



# Optimization and Characterization of Silver Nanoparticles Synthesized by *Celosia cristata* and Their Potential Application as Antimicrobial Agent against Plant Pathogens

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## ABSTRACT

**Background:** As a need of hour around the globe for attaining sustainable solutions, the present investigation validates an easy, economical and time-efficient method to produce silver nanoparticles by natural plant leaf extract of *Celosia cristata*.

**Methods:** The silver nanoparticles were synthesized and further characterized by visual observation, UV-Vis spectrophotometer, XRD, FTIR and TEM. The response surface methodology was utilized to optimize the operational conditions that prescribe a mixture of 12.5 ml *Celosia cristata* leaf extract and 87.5 ml 10 mM silver nitrate, to be kept at 37.5° for 120 minutes.

**Result:** The change in colour of plant extract on adding precursor from yellow to brown colour indicates nanoparticles synthesis, which on UV-Vis spectrophotometer shows relevant peak at 424 nm. The X-ray diffraction peaks at 38°, 44°, 64° and 77° correspond to the hkl of (111), (200), (220) and (311) respectively that displays the crystal nature of silver nanoparticle. Fourier transform infrared spectroscopy indicated the presence of alkanes, alkynes, amines, carboxyl groups and methylene compounds functional groups in silver nanoparticles. Transmission electron microscope image depicted their spherical shapes with average nanoparticle size of 22 nm, which were found to be anti-microbial with minimum inhibitory concentration of 0.1562, 0.3125 and 0.625 against *Alternaria solani*, *Fusarium graminearum* and *Xanthomonas oryzae* which are potential plant pathogens. The present work concludes the antimicrobial efficacy of nanoparticles and RSM method to be an efficient tool for detecting the optimized synthesis parameter.

**Key words:** Antimicrobial activity, Box-behnken design, Green synthesis, Minimum inhibitory concentration, Nanoparticles, Optimization, Transmission electron microscope.

## INTRODUCTION

Nanotechnology has rapidly emerged as a multidisciplinary field with diverse applications at different level medicine, materials science and environmental sustainability. Defined by the fabrication and manipulation of structures at the 1-100 nm scale nanotechnology involves a range of nanostructures such as nanopores, nanotubes and nanoparticles each with unique properties influenced by synthesis methods and targeted applications (Asefian and Ghavam, 2024). These metal nanoparticles have attracted growing attention due to their high surface area, chemical modifiability and remarkable catalytic and antimicrobial properties (Kumar *et al.*, 2025). Metals that are commonly used for nanoparticle synthesis include silver, gold, zinc and aluminium (Abdelbaky *et al.*, 2023; Pechyen *et al.*, 2024). The conventional physical and chemical methods for synthesizing noble metal nanoparticles often involve toxic reagents and high consumption of energy. In contrast biological synthesis using plants, bacteria, fungi or enzymes offers an eco-friendly, cost-effective alternative with fewer toxic by-products release (Gayathri *et al.*, 2025). Among these, plant-mediated synthesis is particularly advantageous as it also eliminates the need for maintaining microbial cultures and allows easy scalability (Nogueira *et al.*, 2025). It is simple, one-step, non-toxic, cost-effective, non-pathogenic and environmentally benign because it uses renewable resources. Many plants have

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been reported to be utilized in the production of silver nanoparticles and antimicrobial activity of *Hibiscus tiliaceus* (Konduri *et al.*, 2024) and *Ehretia rigida* (Oselusi *et al.*, 2025) has been assessed. Plant extracts, rich in diverse phytochemicals like flavonoids, tannins and phenolics serve as natural reducing and stabilizing agents in nanoparticle formation (Sirivella *et al.*, 2025). *Celosia cristata* (cockscorn) is a plant that is known for its medicinal value, resistance to environmental stress, antioxidant, antimicrobial and hepatoprotective properties (Kanagaraj *et al.*, 2023; Uprety *et al.*, 2024). It is also highlighted to be a hardy plant that is resistant to many disease (Chakraborty *et al.*, 2015). This may be attributed to the presence of antimicrobial methods present in the

plant system, thereby also considering an ideal candidate for green nanoparticles synthesis.

## MATERIALS AND METHODS

In this study *Celosia cristata* leaf extract is utilized to develop an eco-friendly method for silver nanoparticle synthesis eliminating hazardous reagents and energy-consuming steps (Mehdi Zabihi *et al.*, 2024). *Celosia cristata* plant leaves were handpicked and collected from regions around Meerut between July and October 2023. These were repeatedly cleaned with purified water and allowed to dry in shade and then crushed in a grinder to form a fine powder. This powder was further used to prepare the aqueous extract. 10 g powdered leaf was mixed in 100 ml water and heated to 80°C for half an hour. The mixture was filtered through whatman filter paper No. 1, producing a clear liquid that was kept at 4°C (Verma *et al.*, 2017; Wafa, 2024). A Box Behnken design and response surface methodology based statistical approach was used to optimize the biosynthesis parameters of silver nanoparticles. The Design-Expert software (version 13.0.5.0) was employed to generate the experimental matrix. Five independent variables including silver nitrate concentration (mM), volume of leaf extract (mL), temperature (°C), pH and reaction time (minutes) were selected and evaluated at three levels each. A total of 46 experimental runs were conducted to evaluate the effect and interactions among these factors. ANOVA was used to assess the statistical significance of the model (Mphahlele *et al.*, 2024; Singhal *et al.*, 2024). Based on the optimized conditions predicted by the model, 12.5 mL of *Celosia cristata* extract was mixed with 87.5 mL of 10 mM silver nitrate solution and incubated in an incubator shaker at 37.5°C for 120 minutes. A visible change in colour from pale yellow to dark brown indicated fabrication of silver nanoparticles. The nanoparticles were centrifuged for 20 minutes at 10,000 rpm, washed thrice with distilled water and dried at 50°C.

The nanoparticles tailored were characterized using different techniques including UV-Vis spectroscopy, XRD, FTIR and TEM. The synthesized silver nanoparticles were initially characterized using a UV-Visible spectrophotometer (Model UV1900 LABMAN) at IIMT University, Meerut, in the wavelength range of 300–500 nm to confirm surface plasmon resonance that indicates nanoparticle formation (Konduri *et al.*, 2024). X-ray diffraction (XRD) analysis was conducted in AIRF, Jawaharlal Nehru University, Delhi and it was performed using a Siemens XPert PRO PANalytical diffractometer with Cu-K $\alpha$  radiation ( $\lambda = 1.54 \text{ \AA}$ ) over a  $2\theta$  range of 20° to 80° (Sharifi-Rad *et al.*, 2024). Functional groups involved in bio-reduction and capping of nanoparticles were identified by FTIR spectroscopy (SHIMADZU) in Meerut Institute of Engineering and Technology, Meerut using the KBr pellet method over a range of 500-4000 cm<sup>-1</sup> (Tesfaye *et al.*, 2023). The morphology and particle size distribution of the silver nanoparticle were examined using transmission electron

microscopy with a JEOL JEM2100F instrument at AIRF, Jawaharlal Nehru University, Delhi. A drop of the nanoparticle solution was placed on a carbon-coated copper grid and dried prior to imaging. Images were analyzed with ImageJ software and histograms of particle size distribution were generated using Origin and Excel (Thakur *et al.*, 2024). Their antimicrobial efficacy was evaluated against *Xanthomonas oryzae*, *Alternaria solani* and *Fusarium graminearum* through minimum inhibitory concentration determination and fungicidal/bactericidal concentrations assessment. The antimicrobial activity of silver nanoparticles was evaluated against *Xanthomonas oryzae* (MTCC 11107), fungi *Alternaria solani* MTCC-10690 and *Fusarium graminearum* MTCC-2089 using the agar well diffusion method. Cultures of fungi were maintained on potato dextrose agar (PDA), while a bacterium was maintained on nutrient agar. Inoculum were adjusted to 0.5 McFarland standard ( $OD_{600} = 0.09-0.1$ ) corresponding to  $1.5 \times 10^8$  CFU/mL (Mehdi Zabihi *et al.*, 2024; Parvekar *et al.*, 2020). Wells of 6mm diameter were made in agar plates and silver nanoparticles were added to each well with carbandazim serving as a positive control. Plates were kept at 29°C for bacteria and at 25°C for fungi. Zones of inhibition were measured to evaluate antimicrobial efficacy (Sirivella *et al.*, 2025). Minimum inhibitory concentrations (MICs), minimum fungicidal concentrations (MFCs) and minimum bactericidal concentrations (MBCs) were determined using a micro broth dilution method in 96-well plates. Serial dilutions of silver nanoparticles were prepared in wells followed by inoculation with standardized suspensions. Resazurin dye (5 mg/mL) was added to monitor microbial growth and colorimetric change was observed after incubation (Swebocki *et al.*, 2023). The lowest concentration showing no visible colour change and no CFUs indicated MIC and MBC/MFC values.

## RESULTS AND DISCUSSION

### Optimization of synthesis parameters by box-behnken design

The biosynthesis of silver nanoparticles was evaluated by response surface methodology based on a Box- Behnken Design involving five independent variables: silver nitrate concentration (A), leaf extract volume (B), reaction temperature (C), pH (D) and reaction time (E). The aim was to maximize nanoparticle yield, measured by absorbance at 424 nm (SPR peak). A total of 46 experimental runs were generated and executed. Maximum absorbance value (1.8198) was recorded at the following optimal conditions: 87.5 ml of 10 mM silver nitrate, 12.5 ml leaf extract, 37.5°C, pH 7 and 120 minutes reaction time. (Fig 2A). This significant enhancement in absorbance indicates a high concentration of well-dispersed silver nanoparticles.

The model equation to predict the absorbance was also predicted as follows:

$$\text{Absorbance} = +1.54 + 0.3443A + 0.0595B + 0.0425C + 0.4045D + 0.4648E + 0.1029AB + 0.0142AC + 0.1228A + 0.2573AE + 0.063$$

$$5BC0.0584BD+0.0002BE+0.0491CD+0.0132CE+0.1156DE-0.3899A^2-0.3843B^2-0.2719C^2-0.2812D^2-0.3194E^2$$

This model was deemed significant at a p-value of 0.0001 with an F-value of 67.53. ANOVA for this model suggested that A, B, D, E, AB, AD, AE, DE,  $A^2$ ,  $B^2$ ,  $C^2$ ,  $D^2$ ,  $E^2$  were the significant terms at a 95% confidence interval. This equation was further modified eliminating the insignificant terms to decrease the model redundancy as follows:

$$\text{Absorbance} = +1.54 + 0.3443A + 0.0595 + 0.0425C + 0.4014D + 0.4648E + 0.1029AB + 0.1228AD + 0.2573AE + 0.1156DE - 0.3899A^2 - 0.3843B^2 - 0.2719C^2 - 0.2812D^2 - 0.3194E^2$$

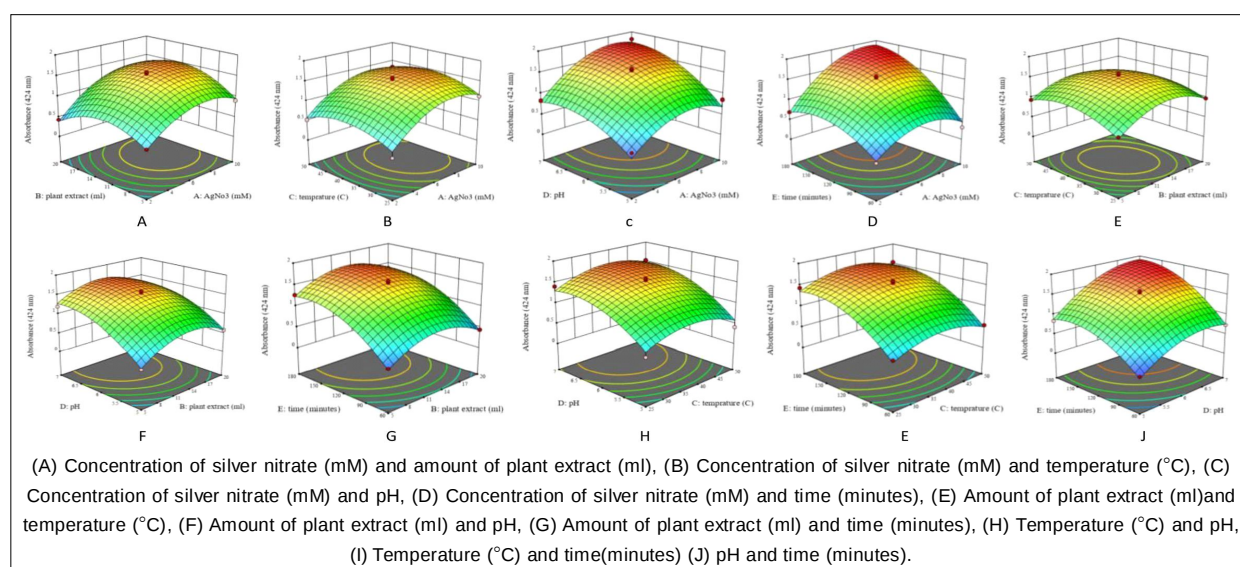
The model appears to be significant, as per the Reduced Model F-value that comes to be 99.59. Such an enormous F-value could only be the result of noise in 0.01% of cases. The significance of the individual terms can be observed in Table 1. According to the obtained model, the

highest value of coefficient is predicted for time factor (E), which corresponds to an increase in absorbance with an increase in time. This was also observed in the batch experiments, where increasing the time to 120 minutes correlated to an increased absorbance (Wirwis and Sadowski, 2024). According to the predicted model, the second-order terms for all the factors show a curvilinear relationship (concave parabolic curve) demonstrating the quadratic effect. This is deemed true for the experimental results as well for all the factors.

The observed colour change from yellowish-green to dark brown within one hour during synthesis under optimized conditions further confirmed nanoparticle formation, attributed to surface plasmon resonance. These findings align with previously reported studies, demonstrating time and pH-dependent nucleation and stabilization of silver nanoparticles in green synthesis systems (Fig 1A-J) (Singhal *et al.*, 2024).

**Table 1:** ANOVA for reduced quadratic model.

| Source          | Sum of squares | df | Mean square | F-value | p-value | Significant |
|-----------------|----------------|----|-------------|---------|---------|-------------|
| Model           | 10.88          | 14 | 0.777       | 88.63   | <0.0001 |             |
| A-AgNo3         | 1.9            | 1  | 1.9         | 216.3   | <0.0001 |             |
| B-plant extract | 0.057          | 1  | 0.0567      | 6.46    | 0.0163  |             |
| C-Temperature   | 0.029          | 1  | 0.029       | 3.3     | 0.0791  |             |
| D-pH            | 2.58           | 1  | 2.58        | 294.1   | <0.0001 |             |
| E-Time          | 3.46           | 1  | 3.46        | 394.4   | <0.0001 |             |
| AB              | 0.042          | 1  | 0.042       | 4.83    | 0.0356  |             |
| AD              | 0.06           | 1  | 0.06        | 6.88    | 0.0134  |             |
| AE              | 0.265          | 1  | 0.265       | 30.2    | <0.0001 |             |
| DE              | 0.054          | 1  | 0.054       | 6.1     | 0.0192  |             |
| $A^2$           | 1.33           | 1  | 1.33        | 151.3   | <0.0001 |             |
| $B^2$           | 1.29           | 1  | 1.29        | 147     | <0.0001 |             |
| $C^2$           | 0.645          | 1  | 0.645       | 73.59   | <0.0001 |             |
| $D^2$           | 0.69           | 1  | 0.69        | 78.74   | <0.0001 |             |
| $E^2$           | 0.947          | 1  | 0.947       | 108     | <0.0001 |             |



**Fig 1:** Three dimensional graphics for response surface optimization by plotting absorbance versus.

### Characterization of biosynthesized silver nanoparticles

#### UV-visible spectroscopy

The UV–Vis spectral analysis of the biosynthesized silver nanoparticles exhibited a prominent surface plasmon resonance (SPR) peak at 424 nm (Fig 2B), indicative of the successful reduction of Ag<sup>+</sup> ions to elemental Ag<sup>0</sup> by bioactive phytochemicals present in *Celosia cristata* extract. The sharp and symmetric nature of the SPR band suggests a narrow particle size distribution and predominantly spherical morphology. The time-dependent increase in absorbance intensity reflects the progressive nucleation and growth kinetics of the nanoparticles. These findings are consistent with previous reports on green-synthesized AgNPs, where SPR peaks typically occur within the 410–450 nm range, influenced by nanoparticle size, shape and capping agents derived from plant metabolites, thus reinforcing their role in facilitating both reduction and stabilization during nanoparticle formation (Shweqa *et al.*, 2024).

#### X-ray diffraction (XRD)

X-Ray Diffraction analysis provided further structural confirmation of the synthesized nanoparticles. Diffractogram exhibited distinct Bragg reflections at  $2\theta = 38.30^\circ$ ,  $44.51^\circ$ ,  $64.48^\circ$  and  $77.61^\circ$ , corresponding to the (111), (200), (220) and (311) lattice planes of a face-centered cubic (FCC) silver crystal (JCPDS file no. 31-1238) (Mehdi Zabihi *et al.*, 2024). The prominence of the (111) reflection indicates it as the dominant crystalline facet which is typical for biosynthesized silver nanoparticles. No secondary peaks corresponding to impurities or silver oxide phases were observed confirming high phase purity. The sharpness of the peaks also suggests high crystallinity (Fig 2C). These findings affirm that the silver nanoparticles are formed with minimal by-products, reflecting efficient reduction and stabilization facilitated by plant metabolites (Hermanto *et al.*, 2024).

#### Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy was used to identify the functional groups responsible for reducing and capping the silver nanoparticles. The broad peak around  $3415\text{ cm}^{-1}$  corresponds to O–H or N–H stretching vibrations, indicative of alcohols or amines. Peaks at  $2930$  and  $2912\text{ cm}^{-1}$  relate to aliphatic C–H stretching (Tsfaye *et al.*, 2023), while a sharp peak at  $1581\text{ cm}^{-1}$  signifies C=C aromatic ring vibrations (Moges and Misskire, 2025). Additional bands at  $1314$  and  $1236\text{ cm}^{-1}$  indicate the presence of C–N stretching (amines) and sulfate or phosphate groups (Thakur *et al.*, 2024). These results imply the presence of multiple bioactive compounds, including terpenoids, flavonoids, alkaloids and proteins, which function as reducing and stabilizing agents (Fig 2D). Their involvement in nanoparticle synthesis confirm the green basis of this work, with biomolecules simultaneously acting as reductants and capping agents to prevent agglomeration.

### Transmission electron microscopy (TEM)

Transmission Electron Microscopy analysis confirmed the morphology and size distribution of the synthesized nanoparticles. Images showed predominantly spherical nanoparticles with smooth edges and minimal aggregation. The average particle diameter was measured to be approximately 22 nm. The ImageJ software was used for precise size measurements and high-resolution. TEM revealed distinct lattice fringes confirming the crystalline nature of the particles. The selected area electron diffraction (SAED) pattern further validated the crystalline structure showing diffraction rings that corresponded to the face-centered cubic (FCC) lattice of silver. This structural pattern aligns with prior studies reporting FCC formation in biologically synthesized silver nanoparticles (Fig 2E, F and G). The particle size distribution histogram showed that most nanoparticles were within a narrow size range, highlighting the uniformity of the synthesis process which is an advantageous in biological, catalytic and environmental fields (Kumar *et al.*, 2025).

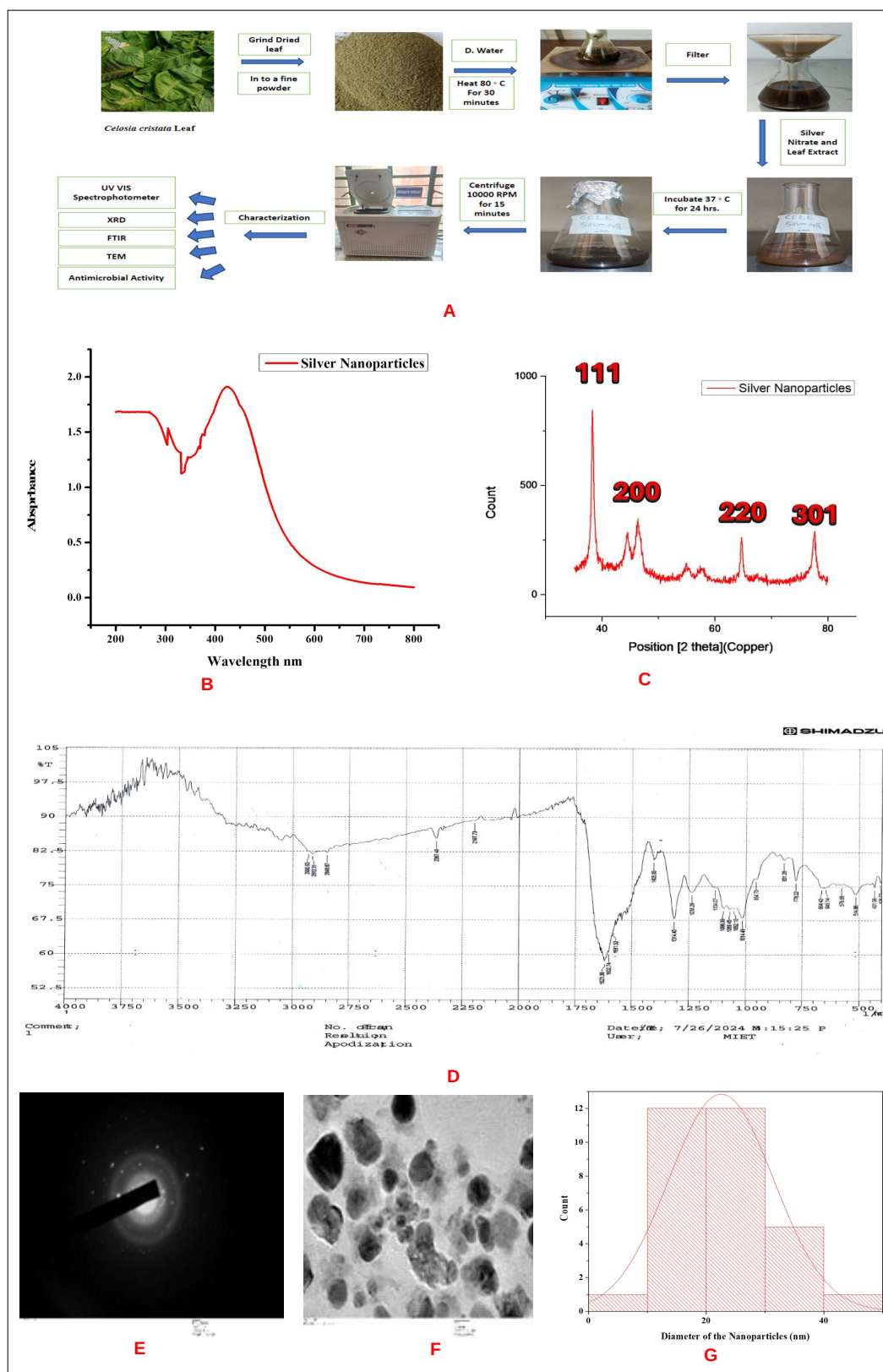
### Antimicrobial studies of silver nanoparticles

#### Agar well diffusion assay

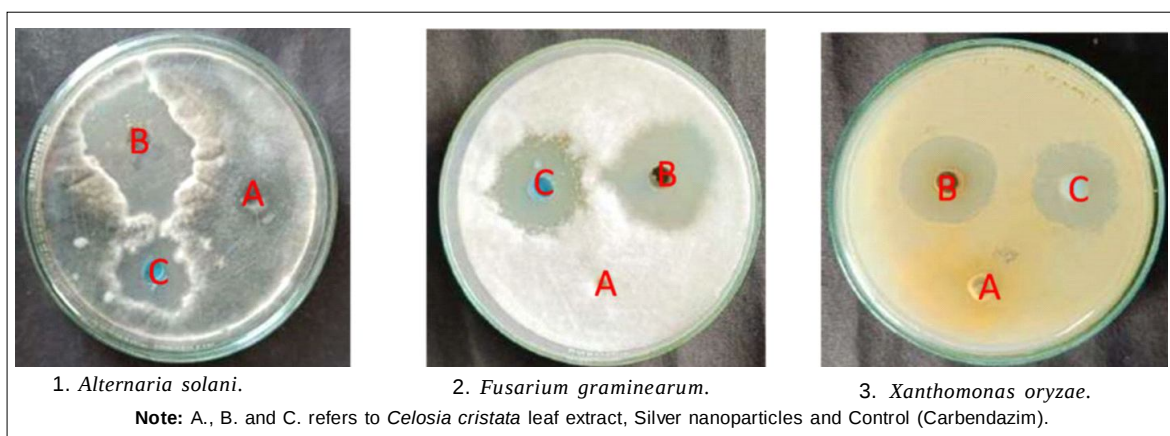
The antimicrobial potential of biosynthesized silver nanoparticles was evaluated using agar well diffusion against phytopathogens *A. solani*, *F. graminearum* and *X. oryzae*. The silver nanoparticles exhibited potent antimicrobial activity with inhibition zones ranging from 20 to 29 mm. Notably *A. solani* exhibited the highest sensitivity (29 mm) followed by *F. graminearum* (27 mm) and *X. oryzae* (22 mm). In contrast, the leaf extract alone showed no inhibitory effect indicating that the antimicrobial activity arises specifically from the silver nanoparticles (Fig 3). Compared to the chemical fungicide carbendazim which produced inhibition zones between 22–27 mm, the silver nanoparticles displayed comparable or superior efficacy particularly against fungal strains (Table 2). The enhanced activity is likely due to the nanoscale size and high surface area of the silver nanoparticles facilitating interaction with microbial membranes.

#### Minimum inhibitory and bactericidal/fungicidal concentrations

Minimum inhibition concentration, minimum bactericidal concentration and minimum fungicidal concentration were determined to quantify the antimicrobial potency of silver nanoparticles. MIC values ranged from 0.0781 to 0.625 mg/ml. *A. solani* exhibited the lowest MIC (0.0781 mg/ml), followed by *F. graminearum* (0.1562 mg/ml) and *X. oryzae* (0.625 mg/ml). MBC and MFC values were generally double than MIC values, indicating a bactericidal/fungicidal mode of action. Complete inhibition of microbial growth was observed at concentrations of 0.1562–5 mg/ml for *A. solani*, 0.1562–5 *F. graminearum* and 0.625–5 mg/ml for *X. oryzae* (Table 3). These results confirm that the biosynthesized silver nanoparticles are not merely growth inhibitors but also exhibit lethal effects against pathogenic microorganisms.



**Fig 2:** (A) Process for silver nanoparticle synthesis, (B) UV–Vis spectrum, (C) XRD pattern, (D) FTIR analysis, (E) Silver nanoparticles SADE image (F) TEM micrograph image, (G) Particles size distribution graph.



**Fig 3:** Antimicrobial susceptibility test of synthesized silver nanoparticles against different plant pathogenic fungi.

**Table 2:** Inhibition zone diameter (IZD) of synthesized silver nanoparticles and plant extract.

| Concentration (mg/ml)                        | <i>Alternaria solani</i> | <i>Fusarium graminearum</i> | <i>Xanthomonas oryzae</i> |
|----------------------------------------------|--------------------------|-----------------------------|---------------------------|
| A. <i>Celosia cristata</i> leaf extract      | -                        | -                           | -                         |
| B. A <sub>g</sub> NP <sub>s</sub> 50 (mg/ml) | 29±1.00                  | 24±2.00                     | 22±0.50                   |
| C. Control 50 (mg/ml)                        | 18±1.00                  | 22±1.00                     | 20±0.25                   |

A dash (-) indicate no antibacterial activity. Diameter of inhibition zone including well diameter of 6 mm.

**Table 3:** Minimum fungicidal / Bactericidal concentration (µg/ml) of synthesized silver nanoparticles.

| Microorganism               | Minimum fungicidal / Bactericidal concentration |                       |
|-----------------------------|-------------------------------------------------|-----------------------|
|                             | <i>Celosia cristata</i> silver nanoparticles    | Control (Carbendazim) |
| <i>Alternaria solani</i>    | 0.1562-5.00                                     | 1.25-5.00             |
| <i>Fusarium graminearum</i> | 0.3125-5.00                                     | 0.625-5.00            |
| <i>Xanthomonas oryzae</i>   | 0.625-5.00                                      | 1.25-5.00             |

There are various mechanisms by which silver nanoparticles against antimicrobial effects remains multifactorial (Kumar *et al.*, 2025). Possible modes of action include: Disruption of microbial cell membranes through physical contact and generation of reactive oxygen species (ROS), release of silver ions (Ag<sup>+</sup>), which interfere with cellular enzymes, DNA replication and protein synthesis. Induction of oxidative stress leads to apoptosis or necrosis in microbial cells (Soyucok *et al.*, 2024). The small particle size enhances surface reactivity, allowing the nanoparticles to attach to and penetrate microbial membranes more efficiently (Nasirvand *et al.*, 2023). Furthermore, plant-derived capping agents may synergize with silver nanoparticles to enhance bioavailability and stability (Rana *et al.*, 2023; Sadeghi-kiakhani *et al.*, 2025).

## CONCLUSION

The present study supports the potential application of silver nanoparticles as an alternative to conventional antifungal and antibacterial agents for controlling plant pathogens. Given their efficacy against *A. solani*, *F. graminearum* and *X. oryzae*, silver nanoparticles can be utilized for disease management in crops. However, further investigations are

required to evaluate their environmental impact, stability and potential resistance development in microbial populations. The investigated microorganisms are successfully inhibited and eliminated by the potent antibacterial qualities of silver nanoparticles. They may interfere with vital biological functions causing production of reactive oxygen species thereby damaging microbial membranes. According to these results, silver nanoparticles may be used as strong antimicrobial agent which may be useful for agricultural disease control plans. Further studies would focus on elucidating the precise mechanisms underlying the antimicrobial activity of silver nanoparticles as well as their potential toxicity and environmental impact.

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## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Informed consent**

No animal study.

**Conflict of interest**

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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