

## Synthesis and characterization of silver nanoparticles using *Datura metel* L. (Solanaceae) leaf extract and its larvicidal activity on *Epilachna vigintioctopunctata* F.

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**ABSTRACT:** Insecticidal activities of synthesized silver nanoparticles of leaf extract of *Datura metel* L. (Solanaceae) (DM) against grubs *Epilachna vigintioctopunctata* F. (Coleoptera, Coccinellidae) at varying levels of concentrations was evaluated. DM leaf extract was used to create AgNPs, and nanoparticle production could be seen after six hours. UV-vis spectrophotometer, Particle size analyzer, FTIR and SEM analysis were used to confirm the synthesis of AgNPs. GCMS spectra of leaf extract of DM showed 20 substances, of which nine were known s phytochemicals and the others were unidentified. UV-visible spectra to analyse the Surface Plasmon Resonance for AgNPs revealed in the range of 366 - 374nm. LC<sub>50</sub> values for the AgNPs synthesized leaf extracts calculated 24 h after treatment against the fourth instar larvae of *E. vigintioctopunctata* using probit analysis revealed the LC<sub>50</sub> of the aqueous leaf extract as 252.31ppm and that of AgNPs synthesized leaf extract as 396.09ppm. When comparing the aqueous and AgNPs synthesized leaf extracts, AgNPs nanoparticles synthesized leaf extracts were more efficient as larvicide than aqueous leaf extract. © 2023 Association for Advancement of Entomology

**KEY WORDS:** AgNPs, aqueous leaf extract, larvicidal activity, LC<sub>50</sub> values

### INTRODUCTION

A common and well-liked vegetable crop growing in the subtropics and tropics is eggplant, *Solanum melongena* L. (Sarkar *et al.*, 2006) and an important vegetable crop in India. According to their size, shape, and color, there are numerous varieties of brinjal available in India (Nisha *et al.*, 2009). The significant losses caused by damage to different agricultural crops are largely attributable to insect pests. According to estimates, insect pests

are responsible for 23 per cent of all agricultural losses (Agarwal, 2011). Pesticides are used often and to control these pests in the vegetable fields, which has led to widespread resistance development, unfavorable impacts on non-target organisms, the presence of hazardous residues in food, and environmental and health risks (Kranthi *et al.*, 2002). All these have highlighted the requirement to create unique, risk-free, and environmentally friendly pest management methods. *Epilachna vigintioctopunctata* F. (Coleoptera,

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Coccinellidae) is a serious pest of solanaceous crops, especially potatoes and aborigines, throughout its range. Adult and grubs feed on the surface of leaves by scraping away the surface cells between the main veins to leave irregular-shaped holes or strips. Heavy feeding damages the leaves, giving them a skeletonized or lace-like appearance. The damaged leaves turn brown and curl as they dry before falling off. *Datura metel* L. (Solanaceae) is a shrub-like, commonly found in India and has pesticide and medicinal properties. An attempt was made to investigate *D. metel* in the creation of silver nanoparticles from its leaf extract and its efficiency as larvicide at varying concentrations on *E. vigintioctopunctata*.

## MATERIALS AND METHODS

Leaves of *D. metel* were gathered in and around Meenampatti, Sivakasi Taluk, between July 2021 and March 2022. The study region is located between latitudes of 9°27'2.6424" from the north and longitudes of 77°48'26.0496" from the east. *D. metel* leaves plucked were cleaned in tap water and allowed to air dry for 5-7 days in the shade. Using an electric blender, the air-dried plant components were powdered. For extraction, 10g of fine leaf powder was collected in a beaker with 100ml of double-distilled water that had been sterilized. After that, it was heated at 60°C for 3 number 1, and the extract was kept at -20°C and used within a week.

**Phytochemical analysis:** Agilent GC 7890A/MS 5975C and a capillary column Agilent DB5MS were used in the GCMS to evaluate the *D. metel* leaf extract. The mass spectrometer was tuned to 70 eV, and the computer library created by WILEY7, NIST05, and NIST05s was used to identify unknown substances using probability-based matching.

**Synthesis of silver nanoparticles from leaf extract:** The precursor used to create silver nanoparticles was silver nitrate. Analytical-grade silver nitrate ( $\text{AgNO}_3$ ) in the amount of 16.961mg was measured out and mixed with 90ml of Milli-Q water. In a 1 Erlenmeyer flask, 90ml of produced 1mM aqueous  $\text{AgNO}_3$  solution was added to 10ml

of aqueous leaf extract and incubated at room temperature in the dark. AgNPs were being produced as a result of the transition from light yellow to dark brown (Lingarao and Savithamma, 2013).

**Characterization of nanoparticles:** Utilizing UV-Visible spectroscopy, the initial characterization of AgNPs was completed. Synthesized silver nanoparticles were subjected to FTIR analysis, particle size analysis and SEM.

**Epilachna beetle rearing:** A mass culture of *E. vigintioctopunctata* was kept in a lab at the Department of Zoology, Ayya Nadar Janaki Ammal College, Sivakasi, Tamilnadu, India, using brinjal leaves as feed, in order to consistently obtain a large number of epilachna larvae for experimental usage. The *E. vigintioctopunctata* infested brinjal plant was grown in the nearby agrifarm of Sivakasi, where the adults of *E. vigintioctopunctata* were collected. The laboratory reared epilachna larvae were used for bioassays and the cultures were maintained throughout the study period. The adults were allowed to breed in the laboratory condition, when the larvae hatch from the egg they were fed with fresh brinjal leaves. They were permitted to develop till becoming third instar stages, and then were used to assess the larvicidal efficiency.

**Larvicidal activity:** The leaf extract's larvicidal activity was tested in the laboratory settings. By dilution with de-ionized water, various amounts of aqueous and synthetic silver nanoparticle leaf extract were created. Each extract had a concentration of 100, 50, 25, 12, 5 and 6.25 mg ml<sup>-1</sup>. To examine leaf extract's larvicidal potential, a spray test was conducted. Different concentrations of extracts were made, sprayed on brinjal leaves, and given to epilachna beetle larvae as feed. To determine the larvicidal activity of each concentration of extracts, ten larvae per concentration were subjected and replicated thrice. Similar to this, three replicates of control group (tap water) for each test was done. After a 24h exposure period, the mortality rate was observed. For each concentration, the percentage of larval mortality from the replicates was calculated.

**Statistical analysis:** Larval mortality analysis assessed using standard deviation and mean separation. Using the program SPSS, 2007, the probit analysis for  $LC_{50}$ , the upper and lower 95 per cent confidence bounds were determined.

## RESULTS AND DISCUSSION

Aqueous *D. metel* leaf extract was used to perform green production of silver nanoparticles. The initial confirmation of the biosynthesis of silver nanoparticles using aqueous leaf extract was the visible observation of colour change. The initial colour of the leaf extract suspension was pale yellow; but, after the addition of silver nitrate and an overnight incubation at room temperature, the colour changed to brown (Fig. 1).

**GCMS analysis of leaf extract:** As many as 20 compounds were detected in GCMS spectra of *D. metel* leaf extract, of which nine were recognized. The remaining 12 substances were unidentified. Retention times, mass spectra, and a library of typical compounds were compared to determine whether phytochemicals were present. Among the nine compounds, L-Arabinitol ( $C_5H_{12}O_5$ ) had 40.57 per cent peak area, with a retention time of 4.887 minutes, having a molecular weight of  $152.15 \text{ g mol}^{-1}$ . The remaining compounds were found in the range of 1 – 7 per cent peak area (Table 1).

**Characterization of leaf extract:** The Surface Plasmon Resonance (SPR) is analyzed using an ultraviolet-visible spectrum. AgNPs UV-visible spectra fell between 366 and 374 nm (Fig. 2a).

**Fourier Transform-Infrared (FTIR) analysis:** FTIR analysis showed the vibrational spectra of AgNPs synthesized leaf extract (Table 2). The alcohol molecule found in the extract was responsible for distinctive peak that occurred at 3453.31. The peak at 760.87 was assigned to the stretching vibration C-CL group. The alkenes group is present in the AgNPs produced extract as indicated by the 1385.76 and 1639.38. Presence of aromatic compound is established by the peak at 1512.09. Alkanes group presence is confirmed by the absorption peaks at 2063.69 and 2885.31 by C-H stretch and C-H stretch respectively. The peak at 2309.6 indicates the presence of alkynes in the AgNPs synthesized extract with Ca C stretch in AgNPs synthesized leaf extracts (Fig. 2b).

**Particles size analysis:** Dynamic light scattering (DLS) was used to establish the intensity-weighted mean diameter (Z-average) of the particle size of DM-AgNPs in the range of 0.5-1nm (Fig. 2c). The particle size and shape of the biosynthesized DM-AgNPs are also determined using SEM examination. The SEM investigation revealed that the produced nanoparticles' mean particle diameters ranged from 20 to 27nm (Fig. 2d).

**Larvicidal activity:**  $LC_{50}$  values for the AgNPs synthesized leaf extracts calculated 24 h after treatment against the fourth instar larvae of *E. vigintioctopunctata* using probit analysis revealed the  $LC_{50}$  of the aqueous leaf extract as 252.31ppm and that of AgNPs synthesized leaf extract as 396.09ppm. Larval mortality improved with

Table 1. GCMS spectra of identified phytochemicals from the extract of *Datura metel*

No	RT (Min)	Compound	Peak area (%)	Molecular formula	Molecular weight ( $\text{g} \cdot \text{mol}^{-1}$ )
1	4.887	L-Arabinitol	40.57	$C_5H_{12}O_5$	152.15
2	5.542	Propanamide	3.41	$C_3H_7NO$	73.095
3	5.709	Benzeneacetaldehyde	7.09	$C_8H_8O$	120.1485
4	7.398	Benzoic acid	1.22	$C_7H_6O_2$	122.12
5	8.464	Silane	4.77	$H_4Si$	32.117
6	11.264	o-Cyanobenzoic acid	1.98	$C_8H_5NO_2$	147.130
7	12.230	Pinacolyl ethyl phosphonofluoridate	2.31	$C_8H_{18}FO_2P$	196.199

Table 2. Functional groups detected in AgNPs synthesized leaf extract of *D. metel* as revealed by FTIR

No	Absorption (cm <sup>-1</sup> )	Class of compounds	Bond
1	743.51	Alkyl halide	C-CL stretch
2	1385.76	Alkenes	C-H bend
3	1512.09	Aromatic	C=C stretch
4	1639.38	Alkenes	C=C stretch
5	2063.69	Alkanes	C-H stretch
6	2309.60	Alkynes	Ca=C stretch
7	2885.31	Alkanes	C-H stretch
8	3453.31	Alcohol	O-H stretch



Fig. 1 Visible colour change of *Datura metel* leaf extract after silver nanoparticles synthesis

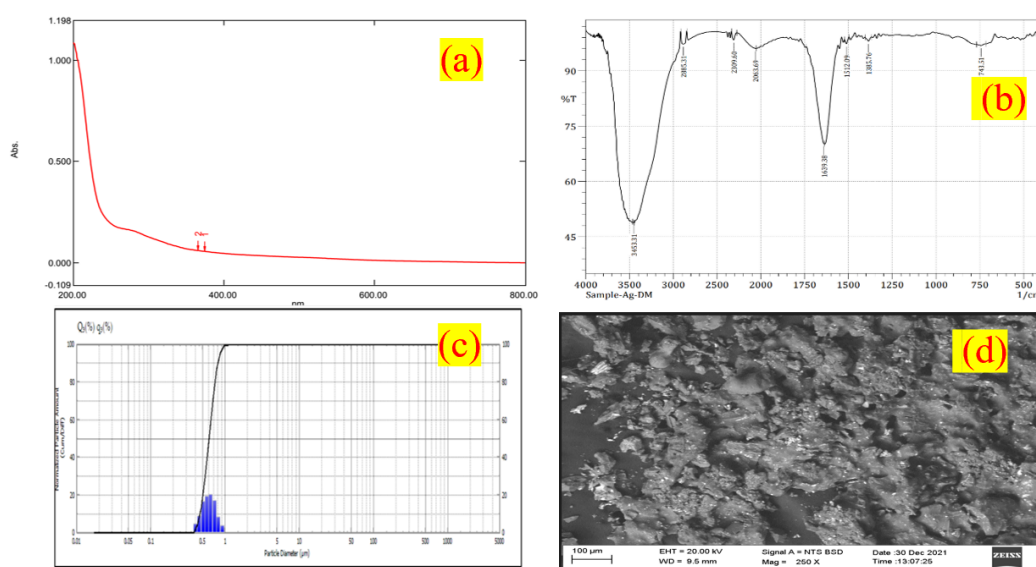


Fig. 2 Characterization of Silver Nanoparticles synthesized (a) UV-Visible Spectral analysis; (b) FTIR analysis; (c) Particle size analysis; (d) SEM analysis

increase in the concentration of silver nanoparticles synthesized leaf extract (Table 3). AgNPs synthesized leaf extract @100 concentration recorded 80 per cent mortality; at the same the aqueous leaf extract showed 10 per cent mortality. When comparing the aqueous and AgNPs synthesized leaf extracts, AgNPs nanoparticles synthesized leaf extracts were more efficient than aqueous leaf extract as larvicide.

Due to the pressing needs to create ecologically friendly technology, biosynthesis of nanoparticles has received a lot of attention. A significant method for creating many types of nanoparticles, such as

copper, iron, platinum, silver, and zinc, has been the biosynthesis of nanoparticles utilizing biological agents (Rasheed *et al.*, 2017; Sharon *et al.*, 2018). Bioresearch in the domain of explaining the mechanism of plant-mediated nanoparticle production holds great promise (Kumar and Yadav, 2009). The synthesis of AgNPs using *D. metel* was verified in the current work utilizing a variety of cutting-edge methods. When *D. metel* leaf extract is added to the silver nitrate solution, the mixture changes color from pale yellow to brown due to the reduction of the silver ion, indicating the creation of silver nanoparticles. The current work provides proof that the extract of *D. metel* has a potential to

Table 3. Silver nanoparticles synthesized leaf extract on the mortality of IV<sup>th</sup> instar larvae of *E. vigintioctopunctata* (@10 larvae/ conc)

Concentration	Mean mortality of IV <sup>th</sup> instar larvae in	
	aqueous	AgNPs
6.25	0	1
12.5	0	2
25	0	2
50	0	5
100	1	8

reduce silver ions (Ag<sup>+</sup> into Ag<sup>0</sup>) and convert silver nitrate to silver nanoparticles while also having larvicidal effects on epilachna beetles. Similar research on *Polyalthia longifolia* samples, whose colour ranges from nearly colorless to brown, was published by Kaviya *et al.*, in 2011.

By comparing mass spectra, retention times, and a library of common compounds, it was possible to confirm that leaf extract contained phytochemical components. In GCMS spectra of *D. metel* leaf extract 20 compounds were detected, of which nine were recognized as phytochemicals. Mishra and Patnaik (2020) obtained comparable findings from a methanol extract of the complete *Withania somnifera* plant. According to the size and polydispersity of NPs, the distinctive peak of AgNPs is located about 430nm (Anandalakshmi *et al.*, 2016). According to Rajagopal *et al.* (2021), the UV-Vis spectra of the CuNPs produced using *Wrightia tinctoria* displayed absorption peak maxima at 357 nm. Plants create phytochemicals either through their primary or secondary metabolism. The majority of the time, they are biologically active in the plant host and aid in plant growth or defense against pests, diseases, or predators.

Measurements using the Fourier transform infrared spectroscopy technique are used to pinpoint the potential biomolecules in charge of the reduction, capping, and effective stability of silver nanoparticles (Padalia *et al.*, 2015). It was demonstrated that NPs were generated by the presence of functional groups like alcohol, halides,

alkanes, alcohol, and aromatic compounds (Prabha *et al.*, 2022).

The larvicidal activity of silver nanoparticles made from *D. metel*'s aqueous extract against the epilachna beetle was observed in this work. The LC<sub>50</sub> values calculated in the present study revealed that both aqueous and AgNPs have remarkable larvicidal effect on epilachna beetle. According to Islam *et al.* (2011), the LC<sub>50</sub> values for three medicinal plants were 18.40, 23.70, and 29.61 per cent, for the epilachna phytophagous pest. When bioefficacy of two indigenous plant products, namely seed extracts of *Strychnos nuxvomica* and *Pachyrrhizus erosus*, and two entomopathogenic fungi, *Beauveria bassiana* and *Metarrhizium anisopliae*, were tested against the epilachna beetle on bottle gourd, the population of the epilachna beetle was significantly reduced (Vishwakarma *et al.*, 2011). According to Ahmed (2007), spraying the castor plant *Ricinus communis* aqueous extract on sunflower, *Helianthus annulus* foliage and capitula, reduced epilachna attacks resulting improved the oil seed harvest.

The present findings clearly show that at certain levels, the suggested green silver nanoparticles have an effect on *E. vigintioctopunctata* that causes mortality. Ag<sup>+</sup> ions are produced from the nanoparticle's surface by oxidation processes, and when they enter an insect's physiological processes, they interact with biological components (such as insect proteins) and induce toxicity (Park *et al.*, 2010). AgNPs are also known to bind with thiol groups in proteins and promote their denaturation, which causes the death of larvae (Johnston *et al.*, 2010). Hence, *D. metel*'s aqueous leaf extract and silver nanoparticles produced leaf extract can be effectively used as larvicide to *E. vigintioctopunctata*.

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