



## PHYTO-SYNTHESIZED SILVER NANOPARTICLES: A POTENT MOSQUITO OVICIDAL AGENT

<sup>1</sup>Kaliyan Veerakumar, \*<sup>1</sup>Marimuthu Govindarajan and <sup>2</sup>Kadarkarai Murugan

<sup>1</sup>Unit of Vector control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India.

<sup>2</sup>Division of Entomology, Department of Zoology, Bharathiar University, Coimbatore-641 046.

**Article History:** Received 11<sup>th</sup> February 2016; Revised 3<sup>rd</sup> April 2016; Accepted 3<sup>rd</sup> April 2016

### ABSTRACT

Green nanoparticle synthesis has been achieved using environmentally acceptable plant extract and eco-friendly reducing and capping agents. The present study to determine the efficacies of synthesized silver nanoparticles (AgNPs) using aqueous leaf extract of *Feronia elephantum* against the eggs of malaria vector, *Anopheles stephensi*, dengue vector, *Aedes aegypti* and filariasis vector, *Culex quinquefasciatus*. Eggs were exposed to varying concentrations of aqueous extract of *F. elephantum* and synthesized Ag NP for 24 h. Ag NP were rapidly synthesized using the leaf extract of *F. elephantum* and the formation of nanoparticles was observed within 6 h. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated six times along with appropriate control. The hatch rates were assessed 48 h post – treatment. The aqueous leaf extract and AgNPs exerted 100% mortality (zero hatchability-12 to 18 hrs eggs) at 250, 300 and 350 µg/mL, and 60, 70 and 80 µg/mL, against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. The results recorded from UV - vis spectrum, Fourier transform infrared, X-ray diffraction (XRD) analysis. Scanning electron microscopy and transmission electron microscopy support the biosynthesis and characterization of AgNPs. This is the first report on ovicidal activity of the plant extract and synthesized AgNPs.

**Keywords:** Green synthesis. Silver nanoparticles (Ag NP), *Feronia elephantum*, leaf extract, ovicides, Mosquitoes.

### INTRODUCTION

Worldwide, various strategies have been developed to reduce the prevalence of various vectors responsible for yellow fever, Chikungunya fever, dengue fever, dengue hemorrhagic fever, dengue shock syndrome, malaria, Japanese encephalitis, and lymphatic filariasis (Ali *et al.*, 1995; Veerakumar *et al.*, 2014a; Govindarajan *et al.*, 2016a). Among 53 anopheline species present in India, nine are vectors of malaria. *Anopheles stephensi* (L.) is the primary vector of malaria in India and other West Asian countries, Malaria remains one of the most prevalent diseases in the tropical world. With 200 million to 450 million infections annually worldwide, it causes up to 2.7 million deaths (World Health Organization, 2010). The disease remains endemic in more than 100 developing tropical countries, and its control is a major goal for improved worldwide health. In India, malaria is still the

most important cause of morbidity and mortality with approximately two to three million new cases arising every year (Sharma *et al.*, 2009; Govindarajan *et al.*, 2016b). *Aedes aegypti*, a vector of dengue that carries the arbovirus responsible for these viral diseases, is widely distributed in the tropical and subtropical zones. Chikungunya, a viral disease caused by both *Ae. aegypti* and *Ae. albopictus*, was recently concerned as an important public health problem in India and other countries like Senegal, West Africa (Yamar *et al.*, 2005). *Culex quinquefasciatus*, a vector of *Wuchereria* species causing lymphatic filariasis, is widely distributed in tropical regions with around 120 million people infected and 44 million people under clinical manifestation (Bernhard *et al.*, 2003).

In India, a total of 553 million people are at risk of infection and there are approximately 21 million people

\*Corresponding author: Assistant Professor, Unit of Vector control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India, , Email: drgovind1979@gmail.com, Mobile: +91 9585265999.

with symptomatic filariasis and 27 million microfilaria carriers. *Wuchereria (W.bancrofti)* is the national burden, widely distributed in 17 states and six union territories (Das *et al.*, 2000). The biosynthesis of nanoparticles is advantageous over chemical and physical methods because it is a cost-effective and environment-friendly method, where it is not necessary to use high pressure, high energy, high temperature, and toxic chemicals (Veerakumar *et al.*, 2014a). Silver nanoparticles (Ag NP) may be released into the environment from discharges at the point of production, from erosion of engineered materials in household products (antibacterial coatings and silver impregnated water filters), and from washing or disposal of silver-containing products (Benn and Westerhoff, 2008). In recent years, the biosynthesis method using plant extracts has received more attention than chemical and physical methods and even more than the use of microbes, for the nano scale metal synthesis, due to the absence of any requirement to maintain an aseptic environment. Nanoparticles have attracted considerable attention because of their various applications. Use of plant extract for the synthesis of nanoparticles could be advantageous over other environmentally benign biological processes because it eliminates the elaborate process of maintaining cell cultures. Recently, green silver nanoparticles have been synthesized using various natural products like *Sida acuta* (Veerakumar *et al.*, 2013).

The larvicidal activity of synthesized Ag NP utilizing aqueous extract from *Eclipta prostrata*, a member of the Asteraceae, has been investigated against fourth instar larvae of filariasis vector, *Cx. quinquefasciatus* Say and malaria vector, *An. subpictus* (Rajkumar and Rahuman, 2011). The larvicidal efficacy of the crude leaf extracts of *Ficus benghalensis*, with three different solvents like methanol, benzene, and acetone, were tested against the early second, third, and fourth instar larvae of *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* (Govindarajan, 2010a). The efficacies of synthesized silver nanoparticles using the aqueous leaf extract of *Mimosa pudica* have been evaluated against the larvae of *An. subpictus*, *Cx. quinquefasciatus*, and *Rhipicephalus microplus* (Marimuthu *et al.*, 2010). The adulticidal activity of silver nanoparticles (AgNPs) synthesized using *Heliotropium indicum* plant leaf extract against adults of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* (Veerakumar *et al.*, 2014b). The leaf extract of *Acalypha indica* with different solvents benzene, chloroform, ethyl acetate, and methanol has been tested for larvicidal-ovicidal activity and oviposition attractancy against *An. stephensi* (Govindarajan *et al.*, 2008). In this study, plant-mediated synthesized Ag NP were evaluated for the first time against ovicidal of dengue (*Ae. aegypti*), malaria (*An. stephensi*), and filariasis (*Cx. quinquefasciatus*) vector mosquitoes.

## MATERIALS AND METHODS

### Materials

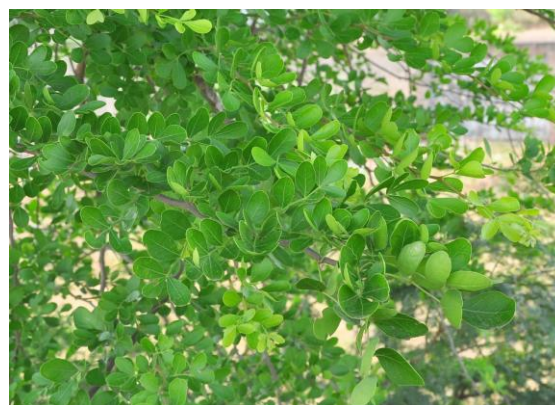
Healthy and Fresh leaves of *F. elephantum* (Rutaceae) were collected from in and around Ammankuppam, Chidambaram area, and Tamil Nadu, and the taxonomic identification was made by Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India. The voucher specimen was numbered and kept in our research laboratory for further reference. Silver nitrate ( $\text{AgNO}_3$ ) was obtained from Qualigens Fine Chemicals, Mumbai, India.

### Preparation of plant leaf extracts

The leaves of *F. elephantum* (Figure 1) were dried in the shade and ground to fine powder in an electric grinder. Aqueous extract was prepared by mixing 50 g of dried leaf powder with 500 mL of water (sterile distilled water) with constant stirring on a magnetic stirrer. The suspension of dried leaf powder in water was left for 3 h and filtered through Whatman no. 1 filter paper, and the filtrate was stored in an amber-colored airtight bottle at 10 °C temperature till use. (Veerakumar *et al.*, 2014c).

### Synthesis of silver nanoparticles

The broth solution of fresh leaves was prepared by taking 10 g of thoroughly washed and finely cut leaves in a 300-mL Erlenmeyer flask along with 100 mL of sterilized double-distilled water and then boiling the mixture for 5 min before finally decanting it. The extract was filtered with Whatman filter paper no. 1 and stored at -15 °C; it could be used within 1 week. The filtrate was treated with aqueous 1 mM  $\text{AgNO}_3$  (21.2 mg of  $\text{AgNO}_3$  powder in 125 mL Milli-Q water) solution in an Erlenmeyer flask and incubated at room temperature. Eighty-eight milliliters of an aqueous solution of 1 mM silver nitrate was reduced using 12 mL of leaf extract at room temperature for 10 min, resulting in a brown-yellow solution indicating the formation of Ag NP.



**Figure 1.** *Feronia elephantum* plant.

### Characterization of synthesized silver nanoparticles

The bioreduction of Ag<sup>+</sup> ion in solution was monitored using UV-visible spectrophotometer (UV-160v, Shimadzu, Japan). The studies on size, morphology, and composition of nanoparticles were performed by scanning electron microscopy (Hitachi S3000 H SEM), transmission electron microscopy (TEM Technite 10 Philips), and energy-dispersive X-ray spectrum (EDX). The purified Ag NPs were examined for the presence of biomolecules using FTIR spectrum (Thermo Scientific Nicolet 380 FT-IR Spectrometer). KBr pellets and crystalline Ag NP were determined by X-ray diffraction (XRD) analysis.

### Mosquito rearing

The laboratory-bred pathogen-free strains of mosquitoes were reared in the vector control laboratory, Department of Zoology, Annamalai University. At the time of adult feeding, these mosquitoes were 3-4 days old after emergences (maintained on raisins and water) and were starved for 12 h before feeding. Each time, 500 mosquitoes per cage were fed on blood using a feeding unit fitted with parafilm as membrane for 4 h. *Ae. aegypti* feeding was done from 12 noon to 4.00 p.m. and *An. stephensi* and *Cx. quinquefasciatus* were fed during 6.00 p.m. to 10.00 p.m. A membrane feeder with the bottom end fitted with parafilm was placed with 2.0 ml of the blood sample (obtained from a slaughter house by collecting in a heparinized vial and stored at 4 °C) and kept over a netted cage of mosquitoes. The blood was stirred continuously using an automated stirring device, and a constant temperature of 37 °C were maintained using a water jacket circulating system. After feeding, the fully engorged females were separated and maintained on raisins. Mosquitoes were held at 28±2 °C, 70-85 % relative humidity, with a photo period of 12-h light and 12-h dark.

### Ovicidal activity

Slightly modified method of Su and Mulla (1998) was performed. Eggs were collected from vector control laboratory, Department of Zoology, Annamalai University. The leaf aqueous extracts and silver nanoparticle were to achieve various concentrations ranging from 50 to 300µg/ml and 10 to 60µg/mL, respectively. Eggs of these mosquito species (100 no. of 0-6, 6-12 and 12-18h old eggs) were exposed to each concentration of leaf aqueous extracts and Ag NP. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated six times along with appropriate control. The hatch rates were assessed 48 h post-treatment by the following formula.

$$\% \text{ of egg hatchability} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} \times 100$$

### Data analysis

The average larval mortality data were subjected to probit analysis for calculating LC<sub>50</sub>, LC<sub>90</sub>, 95 % confidence limits, and chi-square values. The Statistical Package for the Social Sciences 16.0 software was used for all the analyses. Results with p<0.05 were considered significant.

## RESULTS

### Characterization of silver nanoparticles

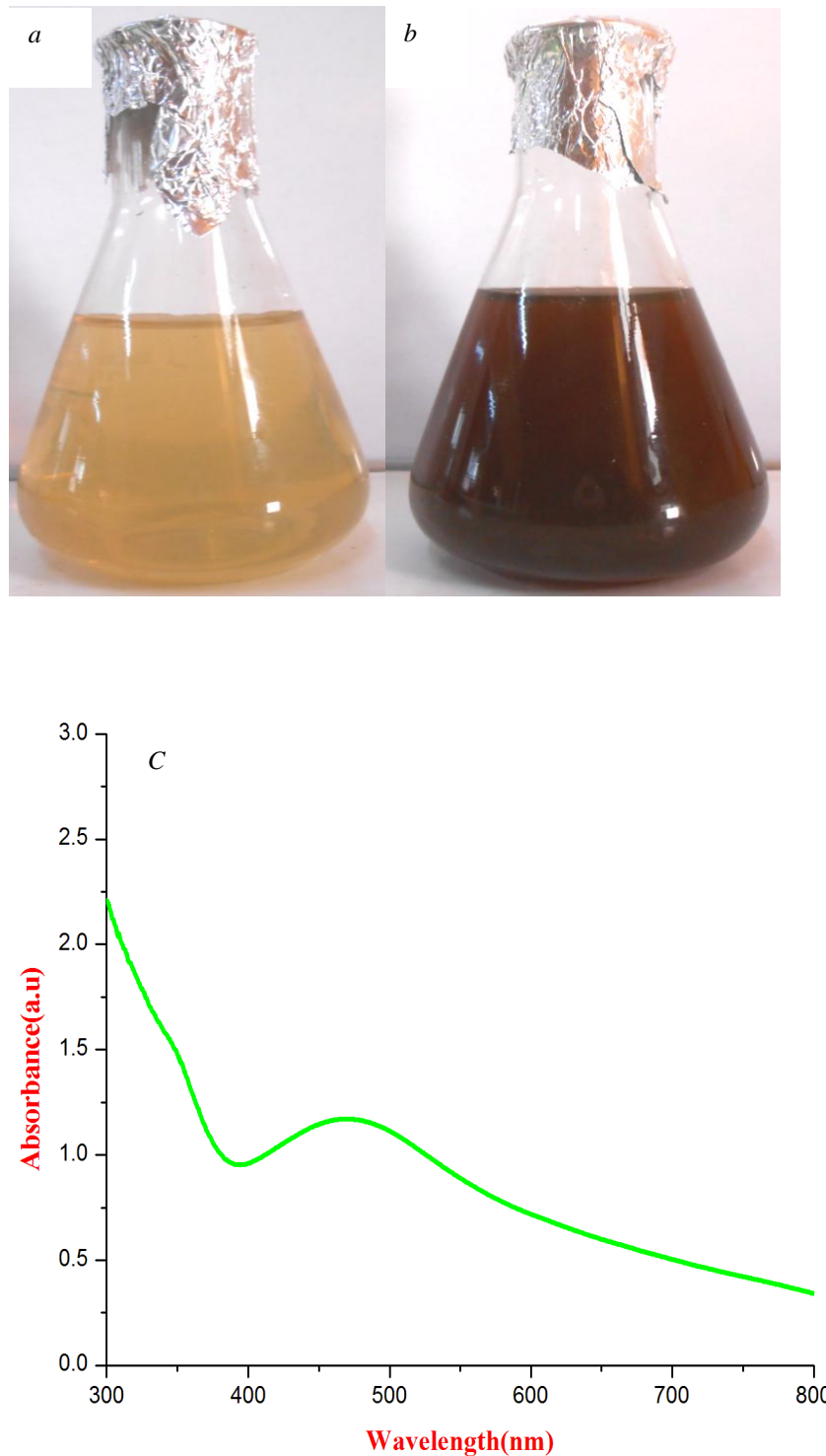
The color change was noted by visual observation of the *F. elephantum* leaf extracts which were incubated with AgNO<sub>3</sub> solution; the *F. elephantum* leaf extract without AgNO<sub>3</sub> did not show any change in color. The color of the extract changed to light brown within an hour, and later, it changed to dark brown during a 6-h incubation period after which no significant change occurred (Figure 2a and b) The absorption spectrum of *F. elephantum* leaf extracts at different wavelengths ranging from 300 to 800 nm revealed a peak at 420 nm (Figure 2c). FTIR analysis of the purified nanoparticles showed the presence of bands due to C–H bending (671.81, 762.21, and 822.47 cm<sup>-1</sup>), C–O stretch (1016.97 and 1,120.07), –C–H bending (1,384.10), C=C bending (1,617.90), C–H stretch (2,849.59), and N–H stretch (3,422.14) (Figure 3). SEM micrographs of the synthesized Ag NP of *F. elephantum* magnified at ×500 and measured at 20 to 60 nm are shown in Figure 4a. The triangular, pentagonal, and hexagonal structures are clear. Energy-dispersive X-ray spectroscopy (EDX) proves the chemical purity of the synthesized AgNPs (Figure 4b). The electron microscopic study of the nanoparticles using TEM revealed that the nano-Ag predominates with spherical, triangle, truncated triangles, and decahedral morphologies ranging from 18 to 45 nm with an average size of 32 nm (Figure 5). The X-ray diffraction pattern of silver nanoparticles produced by leaf extract is shown in (Figure 6). The control thin films of the leaf extract as well as the AgNO<sub>3</sub> did not show the characteristic peaks. The XRD pattern shows four intense peaks in the whole spectrum of 2θ values ranging from 25 to 60. The XRD spectrum compared with the standard confirmed spectrum of silver particles formed in the present experiments were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 38.22°, 44.39°, 64.52° and 77.47° corresponding to 164, 56, 74 and 57 planes for silver, respectively. The XRD pattern clearly shows that the silver nanoparticles formed by the reduction of AgNO<sub>3</sub> ions by *F. elephantum* are crystalline in nature.

### Ovicidal activity of aqueous extract and synthesized AgNPs

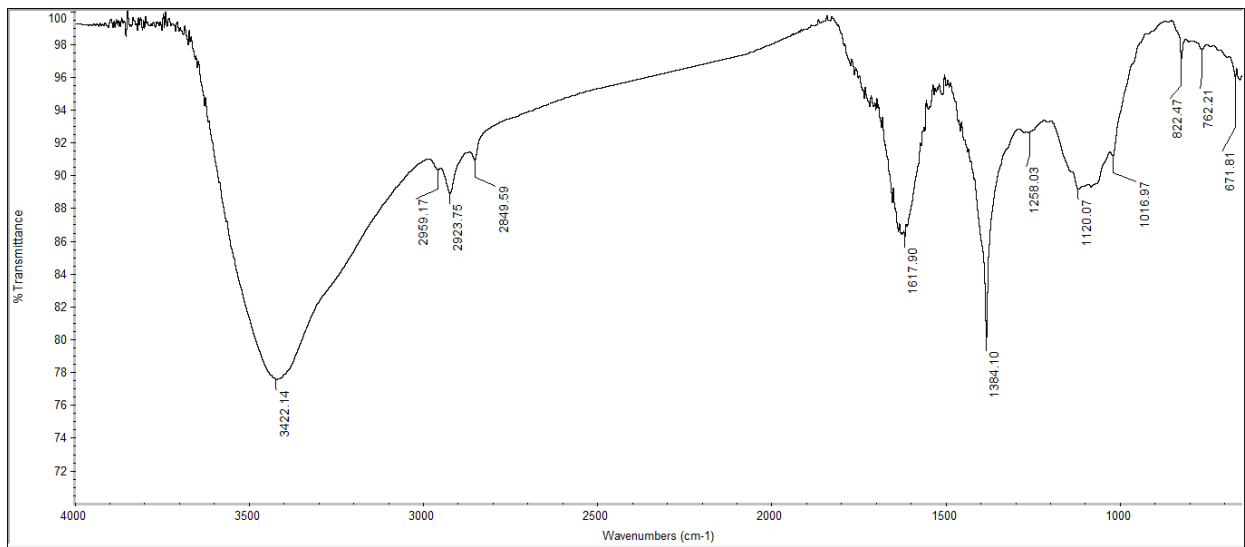
The mean percent of egg hatchability of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* were tested with aqueous leaf extracts and Ag NP at different concentrations of *F. elephantum* leaves, and the results are listed in Tables 1 and 2. The percent hatchability was

inversely proportional to the concentration of extract and directly proportional to the eggs. The Ag NP of *F.elephantum* exerted 100% mortality (Zero hatchability- 12 to 18 hrs eggs) at 50,60 and 70  $\mu\text{g/mL}$ , against *An. stephensi*, *Ae.aegypti* and *Cx. quinquefasciatus*,

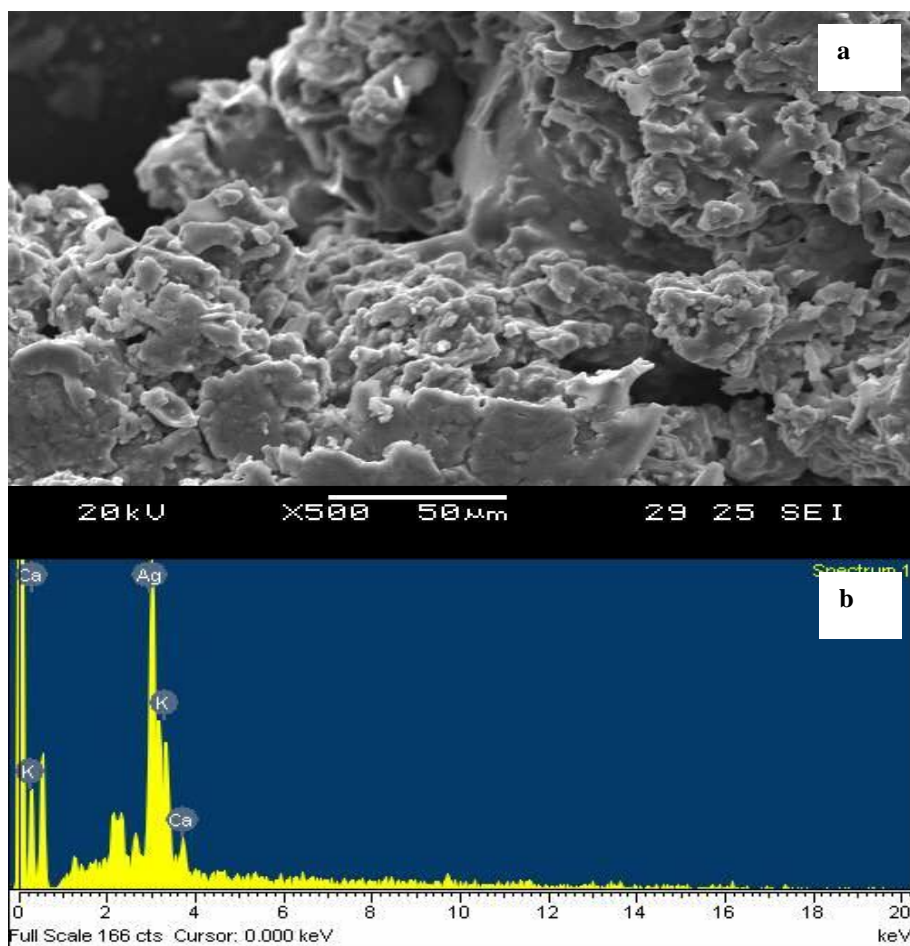
respectively. The Ag NP of *F.elephantum* was found to be most effective than aqueous leaf extracts against eggs of three vector mosquitoes. Control eggs showed the 100% hatchability.



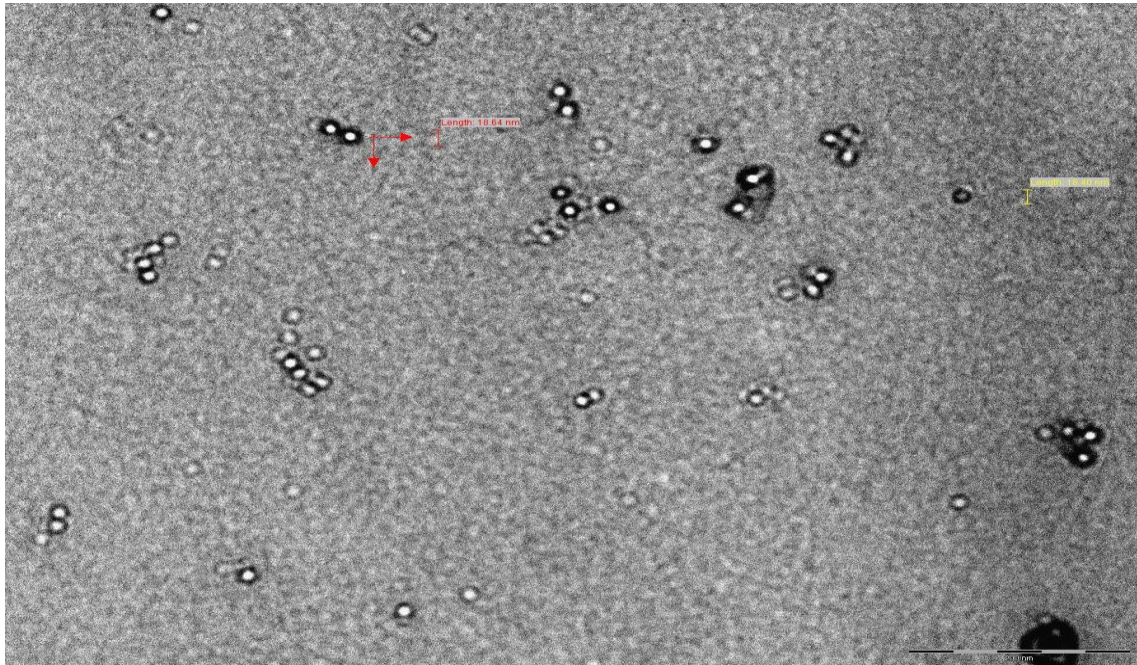
**Figure 2.** (a) Photographs showing change in color after adding  $\text{AgNO}_3$  before reaction. (b) After reaction time of 6 h. (c) UV-Vis spectra of aqueous silver nitrate with *F. elephantum* leaf extract.



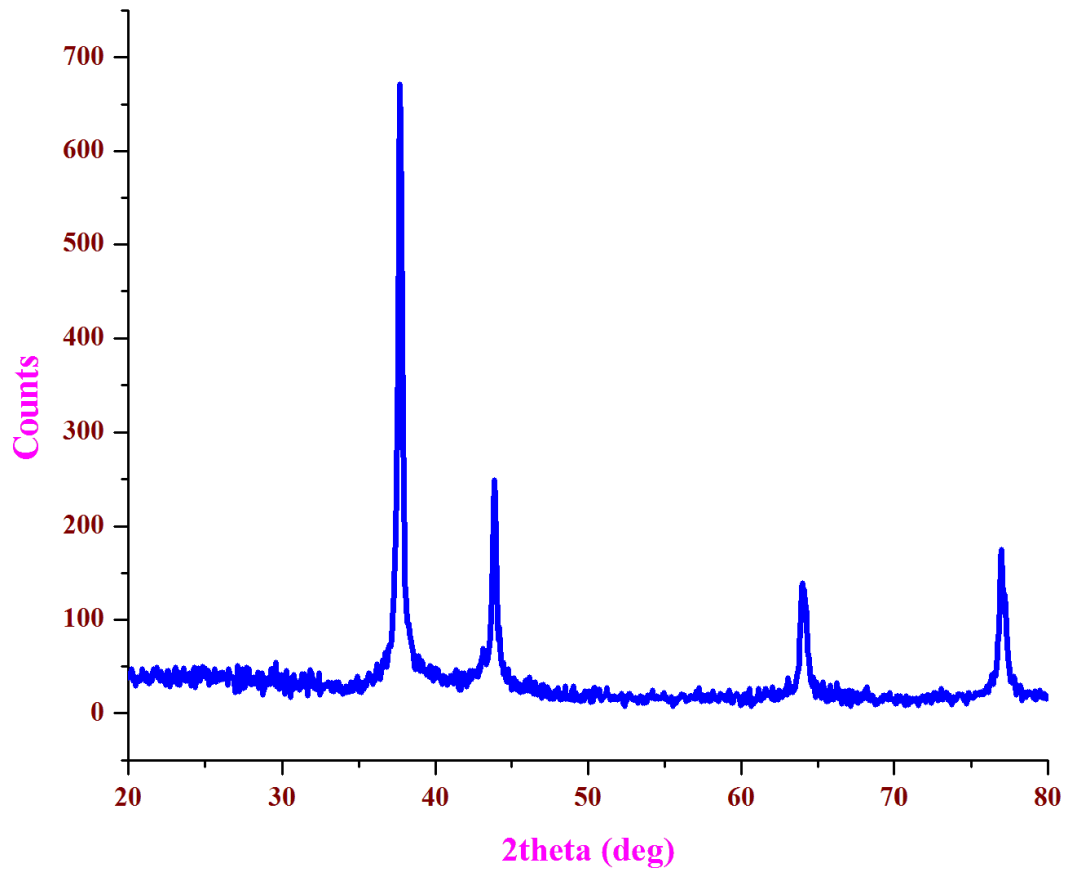
**Figure 3.** FT-IR spectrum of synthesized Ag NP using *F. elephantum* leaf extract.



**Figure 4.** Scanning electron micrographs of AgNPs synthesized with *F. elephantum* leaf extract and 1.0 mM  $\text{AgNO}_3$  solution and incubated at 60 °C for 6 h at pH 7.0. (a) Magnified 500 X, inset bar represents 50 µm; (b) EDX image showing chemical composition.



**Figure 5.** Transmission electron microscopic image and histogram showing synthesized Ag NP from *F. elephantum*.



**Figure 6.** X-Ray diffraction showing synthesized Ag NP from *F. elephantum*.

**Table 1.** Ovicidal activity of *Feronia elephantum* aqueous leaf extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*.

Mosquitoes	Age of the egg raft/eggs (h)	Percentage of egg hatchability						
		Concentration ( $\mu\text{g/mL}$ )						
		Control	50	100	150	200	250	300
<i>An. stephensi</i>	0-6	100 $\pm$ 0.0	21.3 $\pm$ 1.0	16.4 $\pm$ 1.5	NH	NH	NH	NH
	6-12	100 $\pm$ 0.0	46.4 $\pm$ 0.9	27.2 $\pm$ 1.4	19.6 $\pm$ 1.8	NH	NH	NH
	12-18	100 $\pm$ 0.0	62.5 $\pm$ 1.3	41.7 $\pm$ 1.5	34. $\pm$ 1.0	17.2 $\pm$ 1.2	NH	NH
<i>Ae. aegypti</i>	0-6	100 $\pm$ 0.0	39.3 $\pm$ 0.8	27.4 $\pm$ 1.2	19.2 $\pm$ 1.6	NH	NH	NH
	6-12	100 $\pm$ 0.0	53.1 $\pm$ 1.2	43.5 $\pm$ 1.7	36.2 $\pm$ 0.8	21.0 $\pm$ 1.0	NH	NH
	12-18	100 $\pm$ 0.0	73.2 $\pm$ 1.5	62.1 $\pm$ 1.8	54.3 $\pm$ 0.9	32.4 $\pm$ 1.3	17.2 $\pm$ 1.7	NH
<i>Cx. quinquefasciatus</i>	0-6	100 $\pm$ 0.0	56.3 $\pm$ 0.8	49.1 $\pm$ 1.2	33.4 $\pm$ 1.6	19.0 $\pm$ 1.8	NH	NH
	6-12	100 $\pm$ 0.0	71.7 $\pm$ 1.3	63.2 $\pm$ 1.7	52.4 $\pm$ 0.8	38.1 $\pm$ 1.0	17.4 $\pm$ 1.4	NH
	12-18	100 $\pm$ 0.0	96.4 $\pm$ 1.0	85.3 $\pm$ 1.6	72.1 $\pm$ 0.9	54.6 $\pm$ 1.3	37.4 $\pm$ 1.7	18.3 $\pm$ 0.8

NH - No hatchability.

**Table 2.** Ovicidal activity of Ag NP synthesized using *F. elephantum* against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*.

Mosquitoes	Age of the egg raft/eggs (h)	Percentage of egg hatchability						
		Concentration ( $\mu\text{g/mL}$ )						
		Control	10	20	30	40	50	60
<i>An. stephensi</i>	0-6	100 $\pm$ 0.0	28.3 $\pm$ 0.8	16.2 $\pm$ 1.2	NH	NH	NH	NH
	6-12	100 $\pm$ 0.0	44.2 $\pm$ 1.2	31.7 $\pm$ 0.8	19.5 $\pm$ 1.5	NH	NH	NH
	12-18	100 $\pm$ 0.0	58.1 $\pm$ 1.6	47.3 $\pm$ 0.2	39.6 $\pm$ 0.8	17.2 $\pm$ 1.2	NH	NH
<i>Ae. aegypti</i>	0-6	100 $\pm$ 0.0	47.2 $\pm$ 0.8	30.4 $\pm$ 1.2	19.3 $\pm$ 1.6	NH	NH	NH
	6-12	100 $\pm$ 0.0	58.4 $\pm$ 1.5	43.1 $\pm$ 1.0	32.6 $\pm$ 0.8	17.2 $\pm$ 0.6	NH	NH
	12-18	100 $\pm$ 0.0	72.6 $\pm$ 1.4	61.5 $\pm$ 1.8	43.1 $\pm$ 0.4	36.4 $\pm$ 0.8	21.1 $\pm$ 1.2	NH
<i>Cx. quinquefasciatus</i>	0-6	100 $\pm$ 0.0	63.2 $\pm$ 1.6	48.3 $\pm$ 0.4	34.2 $\pm$ 0.8	17.4 $\pm$ 1.2	NH	NH
	6-12	100 $\pm$ 0.0	77.3 $\pm$ 0.6	61.5 $\pm$ 1.2	47.2 $\pm$ 1.8	31.1 $\pm$ 0.6	16.3 $\pm$ 0.8	NH
	12-18	100 $\pm$ 0.0	92.4 $\pm$ 1.6	80.9 $\pm$ 0.4	69.1 $\pm$ 1.8	56.3 $\pm$ 0.4	35.4 $\pm$ 0.8	19.2 $\pm$ 1.2

NH - No hatchability.

## DISCUSSION

Vector control is facing a threat due to the emergence of resistance vector mosquitoes to conventional synthetic insecticides, warranting counter measures such as the development of novel insecticides (Chandre *et al.*, 1998). Botanical insecticides may serve as suitable alternatives to synthetic insecticides as they are relatively safe, degradable and are readily available in many areas of the world. Although several plants form different families have been reported for mosquitocidal activity, only a few botanicals have moved from the laboratory to field use, like neem-based insecticides, which might be due to the light and heat instability of phytochemicals compared to synthetic insecticides (Green *et al.*, 1991). In the present study, the findings indicate the importance of traditional knowledge in science. The plants giving out insecticidal substance is massive. The results of the ovicidal activity of the *F. elephantum* extracts with silver nanoparticles on

*An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* at different time exposures were sustainable.

The highest adult mortality was found in methanol extract of *A. paniculata* against the adults of *Cx. quinquefasciatus* and *Ae. aegypti* with the LC<sub>50</sub> and LC<sub>90</sub> values of 149.81 and 172.37 ppm and 288.12 and 321.01 ppm, respectively (Govindarajan and Sivakumar 2012). The larvicidal activity of Ag NP synthesized using *S. acuta* plant leaf extract against late third-instar larvae of *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti* was determined. The efficacies of synthesized AgNPs (10, 20, 30, 40, and 50  $\mu\text{g mL}^{-1}$ ) and aqueous leaf extract (50, 100, 150, 200, and 250  $\mu\text{g mL}^{-1}$ ) were tested against the larvae of *Cx. quinquefasciatus* (LC<sub>50</sub>, 26.13 and 130.30  $\mu\text{g mL}^{-1}$ ), *An. stephensi* (LC<sub>50</sub>, 21.92 and 109.94  $\mu\text{g mL}^{-1}$ ), and *Ae. aegypti* LC<sub>50</sub> (23.96 and 119.32  $\mu\text{g mL}^{-1}$ ), respectively (Veerakumar *et al.*, 2013).

The mortality was evident after the treatment of *Gomelia asiatica* for all three important vector mosquitoes. The LC<sub>50</sub> and LC<sub>90</sub> values of *G. asiatica* aqueous leaf extract appeared to be effective against *An. stephensi* (LC<sub>50</sub> 113.53 µg/mL and LC<sub>90</sub> 201.56 µg/mL), followed by *Ae. aegypti* (LC<sub>50</sub> 130.19 µg/mL and LC<sub>90</sub> 229.84 µg/mL), and *C. quinquefasciatus* (LC<sub>50</sub> 139.17 µg/mL and LC<sub>90</sub> 243.95 µg/mL). Most considerable mortality was evident after the treatment of silver nanoparticles. Synthesized Ag NP against the vector mosquitoes of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* had the following LC<sub>50</sub> and LC<sub>90</sub> values: *An. stephensi* had LC<sub>50</sub> and LC<sub>90</sub> values of 22.44 and 40.65 µg/mL; *Ae. aegypti* had LC<sub>50</sub> and LC<sub>90</sub> values of 25.77 and 45.98 µg/mL; and *Cx. quinquefasciatus* had LC<sub>50</sub> and LC<sub>90</sub> values of 27.83 and 48.92 µg/mL (Muthukumaran *et al.*, 2015).

The LC<sub>50</sub> and LC<sub>90</sub> values of *H. indicum* aqueous leaf extract appeared to be effective against *An. stephensi* (LC<sub>50</sub>, 68.73 µg/mL; LC<sub>90</sub>, 121.07 µg/mL) followed by *Ae. aegypti* (LC<sub>50</sub>, 72.72 µg/mL; LC<sub>90</sub>, 126.86 µg/mL) and *Cx. quinquefasciatus* (LC<sub>50</sub>, 78.74 µg/mL; LC<sub>90</sub>, 134.39 µg/mL). Synthesized Ag NP against the vector mosquitoes of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* had the following LC<sub>50</sub> and LC<sub>90</sub> values: *An. stephensi* had LC<sub>50</sub> and LC<sub>90</sub> values of 18.40 and 32.45 µg/mL, *Ae. aegypti* had LC<sub>50</sub> and LC<sub>90</sub> values of 20.10 and 35.97 µg/mL, and *Cx. quinquefasciatus* had LC<sub>50</sub> and LC<sub>90</sub> values of 21.84 and 38.10 µg/mL respectively (Veerakumar *et al.*, 2014b).

The synthesized Ag NP using *Chrysosporium tropicum* against the third instars larvae of *Ae. aegypti* with LC<sub>50</sub>= 04 ppm, LC<sub>90</sub>=08.91 ppm, and LC<sub>99</sub>=013.18 ppm. The potential antiplasmodial activity of synthesized silver nanoparticle using *Andrographis paniculata* Neems (Acanthaceae) with the inhibitory concentration (IC<sub>50</sub>) values was 26±0.2 % at 25 µg/mL, whereas it was 83±0.5 % at 100 µg/mL (Panneerselvam *et al.*, 2011). The observed LC<sub>50</sub> values for first, second, third, and fourth instar were 0.65, 0.87, 1.08, and 1.33 ppm, respectively, and the LC<sub>90</sub> values were 2.27, 2.41, 2.76, and 3.24 ppm, respectively. At 0.2 ppm treatment of biosynthesized gold nanoparticles, the pupal mortality was 15 %, where as it has been increased to 75 % at 3.2 ppm. The LC<sub>50</sub> and LC<sub>90</sub> values of biosynthesized gold nanoparticles using *Cymbopogon citratus* for pupae were 1.94 and 4.23, respectively (Naresh Kumar *et al.*, 2011).

The Ag NP synthesized by filamentous fungus *Cochliobolus lunatus* and its larvicidal activity were tested in various concentrations (10, 5, 2.5, 1.25, 0.625, and 0.3125 ppm) against second, third, and fourth instar larvae of *A. aegypti* (LC<sub>50</sub> 1.29, 1.48, and 1.58 ppm; LC<sub>90</sub> 3.08, 3.33, and 3.41 ppm) and against *An. stephensi* (LC<sub>50</sub> 1.17, 1.30, and 1.41 ppm; LC<sub>90</sub> 2.99, 3.13, and 3.29 ppm), respectively (Salunkhe *et al.*, 2011).

The maximum efficacy was observed in crude aqueous and synthesized AgNPs against *Cx. quinquefasciatus* (LC<sub>50</sub> 27.49 and 4.56 mg/L; LC<sub>90</sub>

70.38 and 13.14 mg/L) and against *An. subpictus* (LC<sub>50</sub> 27.85 and 5.14 mg/L; LC<sub>90</sub> 71.45 and 25.68 mg/L), respectively. A biological method has been used to synthesize stable silver nanoparticles that were tested as mosquito larvicides against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* (Arjunan *et al.*, 2012).

The highest mortality of 33, 76, and 100 % was found in aqueous, and 79, 100, and 100 % were observed synthesized AgNPs against the larvae of *An. stephensi* (LC<sub>50</sub> 12.47 and 16.84 mg/mL and LC<sub>90</sub> 36.33 and 68.62 mg/mL) on 48 and 72 h of exposure and against *Cx. quinquefasciatus* (LC<sub>50</sub> 43.80 mg/mL and LC<sub>90</sub> 120.54 mg/mL) on 72-h exposure, and aqueous extract showed 100 % mortality against *An. stephensi* and *Cx. quinquefasciatus* (LC<sub>50</sub> 78.62 and 55.21 mg/mL and LC<sub>90</sub> 184.85 and 112.72 mg/mL) on 72-h exposure at concentrations of 50 mg/mL, respectively. (Subarani *et al.*, 2013). The ethanolic extract of whole plant *Lepiota aspera* against the first to fourth instar larvae and pupae values of LC<sub>50</sub> of I instar was 9.695%, that of II instar was 10.272%, that of III instar was 10.823%, and that of IV instar was 11.303 %, and pupae was 12.732 %, respectively against *An. stephensi* (Kovendan *et al.*, 2012).

Kamakshi *et al.* (2015) reported that the ovicidal activity of *Cereus hildmannianus* extracts on *Ae. aegypti* eggs are present the moderate ovicidal activity was noted only in the petroleum ether extract on the eggs of *Ae. aegypti* with 52.8% EMR at 1000 mg/L at 96 h post treatment period. The lowest concentration (62.5 mg/L) of petroleum ether extract caused 28.8% egg mortality against the eggs of *Ae. aegypti*, the carbon tetrachloride extract showed 38.4% and hexane, ethyl acetate and aqueous extracts recorded egg mortality of 21.6%, 24.8% and 20.0% respectively at 1000 mg/L concentration against *Ae. aegypti*.

Reegan *et al.* (2014) reported that the 500 ppm of the hexane leaf extract of *Limonia acidissima* led to egg mortality of 60 and 79.2 % against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively. The n-butanol and the aqueous fractions have shown little oviposition effective repellence rates against *Ae. albopictus* (100 ppm: 22.72 and 17.06% of effective repellence, respectively). The highest oviposition activity index was achieved by the hexane fraction (-0.82), followed by the ethyl acetate fraction (-0.63) and the methanol fraction (-0.62). A lower oviposition activity index was achieved by the n-butanol fraction (-0.14) and the aqueous fraction (-0.09) (Benelli *et al.*, 2014).

The essential oil from the leaves of *Clausena anisata* exhibited significant larvicidal activity, with 24 h LC<sub>50</sub> values of 140.96, 130.19, and 119.59 ppm, respectively (Govindarajan, 2010b).

## CONCLUSION

In conclusion, our study reveals that the Ag NP of *F. elephantum* has remarkable ovicidal properties. The flora of India has rich aromatic plant diversity with



potential for development of natural insecticides for control of mosquito and other pests. In brief, our findings suggested that the Ag NP from *F. elephantum* may be explored as potential environmental-benign ovicides. These results obtained are useful in search of a more selective, biodegradable, and naturally produced ovicidal compounds.

## ACKNOWLEDGMENTS

The authors would like to thank the Professor and Head of the Department of Zoology, Annamalai University, for the laboratory facilities provided. The authors would also like to acknowledge the cooperation of staff members of the VCRC (ICMR), Pondicherry., thankful to Dr. S. Ramesh, Professor and Head, Veterinary College, Vepery, Chennai for TEM analysis and P.Kolanchinathan, PG and research Lab, Department of Zoology, EVR collage , Tiruchy for XRD analysis.

## REFERENCES

- Ali, A., Nayar, J.K. and Xue, R.D., 1995. Comparative toxicity of selected larvicides and insect growth regulators to a Florida laboratory, population of *Aedes albopictus*. *J. Am. Mosq. Control. Assoc.*, 11(1), 72-76.
- Arjunan, N.K., Murugan, K., Rejeeth, C., Madhiyazhagan, P. and Barnard, D.R., 2012. Green synthesis of silver nanoparticles for the control of mosquito vectors of malaria, filariasis, and dengue. *Vector-Borne Zoonotic Dis.*, 12(3), 262-268.
- Benelli, G., Conti, B., Garreffa, R. and Nicoletti, M., 2014. Shedding light on bioactivity of botanical by-products: neem cake compounds deter oviposition of the arbovirus vector *Aedes albopictus* (Diptera: Culicidae) in the field. *Parasitol. Res.*, 113, 933-940.
- Benn, T. and Westerhoff, P., 2008. Nanoparticle silver released into water from commercially available sock fabrics. *Environ. Sci. Technol.*, 42, 4133-4139.
- Bernhard, L., Bernhard, P. and Magnussen, P., 2003. Management of patients with lymphoedema caused by filariasis in northeastern Tanzania: alternative approaches. *Physiotherapy*, 89, 743-749.
- Chandre, F., Darriet, F., Darder, M., Cuany, A., Doannio, J.M.C., Pasteur, N. and Guillet, P., 1998. Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. *Med. Vet. Entomol.*, 12, 359-366.
- Das, P.K., Pani, S.P. and Krishnamoorthy, K., 2000. Prospects of elimination of lymphatic filariasis in India. *ICMR Bull* 32(5-6), 41-54.
- Govindarajan, M., 2010a. Chemical composition and larvicidal activity of leaf essential oil from *Clausena anisata* (willd.) Hook. F. Benth (Rutaceae) against three mosquito species. *Asian Pacific J. Trop.Med.*, 3, 874-877.
- Govindarajan, M., 2010b. Larvicidal efficacy of *Ficus benghalensis* L. plant leaf extracts against *Culex quinquefasciatus* Say, *Aedes aegypti* L. and *Anopheles stephensi* L. (Diptera: Culicidae). *Eur. Rev. Med. Pharmacol. Sci.*, 14(2), 107-111.
- Govindarajan, M. and Sivakumar, R., 2012. Adulticidal and repellent properties of indigenous plant extracts against *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Parasitol. Res.*, 110, 1607-1620.
- Govindarajan, M., Jebanesan, A., Pushpanathan, T. and Samidurai, K., 2008. Studies on effect of *Acalypha indica* L. (Euphorbiaceae) leaf extracts on the malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitol. Res.*, 103(3), 691-695.
- Govindarajan, M., Rajeswary, M., Hoti, S.L., Murugan, K., Kovendan, K., Arivoli, S. and Benelli, G. 2016a. *Clerodendrum chinense*-mediated biofabrication of silver nanoparticles: Mosquitocidal potential and acute toxicity against non-target aquatic organisms. *J. Asia-Pacific Entomol.*, 19(1), 51-58.
- Govindarajan, M., Rajeswary, M., Veerakumar, K., Muthukumar, U., Hoti, S.L. and Giovanni Benellic, 2016b. Green synthesis and characterization of silver nanoparticles fabricated using *Anisomeles indica*: Mosquitocidal potential against malaria, dengue and Japanese encephalitis vectors. *Exper. Parasitol.*, 161, 40-47.
- Green, M.M., Singer, J.M. Sutherland, D.J. and Hibben, C.R., 1991. Larvicidal activity of *Tagetes minuta* (Marigold) toward *Aedes aegypti*. *J. Amer. Mosq. Control. Ass.*, 7, 282-286.
- Kamakshi, K.T., Raveen, R., Tennyson, S., Arivoli, S. and Reagan, D.A., 2015. Ovicidal and repellent activities of *Cereus hildmannianus* (K.Chum.) (Cactaceae) extracts against the dengue vector *Aedes aegypti* L. (Diptera: Culicidae). *Int. J. Mosq. Res.*, 2(1), 13-17.
- Kovendan, K., Murugan, K., Vincent, S. and Barnard, D.R., 2012. Studies on larvicidal and pupicidal activity of *Leucas aspera* Willd. (Lamiaceae) and bacterial insecticide, *Bacillus sphaericus* against malarial vector, *Anopheles stephensi* Liston. (Diptera: Culicidae). *Parasitol. Res.*, 110, 195-203.
- Marimuthu, S., Rahuman, A.A., Rajakumar, G., Santhoshkumar, T., Kirthi, A.V., Jayaseelan, C., Bagavan, A., Zahir, A.A., Elango, G., Kamaraj, C., 2010. Evaluation of green synthesized silver nanoparticles against parasites. *Parasitol. Res.*, 108(6), 1541-1549.
- Muthukumar, U., Govindarajan, M., Rajeswary, M., Hoti, S.L., 2014. Synthesis and characterization of silver nanoparticles using *Gmelina asiatica* leaf extract against filariasis, dengue, and malaria vector mosquitoes. *Parasitol Res.*, 114, 1817-1827.

- Naresh Kumar, A., Murugan, K., Madhiyazhagan, P., Kovendan, K., Prasannakumar, K., Thangamani, S. and Barnard, D., 2011. Mosquitocidal and water purification properties of *Cynodon dactylon*, *Aloe vera*, *Hemidesmus indicus* and *Coleus amboinicus*. *Parasitol. Res.*, 110(4), 1435-43.
- Panneerselvam, C., Ponarulselvam, S. and Murugan, K., 2011. Potential antiplasmodial activity of synthesized silver nanoparticle using *Andrographis paniculata* Nees (Acanthaceae). *Arch. Appl. Sci. Res.*, 3(6), 208-217.
- Rajkumar, G. and Abdul Rahuman, A., 2011. Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrata* leaf extract against filariasis and malaria vectors. *Acta. Trop.*, 118(3), 196-203.
- Reegan, A.D., Gandhi, M.R., Paulraj, M.G., Balakrishna, K. and Ignacimuthu, S., 2014. Effect of niloticin, a protolimonic acid isolated from *Limonia acidissima* L. (Rutaceae) on the immature stages of dengue vector *Aedes aegypti* L. (Diptera: Culicidae). *Acta. Trop.*, 139, 67-76.
- Salunkhe, R.B., Patil, S.V., Patil, C.D. and Salunke, B.K., 2011. Larvicidal potential of silver nanoparticles synthesized using fungus *Cochliobolus lunatus* against *Aedes aegypti* (Linnaeus, 1762) and *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitol. Res.*, 109(3), 823-831.
- Sharma, P., Mohan, L. and Srivastava, C.N., 2009. *Amaranthus oleracea* and *Euphorbia hirta*: natural potential larvicidal agents against the urban Indian malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitol Res.* 106, 171-176.
- Su, T. and Mulla, M.S., 1998. Ovicidal activity of neem products (Azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). *J. Am. Mosq. Cont. Ass.*, 14, 204-209.
- Subarani, S., Sabhanayakam, S. and Kamaraj, C., 2013. Studies on the impact of biosynthesized silver nanoparticles (AgNPs) in relation to malaria and filariasis vector control against *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol. Res.*, 112, 487-499.
- Veerakumar, K., Govindarajan, M. and Rajeswary, M., 2013. Green synthesis of silver nanoparticles using *Sida acuta* (Malvaceae) leaf extract against *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* (Diptera: Culicidae). *Parasitol. Res.*, 112, 4073-4085.
- Veerakumar, K., Govindarajan, M. and Hoti, S.L., 2014a. Adulticidal activity of silver nanoparticles (AgNPs) synthesized Using *Heliotropium indicum* plant leaf extract against adults of *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. *Parasitol. Res.*, 113(12), 4567-77. doi: 10.1007/s00436-014-4147-7.
- Veerakumar, K., Govindarajan, M. and Rajeswary, M., 2014b. Mosquito larvicidal properties of silver nanoparticles synthesized using *Heliotropium indicum* (Boraginaceae) against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol. Res.*, 113, 2363-2373.
- Veerakumar, K., Govindarajan, M. and Hoti, S.L., 2014c. Evaluation of plant-mediated synthesized silver nanoparticles against vector mosquitoes. *Parasitol Res.*, 113:4567-4577.
- World Health Organisation, 2010. Malaria. Factsheet No.94. Geneva. <http://www.who.int/mediacentre/factsheets/fs094/en/> [Accessed July 2010].
- Yamar, B.A., Diallo, D., Kebe, C.M., Dia, I. and Diallo, M., 2005. Aspects of bioecology of two Rift valley fever virus vectors in Senegal (West Africa) *Aedes vexans* and *Culex poicilipes* (Diptera; Culicidae). *J. Med. Entomol.*, 42(5), 739-750.