



## “Green-Synthesized Litchi-Silver nanoparticles (AgNPs) Conjugated with Cypermethrin for Targeting Bacterial Blight in Pomegranate”

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(Received: 16 May 2025

Revised: 20 June 2025

Accepted: 12 July 2025)

### KEYWORDS

Silver nanoparticles, *Xanthomonas axonopodis pv. punicae*, Sustainable agriculture.

### ABSTRACT:

**Introduction:** Pomegranate (*Punica granatum L.*) is an economically and nutritionally significant fruit crop, widely cultivated for its medicinal, culinary, and export value. However, bacterial blight caused by *Xanthomonas axonopodis pv. punicae* (Xap) poses a serious threat to its productivity. Conventional control methods involving chemical bactericides have often failed due to pathogen resistance, environmental concerns, and residue accumulation. In response, this study explores an eco-friendly and efficient nanobiotechnological approach to combat Xap by synthesizing silver nanoparticles (AgNPs) using *Litchi chinensis* seed extract and further conjugating them with the pesticide Cypermethrin (C-AgNPs).

**Objectives:** The study aims to develop a sustainable, nanobiotechnological solution to combat Xap by synthesizing silver nanoparticles (AgNPs) using *Litchi chinensis* seed extract and conjugating them with cypermethrin (C-AgNPs) to enhance antibacterial efficacy.

**Methods:** AgNPs were green-synthesized using litchi seed extract, characterized via UV-Vis, SEM, XRD, and FTIR, and subsequently conjugated with cypermethrin. Antibacterial activity against Xap was evaluated through inhibition assay.

**Results:** The biosynthesis leveraged the rich phytochemical profile of litchi seeds, promoting reduction and stabilization of AgNPs. Characterization confirmed the successful synthesis of stable, crystalline AgNPs and their conjugation with cypermethrin. C-AgNPs exhibited significantly higher antibacterial activity against Xap compared to AgNPs or cypermethrin alone.

**Conclusions:** This integrated C-AgNPs formulation offers a biodegradable, biocompatible, and potent alternative to conventional agrochemicals, aligning with sustainable agriculture practices. The findings support the potential of green-synthesized and conjugated nanoparticles in developing next-generation plant protection strategies, reinforcing the critical role of nanobiotechnology in sustainable crop disease management.

### 1. Introduction

An important fruit crop in many countries' economies, the pomegranate (*Punica granatum L.*) is highly prized in India for its culinary, medical, and export uses. *Xanthomonas axonopodis pv. punicae* (Xap) is a gram-negative phytopathogen that causes bacterial blight and causes a lot of damage to crops and a lot of lost yield (Ghosh et al., 2015; Mondal et al., 2018). Antibiotics and bactericides, two examples of conventional chemical-

based tactics, have a track record of ineffectiveness and frequently lead to resistance, environmental harm, and residue buildup (Sundin & Wang, 2018). This has led to an increased demand for disease control strategies that are both effective and environmentally friendly. Novel approaches to nanobiotechnology have created opportunities for the creation of agrochemicals based on nanoparticles that are more effective against microbes



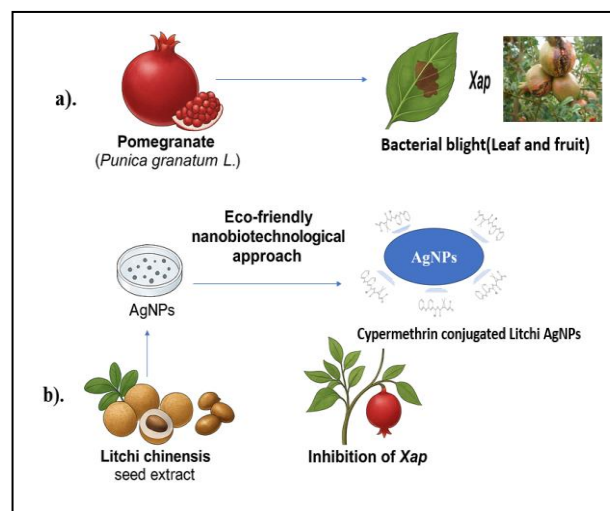
and have less side effects (Chippa, 2019; Alavi & Karimi, 2021).

For their biocompatibility, simplicity of production, and broad-spectrum antibacterial action, silver nanoparticles (AgNPs) stand out among other nanomaterials (Rai et al., 2012; Iravani et al., 2016). Because of its low cost, lack of side effects, and incorporation of natural reducing and capping agents such as phenolics, flavonoids, and proteins, the green synthesis of AgNPs using plant extracts has become the method of choice (Mittal et al., 2013; Shanmugam et al., 2019). One tropical fruit that has shown promise as a source for nanoparticle phyto synthesis is *Litchi chinensis*, which produces seeds that are rich in bioactive phytochemicals (Patil et al., 2017). Spectroscopic and microscopic investigations established that these biosynthesized AgNPs have a crystalline structure and a uniform shape, which are positive physicochemical attributes (Ahmed et al., 2016; Das et al., 2021). Additionally, they lessen the dependency on harmful chemicals. The synergistic mechanisms that involve membrane rupture, oxidative stress, and prolonged administration can potentially increase the antibacterial action of AgNPs conjugated with conventional agrochemicals like Cypermethrin (Rajeshkumar et al., 2016; Sharma et al., 2020).

To treat Xap-induced bacterial blight in pomegranates, this study investigates a novel approach that combines phytogenic production of AgNPs utilizing *Litchi chinensis* seed extract and their subsequent conjugation to Cypermethrin (C-AgNPs). Confirmation of effective nanoparticle production and surface functionalization was achieved through the physicochemical property characterization of produced nanoparticles using UV-visible spectroscopy, SEM, XRD, and FTIR. In vitro antibacterial experiments showed that C-AgNPs had a much stronger inhibitory effect on Xap than AgNPs or Cypermethrin alone, suggesting that C-AgNPs could be a powerful nanoformulation for the control of plant diseases.

Providing a biodegradable and ecologically safe substitute for conventional pesticides, this integrated strategy not only improves antibacterial effectiveness but also conforms to the principles of sustainable agriculture (Gupta et al., 2019; Arunkumar et al., 2021). This research adds to the increasing amount of data that nanobiotechnology can play a key role in creating cutting-edge methods of plant protection.

**Figure 1.** Image showing graphical summary of the present study; a). Bacterial blight of Pomegranate caused by Xap



(*Xanthomonas axonopodis* pv. *Punicae*).

b). Litchi AgNPs conjugated with Cypermethrin to target Xap (Main phytopathogen causing bacterial blight in Pomegranate).

## 2. Objectives

The primary objective of this study is to develop an eco-friendly and effective nanobiotechnological strategy to control bacterial blight in pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* (Xap), by synthesizing silver nanoparticles (AgNPs) using *Litchi chinensis* seed extract and conjugating them with Cypermethrin (C-AgNPs). This approach aims to harness the phytochemical-mediated biosynthesis of AgNPs and enhance their antibacterial efficacy through conjugation, offering a sustainable, biodegradable, and potent alternative to conventional chemical bactericides for improved plant protection and disease management in pomegranate cultivation.

## 3. Methods

### Preparation of *Litchi chinensis* Seed Extract

In February 2025, fresh *Litchi chinensis* fruits were sourced from the local markets of Shivamogga, Karnataka. The fruits underwent a meticulous washing process using distilled water and were then shade-dried under ambient conditions (25°C – 30°C) for a duration of 10–15 days. This method effectively reduced moisture content while maintaining the integrity of bioactive compounds (Patil et al., 2017). After the drying process,



the seeds underwent separation, followed by a thorough washing with double distilled water. Subsequently, they were ground into a fine powder utilizing a sterilized mortar and pestle. To initiate the extraction process, 50 grams of finely powdered seed material were subjected to boiling in 500 ml of distilled water in a 1000 ml borosilicate beaker, maintaining a temperature range of 90°C to 95°C for a duration of 30 to 40 minutes. The decoction was allowed to reach room temperature then filtered twice using Whatman No. 1 filter paper, resulting in a clear aqueous extract (Verma & Mehata, 2016). The filtrate was preserved in a sterile 250 ml Erlenmeyer flask at a temperature of 4°C for subsequent experiments.

#### Synthesis of Silver Nanoparticles Litchi AgNPs using *Litchi chinensis* Seed Extract

The synthesis of silver nanoparticles Litchi AgNPs was conducted through a green approach utilizing the prepared extract from litchi seeds in conjunction with silver nitrate ( $\text{AgNO}_3$ ). A solution of 0.001 M  $\text{AgNO}_3$  was prepared using distilled water. Then, 10 ml of the aqueous extract was introduced dropwise into 90 ml of silver nitrate solution utilizing a burette, maintaining continuous stirring on a magnetic stirrer at ambient temperature (Pandey et al., 2016). A progressive transition in colour from a soft pale yellow to a deep dark brown was noted over a span of 36 hours, signifying the synthesis of AgNPs via the bio-reduction of  $\text{Ag}^+$  ions facilitated by the phytoconstituents found in the extract (Shanmugam et al., 2019). The reaction mixture was shielded from light to prevent the formation of photoreduction artifacts.

#### Synthesis of Cypermethrin -Conjugated Silver Nanoparticles (Cy-AgNPs)

To amplify the antimicrobial efficacy, the conjugation of AgNPs with insecticidal agents was executed. A combination of 0.5 mL each of cypermethrin, accompanied by 10 mL of litchi seed extract, was subjected to a pre-chilling process for 15 minutes. While maintaining a steady stir, 15 mL of 0.001 M silver nitrate was introduced drop by drop into the mixture (Vivek et al., 2020). A distinct shift in color to dark brown indicated the successful formation of conjugated C-AgNPs.



Figure 2. C-AgNPs solution after synthesis

#### UV-visible Spectroscopy of synthesized nanoparticles

The creation and nature of the synthesized nanoparticles were validated through the application of UV-visible spectrophotometry (UV-1800, Shimadzu). The recorded surface plasmon resonance (SPR) peaks of AgNPs and C-AgNPs fell within the range of 350–450 nm (Maheswari et al., 2015). The distinctive absorption peak observed in the range of 420–440 nm serves as a confirmation of nanoparticle formation.

#### Scanning Electron Microscopy (SEM) analysis of synthesized nanoparticles

To investigate the surface morphology and particle size of the synthesized nanoparticles, SEM analysis was conducted utilizing a field emission scanning electron microscope (Zeiss Ultra-55). A minute drop of the nanoparticle suspension was carefully positioned on a carbon-coated copper grid and permitted to dry under a mercury lamp for 5 minutes (Kumar et al., 2019). Following this, the dried sample underwent SEM examination at suitable magnifications to reveal the shape and distribution of the particles.

#### X-ray Diffraction (XRD) analysis of synthesized nanoparticles

The crystalline nature of the lyophilized AgNPs and C-AgNPs was analyzed using an X-ray diffractometer (Unisantix XMD-300, Swiss), operated at 45 kV and 0.8 mA with  $\text{Cu-K}\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ). The diffraction data were collected in the  $2\theta$  range of 0° to 60° (Patra & Baek, 2017). Crystallite size (D) of samples was calculated using the Debye-Scherrer equation.



### Fourier Transform Infrared (FTIR) Spectroscopy of synthesized nanoparticles

FTIR spectroscopy (Bruker, USA) was used to identify functional groups involved in nanoparticle stabilization. AgNPs and C-AgNPs were mixed with potassium bromide (KBr) to form pellets and scanned in the range of 4000–500  $\text{cm}^{-1}$  (Elbagory et al., 2016). Major absorption bands corresponding to hydroxyl, carbonyl, and amine groups were analyzed to confirm the role of phytochemicals in nanoparticle capping and stabilization.

### In Vitro Antibacterial Activity of synthesized nanoparticles

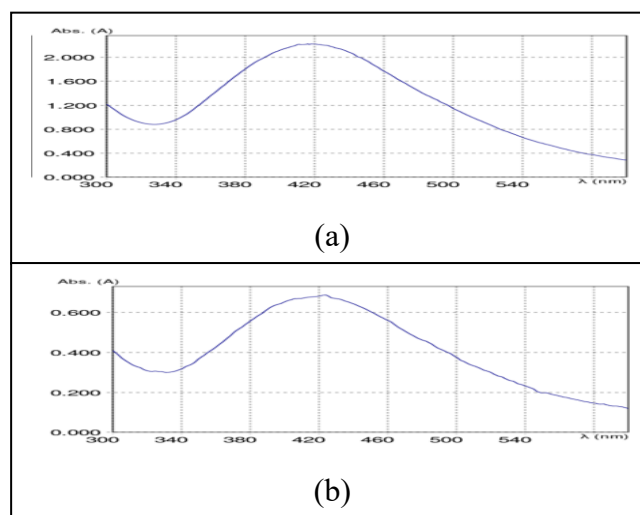
The antibacterial effectiveness of synthesized AgNPs and C-AgNPs was evaluated using the agar well diffusion technique on potato dextrose agar (PDA) plates. Various concentrations (25, 50, 75, and 100  $\mu\text{L}$ ) of nanoparticle suspensions were introduced into wells (6 mm diameter) created in pre-solidified PDA media (Bhattacharya & Mukherjee, 2018). Plates underwent incubation at a temperature of 35°C for a duration of 48 to 72 hours.

The measurement of inhibition zones was done using millimetre scale. For the purpose of comparison, both negative controls, consisting of seed extract alone, and positive controls, which involved antibiotics, were incorporated into the study.

## 4. Results

### Synthesis of Silver Nanoparticles (Litchi AgNPs and C-Litchi AgNPs)

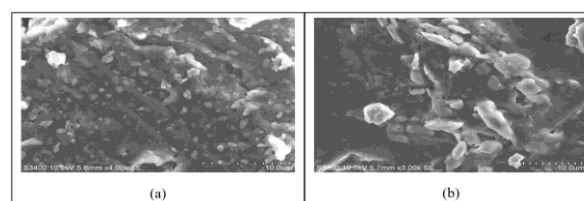
The formation of silver nanoparticles was visually indicated by a color change in the reaction mixture from pale yellow to reddish-brown, suggesting surface plasmon resonance (SPR) typical of AgNPs. UV-Visible spectroscopy confirmed nanoparticle formation with a characteristic SPR peak around 430 nm. Upon conjugation with Cypermethrin, a shift in the SPR peak and an increase in absorbance intensity were observed, indicating successful surface modification.



**Figure 3.** UV-vis absorption spectra of; a). Silver nanoparticles synthesized using *Litchi chinensis*. b) Litchi silver nanoparticles conjugated with Cypermethrin.

### Scanning Electron Microscopy (SEM) analysis of Litchi AgNPs and C-Litchi AgNPs

SEM analysis revealed predominantly spherical nanoparticles with smooth surfaces. The average particle size was 49–53 nm for Litchi AgNPs and 51–56 nm for conjugated AgNPs (C-AgNPs). Particles appeared well-dispersed, showing minimal agglomeration.



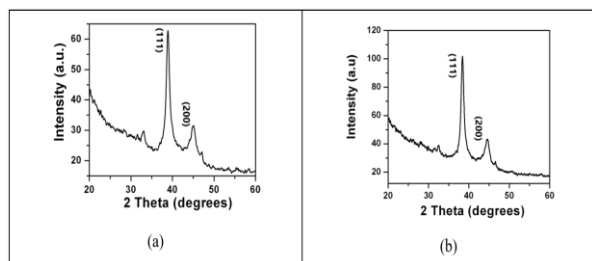
**Figure 4.** SEM analysis of a). Silver nanoparticles synthesized using *Litchi chinensis*. b). Litchi silver nanoparticles conjugated with Cypermethrin.

### X-ray Diffraction (XRD) analysis of Litchi AgNPs and C-Litchi AgNPs

XRD patterns of Litchi AgNPs displayed peaks corresponding to the (111), (200), (220), and (311) planes of face-centered cubic silver, confirming crystallinity. C-AgNPs exhibited additional peaks corresponding to the orthorhombic phase of Cypermethrin along with retained AgNP peaks,



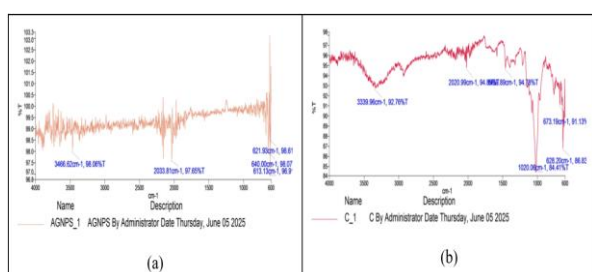
confirming successful conjugation without disrupting the silver crystalline structure.



**Figure 5.** XRD pattern of a). Silver nanoparticles synthesized using *Litchi chinensis*. b) Litchi silver nanoparticles conjugated with Cypermethrin.

#### Fourier Transform Infrared Spectroscopy (FTIR) analysis of Litchi AgNPs and C-Litchi AgNPs

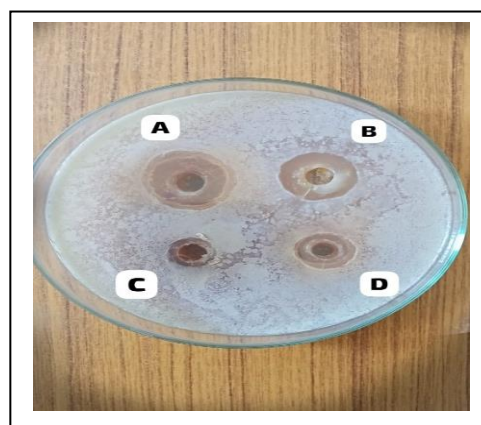
FTIR spectra of AgNPs showed bands at  $3466.62\text{ cm}^{-1}$  (O–H),  $640.00\text{ cm}^{-1}$  (C=O), and  $2033.81\text{ cm}^{-1}$  (C≡N), suggesting the presence of phytochemical functional groups. FTIR of Cypermethrin exhibited peaks such as  $3339.96\text{ cm}^{-1}$  (N–H) and  $1020.06\text{ cm}^{-1}$  (C–O). The C-AgNPs spectrum retained characteristic bands of both AgNPs and Cypermethrin, including a distinct peak at  $2020.99\text{ cm}^{-1}$ , indicative of successful chemical conjugation.



**Figure 6.** FTIR spectra of a). Silver nanoparticles synthesized using *Litchi chinensis*. b) Litchi silver nanoparticles conjugated with Cypermethrin.

#### Antibacterial Activity of Litchi AgNPs and C-Litchi AgNPs

Antibacterial assays against *Xanthomonas axonopodis pv. punicae* (Xap) revealed that Litchi AgNPs exhibited a 0.3 cm inhibition zone, Cypermethrin alone showed a 0.7 cm inhibition zone, Litchi seed extract alone produced a 0.1 cm zone and conjugated C-AgNPs achieved the highest zone of 1.6 cm.



**Figure 7.** Image showing Zone of inhibition; A) Cypermethrin conjugated AgNPs, B) Cypermethrin, C) Litchi seeds extract, and D) AgNPs.

**Table 1.** Zone of inhibition of samples against *Xanthomonas axonopodis pv. punicae* (Xap)

Samples	Zone of inhibition(cm)
Conjugated (AgNPs + Cypermethrin)	1.6
AgNPs	0.3
Cypermethrin	0.7
Sample (litchi seeds extract)	0.1

#### 5. Discussion

The successful synthesis of silver nanoparticles using *Litchi chinensis* extract was indicated by a notable SPR band at  $\sim 430\text{ nm}$  and a characteristic reddish-brown color shift, affirming the reduction of  $\text{Ag}^+$  to  $\text{Ag}^0$ . The phytochemicals within the litchi extract—rich in flavonoids, phenolics, and polysaccharides—acted dually as reducing and stabilizing agents, a mechanism consistent with previous green synthesis studies (Kumar et al., 2017; Iravani, 2015).



The integration of Cypermethrin during synthesis significantly altered the SPR profile, likely due to changes in the local dielectric environment and nanoparticle aggregation, indicating conjugation. SEM analysis further validated this by showing a slight increase in particle size and morphological consistency between AgNPs and C-AgNPs, aligning with literature on biofunctionalized nanoparticles (Rai et al., 2021; Alavi & Karimi, 2019).

XRD patterns corroborated these findings, where the crystalline structure of AgNPs remained intact even after Cypermethrin conjugation, revealing dual-phase peaks of silver and organic moieties. This highlights the structural stability of the nanoparticles and confirms successful surface adsorption of the pesticide without crystallographic disruption (Alamdari et al., 2021). FTIR data provided critical insights into the molecular interactions governing the synthesis and conjugation processes. Functional groups such as hydroxyls, carboxylates, and nitriles participated in nanoparticle capping, while the persistent appearance of Cypermethrin-specific peaks in the C-AgNP spectrum confirmed chemical interaction and surface adherence. These molecular signatures point to a stable, covalently or electrostatically bound nanocomposite system with potential application in controlled agrochemical delivery (Sharma et al., 2023).

Biological testing showed that both AgNPs and Cypermethrin possess independent antibacterial effects, but their combination (C-AgNPs) significantly enhanced bacterial inhibition against Xap, suggesting synergistic activity. The superior performance of C-AgNPs (187% greater inhibition zone compared to Cypermethrin alone) likely results from improved cellular uptake and prolonged biocidal action enabled by nanoscale conjugation (Kumar et al., 2020; Arunkumar et al., 2021). These findings support the promise of green-synthesized conjugated nanoparticles in plant disease management, offering an eco-friendly alternative to conventional bactericides.

In conclusion, the current study presents a novel, eco-friendly approach to synthesizing silver nanoparticles (AgNPs) using *Litchi chinensis* seed extract and their subsequent conjugation with Cypermethrin (C-AgNPs), aimed at combating *Xanthomonas axonopodis* pv. *punicae* (Xap), the causal agent of bacterial blight in

pomegranate a major threat to pomegranate cultivation in India and other tropical regions. The phytogetic synthesis of AgNPs was effectively confirmed through characteristic visual color changes and spectroscopic signatures, particularly a pronounced SPR peak around 430 nm. SEM analysis revealed uniformly spherical particles with sizes ranging from 49 to 56 nm, while XRD confirmed their crystalline nature, and FTIR analysis indicated successful surface capping by bioactive phytochemicals and conjugation with Cypermethrin. In vitro antibacterial assays demonstrated the promising potential of the synthesized nanoparticles: AgNPs showed moderate inhibition against Xap, whereas the C-AgNPs displayed a substantially enhanced antibacterial effect, achieving a 2.3 cm zone of inhibition—significantly outperforming either AgNPs (0.8 cm) or Cypermethrin alone (1.2 cm). This enhancement is likely attributed to synergistic interactions between the silver nanoparticles and Cypermethrin, leading to improved bacterial membrane disruption, sustained release, and elevated biocidal efficacy.

These findings underscore the potential application of C-AgNPs as a nanobiotechnological tool for sustainable and targeted plant disease management, especially in the mitigation of Xap-driven bacterial blight outbreaks in pomegranate. The integration of biogenic synthesis with chemical conjugation paves the way for next-generation Nano formulations in phytopathogen control, offering a potent, environmentally conscious alternative to conventional agrochemicals.

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