the supernatant of both samples, and no precipitate was formed. In contrast, in the control solution of $AgNO_3$ without leaf extract white precipitate was formed on addition of NaCl solution.

Silver nanoparticle-Physical characterisation

UV- visible spectroscopy

The reduction of silver nitrate to AgNPs, facilitated by the aqueous leaf extract, was verified by UV-visible spectroscopy. Treatment of $AgNO_3$ solution with extracts from leaf of *P. nigrum* resulted in an absorption peak at 440 nm (Fig. 3), while the peak shifted to 460 nm with *P. colubrinum* extract (Fig. 4).

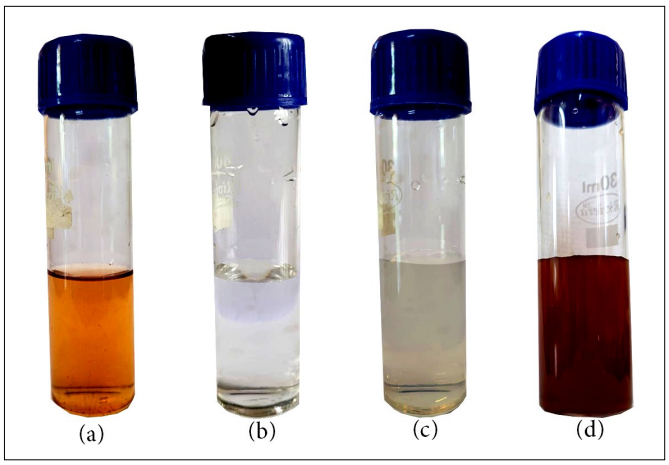

Fig 1. Colour change observed during the green synthesis of AgNPs using *P. nigrum* leaf extract (**a**) *Piper nigrum* leaf extract, (**b**) Silver nitrate, (**c**) Leaf extract + silver nitrate (before incubation), (**d**) Leaf extract + silver nitrate (after incubation)

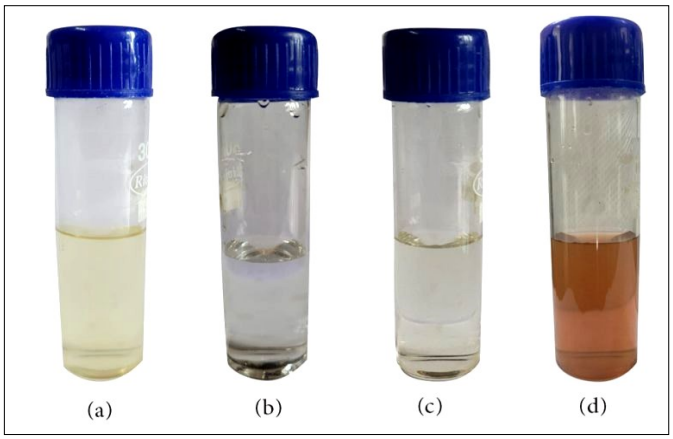

Fig 2. Colour change observed during the green synthesis of AgNPs using *P. colubrinum* leaf extract. (**a**)*Piper colubrinum* leaf extract, (**b**) Silver nitrate, (**c**) Leaf extract + silver nitrate (before incubation), (**d**) Leaf extract + silver nitrate (after incubation).

Field emission scanning electron microscopy

An average size of 57 nm was observed for Pn-AgNPs (Fig. 5), while Pc-AgNPs had an average size of around 56 nm (Fig. 6). FESEM images further confirmed the uniform distribution of the synthesized nanoparticles.

In vitro bioassay of green synthesized silver nanoparticles against the pathogen

Poisoned food technique

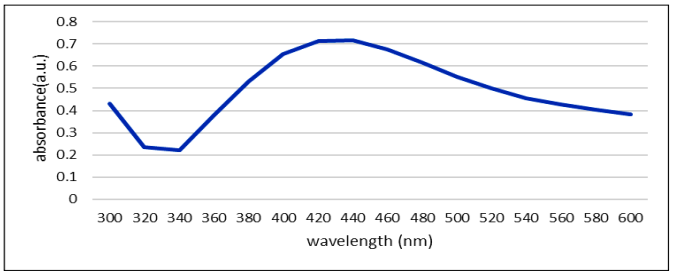

Fig 3. UV–visible absorption spectrum of green synthesized AgNPs using *P. nigrum* leaf extract.

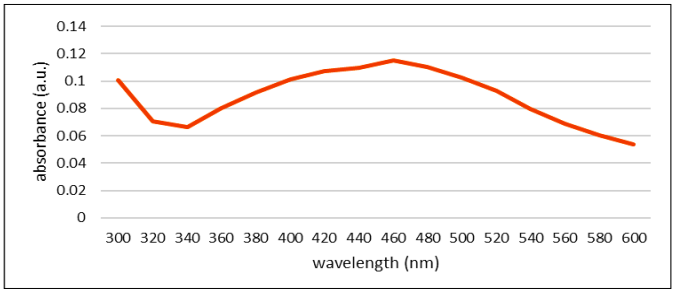

Fig 4. UV–visible absorption spectrum of green synthesized AgNPs using *P. colubrinum* leaf extract.

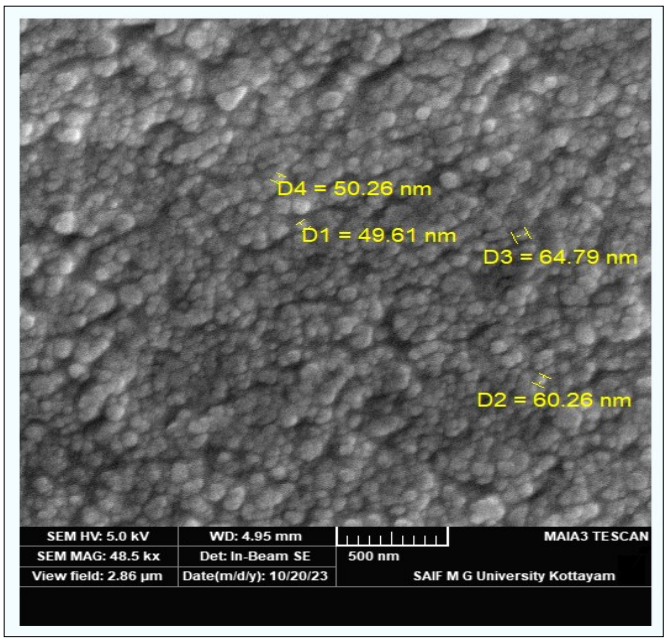

Fig 5. FE SEM image of Pn- AgNPs.

The addition of Copper Oxychloride in the medium exhibited the highest inhibition - 100 % on the 5th day after inoculation. However, little inhibition in mycelial growth of fungus was observed when commercial AgNPs suspension and chitosan nano powder suspension at concentrations of 500 ppm and 750 ppm, as well as leaf extracts from *P. nigrum* and *P. colubrinum* were added to the culture medium. A percentage inhibition (PI) of 70.67 % and 65.33 % was observed for medium amended with 500 ppm of Pn-AgNPs and Pc-AgNPs, respectively. Meanwhile, PI values increased to 76.67 % and 73.33 % when plates were amended with 750 ppm of GSAgNPs from the leaf extracts of *P. nigrum* and *P. colubrinum*, respectively (Fig. 7, Table 1).

Detached leaf test

Healthy leaves of black pepper variety Panniyur-1 inoculated with *P. capsici* without any treatments, resulted in

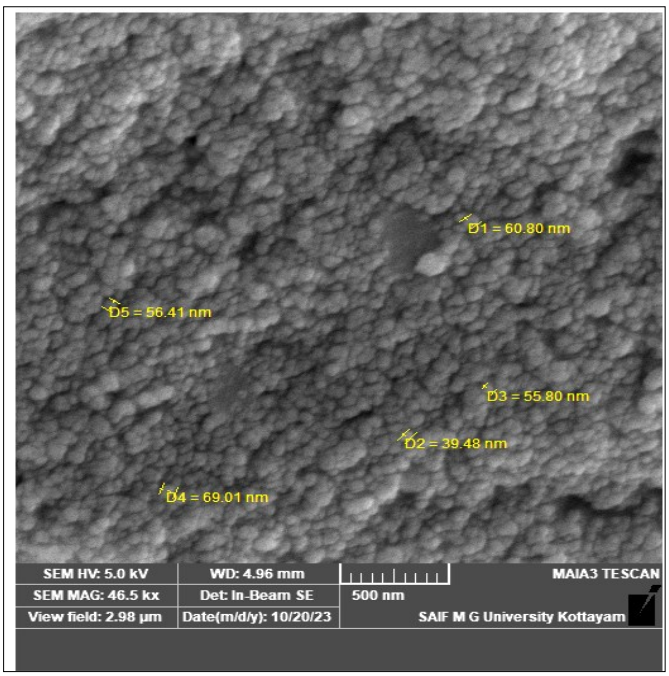

Fig 6. FE SEM image of Pc- AgNPs.

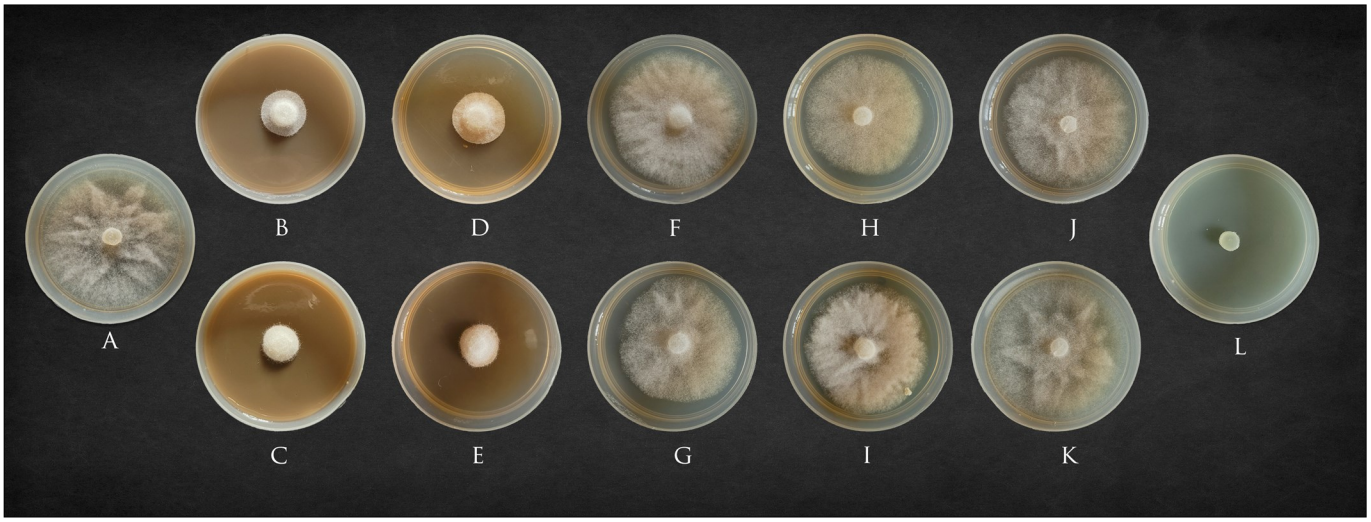

Fig 7. Mycelial growth of *P. capsici* in poisoned PDA medium. (**a**) Control, (**b**)Pn AgNPs at 500 ppm, (**c**) Pn AgNPs at 750 ppm, (**d**) Pc AgNPs at 500 ppm, (**e**) Pc AgNPs at 750 ppm, (**f**) C AgNPs at 500 ppm, (**g**) C AgNPs at 750 ppm, (**h**) Ch AgNPs at 500 ppm, (**i**) Ch AgNPs at 750 ppm, (**j**)*P. nigrum* leaf extract (5 %), (**k**) *P. colubrinum* leaf extract (5 %), (**l**) Chemical control (Copper Oxychloride 0.2 %). **Pn AgNPs** = Green synthesized silver nanoparticles using *Piper nigrum* leaf extract. **Pc AgNPs** = Green synthesized silver nanoparticles using *Piper colubrinum* leaf extract . **C AgNPs** = Commercial silver nanoparticles. **Ch AgNPs** = Commercial chitosan nanoparticles.

Table 1. Mycelial growth inhibition of *P. capsici* in poisoned food technique.

Sl. No	Treatments	Radial growth (cm)	Mycelial growth inhibition (%) *
1.	Pn AgNPs at 500ppm	0.73	70.67± 1.155^{d}
2.	Pn AgNPs at 750 ppm	0.58	76.67 ± 1.155^{b}
3.	Pc AgNPs at 500 ppm	0.86	65.33 ± 1.155^{e}
4.	Pc AgNPs at 750 ppm	0.66	73.33 ± 1.155^{c}
5.	C AgNPs at 500ppm	2.16	13.33 ± 1.155^{h}
6.	C AgNPs at 750 ppm	2.10	16.00 ± 0.000^{g}
7.	ChNPs at 500 ppm	2.06	17.33 ± 1.155^{fg}
8.	ChNPs at 750 ppm	2.05	18.00 ± 0.000^{f}
9.	*P. nigrum* leaf extract (5 %)	2.25	10.00 ± 2.000^{i}
10.	*P. colubrinum* leaf extract (5 %)	2.5	0
11.	Chemical Control (COC 0.2 %)	0	100 ± 0.000^{a}
12.	Control	2.5	0
	SE (m)		0.577

Pn AgNPs = Green synthesized silver nanoparticles using *Piper nigrum* leaf extract, **Pc AgNPs** = Green synthesized silver nanoparticles using *Piper colubrinum* leaf extract, **C AgNPs** = Commercial silver nanoparticles, **Ch AgNPs** = Commercial chitosan nanoparticles, **COC**= Copper Oxychloride, *Mean (±SD) of 3 replications. The percentage mycelial growth inhibition was determined by comparing the radial growth of the fungus in the amended PDA plates to that in the control plate seven days after inoculation. Duncan's multiple range test (DMRT) indicates that there is no significant difference between the figures in a column followed by the same letter (p≤0.05).

typical foot rot symptoms. These symptoms began as translucent black lesions on the third day after inoculation (Fig. 8), eventually spreading to cover the entire leaf lamina, causing leaf drying. On the 5th day after inoculation, lesions approximately 3.5 cm long were observed on pathogen inoculated control leaves. Similar disease symptoms appeared on leaves sprayed with AgNPs, chitosan nanoparticles, and leaf extacts, though there were significant differences in lesion development between treatments using 500 ppm Pn-AgNPs (1.4 cm) and Pc-AgNPs (2.0 cm) compared to those using 750 ppm Pn-AgNPs (no lesion) and Pc-AgNPs (no lesion) (Fig. 9). Severity levels also varied significantly among the treatments (Table 2).

Cost analysis

The estimated treatment cost per plant for managing foot rot using GSAgNPs is calculated to be two rupees, making it a low-cost option for disease management. Cost analysis highlights the affordability of GSAgNP treatment, suggesting its potential for widespread adoption among cost-sensitive farmers. Cost breakdown is given in Table 3.

Discussion

The green synthesis of nanoparticles is often referred to as biosynthesis, involving a range of biological sources. The green synthesis process involves three key components: reducing agents, solvents, and capping agents. Biomolecules, be naturally occur in or produced by microbes and plants, such as polyphenols, terpenoids, flavonoids, alkaloids, proteins, tannins, polysaccharides, vitamins, and amino acids, serve as reducing agents during synthesis of nanoparticles (11). These biomolecules reduce metal ions

Fig 8. Symptom development on detached leaves three days after inoculation with *P. capsici* . (**a**) Pn AgNPs at 500 ppm, (**b**) Pn AgNPs at 750 ppm, (**c**) Pc AgNPs at 500 ppm, (**d**) Pc AgNPs at 750 ppm, (**e**) C AgNPs at 500 ppm, (**f**) C AgNPs at 750 ppm, (**g**) Ch AgNPs at 500 ppm, (**h**) Ch AgNPs at 750 ppm, (**i**) *P. nigrum* leaf extract (5 %), (**j**) *P. colubrinum* leaf extract (5 %), (**k**) *Pseudomomas fluorescens* (2 %), (**l**) Chemical control (Copper Oxychloride 0.2 %), (**m**) Control. **Pn AgNPs** = Green synthesized silver nanoparticles using *Piper nigrum* leaf extract. **Pc AgNPs** = Green synthesized silver nanoparticles using *Piper colubrinum* leaf extract. **C AgNPs** = Commercial silver nanoparticles. **Ch AgNPs** = Commercial chitosan nanoparticles.

Fig 9. Symptom development on detached leaves five days after inoculation with *P. capsici* . (**a**) Pn AgNPs at 500 ppm, (**b**) Pn AgNPs at 750 ppm, (**c**) Pc AgNPs at 500 ppm, (**d**) Pc AgNPs at 750 ppm, (**e**) C AgNPs at 500 ppm, (**f**) C AgNPs at 750 ppm, (**g**) Ch AgNPs at 500 ppm, (**h**) Ch AgNPs at 750 ppm, (**i**) *P. nigrum* leaf extract (5 %), (**j**) *P. colubrinum* leaf extract (5 %), (**k**) *Pseudomomas fluorescens* (2 %), (**l**) Chemical control (Copper Oxychloride 0.2 %), (**m**) Control. **Pn AgNPs** = Green synthesized silver nanoparticles using *Piper nigrum* leaf extract. **Pc AgNPs** = Green synthesized silver nanoparticles using *Piper colubrinum* leaf extract. **C AgNPs** = Commercial silver nanoparticles. **Ch AgNPs** = Commercial chitosan nanoparticles.

Table 2. Size of lesion on detached leaves with different treatments after artificial inoculation with *P. capsici* .

Sl. No	Treatments	Size of lesion (cm)*	
		3 DAI	5 DAI
1.	Pn AgNPs at 500ppm	0.5 ± 0.200^{e}	1.40 ± 0.265^{f}
2.	Pn AgNPs at 750 ppm	0	0
3.	Pc AgNPs at 500 ppm	0.4 ± 0.173^{e}	2.00 ± 0.265^{e}
4.	Pc AgNPs at 750 ppm	0	0
5.	C AgNPs at 500ppm	1.4 ±0.265^{bc}	3.03 ± 0.252^{b}
6.	C AgNPs at 750 ppm	1.3 ± 0.100^{c}	2.70 ± 0.173^{c}
7.	ChNPs at 500 ppm	1.0 ± 0.173^{d}	2.30 ± 0.100^{d}
8.	ChNPs at 750 ppm	0.9 ± 0.100^{d}	2.23 ± 0.115^{de}
9.	*P. nigrum* leaf extract (5 %)	1.4 ±0.265^{bc}	3.00 ± 0.265^{b}
10.	*P. colubrinum* leaf extract (5 %)	1.6 ± 0.0^{b}	3.20 ± 0.100^{b}
11.	*Pseudomonas fluorescens* (2 %)	0.8 ± 0.100^{d}	2.03 ± 0.058^{de}
12.	Chemical Control (COC 0.2 %)	0	0
13.	Control	1.9 ± 0.100^{a}	3.50 ± 0.173^{a}
	SE (m)	0.085	0.097

Pn AgNPs = Green synthesized silver nanoparticles using *Piper nigrum* leaf extract, **Pc AgNPs** = Green synthesized silver nanoparticles using *Piper colubrinum* leaf extract, **C AgNPs** = Commercial silver nanoparticles, **Ch AgNPs** = Commercial chitosan nanoparticles, **COC**= Copper Oxychloride. *Mean (±SD) of 3 replications. The percentage mycelial growth inhibition was determined by comparing the radial growth of the fungus in the amended PDA plates to that in the control plate seven days after inoculation. Duncan's multiple range test (DMRT) indicates that there is no significant difference between the figures in a column followed by the same letter (p≤0.05).

Table 3. Treatment cost breakdown for one plant.

Item	Cost incurred in treating one plant with GSAgNPs (750 ppm)
Reactants	1.6
Energy	0.2
Equipment depreciation	0.1
Others (labour charges, application costs, etc.)	0.1
Total	2.0

to a zero-valence state, and the functional groups within these primary biopolymers and phytochemicals play a role in stabilizing the resulting nanoparticles. Among various biological methods, the use of plant extracts for nanoparticle synthesis is preferred due to their widespread availability, safety, lack of toxicity, and the presence of an extensive array of phytoconstituents which can perform as reducing agents (12).

Metal nanoparticles are usually characterized for tracking the completeness of reduction, identify the functional groups involved in the bio-reduction process, assess purity levels, and analyze their morphological attributes. The commonly employed techniques are X-ray diffraction (XRD), UV–visible spectrophotometry, Scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, Transmission electron microscopy (TEM), etc. (13). Several factors affect the green synthesis of metal nanoparticles, including solution pH, reaction time, temperature, plant extract concentration, concentration and nature of metal salt, pressure etc.

The change in colour of the reaction mixture from light brown to dark brown after incubation for one hour confirmed the nanoparticles formation. The activation of surface plasmon resonance (SPR) bands of silver nanoparticles caused the colour change, which is directly related to the synthesis of AgNPs. The colour change indicates the formation of AgNPs due to the reduction of $Ag^{+}$to zero-valent silver nanoparticles (Ag^{0}). Additionally, the complete conversion of AgNPs could be confirmed by adding 0.1 M NaCl to the supernatant obtained after separating the GSAgNPs, since no precipitate of silver chloride would be produced in the supernatants demonstrating the total change of Ag^{+} ions. UV-visible spectrophotometry tracks the characteristic peaks generated by metal salt-derived nanoparticles (NPs) at various absorption wavelengths. UV -visible spectroscopy is also employed to estimate the aggregation state and size distribution of NPs (14). Besides, the morphological characteristics of nanoparticles are assessed through the use of scanning electron microscopy (SEM). Additionally, SEM analysis can be done to estimate the average size of nanoparticles with the assistance of certain statistical software tools (13).

Green-synthesized nanoparticles (GSNPs) have been used for combatting several phytopathogens. The mechanism of action of nanoparticles can be broadly categorized as disruption of the peptidoglycan layer in bacterial cell walls, toxicity resulting from the toxic metal ions released into the cytoplasm, leading to imbalances in nutrient uptake, impairment of membrane function including membrane damage and the loss of membrane potential, generation of reactive oxygen species (ROS), production of antioxidants, damage to genetic material such as double-helix strand breaks, dysfunction of proteins etc. Apart from the impact of metal ions, different metabolites found in plant extracts have been observed to induce cell death in pathogens and stimulate systemic resistance in plants (15). Alkaloids, phenolics, and natural compounds present in plant extracts have been shown to possess bactericidal and fungicidal properties against plant pathogens, thereby enhancing the efficacy of GSNPs (16).

GSNPs are reported to be effective against several plant pathogens including fungi, bacteria and viruses. AgNPs green synthesized from *Amaranthus dubius* suppressed leaf blight pathogen *Rhizoctonia solani* (17). AgNPs green synthesized using *Piper nigrum* has already been reported effective against phytopathogenic bacterium *Citrobacter freundii* and *Erwinia cacticida* (18). GSAgNPs from the leaf extract of *P. colubrinum* showed antibacterial activity against foodborne pathogens *Staphylococcus aureus* and *Escherichia coli* (9).

The easiest method to evaluate the nanoparticle suspension's antimicrobial efficacy is through growth inhibition experiments on agar plates. This approach provides insights into how the treated materials directly impact the pathogenic organism. Findings from the *in vitro*

assessment of inhibition of mycelial growth demonstrated that AgNPs synthesized using green methods with leaf extracts from *Piper* species effectively restrained the growth of *P. capsici*. Although both *Piper* species' leaf extracts produced nanoparticles with inhibitory effects on the fungal pathogen, AgNPs derived from *P. nigrum* leaves exhibited superior antifungal activity compared to those from *P. colubrinum* leaves. GSNPs have an additional coating from plant extracts. The better antifungal activity of Pn-AgNPs can be attributed to the type of phytochemicals present in *P. nigrum* leaf extract, which could provide better capping and stabilization of the silver nanoparticles. Extracts from the leaves of *Piper* species used in this research were evaluated in order to check whether they contributed to the antifungal action. However, introducing leaf extracts into the growth medium did not impact fungal growth, indicating that they themselves do not have direct inhibitory properties. The detached leaf test serves as more realistic simulation of field conditions by involving interactions among the host plant, fungal pathogen, and antifungal agent. In this assay, the direct inhibitory activity of the antifungal agent can be assessed by observing the reduction of disease symptoms. The assay was conducted using leaves from the foot rot-susceptible black pepper variety Panniyur 1. Results from the detached leaf assay aligned with the findings from the *in vitro* plate assays.

AgNPs synthesized with leaf extracts from *Piper* species effectively suppressed symptom development caused by *P. capsici* on detached leaves of susceptible black pepper. Financial analysis proves that at a treatment cost of just 2 rupees per plant, management of foot rot with green synthesized AgNPs is highly economical, making it accessible for small-scale farmers. This low cost offers a significant advantage by reducing financial strain while providing an effective, sustainable alternative to more expensive chemical treatments. A potential weakness of using green-synthesized AgNPs for plant disease management is the limited understanding of their long-term environmental impact and potential buildup in soil, which may affect soil microorganisms and plant health over time.

Conclusion

This study confirms the potential of GSNPs, particularly those derived from *Piper* spp. leaf extracts, as an economically viable and environmentally sustainable alternative for managing foot rot in black pepper. With an effective, low cost per treatment, the technique demonstrates economic feasibility, especially for small-scale farmers, while minimizing reliance on synthetic pesticides. Although AgNPs have shown significant disease-suppressing capabilities, further exploration of more cost-effective, less phytotoxic metals, such as copper, zinc, and magnesium could enhance the economic appeal and scalability of this approach. These findings strongly support the hypothesis that GSNPs could serve as a practical, industrially scalable solution for plant disease management, contributing to the broader adoption of sustainable agricultural practices.

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Authors' contributions

GSS carried out the investigation, visualization, and wrote the original draft. SST contributed to the conceptualization, methodology, review and editing of the writing, and supervision of the work. KNA provided resources, contributed to the review and editing of the writing, and supervised the work. SD, CBN, and NBJ contributed resources to the study. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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