

# Green synthesis of nanoparticles from plants: Its application in plant pathogen management

A Summary submitted to the  
Babasaheb Bhimrao Ambedkar University, Lucknow  
in fulfilment of Requirement for the Award of Degree of

**Doctor of Philosophy**  
**in Environmental Science**



BY

*Ashvani Kumar Chaudhari*

Enrolment No- 1109/15

SUPERVISOR

*Dr. S. K Dwivedi*

Professor

DEPARTMENT OF ENVIRONMENTAL SCIENCE  
SCHOOL FOR ENVIRONMENTAL SCIENCES  
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY  
(A Central University) (NAAC A++ Accredited)  
RAEBARELI ROAD, LUCKNOW, UTTAR PRADESH-226025, INDIA

**2023**

## *Summary*

---

Pathogens cause a range of diseases on a variety of agronomic, horticultural, ornamental, and forest plant species. Among the methods for the control of plant pathogens, the use of pesticides is the most prevalent. While pesticides have played a crucial role in improving agricultural productivity and controlling disease vectors, they also have various environmental and health effects. The pesticides can leach into groundwater or run-off into surface water, leading to contamination of water bodies. This can harm aquatic ecosystems and affect the organisms living in these environments. The pesticides may also persist in the soil, affecting soil quality and potentially harming non-target organisms like earthworms and beneficial microbes, which can disrupt the balance of the ecosystem. Pesticide residues can remain on crops even after washing and processing, leading to potential human exposure through the consumption of contaminated food. The target pathogen can develop different defenses to pesticides; it means that plant pathogenic pathogen can adapt to pesticides by mutations, leading to the loss of pesticide efficacy. Therefore, it is necessary to develop long-term efficient, eco-friendly, and cost-effective technique against pathogen.

In the last few years, nanotechnology has proposed different tools for solving agricultural problems. Nanotechnology can improve the production of the crop with the development of nano-fertilizers and nano-treatments for plant diseases. Different nanoparticles (NPs) have been studied for the treatment of plant diseases but it has been observed that silver (Ag) NPs can be used as a potent fungicide. For agricultural applications, the Ag-NPs should be synthesized in a cost-effective way. There are many synthesis methods such as hydrothermal, microwave, photochemical, electrochemical, microemulsion, and chemical reduction. Nonetheless, most of them use harmful

chemicals, use critical reaction conditions, organic solvents, and expensive equipment. In this sense, for the synthesis of stable Ag-NPs a green synthesis method that uses environmentally friendly and cost-effective chemicals is needed. In the present study, the silver nanoparticles were green synthesized using leaf and flower extract of different plants as the extract act as a reducing agent of Ag<sup>+</sup> ions.

### **6.1 Isolation of fungus**

- ***Lasiodiplodia parva:***

Symptomatic branches were initially washed with tap water. Pieces from the edges of the symptomatic and symptomless tissues were cut and surface-sterilized with sodium hypochlorite (1%) for 2 min. The pieces were washed in distilled water, placed on filter paper, then transferred onto 2.5% potato dextrose agar (PDA) amended with 10 mg l<sup>-1</sup> rifampicin. The plates were incubated at room temperature (25 °C-28 °C) for 7 days. Eventually, hyphal tips of the isolates were transferred onto fresh PDA to obtain pure cultures.

- ***Aspergillus niger & Aspergillus terreus:***

The cultures of *Aspergillus terreus* and *Aspergillus niger* were collected from our laboratory (Singh and Dwivedi, 2020, 2022).

#### **6.1.1 Molecular Identification of Fungal Isolates**

- For molecular identification, internal transcribed spacer region sequencing was done. For identification, blast tool, NCBI database was used to identify the fungal species using sequenced data.
- Isolate A showed maximum similarities with *Lasiodiplodia parva* species.
- Isolate B showed maximum similarities with *Aspergillus terreus* species.

- Isolate C showed maximum similarities with *Aspergillus niger* species.

## 6.2 Green synthesis of silver nanoparticles from *Ziziphus mauritiana* (Zm)

- The leaves of *Ziziphus mauritiana* were collected from the BBAU campus and were washed and dried. The dried leaves were grinded into a fine powder. 10g of leaf powder was mixed with 100mL of distilled water and was heated to 70-90°C for an hour. The mixture was centrifuged at 6500 rpm for 20 minutes and the leaf extract was obtained.
- 10mL of the leaf extract was added to 90 mL of water containing 0.1M AgCl<sub>2</sub> solution. The resulting solution was then stirred and heated to 95°C for 30 minutes, the solution was then incubated at 85°C for 15 minutes. The solution after incubation turned dark brown, indicating the synthesis of Ag nanoparticles. The solution was then centrifuged at 10,000 rpm for 10 minutes to obtain the precipitate (AgNPs), which was washed twice with double-distilled water and centrifuged again at 10,000 rpm for 10 minutes. The resulting mass was collected and dried at 30-43°C in a hot air oven. The dried Zm-Ag NPs were then scraped and stored.

### 6.2.1 Characterization of Zm-Ag NPs

- The UV-Vis spectroscopy results showed a sharp peak at 460nm which is indicative of the synthesis of Zm-Ag NPs.
- The SEM analysis showed both individual Zm-Ag NPs and aggregates, with particle sizes ranging from 67-120nm. The dominant size was 120nm for individual cubical particles. The analysis of EDX indicated a significant rise in peak near 3.0 keV, corresponding to Ag's binding energies, suggesting that the high-purity Ag were present in synthesized nanoparticles. The weight % of Ag

was determined to be 98.20, confirming the existence of Zm-Ag NPs in the synthesized sample of NPs.

- The FTIR analysis showed peaks at 3387, 1610, 1382.6, 1036.2 and 635.8  $\text{cm}^{-1}$ . The presence of functional groups like C=O, N-H and COOH have played a significant part in the bio-reduction of  $\text{Ag}^+$  to Ag NPs
- The XRD analysis showed that Zm-Ag NPs had a cubic lattice. The presence of 5 distinct peaks in the pattern of XRD provided evidence like,  $27.77^\circ$  (111),  $32.29^\circ$  (200),  $37.10^\circ$  (111),  $43.44^\circ$  (200),  $46.15^\circ$  (220),  $50.10^\circ$  (311), and  $57.44^\circ$  (222) as well as some major peaks at 2 $\theta$  position  $27.77^\circ$ ,  $32.29^\circ$  and  $35.10^\circ$  denoting the different plane (111), (200), (111) respectively. These results showed that Zm-Ag NPs are crystalline in nature.

### 6.2.2 Antimicrobial and Enzymatic activity of Zm-Ag NPs

- The biosynthesized Zm-Ag NPs at a dilution of 4 mg/L showed the highest zone of inhibition against *A. terreus* (53.5%), *L. parva* (56.2%) and *A. niger* (65.1%) compared to other dilutions. The decrease in ZM-Ag NPs concentration led to the decrease in the zone of inhibition.
- The activity of SOD increased in the presence of Zm-Ag NPs and raised with rise in the concentration of Zm-Ag NPs. The maximum SOD activity was recorded at 1 mg/L of Zm-Ag NPs which was 15% higher as compared to control in *A. niger*. Similarly, in *L. parva* and *A. terreus*, the activity level of SOD was 10% and 11% respectively as compared to the control.
- When exposed to Zm-Ag NPs, the CAT activity in all the fungus increased significantly. The activity of CAT at 1 mg/L of Zm-Ag NPs was 18%, 22%, and 25% for *A. niger*, *L. parva* and *A. terreus* respectively as compared to the control.

- The trend in POD action was identical to that of CAT action in fungal isolates exposed to Zn-Ag NPs. At 1 mg/L of Ag NPs, POD activity in *L. parva* increased by two-fold while it was 8% and 5% for *A. terreus* and *A. niger* respectively as compared to the control.

### 6.3 Green synthesis of silver nanoparticles from *Cassia fistula* (Cf)

- The flowers of *Cassia fistula* were collected from the BBAU campus and were washed and dried. The dried flowers were grinded to a fine powder. 10g of dried flower powder was mixed with 100mL of distilled water and was heated to 70-90°C for an hour. The mixture was centrifuged at 6500 rpm for 20 minutes and the flower extract was obtained.
- 10mL of the flower extract was added to 90mL of water containing 0.1M AgCl<sub>2</sub> solution. The resulting solution was then stirred and heated to 95°C for 30 minutes, the solution was then incubated at 85°C for 15 minutes. The solution after incubation turned darkish brown, indicating the synthesis of Ag nanoparticles. The solution was then centrifuged at 10,000 rpm for 10 minutes to obtain the precipitate (Ag NPs), which was washed twice with double-distilled water and centrifuged again at 10,000 rpm for 10 minutes. The resulting mass was collected and dried at 30-43°C in a hot air oven. The dried Cf-Ag NPs were then scraped and stored.

#### 6.3.1 Characterization of Cf-Ag NPs

- The UV-Vis spectroscopy analysis showed a distinct peak at 462 nm which suggested the synthesis of Cf-Ag NPs through the Surface Plasmon Resonance (SPR) phenomenon.
- The obtained images of SEM disclosed the existence of both individual particles and aggregates, with size ranging from 78-120 nm. The majority of the

individual particles had a spherical shape with an uneven surface, measuring 120 nm in size. EDX analysis displayed a significant peak near 3.0 keV, corresponding to Ag's binding energy, indicating the presence of high-purity Ag in the synthesized NPs. The weight % of Ag was found to be 98.20, thus confirming the presence of Cf-Ag NPs in the sample.

- The FTIR analysis showed peaks at 3387, 2921, 1610, 1248, 1383.2, 1069.2 and 635.8  $\text{cm}^{-1}$ . The presence of functional groups like C=O, N-H and COOH have played a vital part in the bio-reduction of  $\text{Ag}^+$  to Ag NPs.
- The XRD pattern of Cf-Ag NPs showed the presence of five distinct peaks at  $27.50^\circ$ ,  $31.93^\circ$  (200),  $37.84^\circ$  (111),  $44.06^\circ$  (220), and  $64.25^\circ$ . Additionally, two prominent peaks were observed at  $2\theta$  positions of  $32.29^\circ$  and  $37.10^\circ$ , corresponding to the (111) and (200) planes, respectively. These results provide compelling evidence that the biosynthesized Cf-Ag NPs possess a crystalline structure.

### 6.3.2 Antimicrobial and Enzymatic activity of Cf-Ag NPs

- The maximum inhibition percentage of *A. terreus* by biosynthesized Cf-Ag NPs was 58.1% at 4 mg/L followed by 41% and 33.1% at 2 mg/L and 1 mg/L respectively. In case of *A. niger*, the highest inhibition percentage was at 4 mg/L which was 62.3% followed by 48.4% and 45.3% at 2 mg/L and 1mg/L respectively. Likewise, the highest inhibition of *L. parva* was recorded at 4 mg/L which was 52.4% followed by 49% and 45% at 2 mg/L and 1 mg/L respectively. The increase in concentration of Cf-Ag NPs led to the increase in the zone of inhibition of fungal isolates.
- The activity of SOD increased in the presence of Ag NPs and decreased with the decrease in concentration Cf-Ag NPs. At the concentration of 1 mg/L of Cf-

Ag NPs, the activity of SOD increased by almost two fold in case *L. parva* as compared to control while it was 70 % for *A. terreus* and 100 % for *A. niger* at 1 mg/L of Cf-Ag NPs as compared to control.

- The CAT activity in all the fungus increased significantly in the presence of Cf-Ag NPs. The activity of CAT at 1 mg/L of Cf-Ag NPs was 45.3%, 60.3%, and 45.3% for *A. niger*, *L. parva* and *A. terreus* respectively as compared to control.
- Similar trend of CAT action was followed in POD of fungus isolates exposed to Cf-Ag NPs. At 1 mg/L of Cf-Ag NPs, the POD activity was 56.2%, 38.4% and 27.4% for *A. terreus*, *A. niger*, and *L. parva* respectively as compared to control.

#### **6.4 Green synthesis of silver nanoparticles from *Dracaena trifasciata* (Dt)**

- The leaves of *Dracaena trifasciata* were collected from the BBAU campus and were washed and dried. The dried leaves were grinded into a fine powder. 10g of leaf powder was mixed with 100mL of distilled water and was heated to 70-90°C for an hour. The mixture was centrifuged at 6500 rpm for 20 minutes and the leaf extract was obtained.
- 10mL of the leaf extract was added to 90mL of water containing 0.1M AgCl<sub>2</sub> solution. The resulting solution was then stirred and heated to 95°C for 30 minutes, the solution was then incubated at 85°C for 15 minutes. The solution after incubation turned dark brown, indicating the synthesis of Ag nanoparticles. The solution was then centrifuged at 10,000 rpm for 10 minutes to obtain the precipitate (Ag NPs), which was washed twice with double-distilled water and centrifuged again at 10,000 rpm for 10 minutes. The resulting mass was collected and dried at 30-43°C in a hot air oven. The dried Dt-Ag NPs were then scraped and stored.

#### 6.4.1 Characterization of Dt-Ag NPs

- The UV-Vis spectroscopy analysis showed a distinct peak at 465 nm which suggested the synthesis of Dt-Ag NPs through the Surface Plasmon Resonance (SPR) phenomenon.
- The SEM analysis showed that the size of the biosynthesized Dt-Ag NPs ranged from 10 nm to 150 nm while the average size was 53 nm. However, the surface morphology of nanoparticle was not very clear and the particles were present in the agglomerated form.
- The FTIR analysis showed peaks at 3389.8, 1646, 1567, 1385.9, 1069.6 and 661.1  $\text{cm}^{-1}$ . The silver oxide was formed with substitution reaction, where first H atom of HO-R (R= alkyl group) was replaced by silver atom (Ag) to form intermediate Ag-O-R then R substituted by Ag to form Ag-O-Ag.
- The x-ray diffraction analysis data was recorded in  $2\theta$  range from  $20^\circ$  to  $80^\circ$ . The multiple peaks were recorded at  $2\theta$  value of  $28^\circ$ ,  $32^\circ$ ,  $34^\circ$ ,  $38^\circ$  and  $46.2^\circ$  in the analysis. The obtained results demonstrated that Dt-Ag NPs were highly crystalline in nature.

#### 6.4.2 Antimicrobial and Enzymatic activity of Dt-Ag NPs

- With the rise in concentration of Dt-Ag NPs, the inhibition percentage also increased gradually for all the tested fungi. The maximum inhibition percentage of *A. terreus* by biosynthesized Dt-Ag NPs was recorded at 4 mg/L which was 58%. while *A. niger* showed highest inhibition percentage of 65% at 4 mg/L which was higher as compared to *A. terreus*. The fungus *L. parva* also showed maximum inhibition of 51% at the concentration of 4 mg/L which was lower than *A. terreus*.

- The SOD activity increased in the presence of Dt-Ag NPs. The maximum SOD activity was recorded at 1 mg/L of Dt-Ag NPs which was 18% higher as compared to control in *A. terreus*. Likewise, in *A. niger* and *L. parva*, the SOD activity was 20% and 11% respectively as compared to the control.
- The activity of CAT increased significantly in the presence of Dt-Ag NPs and it was 45%, 34%, 36% for *A. terreus*, *A. niger*, and *L. parva* respectively as compared to the control.
- The Dt-Ag NPs have potential impact on the fungal isolates as a significant increase in POD activity was recorded as compared to control. In the presence of 1 mg/L of Dt-Ag NPs, the POD activity increased significantly by 50%, 20% and 50% for *A. terreus*, *A. niger*, and *L. parva* respectively as compared to the control.