INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

OPEN ACCESS

Received: 22-03-2023

Accepted: 11-09-2023

Published: 17-10-2023

Citation: Yuvarajan R, Natarajan D, Karthika R (2023) Larvicidal Efficacy and Characterization of Silver Nanoparticles Derived from Aqueous Leaves Extracts of *Trichosanthes tricuspidata* against Dengue Vector *Ades aegypti*. Indian Journal of Science and Technology 16(39): 3258-3266. https://doi.org/10.17485/IJST/v16i39.664

* **Corresponding author**.

karthibtanish@gmail.com

Funding: None

Competing Interests: None

Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Larvicidal Efficacy and Characterization of Silver Nanoparticles Derived from Aqueous Leaves Extracts of *Trichosanthes tricuspidata* against Dengue Vector *Ades aegypti*

R Yuvarajan[1], D Natarajan[2], R Karthika[3]*

1 PG and Research Department of Biotechnology, Mahendra Arts and Science College (Autonomous), Kalipatti, Namakkal, 637501
2 Natural Drug Research Laboratory, Department of Biotechnology, Periyar University, Salem, 636 011
3 Department of Biotechnology, K.S. Rangasamy College of Arts and Science (Autonomous), Tiruchengode, 637 215, India

Abstract

Objectives: The present investigation aims to analyze the larvicidal activity of *T. tricuspidata* plant extract and silver nanoparticles from aqueous extracts of *T. tricuspidata*. **Methods:** silver nanoparticles synthesized from *T. tricuspidata* leaf extract, were evaluated against larvae of *A. aegypti*. Synthesis of AgNPs was confirmed using UV–Vis spectroscopy, and characterized using powdered X-ray diffraction, scanning electron microscopy and transmission electron microscopy. **Findings:** For characterization of green silver nanoparticle UV-visible spectroscopy has been used which yield pointed peak at 454nm. A particle size analyzer was used to characterization of the average size (181nm) distributions in the intensity (57.7%) of silver nanoparticles, and X-ray diffraction (XRD) of planes (111, 200, 220, and 311) intensity (2θ) peaks validated the silver ion's cubic structure. The highest mortality was determined from the silver nanoparticles against *A. aegypti* in the LC_{50} value of 30.928. **Novelty:** The nanoparticle synthesized from *T. tricuspidata* leaf extract was found to excellent toxic effect against the disease transmitting vector mosquitoes. The silver nanoparticles is a rapid, eco-friendly, and a single-step approach and the AgNps formed can be potential mosquito larvicidal agents.

Keywords: Larvicidal Activity; Silver Nanoparticles; SEM; T. Tricuspidata; A. Aegypti

1 Introduction

Mosquitoes spread Malaria, Dengue, Chikungunya, Filariasis, and Japanese encephalitis, creating a major public health problem among the other diseases that result in significant morbidity and mortality each year around the world [1]. Dengue fever presently threatens around 2500 million people, or two-fifths of the world's population. Accord ing to World Health Organization it is estimated that more than 50 million people affected by Dengue virus. It causes a severe social health issue around the globe, commonly in equatorial regions where the vector, *Aedes aegypti*, an anthropophillic species which thrives due to favorable environmental circumstances and diverse kinds of arboviruses are found in India. *Aedes aegypti* is a type of mosquito that breeds in freshwater, controlling it during the rainy season is extremely challenging and it is accountable for spreading vector-borne diseases in humans all over the world[(2)].

Synthetic chemical pesticides are widely used to control mosquitoes, but as these pesticides are non-biodegradable, they cause pollution and have a negative impact on non-target organisms. Thus, plant-based biopesticides with promising effects are an alternative for these pesticides. Several studies revealed that the use of plant based nanoparticles to control these type of mosquito larvae and that are easily biodegradable, no impact on non – target organisms and very safe to environment[(3)]. *Trichosanthes tricaspidatar*, often known as Indrayan, is a medicinally significant plant and various plant parts, such as the fruits, bark, roots have medicinal value such as carminative, purgative, abortifacient, and violent hydragogue; useful in asthma, earache, ozoena, inflammations, epilepsy, and rheumatism; cures hemicrania, limb weakness, ophthalmic, and leprosy; useful in asthma, earache, ozoena, inflammations, epilepsy, and rheum[(4)].

A promising substitute for the direct use of plant-derived bioactive compounds as larvicides is the production of silver nanoparticles (AgNPs) from plant extracts. Plant material is suitable for the synthesis of AgNPs because it can contain secondary metabolites that act as reducing, capping, and stabilising agents, resulting to related shortened reaction times. Green nanoparticles can be created utilising affordable, biocompatible technologies. After attaching to proteins or DNA, phyto-synthesized AgNPs have the capacity to enter the mosquito's cells through the exoskeleton and cause mortality. They also alter DNA and alter enzymes, but their impact on non-target animals like fish and helpful arthropods is minimal[(5)]. Therefore, the present study is intended to explore the nanoparticle synthesizing ability of *T. tricuspidata* species and its efficacy is evaluated against of *Ades aegypti* under laboratory condition.

2 Methodology

2.1 Collection of Plant Materials

Fresh leaves of *T. tricuspidata* were collected from high altitudes (2000 to 3000 feet height) of Kalvarayan hills (latitude 11.738187, longitude 78.963882), Salem District, Tamil Nadu, India. The collected plant material was authenticated by the Botanical Survey of India (reference number: BSI/SRC/5/23/2016/Tech./454), Coimbatore, Tamil Nadu, India. The voucher specimen (specimen number: PU/BT/NDRL/2016/201) was deposited in Natural Drug Research Laboratory (NDRL), Department of Biotechnology, Periyar University, Salem, Tamil Nadu, India for future reference. The collected plant leaves were checked for microbial infections, washed with running tap water to get eliminate the solid dirt and dried over shadow at room temperature for 2 weeks. A commercial electric blender was used to powder the dried plant material.

2.2 Preparation of Extracts

The powdered plant material (500 g) was extracted in an increasing polarity manner using different solvents such as petroleum ether, chloroform, methanol, and water for 48 to 72 hours till the efflux solvent turned colorless. The extracts were purified using Whatman filter paper No. 1 before being concentrated at 40°C under decreased pressure. All of the extracts were placed in an airtight container and stored at 4°C for cognitive testing.

2.3 Synthesis and Characterization of Silver Nanoparticles

The methanolic crude extracts of *T. tricuspidata* were elected for the synthesis of AgNPs which harbor superior larvicidal potential with the lowest LC_{50} value. 10 ml of 5% (w/v) *T. tricuspidata* leaf methanol extract (as a reducing agent) was blended with 1 mM of silver nitrate solution (90 ml) in a glass container and kept in constant stirring (400 rpm) condition using a magnetic stirrer for 12 hours in the dark at room temperature to synthesize AgNPs. After the incubation period, color changes (greenish-yellow to dark brown) were examined in the reaction mixture which shows the formation of AgNPs. Furthermore, 1 ml of the reaction mixture were subjected to UV–Visible absorption maxima scanning analysis at 300 to 700 nm using a UV–Visible spectrophotometer (Beckman Du 640 UV Vis Spectrophotometer T90 UV–Vis spectrophotometer, PG instruments Ltd, India) to confirm the presence of AgNPs in the reaction mixture. Synthesized AgNps were spirited from the reaction mixture by using repeated centrifugation process and spin at 10,000 rpm for 15 min, and the pellet were re-dispersed with double distilled water and subsequently with methanol.

The purified pellet was dried at 60°C in an oven and stored in an airtight container for further investigations. The shape, size and elemental composition of synthesized AgNPs were investigated using various techniques such as SEM (TESCAN, VEGA3, Czech Republic) and TEM. The surface of AgNPs bioactive functional groups were determined by FT-IR analysis (FT/IR 4000 series, JASCO). The crystalline nature of the AgNPs was studied by XRD (Rigaku Miniflex II desktop X-ray diffractometer instrument, Japan) analysis.

2.4 Mosquito Source

The larvae of *Aedes aegypti* were collected from the paddy fields in and around Omalur region, Salem (Dt), Tamil Nadu, India, which are accompained in the Natural Drug Research Laboratory, Department of Biotechnology, Periyar University, Salem. The larvae was kept in plastic trays filled with tap water, fed a 3:1 diet of Brewer's yeast and powdered dog biscuits daily, and conserved at 27°C with 75-85 percent relative humidity and a 14:10 light/dark photoperiod. The late third instar and early fourth instar *Aedes aegypti* larvae were chosen for the larvicidal bioassay from the Laboratory's stock cultures.

2.5 Larvicidal Assay

The larvicidal potential of various solvent crude extracts of T. tricuspidata and AgNP's were assessed as per the standard protocol of[6] WHO (2005). Briefly, in a plastic container 25 early fourth instar larvae of *A. aegypti* were conserved in 249ml of double distilled water with 1ml of various concentrations (100, 200, 300, 400 and 500 mg/L) of plant crude extracts and AgNPs (100, 200, 300, 400 and 500 mg/L) which were dissolved in the DMSO. A 0.02% (v/v) tween-80 and 1ml of DMSO served as an emulsifier and negative control respectively. The total number of dead larvae was documented after 24 hours of incubation and the mortality rate was amended with Abbott's formula[7]. The percentage of mortality was calculated from three independent experiments of triplicates.

2.6 Statistical Analyses

The standard deviation (SD) of all experiments was used to represent all data given in this study, which was derived from triplicates. SPSS software was used for statistical analysis (16.0 version). Tukey's multiple range tests with 95 percent confidence limits at p0.05 were used to compare any significant differences between samples, and analysis of variance (ANOVA) was utilised for all data in a randomised design. Probit analysis was used to determine the lethal concentrations (LC50, LC90, and LC99) at 95 percent upper and lower confidence levels (UCL and LCL), as well as chi-square values, from the average larvicidal data.

3 Result and Discussion

Researchers are evaluating the effectiveness of silver nanoparticles against the parasites that cause the two most dangerous diseases in the world, malaria and dengue, due to their broad spectrum antibacterial characteristics. As opposed to other techniques, the synthesis of AgNps employing microorganisms such bacteria, fungus, actinomycetes, and extracts of different plant sections is more environmentally friendly. After addition of 10ml of *T. tricuspidata* (100g/ml) to the silver nitrate solution (90ml) resulted in light yellow hue shifting to reddish brown, indicating the presence of green based silver nanoparticles.

3.1 Characterization

3.1.1 UV Visible Spectroscopy

The silver nanoparticles were confirmed by the formation of yellow colour to reddish brown. The UV-Vis spectra taken at various stages during the reaction from the flask containing brown-colored colloidal silver reveal increasing colour intensity. The SPR band of green silver nanoparticles synthesized from the extract of *T. tricuspidata* showed maximum absorbance 420 nm which showed in Figure 1. This result confirming the synthesis of silver nanoparticles due to reduction of Ag ions by the phytochemicals present in the *T. tricuspidata* extract. Similar results were observed from the plant extracts of *Momordica charantia*[8] and *Lagenaria siceraria*[9]. Due to the excitation of surface plasmon vibrations in the silver nanoparticles the color changes denoted silver nanoparticles formation[10].

3.1.2 FTIR spectroscopy

The possible functional group of methanolic extracts of *T. tricuspidata* was identified using FT-IR analysis. 11 groups were detected from the wavelength ranged from 500 to 3500cm^{-1}. Briefly, 3447.76 cm^{-1} was denoted that alcohol or phenolic groups (OH stretching), 2999.34 and 2914.11 cm^{-1} indicates the alkanes groups (CH stretching) cm^{-1}, the amine acted as the functional group was detected in the ranges of 2110.45 and 1436.84 cm^{-1} (NH stretching), The sulfur derivatives of the functional group was detected in the range of 1314.13cm^{-1} (S-O stretching), the primary alcoholic groups expressed in the range of 1025.47cm^{-1}

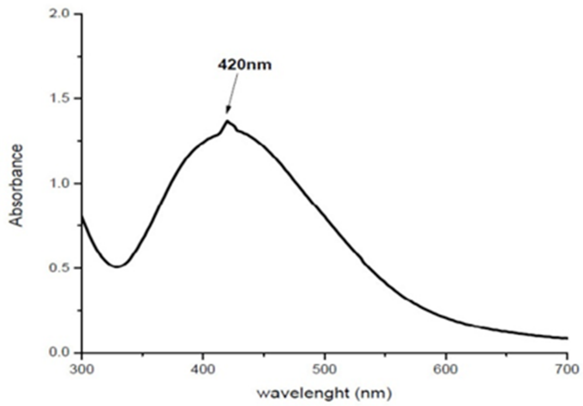

Fig 1. UV–Vis spectrum of AgNPs synthesized using *T. tricuspidata* extract

(C- OH stretching), alkenes group of compounds were identified from the wavelength range of 954.43 (C-O strecting) and rest of the wavelength 703.84 and 670.71cm^{-1} denoted that halogen compounds (C-Br stretching) (Figure 2). The band at 3315 cm^{-1}corresponds to O-H or N-H stretch contains alcohol, phenols, primary, secondary amines or amides, respectively[11]. The peak at 3199 cm^{-1} belongs to O-H stretch of carboxylic acid. The prominent band obtained at 1672 cm^{-1} assigned to carbonyl peak C=O stretching indicate carboxylate content in plant samples ([11]).

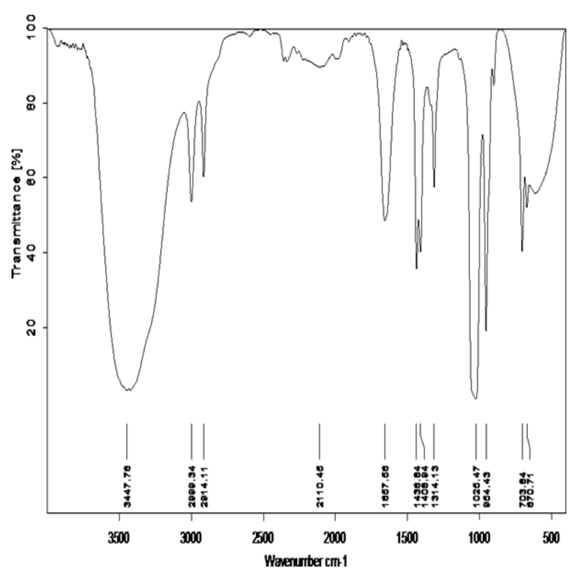

Fig 2. FT-IR Spectrum of synthesized silver nanoparticles

3.1.3 Particle Size Distribution

The distribution of particle size in fraction *T. tricuspidata* of green produced silver nanoparticles was investigated, and the results revealed that it was dispersed in varied sizes in a poly-dispersible mode showed in Figure 3. The particle size in colloidal solution was measured using DLS. The first and second peaks showed the particle size, which were found to be 119.6 and 4350 nm, respectively, with diameters of 60.59 and 941.3 nm, and peak intensity of 94.6 percent and 5.4 percent. Silver nanoparticles had an average size of 101.2 nm. It was discovered that the polydispersity index was 0.307. Similar kinds of peaks are reported by Jeyanthi[10], the size distribution of silver nanoparticles was also reported in plant extracts of *Dracaena mahatma*[12].

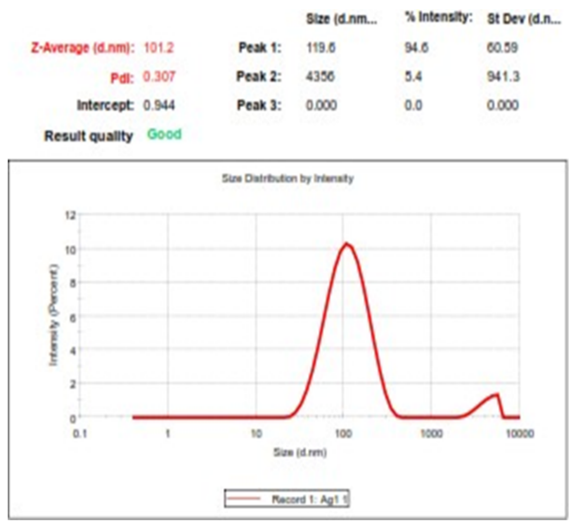

Fig 3. Particle Size Analysis of AgNPs from fraction 2 of *T. tricuspidata*

3.1.4 X-ray diffraction

The associated Bragg reflections are also visible in the intensity peaks of the XRD pattern produced for the plant silver nanoparticles (111), (200), and (220) sets of lattice planes, as evidenced by varied peak ranges at 38.87, 44.96, 47.07, and 58.07, which correspond to silver peaks showed in Figure 4. These results match the standard joint committee for powder diffraction set (JCPDS) (File No: 040783), indicating that silver nanoparticles have a face-centered cubic lattice. The nano dimensional condition of the synthesised system is inferred by the crisp and broad diffraction pattern. Thus, XRD spectrum confirmed the formation of silver nanoparticles. The particles' numerous peaks refer to their multi-faceted development orientation as compared to discussion by Shankar [13]. The same kind of peaks visualized in the leaf extracts from *Erythrina indica* and *Eclipta alba* also showed broad-spectrum activity [14].

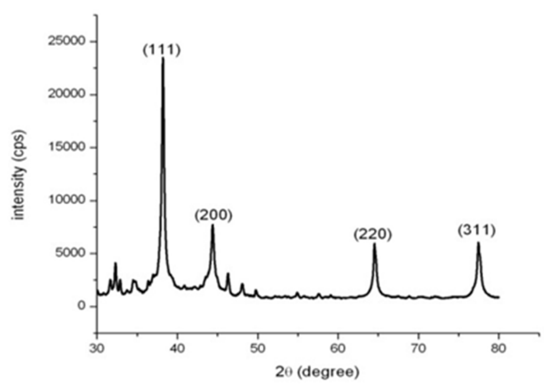

Fig 4. X-ray diffraction pattern of AgNPs synthesized using *T. tricuspidata* extract

3.1.5 Scanning Electron Microscopy

Surface morphology includes size, shape and surface of the AgNps was characterized by SEM examination (Figure 5). The AgNPs were produced and evaporated onto a clean glass slide, which was then covered and allowed to dry completely at room temperature. The produced silver nanoparticles are spherical in shape, with diameters ranging from 12 to 18 nm, according to the SEM micrograph. The micrograph shows silver aggregates on *T. tricuspidata*; silver nanoparticles was characterized using

SEM by various researchers discovered spherical silver nanoparticles from plant extracts of various sizes, including *Padina tetrastromatica*[15], *Mukia maderaspatana*, *Kedrostis foetidissima*, and *Cayra tiapedata*[16].

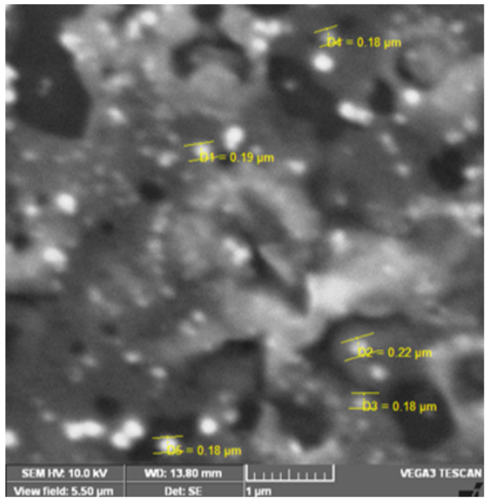

Fig 5. SEM image of AgNPs synthesized using *T. tricuspidata* extract

3.1.6 High Resolution Transmission Electron Microscope

The HR-TEM images of AgNPs derived from methanol (fraction 2) extract of *T. tricuspidata* showed the morphology of the AgNPs was almost spherical in nature(Figure 6). But some of the AgNPs was found to be oval or elliptical. It was noticeable that the edges of the particles were lighter than the centers, it might be suggesting that there is presence of some biomolecules in that location. TEM analysis showed that most particles had a size between 7-10nm. The results were similar to the size of silver nanoparticles determined in *Berberis aristata*[17], *Aloe vera*[18], *Origanum vulgare*[19]. *Musa balbisiana*, *Azadirachta indica* and *Ocimum tenuiflorum*[20].

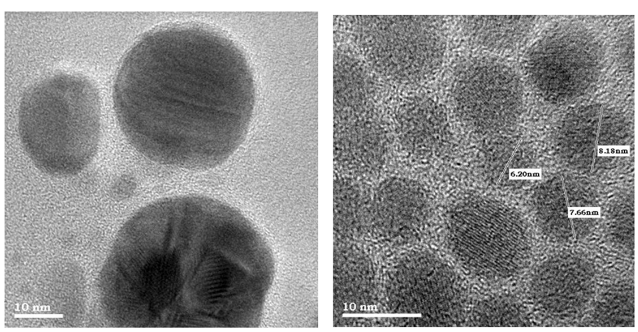

Fig 6. HRTEM image of AgNPs synthesized using *T. tricuspidata* extract

3.1.7 Larvicidal activity

Plant-based Silver nanoparticles are a potential source of microbial, insecticidal and also larvicidal compounds and the present investigation deals with the larvicidal potential of silver nanoparticles from the aqueous leaves extract of *T. tricuspidata* along with different crude extracts of methanol, water, chloroform and petroleum ether. The larvicidal potential of silver nanoparticles showed significant activity against dengue vector in the lowest LC_{50} value of 30.93μg/ml. Similarly, in this study methanolic extracts of *T. tricuspidata* also exposed the mortality in the LC_{50}(42.746mg/ml) in the highest concentrated level than silver nanoparticles showed in Figure 7. The earlier report stated that the different solvents extract showed moderate mortality against *A. aegypti* and the LC_{50} values were 112.19, 137.48, 118.67, 102.05, and LC_{90} values of 91.20 and 119.58, 146.34, 124.24, 110.12, and 99.54 ppm, respectively[21]. Adult mortality was highest in the methanol extract of *Aedes paniculata* against *Aedes aegypti* adults, with values of 172.37 ppm (LC_{50}) and 321.01 ppm (LC_{90})[22]. *Cassia tora* leaf extracts (hexane, chloroform, benzene, acetone, and methanol) had LC_{50} and LC_{90} values of 329.82, 307.3, 287.15, 269.57, and 252.03 ppm, respectively, against *Aedes aegypti*, and LC90 values of 563.24, 528.33, 496.92, 477.61, and 448.05 ppm,[23]. Similarly, when the leaves extracts of *C. asiatica* were tested against third instar larvae of *Anopheles stephensi*, *Aedes aegypti*, and *Cx. quinquefasciatus* with a range of varying concentrations of synthesised AgNPs like 8, 16, 24, 32, and 40 g/mL were compared to aqueous leaf extract at concentrations of 40, 80, 120, 160, and 200 g/mL[24]. The researchers tested the larvicidal activity of leaf extracts of *Ambrosia arborescens* and green-synthesized silver nanoparticles (AgNPs) against third instar larvae of *Aedes aegypti* after exposing them for 24 hours to aqueous plant extracts at various concentrations such as 1500, 3000, 4500, and 6000 ppm, as well as plant-synthesized AgNPs at 0.2 AgNPs were more hazardous in the LC50 = 0.28 ppm and LC_{90} = 0.43 ppm ranges than the plant extract in the LC_{50} = 1844.61 ppm and LC_{90} = 6043.95 ppm ranges[24,25]. The LC_{50} and LC_{90} values of synthesised AgNPs against *Aedes aegypti* larvae were (37.87 and 132.86 ppm) and *C. quinquefasciatus* larvae were (14.70 and 28.96 ppm), respectively. *P. virens* AgNPs were found to have the highest mortality rate against *Aedes aegypti* and *C. quinquefasciatus* larvae[26]. The production of silver nanoparticles (AgNPs) with *Plumbago auriculata* aqueous extract and evaluation of larvicidal properties. At concentrations of 45.1 and 41.1 g/mL, produced nanoparticles suppressed the fourth instar larvae of *Aedes aegypti* and Culex quinquefasciatus, respectively. Acetylcholinesterase (AChE) is an enzyme that breaks down acetylcholine (AChE), an important neurotransmitter in the central nervous system (CNS) of insects. Nanoparticles may bind to and suppress the action of this enzyme. Dose-dependent experiments revealed that manufactured nanoparticles were effective at low doses as well. As a result, the findings suggest that plant extracts and nanoparticles may be a better alternative to currently available insecticides for mosquito control.

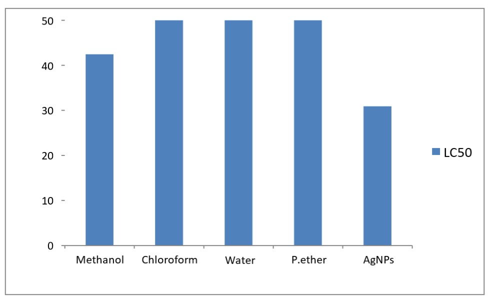

Fig 7. Larvicidal potential of plant extracts and AgNPs from *T. tricuspidata*

An objective of this study was compare to synthesised garlic AgNPs with published data evaluating the potential insecticidal activity of other candidate AgNPs (synthesised from other plant species), in order to support the selection of the best formulations for further development work. This was done due to the different toxicity mechanisms discussed above. As a result, the current work chose to use two instars that were frequently examined in prior studies. Larvicidal efficacy increased directly with exposure duration within the dose range utilised in this study, with a commensurate decline in LC50 values. In preliminary analyses, a greater mortality was noted for 2nd instar larvae than for 3rd instars. Tests on seven indigenous plants, including *A. sativum* and *Zingiber officinale* (Zingeraceae), revealed a strong concentration-dependent link with larval mortality in *Culex quinquefasciatus* (Say) larvicidal efficacy. Thus, the results of the current research were mirrored in their study[27].

They exhibit considerable potential in these areas as an alternative control agent for disease vectors. They therefore require additional research, including the possibility of reducing the doses utilised in the field, as they serve as the foundation for potentially valuable improvements to sustainable pest management methods for insect vectors of human diseases like mosquitoes.

4 Conclusion

In conclusion, the results of the current green synthesis demonstrate that the extract of *T. tricuspidata* leaves can function as a powerful reducing agent when creating silver nanoparticles. The creation of silver nanoparticles that are hydrophilic in nature, disperse uniformly in water, are highly stable, and have significant mosquito larvacidal activity against *Aedes aegypti* would be a major benefit of this biological reduction of metal. The current work demonstrates that *T. tricuspidata*'s silver nanoparticles are an efficient, environmentally friendly technique for managing mosquitoes that transmit diseases to humans.

Acknowledgment

The authors sincerely thank the Department of Biotechnology, Periyar University, Salem for providing the laboratory facilities to carry out toxicity assays and thankful to the Centre for Nanoscience and Technology, Anna University, Chennai to provide characterization facilities for nanomaterials.

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