

Larvicidal and pupicidal activity of synthesized silver nanoparticles using *Leucas aspera* leaf extract against mosquito vectors, *Aedes aegypti* and *Anopheles stephensi*

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Abstract

Mosquitoes are one of the most medically significant groups of vectors, having an ability to transmit parasites and pathogens that can have devastating impacts on humans. The development of reliable and eco-friendly processes for the synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology. In this study, we address the biosynthesis of silver nanoparticles (AgNPs) using *Leucas aspera* leaf extract, and evaluate its lethal concentration (LC₅₀ and LC₉₀) values against first to fourth instar larvae and pupae of the mosquito vectors, *Aedes aegypti* and *Anopheles stephensi*. The nanoparticles were characterized by UV-Vis spectrum, scanning electron microscopy, energy-dispersive X-ray spectroscopy, and Fourier-transformed infrared spectroscopy analysis. Larvae and pupae were exposed to varying concentrations of aqueous extracts of synthesized AgNPs for 24 h. The maximum mortality was observed from synthesized AgNPs,

with LC₅₀ values for I-IV instars and pupae ranging from 13.06 to 25.54, and LC₉₀ values ranging from 24.11 to 47.34 for *Ae. aegypti*; for *An. stephensi*, the corresponding LC₅₀ values ranged from 12.45 to 22.26, and the LC₉₀ values ranged from 23.50 to 42.95. With methanol leaf extract of *L. aspera* against *Ae. aegypti*, the LC₅₀ values ranged from 174.89 to 462.96 and the LC₉₀ values ranged from 488.16 to 963.74; for *An. stephensi*, the corresponding LC₅₀ values ranged from 148.93 to 417.07 and the LC₉₀ values ranged from 449.72 to 912.94. The study suggests that nanoparticles could be a preferred alternative to the most hazardous existing chemical pesticides, contributing to a more healthy environment by providing an ideal ecological and user-friendly vector control strategy for managing malaria and dengue, and contributing to their eventual elimination in the near future.

Introduction

Mosquitoes are the principal vectors of many vector-borne diseases affecting human beings and animals, in addition to being nuisance pests. Vector-borne diseases in India, *e.g.*, malaria, dengue, chikungunya, filariasis, Japanese encephalitis, and leishmaniasis, cause thousands of deaths per year. India reports 1.48 million malarial cases and about 1173 deaths, 1.4 million suspected and 1985 confirmed chikungunya cases, 5000 Japanese encephalitis cases and approximately 1000 deaths, and 383 dengue cases and 6 deaths during 2006 and 2007 (Kumar *et al.*, 2007; WHO, 2007; Gopalan & Das, 2009; Dhiman *et al.*, 2010). Malaria infects more than 500 million people each year, killing approximately 1.2 to 2.7 million annually. Among the 109 malaria endemic countries, India had 1.5 million confirmed malaria cases in 2009 with over 1000 deaths (WHO, 2010). *Anopheles stephensi* is the primary vector of malaria in India and other West Asian countries, and improved methods of control are urgently needed (Burfield & Reekie, 2005; Mittal *et al.*, 2005). Malaria, caused by *Plasmodium falciparum*, is one of the leading causes of human morbidity and mortality from infectious diseases, predominantly in tropical and subtropical countries (Snow *et al.*, 2005). The highest number of malaria (*P. falciparum*) cases and malaria-related deaths are recorded from the state of Orissa located in the eastern part of India (Sharma *et al.*, 2010).

Dengue fever/dengue hemorrhagic fever (DHF) continues to be of major public health importance in countries of the Western Pacific and Southeast Asia. These regions are experiencing an increase in the frequency of epidemics. Since 1963, outbreaks of dengue/DHF have been recorded in almost all parts of India. The increasing trend of dengue outbreaks accompanied by DHF is posing a problem of utmost importance to the public health of India (WHO, 1999). Botanical and microbial insecticides have been increasingly used for mosquito control

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because of their efficacy and documented non-toxic effects on non-target organisms (Ascher *et al.*, 1995).

Biosynthesis of nanoparticles using plant extracts is currently under development. Use of plants for the synthesis of nanoparticles could be advantageous over other environmentally benign biological processes, as this eliminates the elaborate process of maintaining cell cultures. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracellularly using plants or their extracts in a controlled manner according to their size, shape and disparity (Kumar & Yadav, 2009). In recent years, plant-mediated biological synthesis of nanoparticles has gained importance due to its simplicity and eco-friendliness.

The plant, *Leucas aspera* Wild., a member of the Lamiaceae family and commonly known as *Thumbai*, is distributed throughout India. It is pungently aromatic and commonly used as an antipyretic herb in South India. The juices from the leaves are used as an external application for psoriasis and painful swelling. The flowers are used with honey to treat coughs and cold in children. The leaves are useful for the treatment of chronic rheumatism. Crushed leaves are applied to the bites of snakes, poisonous insects, and scorpion stings (Srinivasan *et al.*, 2011). The plant is used traditionally as an antipyretic and its insecticidal flowers are valued as a stimulant, diaphoretic, insecticide and emmenagogue. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. *L. aspera* leaves are used as an insecticide and mosquito repellent in rural areas (Sadhu *et al.*, 2003; Maheswaran *et al.*, 2008). Many researchers have reported on the effectiveness of plant extracts against mosquito larvae (Govindarajan *et al.*, 2008; Kovendan *et al.*, 2012b, 2012c, 2012d). In the present study, to evaluate the larvicidal and pupicidal activity of AgNPs synthesized using *L. aspera*, leaf extract was assessed under laboratory conditions against the target species of *An. stephensi* and *Ae. aegypti*.

Materials and methods

Collection of plants and preparation of extracts

L. aspera plants were collected from in and around Bharathiar University Campus, Coimbatore. *L. aspera* leaves were washed with tap water and shade-dried at room temperature (28±2°C) for 5 to 10 days. The air-dried leaves were powdered using an electric blender; 500 g of the powder was gradually macerated in 1.5 L of methanol over a period of 72 h and filtered. The total yield of the *L. aspera* crude extract using the methanol extraction method was 13.6 g. The extracts were concentrated at a reduced temperature on a rotary vacuum evaporator and stored at 4°C. One gram of the plant residue was dissolved in 100 mL of acetone, which was considered a 1% stock solution. From this stock solution we prepared various concentrations (50, 150, 250, 350 and 450 ppm). AgNO₃ was purchased from the Precision Scientific Company (Pvt Ltd., Coimbatore, Tamil Nadu, India).

Synthesis of silver nanoparticles

Leaves were washed with distilled water and dried for 3 d at room temperature. A plant leaf broth was prepared by placing 10 g of the leaves (finely cut) in a 300 mL flask with 100 mL of sterile distilled water. This mixture was boiled for 5 min, decanted, stored at -4°C, and used in our tests within 1 week. The filtrate was treated with aqueous 1 mM AgNO₃ solution in an Erlenmeyer flask and incubated at room temperature. As a result, formation of a brown-yellow solution, indicating the formation of AgNPs, it was found that aqueous silver ions can be reduced by an aqueous extract of plant parts to generate extremely stable silver nanoparticles in water.

Characterisation of silver nanoparticles

Presence of synthesized silver nanoparticles was confirmed by sampling the reaction mixture at regular intervals, and the absorption maxima was scanned by UV-Vis spectra, at a wavelength of 200-700 nm using a UV-3600 Shimadzu spectrophotometer at 1 nm resolution. The reaction mixture was then centrifuged at 15,000 rpm for 20 min, and the resulting pellet was dissolved in deionized water and filtered through a Millipore filter (0.45 µm). An aliquot of this filtrate containing silver nanoparticles was used for scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS) and Fourier transformed infra red spectroscopic (FTIR) studies. The structure and composition of freeze-dried purified silver particles was analysed by using a 10 kV ultra high resolution scanning electron microscope; 25 µL of the sample was spatter-coated on a copper stub and the images of nanoparticles were studied using FEI QUANTA-200 SEM. The surface groups of the nanoparticles were qualitatively confirmed by using FTIR spectroscopy (Stuart, 2002), with spectra recorded by a Perkin-Elmer Spectrum 2000 FTIR spectrophotometer. In addition, the presence of metals in the sample was analysed by EDS.

Mosquito culture

An. stephensi and *Ae. aegypti* eggs were collected from the National Centre for Disease Control field station Mettupalayam, Tamil Nadu, using an *O* type brush. These eggs were brought to the laboratory and transferred to 18×13×4 cm enamel trays containing 500 mL of water for hatching. The mosquito larvae were fed pedigree dog biscuits and yeast in a 3:1 mixture. The feeding was continued until the larvae developed to the pupal stage. The pupae were collected from the culture trays and transferred to plastic containers (12×12 cm) containing 500 mL of water using a dipper. The plastic jars were kept in a 90×90×90-cm mosquito cage for adult emergence. Mosquito larvae were maintained at 27±2°C, 75-85% relative humidity, under a photoperiod of 14:10 (light/dark). A 10% sugar solution was provided for a period of 3 days before blood feeding. The adult female mosquitoes were allowed to feed on the blood of a rabbit (one rabbit per day, exposed on the dorsal side) for 2 days, to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

Larval and pupal toxicity test

The larvicidal activity was assessed by the procedure of WHO (1999) with some slight modification and as per the method of Kovendan *et al.* (2012a). Twenty-five specimens of first to fourth instar larvae and pupae were introduced into a 500-mL glass beaker containing 249 mL of dechlorinated water, and 1 mL of the desired concentrations of leaf extract and synthesized silver nanoparticles (10, 15, 20, 25 and 30 ppm) were added. Larval food was provided also for the test larvae. At each tested concentration, two to five trials were conducted and each trial consisted of five replicates. The control was set up by mixing 1 mL of acetone with 249 mL of dechlorinated water. The larvae and pupae were exposed to dechlorinated water without acetone, which served as a control. The control mortalities were corrected by using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control} \times 100}{100 - \text{Control mortality}} \quad (1)$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae/pupae} \times 100}{\text{Number of larvae/pupae introduced}} \quad (2)$$

Statistical analysis

The average larval and pupal mortality data were subjected to probit analysis for calculating the LC₅₀, LC₉₀, and other statistics at the 95% upper and lower fiducial limits, and Chi-square values were calculated by using the method of Finney (1971). The statistical software package SPSS (2007) version 16.0 was used. Results with P<0.05 were considered to be statistically significant.

Results and discussion

The results of the larvicidal and pupicidal mortality caused by leaf extracts of *L. aspera* and synthesized AgNPs against *Ae. aegypti* and *An. stephensi* are presented in Tables 1-4 and Figures 1 and 2. The LC₅₀ and LC₉₀ values of *L. aspera* and synthesized AgNPs against first to fourth instars larvae and pupae of *An. stephensi* and *Ae. aegypti* can be summarized as follows: the LC₅₀ values of the methanol extracts of *L. aspera* against instars I-IV and pupae of *Ae. aegypti* ranged from 174.89 to 462.96, and the LC₉₀ values ranged from 488.16 to 963.74; for *An. stephensi*, the LC₅₀ and LC₉₀ values ranged from 148.93 to 417.07 and 449.72 to 912.94, respectively. The corresponding LC₅₀ and LC₉₀ values

of the synthesized AgNPs against *Ae. aegypti* ranged from 13.06 to 25.54 and 24.11 to 47.34, respectively; against *An. stephensi*, the LC₅₀ and LC₉₀ values ranged from 12.45 to 22.26 and 23.50 to 42.95, respectively.

All the larvae and pupae tested showed evidence of a lethal effect, and mortality was positively dose-dependent against mosquito vectors, *An. stephensi* and *Ae. aegypti*.

Larvicides play a vital role in controlling mosquitoes in their breeding sites. The present study was carried out to establish the larvicidal activities of biosynthesized silver nanoparticles (AgNPs) against *An. stephensi* responsible for diseases of public health importance. The use of natural product chemistry coupled with nanotechnology that reduces mosquito populations in the larval stage can provide many associated benefits to vector control. Since silver nanoparticles are considered to be potential agents for various biological applications including antimicrobials, their application as a mosquito larvicide was investigated. Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest control agents. The botanical extracts from the plant leaves, roots, seeds, flowers and bark in their crude form have been used as conventional insecticides for centuries. Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality to target organisms to be effective (Kabaru & Gichia, 2001).

The potential uses and benefits of nanotechnology are numerous.

Table 1. Larval and pupal toxicity effect of *Leucas aspera* methanol leaf extract against malaria vector, *Anopheles stephensi*.

Mosquito life stages	Percentage of larval and pupal mortality±SD*					LC ₅₀ (ppm) (LFL-UFL)	LC ₉₀ (ppm) (LFL-UFL)	χ ² (df=4)
	50	150	250	350	450			
I instar	36±0.81	49.1±0.65	65.1±0.18	77.6±0.47	92.8±0.58	148.93 (111.96-178.75)	449.72 (402.39-518.49)	1.76 ^a
II instar	31.3±0.94	41±0.52	59.6±1.24	67.3±0.47	81.6±2.05	199.44 (160.57-232.97)	571.90 (500.32-685.50)	0.90 ^a
III instar	28.3±1.95	35.1±0.83	52.3±0.94	60.1±0.62	72.6±1.69	252.88 (213.64-292.55)	681.44 (582.66-850.45)	0.75 ^a
IV instar	23.7±0.52	29.3±1.24	43.9±0.77	50.9±0.89	57.7±1.79	349.99 (300.35-423.80)	888.00 (722.54-1219.62)	0.87 ^a
Pupa	17±1.41	23.2±2.44	35.1±1.89	44.2±2.05	52.1±1.32	417.07 (362.34-507.41)	912.94 (749.58-1228.95)	0.36 ^a

Control nil mortality; SD, standard deviation; LC₅₀, 50% lethal concentration; LFL, lower fiducial limit; UFL, upper fiducial limit; LC₉₀, 90% lethal concentration; χ², chi-square value; df, degrees of freedom. *Each mean±SD value is of five replicates. ^aP<0.05, significance level.

Table 2. Larval and pupal toxicity effect of *Leucas aspera* methanol leaf extract against dengue vector, *Aedes aegypti*.

Mosquito life stages	Percentage of larval and pupal mortality±SD*					LC ₅₀ (ppm) (LFL-UFL)	LC ₉₀ (ppm) (LFL-UFL)	χ ² (df=4)
	50	150	250	350	450			
I instar	31±1.34	46.7±1.57	61.4±2.01	73.4±0.61	89.1±1.41	174.89 (139.80-204.39)	488.16 (435.69-565.53)	0.92 ^a
II instar	26.3±1.19	38.7±1.80	55.6±0.48	63.2±1.39	72.6±1.76	238.95 (199.94-276.40)	653.05 (561.99-805.78)	1.01 ^a
III instar	22.2±1.90	31.3±1.63	47.6±0.83	55.2±1.39	63.1±1.30	309.54 (268.94-359.92)	766.25 (645.69-981.96)	1.12 ^a
IV instar	18.0±2.67	25.2±2.05	39.2±1.75	46.3±1.69	52.8±1.44	398.16 (345.23-483.83)	906.52 (742.32-1227.48)	1.06 ^a
Pupa	14.6±2.22	20.3±2.09	30.2±1.89	39.2±0.71	48±1.32	462.96 (399.59-575.05)	963.74 (785.17-1317.54)	0.11 ^a

Control nil mortality; SD, standard deviation; LC₅₀, 50% lethal concentration; LFL, lower fiducial limit; UFL, upper fiducial limit; LC₉₀, 90% lethal concentration; χ², chi-square value; df, degrees of freedom. *Each mean±SD value is of five replicates. ^aP<0.05, significance level.

These include agricultural productivity enhancement involving nanoporous zeolites for slow release and efficient dosage of water and fertilizer, nanocapsules for herbicide delivery, vector and pest management, and nanosensors for pest detection (Scrini & Lyons, 2007; Scott, 2007; Joseph & Morrison, 2006; Liu & Du, 2004). The utilization of biological systems for nanoparticle synthesis is a notable alternative for the advancement of this multifaceted approach. Biological systems have shown the ability to interact with metal ions and reduce them to form metallic nanoparticles (Beveridge *et al.*, 1997). Plants are widely used for nanoparticle synthesis, and the use of plants for the fabrication of nanoparticles is a rapid, low-cost, eco-friendly single step method for the biosynthesis process (Huang *et al.*, 2007).

The colour intensity of the leaf extracts of *L. aspera* incubated with silver ions at the beginning of the reaction and after 2 h of reaction is shown in Figure 3A. AgNPs were observed to be stable in solution and show very little aggregation. The appearance of the yellowish brown colour was an indication of formation of colloidal silver nanoparticles in the medium. The brown colour could be due to the excitation of surface plasmon vibrations, typical of the silver nanoparticles (Ahmad *et al.*, 2003; Krishnaraj *et al.*, 2010). It is generally recognized that UV-Vis spectroscopy can be used to examine size and shape-controlled nanoparticles in aqueous suspensions (Shrivastava & Dash, 2010). Figure 3B displays the UV-Vis spectra of the leaf solution as a function of reaction time. The

strong resonance centred at 400 nm was clearly observed and increased in intensity with time, and could arise from the excitation of longitudinal Plasmon vibrations in silver NPs in the solution. These changes have been attributed to the excitation of surface plasmon resonance in the metal nanoparticles (Natarajan *et al.*, 2010). Reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the leaves of *L. aspera* extract observed by the UV-Vis spectroscopy revealed that the presence of silver nanoparticles may be correlated with the UV-Vis spectra.

For the SEM studies, reaction mixtures were air-dried on silicon wafers. As a result, a coffee-ring phenomenon was observed. It is well known that when liquids that contain fine particles are evaporated on a flat surface, the particles accumulate along the outer edge and form typical structures (Chen & Evans, 2009). A representative SEM micrograph (Figure 4A) of synthesized nanoparticles shows spherical and cubic structures with a size range of 30-37 nm. The rough morphology of the AgNPs provides excellent larvicidal activity of the synthesized nanoparticles. In addition, the capped silver particles were stable in solution for at least 8 weeks. The EDS attachment of the SEM can provide information on the chemical analysis of the fields being investigated or the composition at specific locations (spot EDS). Figure 4B shows a representative profile of the spot EDS analysis, obtained by focusing on AgNPs. The silver nanoparticles formed were predominant-

Table 3. Larval and pupal toxicity effect of synthesized AgNPs against malaria vector, *Anopheles stephensi*.

Mosquito life stages	Percentage of larval and pupal mortality±SD*					LC ₅₀ (ppm) (LFL-UFL)	LC ₉₀ (ppm) (LFL-UFL)	χ ² (df=4)
	Concentration of AgNPs (ppm)							
	10	15	20	25	30			
I instar	41.2±2.62	60.2±1.22	79.8±1.71	90.4±1.38	100.0±0.0	12.45 (10.80-13.76)	23.50 (21.96-25.56)	3.34 ^a
II instar	35.2±1.15	49.2±1.37	63.1±2.27	81.2±1.22	96.2±0.0	15.01 (13.40-16.35)	28.37 (26.34-31.21)	3.98 ^a
III instar	32.6±1.71	43.0±1.82	56.6±1.30	76.4±0.82	89.4±1.22	16.55 (14.89-17.98)	31.77 (29.22-35.49)	2.18 ^a
IV instar	26.8±1.25	39.4±1.32	48.8±1.70	65.4±1.10	79.7±1.70	19.17 (17.46-20.81)	37.23 (33.59-42.95)	0.81 ^a
Pupa	21.5±1.10	34.2±1.18	45.4±1.85	53.8±1.20	69.7±0.55	22.26 (20.42-24.40)	42.95 (37.89-51.50)	0.62 ^a

Control nil mortality; SD, standard deviation; LC₅₀, 50% lethal concentration; LFL, lower fiducial limit; UFL, upper fiducial limit; LC₉₀, 90% lethal concentration; χ², chi-square value; df, degrees of freedom. *Each mean±SD value is of five replicates. ^aP<0.05, significance level.

Table 4. Larval and pupal toxicity effect of synthesized AgNPs against dengue vector, *Aedes aegypti*.

Mosquito life stages	Percentage of larval and pupal mortality±SD*					LC ₅₀ (ppm) (LFL-UFL)	LC ₉₀ (ppm) (LFL-UFL)	χ ² (df=4)
	Concentration of AgNPs (ppm)							
	10	15	20	25	30			
I instar	39.2±1.62	57.2±0.80	76.8±1.25	89.4±1.38	100±0.0	13.06 (11.50-14.32)	24.11 (22.56-26.21)	4.02 ^a
II instar	33.2±2.05	48.8±1.90	61.2±1.87	72±1.72	88.0±1.10	15.88 (13.90-17.48)	32.92 (29.98-37.41)	1.20 ^a
III instar	29.6±1.47	37.4±1.75	54.9±1.80	64.2±0.59	82.2±1.21	18.52 (16.77-20.14)	36.47 (32.95-41.98)	1.70 ^a
IV instar	20.8±0.90	31.4±1.63	46.8±1.90	54.4±1.10	71.4±1.30	22.10 (20.39-24.06)	41.28 (36.81-48.55)	0.75 ^a
Pupa	18.5±1.60	26.2±2.11	37.4±1.35	48.2±1.50	60.8±0.55	25.54 (23.42-28.51)	47.34 (41.18-58.20)	0.05 ^a

Control nil mortality; SD, standard deviation; LC₅₀, 50% lethal concentration; LFL, lower fiducial limit; UFL, upper fiducial limit; LC₉₀, 90% lethal concentration; χ², chi-square value; df, degrees of freedom. *Each mean±SD value is of five replicates. ^aP<0.05, significance level.

ly cubical with a uniform shape. It is known that the shape of metal nanoparticles considerably changes their optical and electronic properties (Xu & Käll, 2002). The particle shape of plant-mediated AgNPs are mostly spherical, with the exception of neem (*Azadirachta indica*), which yields poly-dispersed particles with both spherical and flat plate-like morphology, 5-35 nm in size (Shankar *et al.*, 2004). The overall morphology of AgNPs is more clearly seen, and the particles are mono-dispersed, having circular and flower shapes, respectively. Strong signals from the silver atoms in the nanoparticles were observed, and signals from Si, K, C, and Pt atoms were also recorded. The C and K signals were likely due to X-ray emission from carbohydrates, proteins, and enzymes present in the cell wall of the biomass.

The FTIR spectra of aqueous silver nanoparticles prepared from the *L. aspera* leaf extract (Figure 5) show transmittance peaks. Further, the results of the FTIR values of synthesized silver nanoparticles showed the presence of various functional groups such as alkane groups, methylene groups, alkene groups, amine groups and carboxylic acids. These functional groups are the major classes in many of the chemical groups, which have been previously proven to have potential as reducing agents in the synthesis of silver nanoparticles (Cho *et al.*, 2005). Studies indicate that the carboxyl ($-C=O$), hydroxyl ($-OH$) and amine ($-NH$) groups in leaf extracts are the ones mainly involved in the fabrication of silver nanoparticles, and for which bands were reported (Vigneshwaran *et al.*, 2007).

Mahesh Kumar *et al.* (2012) reported that 43% mortality was noted for first to fourth instars larvae and pupae of *Culex quinquefasciatus* treated with *S. xanthocarpum*, the LC_{50} =155.29, 198.32, 271.12, 377.44

and 448.41 ppm; the LC_{90} =687.14, 913.10, 1011.89, 1058.85, and 1141.65 ppm, respectively. Results of the present study are consistent with the earlier findings of Muthukrishnan *et al.* (1997), who observed that the LC_{50} values of ethyl acetate extract of *L. aspera* were 75.40, 93.09, 132.20, and 138.60 ppm against the first, second, third, and fourth larval instars, respectively of *C. quinquefasciatus*. Mwangi & Rembold (1998) reported that the leaf extracts of *L. aspera* exhibited high mortality, especially during the moulting process or the subsequent process of melanization and tanning. Murugan & Jayabalan (1999) reported that 90% larval mortality was exhibited at 4% concentration of the leaf extract of *L. aspera* against the fourth instar larvae of *An. stephensi*. The ethanolic extract of whole plant *L. aspera* against the first to fourth instar larvae and pupae had LC_{50} =9.70, 10.27, 10.82, 11.30, and 12.73 ppm, respectively, against *An. stephensi* (Kovendan *et al.*, 2012a). In the present findings, the values for LC_{50} were 168.41, 38.95, 309.54, 398.16 and 475.83 ppm; the LC_{90} values were 471.15, 653.05, 766.25, 906.52 and 991.55 ppm, respectively against *An. stephensi*.

The larvicidal effect of aqueous crude leaf extracts, silver nitrate solution, and synthesized silver nanoparticles (Ag-NPs) of *Mimosa pudica* showed that the highest mortality against the larvae of *A. subpictus* was found in synthesized AgNPs (LC_{50} =8.89, 11.82, and 0.69 ppm) and against the larvae of *C. quinquefasciatus* (LC_{50} =9.51, 13.65, and 1.10 ppm) (Marimuthu *et al.*, 2011). The highest mortality was found in methanol, aqueous, and synthesized AgNPs, which used *Nelumbo nucifera* plant extract against the larvae of *A. subpictus* (LC_{50} =8.89, 11.82, and 0.69 ppm; LC_{90} =28.65, 36.06, and 2.15 ppm) and against the larvae of *C. quinquefasciatus* (LC_{50} =9.51, 13.65, and 1.10 ppm; LC_{90} =28.13, 35.83,

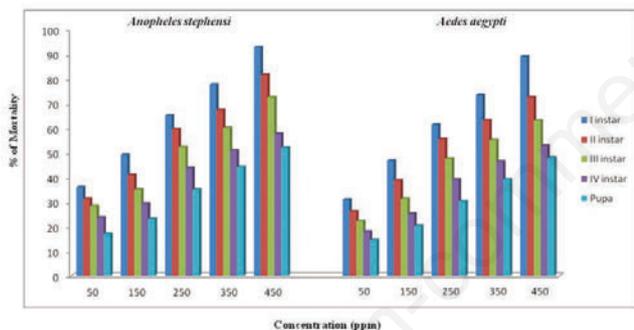


Figure 1. Larvicidal and pupicidal activity of *L. aspera* methanol leaf extract against malarial vector, *An. stephensi* and dengue vector, *Ae. aegypti*.

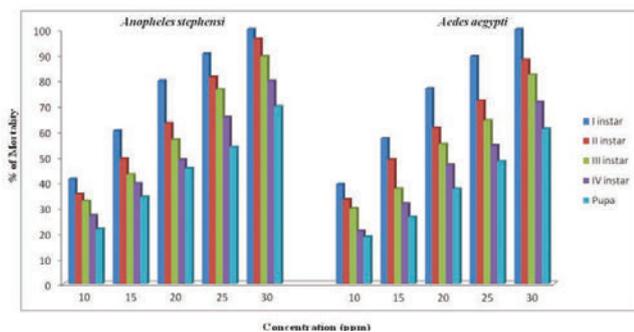


Figure 2. Larvicidal and pupicidal activity of synthesized AgNPs against malarial vector, *An. stephensi* and dengue vector, *Ae. aegypti*.

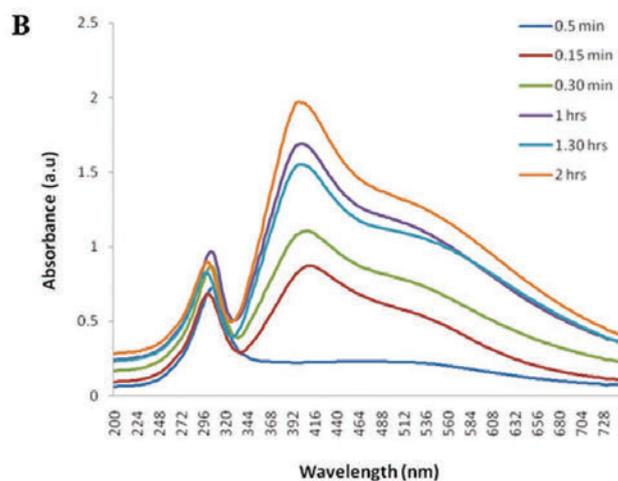


Figure 3. A) Change in colour after adding $AgNO_3$ before and after 30 min reaction time; B) UV vis spectra of aqueous silver nitrate with *L. aspera* leaf extract at different time intervals.

and 3.59 ppm), respectively (Santhoshkumar *et al.*, 2011). The synthesized zinc oxide nanoparticles showed the LC_{50} and r^2 values against the *Rhipicephalus microplus* (13.41 mg/L; 0.982), *Pediculus humanus capitis* (11.80; 0.966 mg/L), and the larvae of *A. subpictus* (3.19; 0.945 mg/L) and *C. quinquefasciatus* (4.87; 0.970 mg/L), respectively (Kirthi *et al.*, 2011). In the present study, the LC_{50} =13.06, to 25.54 ppm; the LC_{90} =24.11, to 47.34 ppm, respectively against *Ae. aegypti*.

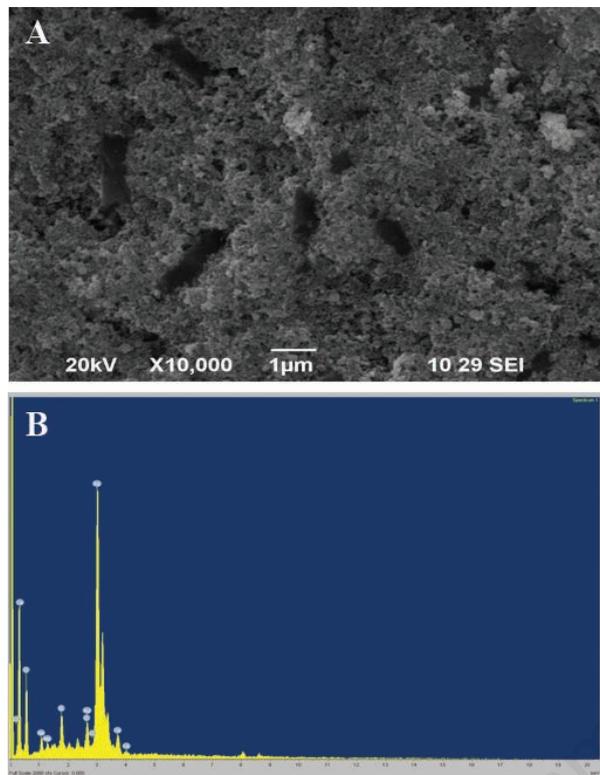


Figure 4. Image of scanning electron microscopic observation of synthesized silver nanoparticles. A) Higher magnification (1 μ m); B) Energy dispersive X-ray spectroscopy image showing the chemical composition of the particles.

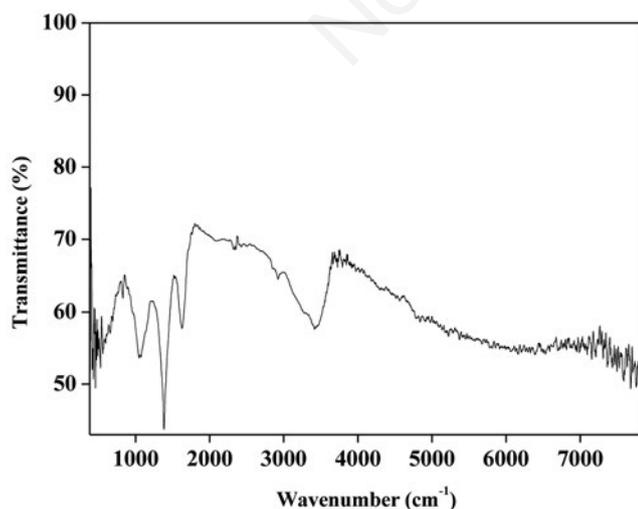


Figure 5. Fourier transformed infra red spectroscopic spectrum of silver nanoparticles synthesized using *L. aspera* leaf extract.

Priyadarshini *et al.* (2012) have reported on the AgNPs synthesized using *Euphorbia hirta* plant leaf extract against the malarial vector *An. stephensi* first to fourth instar larvae and pupae. Their LC_{50} values were 10.14, 16.82, 21.51, and 27.89 ppm, for first to fourth instars (respectively), and the LC_{90} values were 31.98, 50.38, 60.09, and 69.94 ppm, for first to fourth instars (respectively); the LC_{50} and LC_{90} values for pupae were 34.52 and 79.76 ppm, respectively. The bioactivity of the latex-producing plant *Pergulariadaemia* as well as AgNPs against the larval instars of *Ae. aegypti* and *An. stephensi* mosquito larvae was determined, the results AgNPs shows excellent larvicidal activity of first, second, third and fourth instars larvae exhibit noticeable effects after 24 or 48 h of exposure at their LC_{50} and LC_{90} values (Patil *et al.*, 2012). Potential antiplasmodial activity of synthesized silver nanoparticles was reported using *Andrographis paniculata* Nees (Acanthaceae), with inhibitory concentration (IC_{50}) values of $26 \pm 0.2\%$ at 25 μ g/mL, and $83 \pm 0.5\%$ at 100 μ g/mL (Panneerselvam *et al.*, 2011). Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* Linn. G. Don and their antiplasmodial activities against *Plasmodium falciparum* have been reported by Ponarulselvam *et al.* (2012).

In another study, synthesized AgNPs from *Murraya koenigii* against first to fourth instar larvae and pupae of *An. stephensi* had LC_{50} values of 10.82, 14.67, 19.13, 24.35, and 32.09 ppm and LC_{90} values of 32.38, 42.52, 53.65, 63.51, and 75.26 ppm, respectively (Suganya *et al.*, 2013). Larvicidal studies were carried out against *C. quinquefasciatus* and the results were compared with bulk permethrin. The LC_{50} of nanopermethrin and bulk permethrin to *C. quinquefasciatus* was 0.117 and 0.715 mg/L, respectively (Anjali *et al.*, 2010). The mosquito larvicidal activity of UV irradiation-induced AgNPs was found to decrease the survival of fourth instar larvae of *Ae. aegypti* by 88% after 24 h of exposure at 1 ppm concentration. According to Sap-lam *et al.* (2010), 5-10 nm poly-methacrylic acid capped silver nanoparticles may penetrate the larval membrane, which causes mortality of mosquito larvae.

Conclusions

In the present investigation, silver nanoparticles were synthesized using *L. aspera*. The synthesized silver nanoparticles were tested for their larvicidal activity against mosquito larvae and pupae of *An. stephensi* and *Ae. aegypti*. The prospect of utilising natural products for synthesizing silver nanoparticles and testing their efficacy in controlling mosquitoes larvae is a modern approach facilitating the development of a more potent and environmentally safe biopesticide. This biological reduction of metal could be a boon for the development of clean, nontoxic, and environmentally acceptable control agents. The synthesized silver nanoparticles are hydrophilic in nature, disperse uniformly in water, are highly stable, and exhibit significant mosquito larvicidal and pupicidal activity against *An. stephensi* and *Ae. aegypti* as target species in vector control strategies.

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