

Research Article

Tradescantia pallida*-derived silver nanoparticles for the management of *Aedes aegypti

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Abstract

Aedes aegypti is a cosmopolitan vector of arboviral diseases like dengue, Zika, chikungunya, and yellow fever. The management of these diseases requires effective mosquito control strategies due to the lack of suitable medications and vaccines. In addition, the inefficacy of insecticides due to multiple resistances and environmental safety concerns has raised interest in the use of botanicals for this purpose. Hence, the present study synthesized silver nanoparticles (AgNPs) at 3 mM AgNO₃ using varying volumes (0.5, 1.0, 1.5, and 2.0 mL) of aqueous leaf extract of *Tradescantia pallida* (*Tp*-AgNPs) and estimated their efficacy against *Ae. aegypti* larvae. The results showed the highest larvicidal potential of *Tp*-AgNPs at 3 mM of silver nitrate, the efficacy increasing with exposure time. The larval mortality increased by 9.31–34.58% after 48 h of treatment as compared to 24 h, while a noticeable increase of 45.15–53.53% was observed after 72 h of treatment. The efficient *Tp*-AgNPs were characterized using various biophysical techniques, including UV-Vis spectroscopy, Field Emission Scanning Electron Microscopy (FESEM), Transmission Electron Microscopy (TEM), Energy Dispersive X-ray (EDX) Spectroscopy, X-ray diffraction (XRD), and Fourier Transform Infrared (FT-IR). The results showed that they were synthesized at 312 nm. The nanoparticles were mostly spherical, with a solid face-centred cubic structure and were widespread, ranging in size from 15 to 40 nm. The FT-IR bands indicated that plant phytochemicals were involved in both breaking down and stabilizing AgNPs. Overall, the results show that *T. pallida*-mediated silver nanoparticles are cost-effective and efficient larvicidal agents against *Ae. aegypti*. The study recommends using *Tp*-AgNPs as an alternate to traditional chemical insecticides for controlling mosquito vectors.

Keywords: *Aedes aegypti*, Larvicidal activity, Mosquito control, Silver nanoparticles, *Tradescantia pallida*

INTRODUCTION

Aedes aegypti L. is one of the most prevalent vectors that transmit diseases such as dengue, chikungunya, yellow fever, and Zika. In 2025, the WHO reported more than 7.6 million dengue cases, including over 3.4 million confirmed cases, 16,000 severe cases, and 3,000 fatalities (WHO, 2025). According to modelling studies, there are an estimated 390 million infections/year worldwide, with approximately 96 million symptomatic diseases (Bhatt *et al.*, 2013). For a short period in 2020–2021, reported cases reduced which has been ascribed to the COVID-19 pandemic, making it difficult to monitor and control the vectors (WHO, 2022). How-

ever, in 2022, dengue started spreading rapidly again, and by 2023, over 6 million cases were reported worldwide (WHO, 2024). In 2024, the situation continued to deteriorate, with the WHO reporting approximately 13.9 to 14 million infections in 120 countries; the highest number of dengue cases ever recorded (PAHO, 2024; WHO, 2025). In India, according to National Vector Borne Disease Control Programme (NVBDCP), a total of 2,33,519 cases and 297 deaths were reported in 2024 (NVBDCP, 2025).

Several control strategies have been attempted to control these mosquitoes, among which use of synthetic insecticides is the most widely used vector control program. Despite these, the *Aedes*-borne diseases are

rising every year, primarily due to the development of insecticide resistance. Resistance in *Ae. aegypti* has been reported from Indonesia, India, Malaysia, Thailand, China, Mexico, Columbia, and Brazil, and several other countries (Pintong *et al.*, 2020). Besides, harmful effects of synthetic chemicals on humans, other organisms and the ecosystem have raised grave concerns. Hence, researchers have diverted their attention to botanicals as suitable alternates and started exploring phytochemicals for controlling mosquitoes (Mehlhorn *et al.*, 2005; Amer and Mehlhorn 2006; Elango *et al.*, 2009; Ghosh *et al.*, 2012; Warikoo and Kumar, 2014; Sharma *et al.*, 2016; Campos *et al.*, 2020). Shaalan *et al.* (2005) conducted a thorough review of several plant species, including their effects on mosquitoes, extraction procedures, bioactive constituents, and possible synergistic and antagonistic effects of extracts. The advent of nanotechnology resulted in enhanced efficacy of these extracts by making them easier to spread and penetrate (Benelli *et al.*, 2019; Radwan *et al.*, 2024). Such nanoparticles are regarded as a possible alternative to chemical insecticides due to their higher efficacy and environmental safety.

Earlier, different biological agents have been studied to make simple, cost-effective, and eco-friendly metallic nanoparticles, using silver, gold, titanium, and platinum (Thakkar *et al.*, 2009; Salunkhe *et al.*, 2011; Tran *et al.*, 2013; Shah *et al.*, 2015; Ahmed *et al.*, 2016). Silver nanoparticles (AgNPs) have been formulated using various botanicals, like neem (*Azadirachta indica*), green tea (*Camellia sinensis*), *Sesbania drummondii* shrub, natural rubber, starch, aloe vera plant extract, and lemongrass leaf extract (Vidyasagar *et al.*, 2023; Arya *et al.*, 2024). The AgNPs made from the leaf extract of *Mimosa pudica* and *Nelumbo nucifera* showed high efficacy against fourth instars of the *Culex quinquefasciatus* and *Anopheles subpictus* Grassi mosquitoes (Marimuthu *et al.*, 2011; Santoshkumar *et al.*, 2011). AgNPs synthesized using *Moringa oleifera*, *Aloe barbadensis*, *Eucalyptus globulus*, *Clitoria ternatea*, *Pongamia pinnata*, *Curcuma longa*, *Zingiber officinale*, *Ageratum conyzoides*, and *Ocimum basilicum* have shown larvicidal, pupicidal and morphological aberration effects against *Ae. aegypti* at low concentrations. Reports have suggested that green-synthesized nanoparticles have better surface reactivity, bioavailability, and enhanced interactions with larval tissues than mere plant extracts making them more effective. In addition, plant-based silver nanoparticles are safer to the environment, cost-effective, and can be controlled to obtain the right shape, size, and sensitivity (Gnanadesigan *et al.*, 2011; Elumalai *et al.*, 2016; Poudel *et al.*, 2022; Arshad *et al.*, 2023).

Tradescantia pallida, belonging to the Commelinaceae family and commonly referred to as purple queen, is an

ornamental plant with easy propagation, fast growth, and low maintenance requirements making it suitable for scalable biomass sourcing. Use of this plant does not compete with food crops or medicinal plants, thereby supporting its cost-effective use. It possesses significant traditional therapeutic benefits as an anti-inflammatory and antitoxic agent to enhance blood circulation (Li, 2006). Prior investigations into the phytochemical composition of *T. pallida* have identified pharmacologically relevant constituents, including alkaloids, flavonoids, phenolics, tannins, and saponins (Huq *et al.*, 2016), as well as a substantial reservoir of naturally occurring pigments, particularly anthocyanins (Jabli, 2018). The chemicals derived from *T. pallida* extract demonstrate significant antioxidant, antibacterial (Tan *et al.*, 2014), anticancer, cytotoxic, thrombolytic, *in vivo* analgesic, and membrane-stabilizing effects (Huq *et al.*, 2016; Dash *et al.*, 2020). Silver nanoparticles have been synthesized from the extract of *T. pallida* (Hussain *et al.*, 2016) to estimate their activity against the microbial world (Naaz *et al.*, 2024); however, to our knowledge, their effects have not been assessed against *Ae. aegypti* larvae. Hence, the present study was conducted to formulate *T. pallida* extract silver nanoparticles from AgNO₃ precursors, characterize them using multiple biophysical techniques, and assess their effects on early fourth instars of *Ae. aegypti*.

MATERIALS AND METHODS

Test organism - *Aedes aegypti*

Current research study employed early fourth instars of the dengue vector *Ae. aegypti* that were reared in an insectary at a controlled temperature of 28 ± 1 °C, the relative humidity of $80 \pm 5\%$, and a light-dark cycle of 14:10 h L/D, following the steps laid out by Sharma *et al.* (2019).

Plant collection

The fresh and healthy leaves of the *T. pallida* were collected from the premises of Acharya Narendra Dev College, University of Delhi, New Delhi, India (Coordinates: 28°54'N, 77°26'E) in the month of June 2025.

Preparation of aqueous plant extract

The leaves of *T. pallida* were cleaned thoroughly with tap water followed by double distilled water to remove dust and dirt. The leaves were cautiously examined for any disease and cut into small pieces. The 20 g of leaves were transferred into a 250 mL glass beaker containing 100 mL double distilled water. A leaf broth was set by boiling the contents at 60 °C for 15 to 20 min (Elumalai *et al.*, 2016; Sharma *et al.*, 2017) and kept undisturbed for 2–3 h. The extract was filtered

through a fine, clean muslin cloth, followed by a Whatman No. 1 filter paper to eliminate particulate matter. The resultant clear extract was stored in amber-coloured culture bottles at 4 °C for more investigations.

Synthesis and optimization of silver nanoparticles

Primary synthesis of nanoparticles was carried out in accordance with Sharma *et al.*, (2019) to optimize the volume of aqueous leaf extracts of *T. pallida*. Varying volumes of leaf extract (0.5, 1.0, 1.5, and 2.0 mL) were added to 10 mL of 3 mM silver nitrate (AgNO₃), and the synthesis was monitored. The mixture was incubated at 80-90 °C for 30 min, then allowed to cool before being examined intermittently to evaluate the bio-reduction of Ag⁺ in aqueous solution. The change in colour from light lavender to dark brown was used as an indicator of nanoparticles biosynthesis.

Primary characterization: UV-Vis spectral analysis

The intensity of the *Tp*-AgNPs was scanned and recorded in UV-Visible spectra from 200 to 700 nm, using a UV-Vis double-beam spectrophotometer (Lmspuv1900 Labman) with a resolution of 1 nm. An aqueous solution of AgNO₃ was used as a control during the spectrophotometric analysis.

Mosquito larvicidal bioassay

The larvicidal activity of the aqueous plant extracts of *T. pallida* and *Tp*-AgNPs against the early fourth instars of *Ae. aegypti* was estimated using the standard WHO procedure (2005), with minor adjustments. To evaluate the larvicidal potential, a larvicidal bioassay was performed using centrifuged and purified nanoparticles in a range of *Tp*-AgNP concentrations (from 40 µg/mL to 200 µg/mL). 1 mL of *Tp*-AgNPs and 199 mL of dechlorinated water were thoroughly mixed with 20 active early fourth instars of *Ae. aegypti*. For every concentration, five replicates were run concurrently. Control groups were subjected to the AgNO₃ solution in water. *Ae. aegypti* larval mortality was measured at 24h, 48h, and 72h.

Characterization of silver nanoparticles

The formulated *Tp*-AgNPs were characterized using several biophysical approaches (Sharma *et al.*, 2017, 2019). These included Field Emission Scanning Electron Microscopy (FESEM) to identify the size and morphology of the synthesized AgNPs; Transmission Electron Microscopy (TEM) to analyze the internal structure, shape and precise size of synthesized NPs; Energy Dispersive X-ray (EDX) spectroscopy to determine the purity and the chemical composition of the NPs; X-ray Diffraction (XRD) for phase crystal size and Fourier Transform Infrared Radiation (FT-IR) spectroscopy for molecular fingerprinting of the *Tp*-AgNPs.

Field emission scanning electron microscopy (FESEM)

The aqueous solution of *Tp*-AgNPs was centrifuged repetitively and positioned on the carbon-coated copper grids to prepare thin films. A blotting sheet was used to absorb the extra solution and a mercury lamp was used to dry the film five min. The morphology of the nanoparticles was characterized using FESEM (Field Emission Scanning Electron Microscopy) GEOL Model: JSM-7610FPlus U.S (Sigma) at accelerating voltage of 10 keV.

Energy dispersive X-ray (EDX) spectroscopy

An AMETEK instrument was used to analyse the sample at an accelerating voltage of 20.0 kV in order to estimate both qualitatively and quantitatively the elements that might be involved in the creation of silver nanoparticles.

Transmission electron microscopy (TEM)

On a piece of parafilm of the carbon-coated copper grid, a droplet of *Tp*-AgNPs was deposited, and it was let to settle for five to ten min. The grid was left undisturbed for 10 to 20 min. to allow for absorption after the blotting sheet had soaked the surplus fluid. After that, the nanoparticles were examined using a Thermo Fisher TECNAI G2 F20 transmission electron microscope running at a 200 kV accelerating voltage.

Fourier transform infrared radiation (FT-IR)

Using a Bruker Optik V-70V, FT-IR analysis was carried out to assess the functional groups on the surface of silver nanoparticles, which are most likely in charge of stabilising and lowering silver ions.

X-ray diffraction (XRD) analysis

XRD spectrum was recorded using Bruker D2 Phaser XRD to ensure the crystalline nature of synthesized silver nanoparticles.

Data analysis

The Statistical Package for the Social Sciences (SPSS Version 19.0) was used to do a probit analysis on the collected data. Estimates of the Lethal Concentration (LC) were made at 30, 50, and 90 levels. To determine significance and analyse variations among test samples, other statistical parameters were computed, such as regression coefficient, chi-square, standard deviation, and 95% confidence limits.

RESULTS AND DISCUSSION

The present study has been carried out to evaluate the efficiency of silver nanoparticles, synthesized using a single batch of *T. pallida* aqueous leaf extracts collect-

ed during June 2025, as a possible control agent for *Ae. aegypti*. Nevertheless, the phytochemical profiles of the plant may vary with seasonality, soil conditions, and geographic location, which could influence the reduction and capping efficiency during nanocomposite synthesis (Khan and Ali, 2020). The NPs were synthesized with varying amounts of silver nitrate solution, assessed for larvicidal efficacy, and characterized using various techniques.

***Tp*-AgNPs synthesis: UV-Vis spectroscopic analysis**

The silver nanoparticles (AgNPs) were formulated by adding different volumes of aqueous plant extract of *T. pallida* (*Tp*-AgNPs) to 10 mL of 3 mM silver nitrate. The mixtures showed a progressive colour transition of the solution from lavender to orangish, then to dark brown, over 30–45 minutes during the synthesis of 3 mM AgNPs. The appearance of dark brown colour indicated the formation of AgNPs (Fig. 1). The biosynthesis of silver nanoparticles at 3 mM was monitored by UV-Vis spectral scans at 303 nm, 312 nm, 313 nm, and 325 nm (Fig. 2). Peaks attained at different wavelengths are summarized in Table 1. UV-Vis results revealed the most prominent, narrow and highest peak at 312 nm obtained with NPs formed with 1 mL of leaf extract (Fig. 2 and Table 1). The appearance of a sharp peak indicates a strong, stable SPR (Surface Plasmon Resonance) band, suggesting the maximal reduction of silver nitrate and validating the synthesis of nanoparticles. Additionally, the narrowness of the peak indicated similar-sized, shape-matched nanoparticles, while the highest absorbance indicated well-dispersed nanoparticles in solution. These results are in accordance with Peddi and Sadeh (2015), who synthesized AgNPs from *Achyranthes aspera* stem extract at the same wavelengths. Based on the above results, 1 mL volume of *Tp*-ALE was selected for synthesis of NPs and further characterization.

Mosquito larvicidal bioassay

The larvicidal bioassay with 1000 µg/mL aqueous leaf extract of *T. pallida* against early fourth instars of *Ae.*

aegypti showed very low larvicidal activity, resulting in only 3% mortality at 24 h, 6% at 48 h, and 13% at 72 h. Whereas, the larvicidal assessment of *T. pallida* aqueous leaf extract (*Tp*-ALE)-mediated AgNPs synthesized at 3 mM against early fourth instars of *Ae. aegypti* (Table 2) resulted in LC₃₀, LC₅₀, and LC₉₀ values of 45.64 µg/mL, 61.64 µg/mL, and 128.4 µg/mL, respectively, after 24 h of treatment. Additionally, treatment with the *Tp*-AgNPs resulted in markedly higher, time-dependent larvicidal activity, even at substantially lower concentrations. Increasing the treatment duration to 48 h resulted in a 12.77% decline in the LC₅₀ value, indicating higher toxicity. Likewise, 37.12% decrease in LC₅₀ value was observed by extending the time of exposure till 72 h showing the delayed lethal impact of nanoparticles. This may be plausibly due to the accumulation of silver ions or physiological stress imposed on the larvae, suggesting their efficient use in mosquito management. It is important to note that the larval treatment with AgNO₃ alone did not result in any mortality negating the effects of silver ions. On the other hand, commonly used mosquito larvicide, temephos, has been reported to induce significant toxic effects on *Ae. aegypti*, resulting in LC₅₀ values of 0.0022 µg/mL (Pandey et al., 2025), though these values may vary depending on the geographical location, strain used, and its susceptibility status. Present studies showed that the LC₅₀ value of *Tp*-AgNPs is ~28,000 times higher than that of temephos, indicating a significantly lower intrinsic toxicity. However, high levels of temephos resistance in the mosquito population and the environmental and health hazards associated with it suggest the use of nanomaterial-based larvicides as promising alternatives for controlling mosquitoes in insecticide-resistant populations, even though AgNPs require higher concentrations to remain physiologically relevant. Currently, researchers are investigating phytochemicals in the nanoform to combat mosquitoes (Li et al., 2011; Gope et al., 2023). Various plants have been investigated for the eco-friendly production of silver nanoparticles (AgNPs) demonstrating their higher efficacy due to the amalgamation of nanoparticles with bioactive constituents of plant extracts (Benelli, 2016; Lade 2017). The

Table 1. Screening of different ratios of aqueous leaf extract of *Tradescantia pallida* with 3 mM of silver nitrate for the optimum synthesis of silver nanocomposites using UV-Vis Double beam spectrophotometer

S. No.	Volume of <i>Tp</i> -ALE (mL) + 3mM AgNO ₃ (10 mL)	Wavelength (nm)	Absorbance (au)
	0.5	303	0.43
	1.0	312	1.81
	1.5	313	1.59
	2.0	325	1.60

Tp-ALE: *Tradescantia pallida*-Aqueous Leaf Extract; AgNO₃: Silver Nitrate

Table 2. Larvicidal activity of the silver nanoparticles synthesized from leaf extract of *Tradescantia pallida* at 3 mM concentration of silver nitrate against early fourth instars of *Aedes aegypti* at 24 h, 48 h and 72 h.

LC levels	Lethal concentration (95% Lower- upper fiducial limit)	S.E.	χ^2 (df)	RC
After 24 h of treatment				
LC ₃₀	45.64 (32.88- 55.15)			
LC ₅₀	61.64 (49.91-72.700)	1.353	2.069 (4)	4.019
LC ₉₀	128.47 (105.58-181.62)			
After 48 h of treatment				
LC ₃₀	41.39 (28.13- 49.77)			
LC ₅₀	53.77 (44.53-61.52)	0.474	0.970 (5)	0.42
LC ₉₀	84.03 (74.65-100.21)			
After 72 h of treatment				
LC ₃₀	23.22 (8.37- 32.14)			
LC ₅₀	33.81 (23.01-41.79)	0.441	5.180 (5)	0.50
LC ₉₀	59.70 (51.25-72.92)			

Tp-AgNPs = *Tradescantia pallida* aqueous leaf Extract-Silver Nanoparticles. LC₃₀ = lethal concentration that kills 30% of the exposed larvae, LC₅₀ = lethal concentration that kills 50% of the exposed larvae, LC₉₀ = lethal concentration that kills 90% of the exposed larvae. S.E. = Standard Error of the regression coefficient (slope), χ^2 = chi-square, df = degree of freedom, RC = Regression Coefficient (slope). Test samples were transformed into log covariant (\log_{10}), $p < 0.05$, level of significance, non-overlapping 95% confidence limits of 72 h LC₅₀ with both 24 h and 48 h indicating a significant difference, while the 24 h and 48 h LC₅₀ values were overlapped and not significantly different. Values are mean of five replicates.



Fig. 1. Colour change in *Tradescantia pallida* aqueous leaf extract (*Tp*-ALE)-Silver nitrate mixtures at 3 mM of concentration indicating synthesis of silver nanoparticles. *te*: Volume of plant extract (a to d): 0.5, 1.0, 1.5 and 2 mL; e: Control of silver nitrate (3 mM)

larvicidal efficacy of leaf extract-mediated synthesis of silver nanoparticles (AgNPs) from *Mukia maderaspatana*, *A. aspera* (Sharma et al., 2019, 2020), *Gmelina asiatica*, and *Chomelia asiatica* (Kabtiyal et al., 2022; Okeke et al., 2023; Rajesh and Madhumitha, 2023), as well as *Malva sylvestris*, *Quisqualis indica*, and *Carissa* (Dass et al., 2024; Ekpoma et al., 2022; Yagoo et al., 2025) has been investigated against various mosquito species.

Previous research supports our results, showing the effects of silver nanoparticles against mosquito larvae.

In 2025, Kumari et al. synthesized silver nanoparticles (AgNPs) using aqueous extract of *Plumbago auriculata*, and evaluated their larvicidal activities against fourth instars larvae of *Ae. aegypti* and *Cx. quinquefasciatus* and reported LC₅₀ value of 45.1 and 41.1 $\mu\text{g}/\text{mL}$ respectively at 24 h of exposure. In another study, the larvicidal efficacy of silver nanoparticles synthesized from *Coleus aromaticus* and *Wrightia tinctoria* was assessed against *An. stephensi* larvae. The 24-hour LC₅₀ and LC₉₀ values of *C. aromaticus* were 10.11 ppm, 15.12 ppm, and 27.65 ppm, respectively, while the LC₅₀ values for *W. tinctoria* were 18.12 ppm, 21.20 ppm, and 33.26 ppm against II, III, and IV larvae, respectively (Karthika et al., 2025). Furthermore, AgNPs derived from *Acacia sinuata* seed extract showed moderate larvicidal efficacy against third-instar *Ae. aegypti*, with reported LC₅₀ of 23.03 ppm and LC₉₀ value of 38.00 ppm, respectively, after the exposure of 24 h (Sharma et al., 2024).

In comparison, AgNPs synthesized from *Alternanthera sessilis* exhibited much higher toxicity, with LC₅₀ and LC₉₀ values of 2.93 ppm and 7.63 ppm after 24 h (Kumar et al., 2023). Recently, Kumari et al. (2025) synthesized silver nanoparticles using aqueous neem (*Azadirachta indica*) leaf extract and recorded 100% mortality against *Ae. aegypti* larvae @ 25 ppm after 96

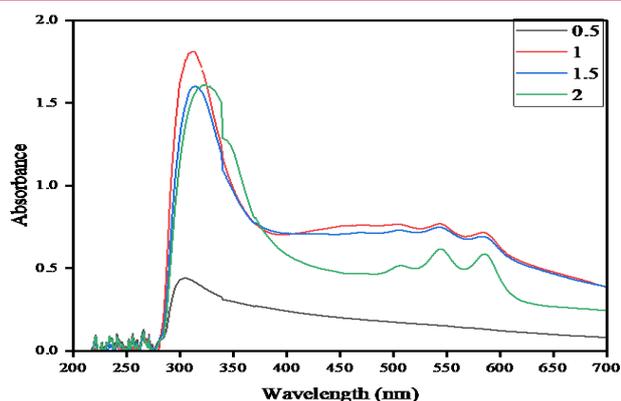


Fig. 2. UV-Vis spectra of *Tp*-AgNPs (*Tradescantia pallida* silver nanoparticles) synthesized using different volumes of *Tp*-ALE at 3 mM of silver nitrate

h demonstrating LC₅₀ value as 3.22 ppm for AgNPs and 381.94 ppm for crude extract. The time-dependent decrease in LC₅₀ values for *Tp*-AgNPs observed in the present study coincides with prior findings, indicating that prolonged exposure (48–72 h) considerably increases the larvicidal effectiveness of plant-mediated silver nanoparticles against *Ae. aegypti*.

Insecticides formulated as nanoparticles have been recognized as a credible and appropriate alternative to traditional insecticides for mosquito control, as they are known to cause less ecological harm (Kumar *et al.*, 2020). Various studies on green-synthesized silver nanoparticles (AgNPs) have identified several mechanisms by which these nanoparticles can affect mosquito physiology. Previous studies have documented that penetration of silver nanoparticles into the larval system triggers significant morphological and physiological changes, including damage to the epithelial lining and midgut cells, ultimately leading to larval death (Sharma *et al.*, 2022). The increased penetration of nanoparticles is attributed to the small spherical shape of nanoparticles and high reactivity, which enable them to easily cross the cellular membrane barriers without hindrance, a property that makes green-synthesized silver nanoparticles more effective than plant extracts alone, although penetration capacity may differ depending on particle size (Parthiban *et al.*, 2019).

Once internalized, these nanoparticles have been shown to bind to sulfur-rich proteins and DNA, ultimately disrupting vital homeostatic and physiological processes in larvae (Parthiban *et al.*, 2019; Kojom Foko *et al.*, 2023). Additionally, the deposition of these nanoparticles within the midgut area has been linked with severe cellular damage, including breakdown of the epithelial lining, cell lysis, and damage to the peritrophic membrane and microvilli, as reported in studies involving *Achyranthes aspera*-mediated nanoparticles (Sharma *et al.*, 2022), further leading to the breakdown of nutrient absorption and integrity in the gut, thus contributing significantly to larval mortality (de Assunção *et*

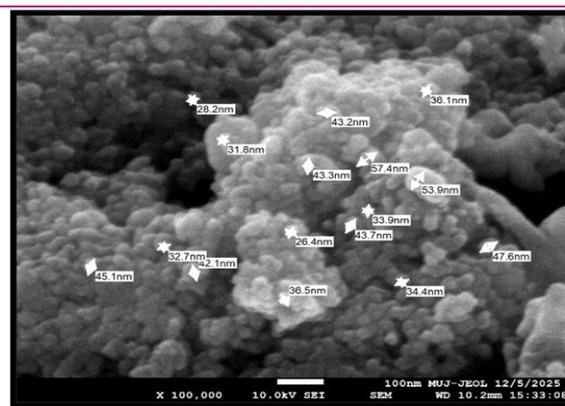


Fig. 3. Field emission scanning electron microscopy (FESEM) images of *Tradescantia pallida* derived silver nanoparticles

al., 2024).

In addition, plant-mediated AgNPs have been found to cause oxidative stress in mosquito larvae by producing reactive oxygen species (ROS) that exceed the antioxidant defence system's capacity and damage cellular components (Malla and Chandra, 2023). This further leads to oxidative damage, which is associated with significant changes in antioxidant enzymes and detoxification mechanisms, further impairing larval survival. Moreover, the ability of green-synthesized nanoparticles to affect crucial physiological enzymes, such as those involved in nervous system physiology and metabolism, has also been identified, further increasing larval stress and mortality (Pilaquinga *et al.*, 2024). Studies have also elucidated that silver nanoparticles can act through multiple, non-specific mechanisms (e.g., disruption of cellular membranes, oxidative stress, and protein denaturation), which may bypass conventional resistance pathways such as target-site mutations or enhanced detoxification commonly associated with pyrethroid resistance.

Characterization of silver nanoparticles

The *Tp*-AgNPs synthesized at 3 mM showed promising larvicidal potential and were selected for further biophysical characterization by SEM, EDAX, TEM, FT-IR, and XRD.

Field-emission scanning electron microscopy (FESEM)

FESEM analysis revealed the successful synthesis of *Tp*-AgNPs with a primarily quasi-spherical morphology with rough and granular surface texture. The particle size ranged from 26 to 57 nm, with most particles falling between 30 and 45 nm (Fig. 3), thereby indicating a narrow size distribution, as previously confirmed by UV spectral studies. SEM image also showed substantial agglomeration, a feature of nanocomposite systems. Collectively, these morphological characteristics confirmed homogeneous particle production, good disper-

sion, and efficient nano synthesis.

Several studies on biosynthesized nanoparticles have shown that the phytochemicals in the plant extract, which serve as reducing agents and contribute to the crystalline structure and spherical morphology of the nanoparticles, are responsible for the durability of silver nanoparticles (Sharma *et al.*, 2019, 2020). The inclusion of capping agents from plant extract makes silver nanoparticles more stable, which affects their spherical shape and crystalline structure—a phenomenon that has been well studied in a number of biosynthesized nanoparticles. The surface area and dimensions of silver nanoparticles significantly influence their biological activity; smaller nanoparticles have a greater surface area than larger ones. Previous studies indicated that spherical nanoparticles exhibit greater efficiency than other nanocomposite forms (Zhao *et al.*, 2017; Shahzadi *et al.*, 2022).

Transmission electron microscopy (TEM)

The presence of AgNPs with an average size of about 39 nm was further confirmed by the *Tp*-AgNPs TEM micrograph. Additionally, it was shown that AgNPs were long-lasting and polydispersed without direct con-

tact (Fig. 4a-c). This may result from a thin layer of biomolecule coating on their surface, which functions as a stabilizing agent (Vignesh *et al.*, 2013). Fig. 5 (d) represented selected area (electron) diffraction pattern of the *Tp*-AgNPs that showed the distinct, clear concentric diffraction rings indicating their polycrystalline nature. From the prominent diffraction ring, the reciprocal lattice spacings (g) were calculated as 6.74, 8.09, 9.44, 11.48, 12.78, and 14.50 nm^{-1} , corresponding to interplanar spacings (d) of approximately 0.148, 0.124, 0.106, 0.087, 0.078, and 0.069 nm, respectively. Furthermore, the presence of multiple concentric rings corresponding to the (111), (200), (220), and (311) planes confirmed the crystalline nature of AgNPs in the FCC structure.

Energy dispersive X-ray (EDX)

Metal nanoparticles' optical and elemental properties are strongly influenced by their shape, which can be evaluated using EDX (Xu and Käll 2002; Suman *et al.*, 2013). The EDX spectra of *Tp*-AgNPs showed distinct characteristic peaks for silver (Ag), confirming the successful incorporation of silver into the synthesized nanoparticles. The strong Ag L peak around ~ 3.0 keV and

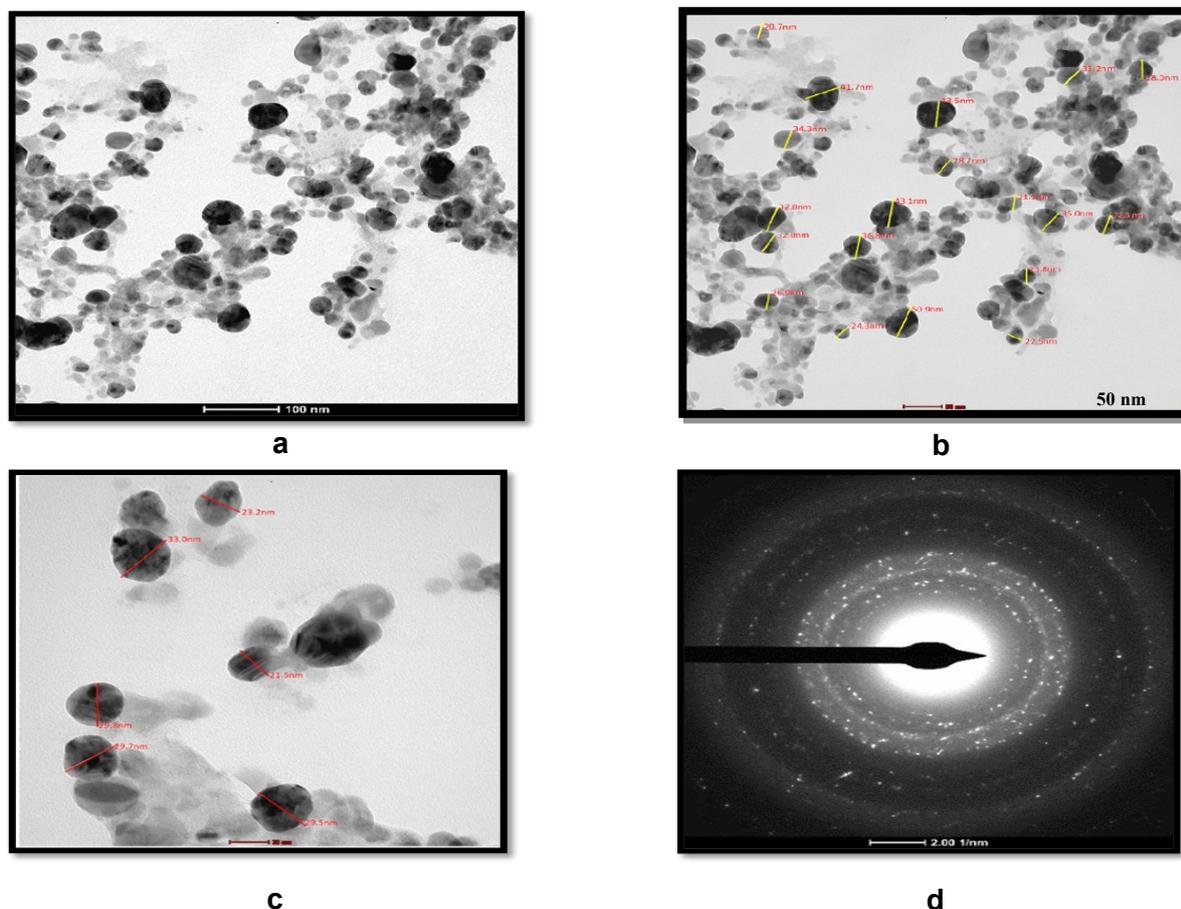


Fig. 4. Transmission electron microscopy (TEM) images of silver nano particles derived from aqueous leaf extract of *Tradescantia pallida*; [a] 100 nm; [b] 50 nm; [c] 20 nm and; [d] Selected area (electron) diffraction pattern

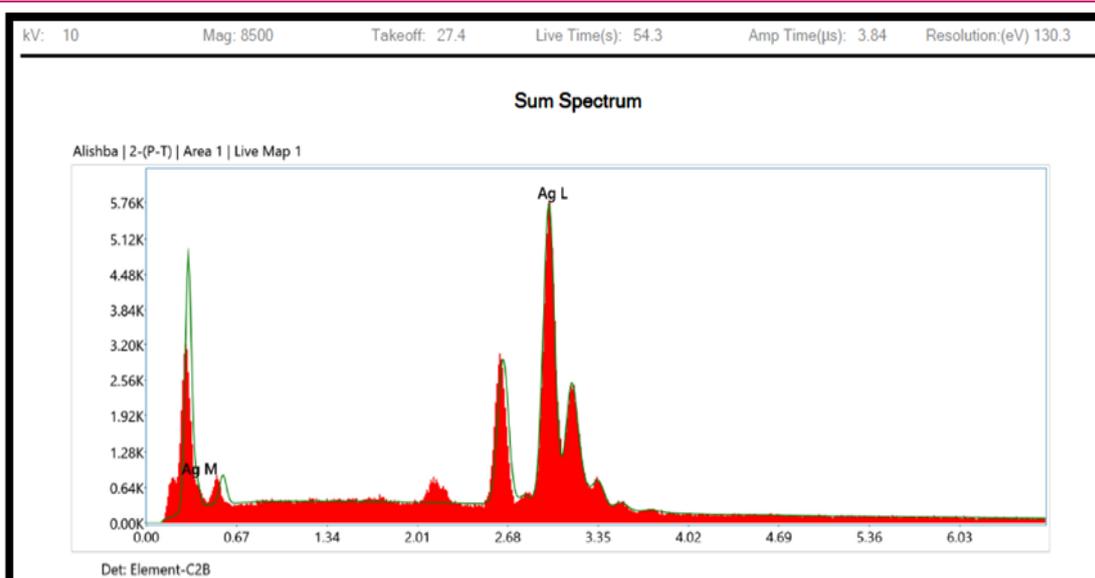


Fig. 5. Energy dispersive X-ray (EDX) spectrum of silver nanocomposites synthesized from aqueous leaf extract of *Tradescantia pallida*

the Ag M peak at lower energy (~0.3 keV) demonstrated substantial silver content and good signal intensity (Fig. 5). The presence of low-intensity peaks (impurity peaks) suggests that carbon (C) and oxygen (O) may be arising from supporting matrix or sample container peaks, indicating that the synthesis was free of significant contamination from other metallic elements. Our findings are supported by those of Okaiyeto *et al.* (2021), who reported that the XRD peaks of biosynthesized silver nanoparticles resembled those of cubic silver.

X-ray diffraction (XRD)

XRD analysis was employed to examine the crystalline structure of silver nanoparticles. The presence of eight diffraction peaks in the XRD pattern (Fig. 6) of *Tp*-AgNPs were seen at Bragg angles of 27.8°, 32.2°, 38.1°, 46.3°, 54.8°, 57.4°, 67.4°, and 76.9° corresponding to the (110), (111), (111), (200), (110), (311), (220), and (311) planes, respectively suggesting the crystalline and face-centred-cubic (FCC) structure of nanoparticles. The average crystallite size of *Tp*-AgNPs was estimated by using the Scherrer equation $D = K\lambda/\beta\cos\theta$. According to the graph, the most intense peak (111) was observed at $2\theta = 32.25^\circ$, and the FWHM of the (111) peak was 0.50. Accordingly, the size of crystallite was found to be 16.22 nm. The presence of certain phytochemical or organic compounds in the plant extract that coats and stabilizes the synthesized silver nanoparticles may explain the repeated, weak peaks observed in the XRD spectra (Krishnaraj *et al.*, 2010). Similar reflections at (111), (200), (220), and (311) of silver metal, confirming face-centred cubic symmetry, have been identified in the silver nanoparticles synthe-

sized from *T. pallida* leaf extracts in earlier studies by Shahzadi *et al.* (2022). They also documented the formation of primarily spherical AgNPs with a consistent morphology in the 30–80 nm size range, which aligns with our findings.

Fourier transform infrared (FT-IR) spectroscopy

The FT-IR spectra of AgNPs synthesized by aqueous leaf extract of *T. pallida* exhibited a total of eight peaks, stretches, and vibrations, suggesting the presence of several functional groups (Fig. 7). The spectra showed discernible peaks at 3247, 2947, 2118, 1846, 1636, 1606, 1417, and 625 cm^{-1} , indicating the presence of different phytochemicals in the *T. pallida* leaf extract, which are likely involved in reducing and stabilizing the AgNPs. A broad, intense peak was observed at 3247 cm^{-1} , which can be assigned to the O-H stretching vibration of the hydroxyl group present in phenols, alcohols, and flavonoids. The absorption band at 2947 cm^{-1} corresponds to C-H stretching vibrations of aliphatic CH_2 and $-\text{CH}_3$ groups, and suggests the presence of organic biomolecules such as terpenoids and alkyl chains from the plant extract. A weak band appearing at 2118 cm^{-1} may be assigned to $\text{C}\equiv\text{C}$ stretching vibrations indicating the presence of unsaturated phytochemicals, although this peak is typically weak and secondary in biosynthesized silver nanoparticles. The peak observed at 1846 cm^{-1} can be attributed to $\text{C}=\text{O}$ stretching vibrations, possibly arising from carbonyl groups of aldehyde, ketone, or ester functionalities present in plant metabolites (Dzhagan *et al.*, 2022; Kumar *et al.*, 2024).

A prominent absorption peak at 1636 cm^{-1} corresponding to $\text{C}=\text{O}$ stretching or amide vibrations, may be asso-

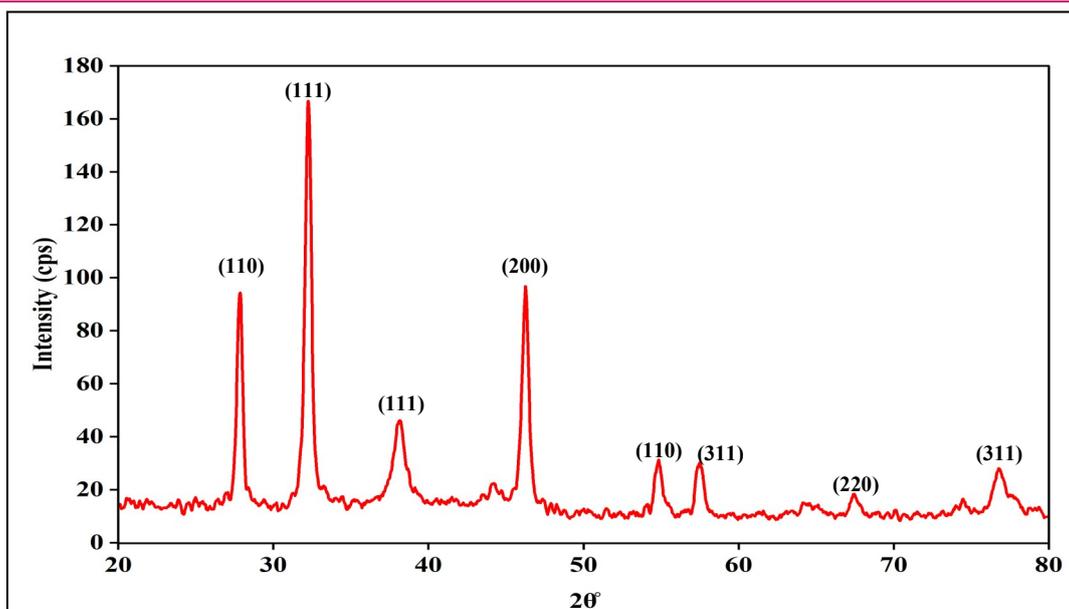


Fig. 6. X-ray Diffraction (XRD) pattern of *Tradescantia pallida*-mediated silver nanoparticles (*Tp*-AgNPs)

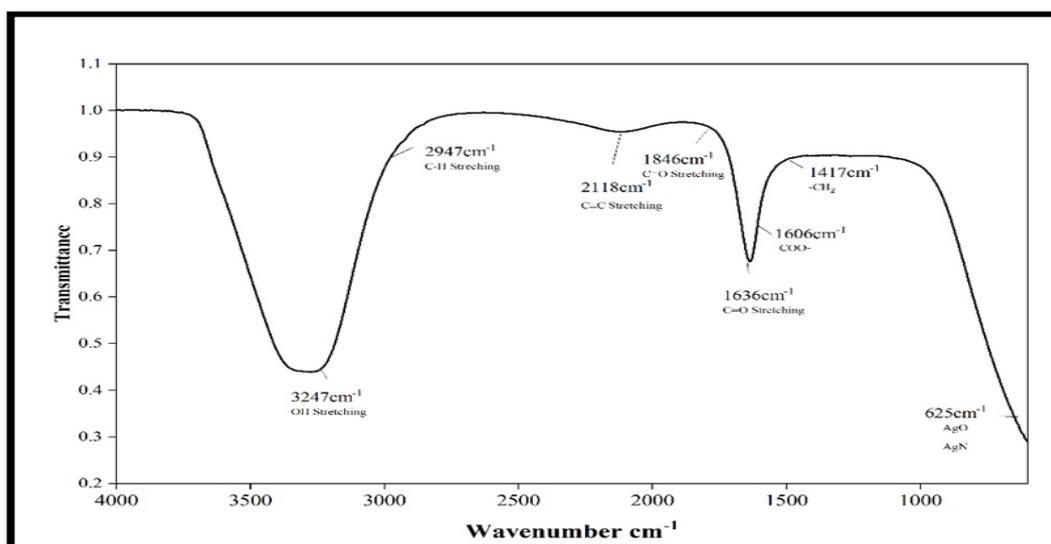


Fig. 7. Fourier transform infrared (FT-IR) spectra of silver nanoparticles prepared using aqueous leaf extract of *Tradescantia pallida*

ciated with proteins or polyphenolic compounds bound to the nanoparticle surface. Additionally, the band at 1606 cm^{-1} can be attributed to the asymmetric stretching of carboxylate ($-\text{COO}^-$) groups, further confirming the involvement of plant biomolecules in the stabilisation of the nanoparticles. The band at 1417 cm^{-1} , assigned to $-\text{CH}_2$ bending vibrations, supports the presence of aliphatic compounds and organic residues in the plant extracts (Madbouly *et al.*, 2015; Zenkin *et al.*, 2024). Finally, a low-frequency peak at 625 cm^{-1} may correspond to Ag-O or Ag-N vibrations, indicating successful interactions between silver ions and oxygen or nitrogen-containing functional groups, thereby confirming the formation of AgNPs (Aabed and Mohammad 2021; Sulaiman *et al.*, 2024). The FT-IR spectral analysis indicates the presence of several organic mole-

cules, including alkanes, alkenes, amines, aromatic amines, phenols, and halogenated compounds in the formulated nanoparticles, which are primarily attributed to flavonoids, glycosides, saponins, and proteins. These compounds are well known as reducing and stabilizing agents in the green synthesis of nanoparticles (Widatalla *et al.*, 2022; Sharifi *et al.*, 2023). Although similar plant-based AgNPs have been reported, this is the first study to demonstrate that *T. pallida*-derived silver nanoparticles exhibit strong, time-dependent larvicidal activity against *Aedes aegypti*. A few studies on *T. pallida* silver nanoparticles limited to cytotoxic, antioxidant, and antibacterial applications, and absence of research on mosquito vectors with this plant, provides a novel, environmentally friendly alternative for controlling insecticide-resistant mosquito pop-

ulations.

Conclusion

The synthesis of AgNPs is currently regarded as an environmentally sustainable, economical, and effective alternate to chemical and microbial insecticides. The present study revealed that AgNPs synthesised from aqueous leaf extracts of *T. pallida* exhibited effective larvicidal activity *in vitro* against *Ae. aegypti*, though substantially less than the commonly used chemical larvicides. The characterization of NPs by various techniques validated the existence of polydisperse AgNPs with an average diameter of ~41 nm. It was also observed that AgNPs exhibited polydispersity without direct established contact and maintained stability for an extended duration. The research showed that *T. pallida*-synthesised AgNPs are facile and rapid to synthesize, exhibit time-dependent stability over the experimental timeframe, and may be utilized sustainably against *Ae. aegypti* larvae, thereby qualifying them as potential mosquito intervention targets. However, further studies are required to identify the bioactive constituents in the extract and to assess their safe use in the field by assessing their effects on non-target organisms.

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Conflict of interest

The authors declare that they have no conflict of interest.

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