International Journal of Zoology and Applied Biosciences
Volume 1, Issue 2, pp: 118-129, 2016
https://doi.org/10.5281/zenodo.1309660

ISSN: 2455-9571
http://www.ijzab.com

Research Article

ADULTICIDAL ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLES USING *CHOMELIA ASIATICA* LINN. (FAMILY: RUBIACEAE) AGAINST *ANOPHELES STEPHENSI, AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE)

Udaiyan Muthukumaran[1], Marimuthu Govindarajan[1]*, Mohan Rajeswary[1], Kaliyan Veerakumar[1] A. Amsath[2] and K. Muthukumaravel[2]

[1]Unit of Vector control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalai Nagar-608 002, Tamilnadu, India
[2] Department of Zoology, Khadir Mohideen College, Adirampattinam-614 701, Tamilnadu, India

Article History: Received 26th February 2017; Revised 22nd April 2016; Accepted 22nd April 2016

ABSTRACT

Vector-borne diseases caused by mosquitoes are one of the major economic and health problems in many countries. Vector control methods involving use of chemical insecticides are becoming less effective due to the development of insecticides resistance, biological magnification of toxic substances through the food chain, and adverse effects on environmental quality and nontarget organisms including human health. Today, nanotechnology is a promising research domain which has a wide ranging application in vector control programs. In the present study, the mosquito adulticidal activity of silver nanoparticles (AgNPs) synthesized using *Chomelia asiatica* plant extract against three important adult female mosquitoes of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* was determined. Range of concentrations of synthesized AgNPs (12, 24, 36, 48, and 60 ppm) and leaf extract (70, 140, 210, 280, and 350 ppm) were tested against the adult mosquito of *An. stephensi* and *Ae. aegypti* and *Cx. quinquefasciatus*. AgNPs were rapidly synthesized using the leaf extract of *C. asiatica*, and the formation of nanoparticles was observed within 6 h. The results were recorded from UV–visible spectroscopy, fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX) and X- ray diffraction (XRD) analysis support the biosynthesis and characterization of AgNPs. The maximum efficacy was observed in synthesized AgNPs against the adult of *An. stephensi* (lethal dose) (LD_{50}=26.60 µg/mL; LD_{90}= 48.34 µg/mL), *Ae. aegypti* (LD_{50}=29.16 µg/mL; LD_{90}= 52.84 µg/mL), and *Cx. quinquefasciatus* (LD_{50}=32.23 µg/ mL; LD_{90}=58.24 µg/mL) respectively. No mortality was observed in the control. These results suggest that the leaf extracts of *C. asiatica* and green synthesis of AgNPs have the potential to be used as an ideal eco-friendly approach for the control of the *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. This is the first report on the adulticidal activity of the plant extracts and AgNPs.

Keywords: Green synthesis, Adulticidal activity, *Chomelia asiatica*, AgNPs, Mosquitoes.

INTRODUCTION

Mosquitoes (Diptera: Culicidae) pose the greatest threat to public health since of their ability to act as vectors of pathogens causing malaria, lymphatic filariasis, dengue, chikungunya, yellow fever, and Japanese encephalitis. These diseases are also responsible for the mortality, morbidity, economic loss and social disruption (Govindarajan *et al*., 2012). Malaria now is responsible for the estimated more than 300 million people falling ill, and there are one million deaths per year (World Health Organization, 2007). *Anopheles stephensi* is accepted as a major vector for urban malaria in India. This species prefers to breed in small synthetic water collections and is responsible for frequent outbreaks of malaria, particularly at construction sites in urban areas (Mittal *et al*., 2005). Among 53 anopheline species present in India, nine are vectors of malaria. In India, malaria is still the most important cause of morbidity and mortality with approximately 2–3 million new cases arising every year (Sharma *et al*., 2009). Dengue fever, dengue hemorrhagic

*Corresponding author: Dr. M. Govindarajan, Assistant Professor, Department of Zoology, Annamalai University, Annamalai Nagar-608 002, Tamilnadu, India, Email: drgovind1979@gmail.com, Mobile: +91 9585265999.

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). Dengue is one of the most important emerging tropical diseases (Gubler, 2002). Dengue epidemics have been reported in over 100 countries and 2.5 billion people live in areas where dengue is endemic (Guzman and Kouri 2002). The dengue viruses are spread and maintained by *Aedes aegypti*, the principal vector of dengue. *Ae.aegypti* is an anthropophilic mosquito, which has evolved intimate relationship with humans and exhibits several behavioral traits like oviposition in man-made and man-used natural and artificial containers (Trpis and Hausermann 1978). Although the vector–man–vector transmission is a well understood mechanism of transmission of dengue viruses under natural conditions (Govindarajan *et al*., 2013). *Culex quinquefasciatus* is a predominant house-resting mosquito in many tropical countries. It is important as a vector of filariasis in some countries as well as a nuisance mosquito. Mosquitoes breed in polluted waters such as blocked drains, damaged septic tanks, or soak age pools close to human habitations. Lymphatic filariasis is probably the fastest spreading insect borne disease of man in the tropics, affecting about 146 million people (WHO, 1992). *Cx.quinquefasciatus* is the most widely distributed mosquito in India, mainly found in urban and suburban areas. The most efficient approach to control the vector is to target the immature stages of the life cycle. Lymphatic filariasis is a mosquito-borne disease caused by mosquito-transmitted filarial nematodes, including *Wuchereria bancrofti* and *Brugia malayi*. The infected people carry the nocturnally periodic *W. bancrofti*, which has *Cx. quinquefasciatus* as the main mosquito vector. *Cx.quinquefasciatus* is a vector of lymphatic filariasis, which is a widely distributed tropical disease with around 120 million people infected worldwide, and 44 million people have common chronic manifestation (Bernhard *et al*., 2003).

Use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future (Elango *et al*., 2010). Recently, there has been a growing interest in investigating the insecticidal potential of extracts from plants used in folk medicine. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against parasites (Mehlhorn *et al*., 2005). Mosquito control is being strengthened in many areas, but there are significant challenges, including an increasing mosquito resistance to insecticides and a lack of alternative, cost-effective, and safe insecticides. Increasing insecticide resistance requires the development of strategies for prolonging the use of highly effective vector control compounds. The use of combinations of multiple insecticides and phytochemicals is one such strategy that may be suitable for mosquito control. Thus, attempts to develop novel materials as mosquito larvicides are still necessary. With the progress of nanotechnology, many laboratories around the world have investigated silver nanoparticle (AgNPs) production. In recent years, nanoparticle polymer composites have become important owing to their small size and large surface area and because they exhibit unique properties not seen in bulk materials. As a result, nanoparticles (NPs) have useful applications in photovoltaic cells, optical and biological sensors, conductive materials, and coating formulations (Templeton *et al*., 2000).

Phytochemicals are botanicals which are naturally occurring insecticides obtained from floral resources. Applications of phytochemicals in mosquito control were in used since the 1920s (Shahi *et al*., 2010), but the discovery of synthetic insecticides such as DDT in 1939 sidetracked the application of phytochemicals in mosquito control programmes. After facing several problems due to injudicious and over application of synthetic insecticides in nature, refocus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Since then, the search for new bioactive compounds from the plant kingdom and an effort to determine its structure and commercial production has been initiated. At present, phytochemicals make up to 1 % of the world's pesticide market (Isman, 1997). Insecticidal effects of plant extracts vary not only according to plant species, mosquito species, geographical varieties and parts used, but also due to extraction methodology adopted and the polarity of the solvents used during extraction. A wide selection of plants from herbs, shrubs and large trees was used for extraction of mosquito toxins. Phytochemicals were extracted either from the whole body of little herbs or from various parts like fruits, leaves, stems, barks, roots, etc. of larger plants or trees. In all cases where the most toxic substances were concentrated upon, they were found and extracted for mosquito control. Plants produce numerous chemicals, many of which have medicinal and pesticidal properties. More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes. Members of the plant families-Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae-have various types of larval, adulticidal or repellent activities against different species of mosquitoes (Shaalan *et al*., 2005).

Nanoparticles and their applications are one of the recent approaches among them. Nanometer-size metallic particles show unique and considerably altered physical, chemical and biological properties compared to their macro scaled counterparts. The difference is mostly due to their high surface-to-volume ratio (Ansari *et al*., 2010). The collected information in the current situations paved the way to develop an alternative ecofriendly biomolecules as a potential biocontrol agent. In this regard, development of reliable processes for the synthesis of nanoparticles with unique properties is an important aspect of nanotechnology.

During synthesis, physical and chemical procedures could be followed for metallic nanoparticles; however, these method posses many problems including the use of toxic solvents, generation of hazardous by-products, high energy consumption, pressure, and are also expensive. The biological methods for synthesis of nanoparticles using plants and microorganisms have been investigated as possible ecofriendly alternatives to physical and chemical methods (Mohanpuria *et al*., 2008). Plants and microbes are currently used for nanoparticle synthesis. The use of plants for synthesize of nanoparticles are rapid, low cost, eco-friendly, and a single-step method for biosynthesis process (Huang *et al*., 2007). Silver nanoparticles (AgNPs) 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). It has been reported that medicinally valuable angiosperms have the greatest potential for synthesis of metallic nanoparticles with respect to quality and quantity (Song and Kim 2009). Among the various known synthesis methods, plant-mediated nanoparticle synthesis is preferred as it is cost-effective, environmentally friendly, and safe for human therapeutic use (Kumar and Yadav 2009).

Reasonable knowledge is available about the adulticidal properties of plant-synthesized metal nanoparticles. Adulticidal activity of synthesized silver nanoparticles from *M. elengi* was highly effective against *An. stephensi* and *Ae. albopictus* (Subramaniam *et al*., 2015). Suresh *et al.* (2015) evaluated the adulticidal activity of synthesized silver nanoparticles using *P. niruri* were toxic against *Ae. aegypti.* Roni *et al*. (2015) reported that fabricated silver nanoparticles using *H. musciformis* were toxic against female adult of *Ae. aegypti*. AgNPs synthesized using *Feronia elephantum* leaf extract against adults of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* (Veerakumar and Govindarajan 2014). The adulticidal activity of silver nanoparticles synthesized using *H. indicum* leaf extract has been evaluated against adults of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* (Veerakumar *et al*., 2014).

In addition recently, a growing number of plants have been successfully used for efficient and rapid extracellular synthesis of silver, copper, and gold nanoparticles (Benelli, 2016; Govindarajan, 2016). Good examples include cheap extracts of *Chomelia asiatica* (Muthukumaran *et al*., 2015a), *Sida acuta* (Veerakumar *et al*., 2013), *Gmelina asiatica* (Muthukumaran *et al*., 2015b), *Clerodendrum chinense* (Govindarajan *et al*., 2016a), *Anisomeles indica* (Govindarajan *et al*.,2016b), *Bauhinia variegata* (Govindarajan *et al*., 2016c).

In the present study, the adulticidal activity of AgNPs synthesized using *C. asiatica* leaf extract was assessed under laboratory conditions. We report the synthesis of AgNPs, reducing the silver ions present in the solution of silver nitrate by the cell-free aqueous leaf extract of *C. asiatica.* However, these biologically synthesized nanoparticles (AgNPs) and aqueous extract of *C. asiatica* were found to produce a significant mosquito adulticidal activity against target species.

MATERIALS AND METHODS

Collection of materials

Fresh leaves of *C. asiatica* (L.) Kuntze (Figure 1) were collected from Kodiyakarai, Tamil Nadu, India, and the taxonomic identification was made by Dr. V. Vengatesalu, Professor, 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 was obtained from Qualigens Fine Chemicals, Mumbai, India.

Figure 1. *Chomelia asiatica* plant.

Mosquitoes

The mosquitoes, *An.stephensi*, *Ae.aegypti*, and *Cx.quinquefasciatus* 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.

Preparation of plant extracts

The leaves (*C. asiatica*) were dried in 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 (boiled and cooled distilled water) with constant stirring on a magnetic stirrer. The suspension of dried leaf powder in water was left for 3 h, filtered through Whatman no. 1 filter paper, and the filtrate was stored in amber-colored air-tight bottle at 10 °C temperature until use.

Synthesis of silver nanoparticles

The fresh leaf of *C. asiatica* broth solution 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 be used within 1 week. The filtrate was treated with aqueous 1mM $AgNO_3$ (21.2 mg of $AgNO_3$ powder in 125 mL Milli-Q water) solution in an Erlenmeyer flask and incubated at room temperature. Eighty-eight-milliliter aqueous solution of 1 mM of 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 AgNPs.

Characterization of the synthesized AgNPs

Synthesis of AgNPs solution with leaf extract may be easily observed by UV–Vis spectroscopy. The bioreduction of the Ag+ ions in solutions was monitored by periodic sampling of aliquots (1 mL) of the aqueous component after 20 times dilution and measuring the UV–Vis spectra of the solution. UV–Vis spectra of these aliquots were monitored as a function of time of reaction on a Shimadzu 1601 spectrophotometer in the 300–800-nm range operated at a resolution of 1 nm. Further, the reaction mixture was subjected to centrifugation at 60,000×g for 40 min; the resulting pellet was dissolved in deionized water and filtered through Millipore filter (0.45 µm). An aliquot of this filtrate containing silver nanoparticles was used for X-ray diffraction (XRD), Fourier transform infrared (FTIR). For electron microscopic studies, 25 µL of sample was sputter-coated on a copper stub and the images of the nanoparticles were studied using scanning electron microscopy (SEM; JEOL, Model JFC-1600). FTIR spectra of the samples were measured using Perkin- Elmer Spectrum One instrument in the diffuse reflectance mode at a resolution of 4/cm in KBr pellets.

Adulticidal activity

Adulticidal bioassay was performed by slightly modified method of World Health Organization (1981) and Veerakumar and Govindarajan (2014). Based on the wide range and narrow range tests, aqueous crude extract was tested at 70, 140, 210, 280, and 350 µg mL−1 concentrations, and AgNPs were tested at 12, 24, 36, 48, and 60 µg mL−1 concentrations. Aqueous crude extract and AgNPs were applied on Whatman no. 1 filter papers (size 12×15 cm). Control papers were treated with silver nitrate and distilled water. Twenty female mosquitoes were collected and gently transferred into a plastic holding tube. The mosquitoes were allowed to acclimatize in the holding tube for 1 h and then exposed to test paper for 1 h. At the end of exposure period, the mosquitoes were transferred back to the holding tube and kept for 24-h recovery period. A pad of cotton soaked with 10 % glucose solution was placed on the mesh screen. Each test included a set control groups (silver nitrate and distilled water) with five replicates for each individual concentration. The lethal concentrations (lethal dose (LD_{50}, LD_{90}) were calculated by probit analysis (Finney 1971).

Statistical analysis

The average adult mortality data were subjected to probit analysis for calculating LD_{50}, LD_{90}, and other statistics at 95 % confidence limits of upper confidence limit and lower confidence limit, and chi-square values were calculated using the Statistical Package of Social Sciences 12.0 software. Results with $p<0.05$ were considered to be statistically significant.

RESULTS

Adulticidal activity of aqueous crude extract and synthesized AgNPs

The results of the adulticidal activity of aqueous crude extract and synthesized AgNPs against the adult of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* are presented in Tables 1 and 2 Considerable mortality was evident after the treatment of *C. asiatica* for all three important vector mosquitoes. At higher concentrations, the adult showed restless movement for some times with abnormal wagging and then died. The rates of mortality were directly proportional to concentration. The maximum efficacy was observed in synthesized AgNPs against the adult of *An. stephensi* (LD_{50} = 26.60 µg/mL; LD_{90} = 48.34 µg/mL), *Ae. aegypti* (LD_{50}=29.16 µg/mL; LD_{90}=52.84 µg/mL), and *Cx. quinquefasciatus* (LD_{50}=32.23 µg/mL; LD_{90}=58.24 µg/mL), respectively. No mortality was observed in the control. χ^2 value was significant at the $p\leq0.05$ level.

Characterization of silver nanoparticles

Color change was noted by visual observation in the *C. asiatica* leaf extracts when incubated with $AgNO_3$ solution. *C. asiatica* leaf extract without $AgNO_3$ 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). The absorption spectrum of *C. asiatica* leaf extracts at different wavelengths ranging from 300 to 800 nm revealed a peak at 450 nm (Figure 2b). FTIR analysis of the purified nanoparticles showed the presence of bands due to O–H group C=H bending (872.03), N=H bending (1111.93), –C=O stretch (1384.00), C–H stretch

(2849.30), C–H stretch (2924.25), and O–H stretch (3423.73) (Figure 3). SEM micrographs of the synthesized AgNPs of *C. asiatica* magnified at ×4000 and measured at 10 µm are shown in Fig. 4a. The triangular, pentagonal, and hexagonal structures are clear. EDX proves the chemical purity of the synthesized AgNPs (Figure 4b). XRD analysis (Figure 5) showed intense peaks at 2θ values of 38.025°, 44.20°, 64.38°, and 77.34° corresponding to (27), (32), (15), and (34) Bragg's reflection based on the face-centered cubic structure of silver nanoparticles.

Table 1. Adulticidal activity of Chomelia asiatica aqueous leaf extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*.

Mosquitoes	Concentration (µg/mL)	24 h mortality[a] (%) ± SD	LD_{50}(µg/mL) (LCL-UCL)	LD_{90}(µg/mL) (LCL-UCL)	χ^2
An.stephensi	Control	0.0±0.0	158.34 (118.67-196.47)	281.25 (235.51-369.94)	17.199*
	70	27.2±1.4			
	140	45.8±1.2			
	210	63.1±0.6			
	280	87.4±1.8			
	350	100.0±0.0			
Ae. aegypti	Control	0.0±0.0	172.83 (123.43-221.64)	310.18 (253.46-437.72)	22.383*
	70	24.6±1.2			
	140	45.2±1.4			
	210	57.7±0.8			
	280	76.3±1.6			
	350	99.5±1.5			
C.quinquefasciatus	Control	0.0±0.0	184.70 (141.02-229.54)	325.63 (271.03-439.42)	18.304*
	70	20.1±1.6			
	140	42.6±1.2			
	210	54.9±0.8			
	280	72.2±1.4			
	350	97.5±1.8			

SD standard deviation, LCL lower confidence limits, UCL upper confidence limits, χ^2 Chi-square test.

*p<0.05, level of significance.

[a] Values are mean ± SD of five replicates.

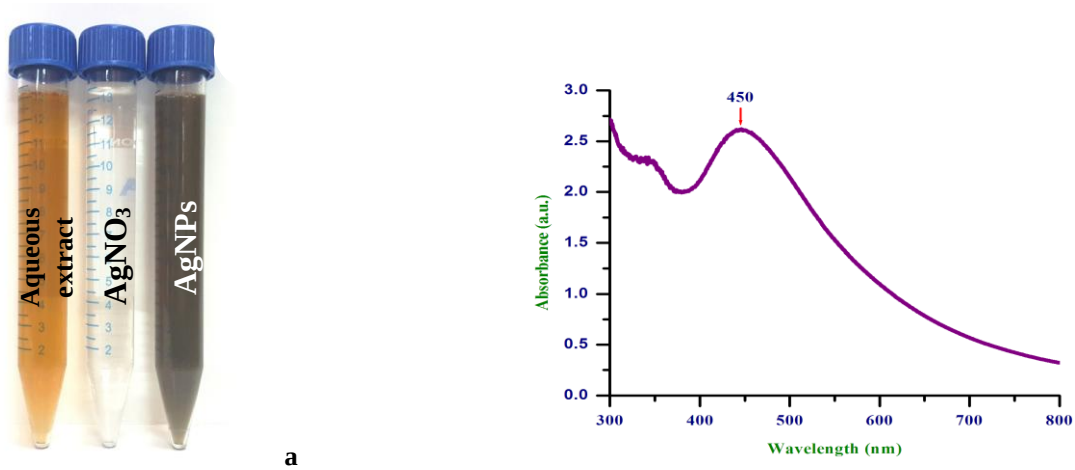

Figure 2a. Color intensity of *C. asiatica* aqueous extract before and after the reduction of silver nitrate (1 mM). The color change indicates Ag^+ reduction to elemental nanosilver. **Figure 2b.** UV-visible spectrum of silver nanoparticles after 180 min from the reaction.

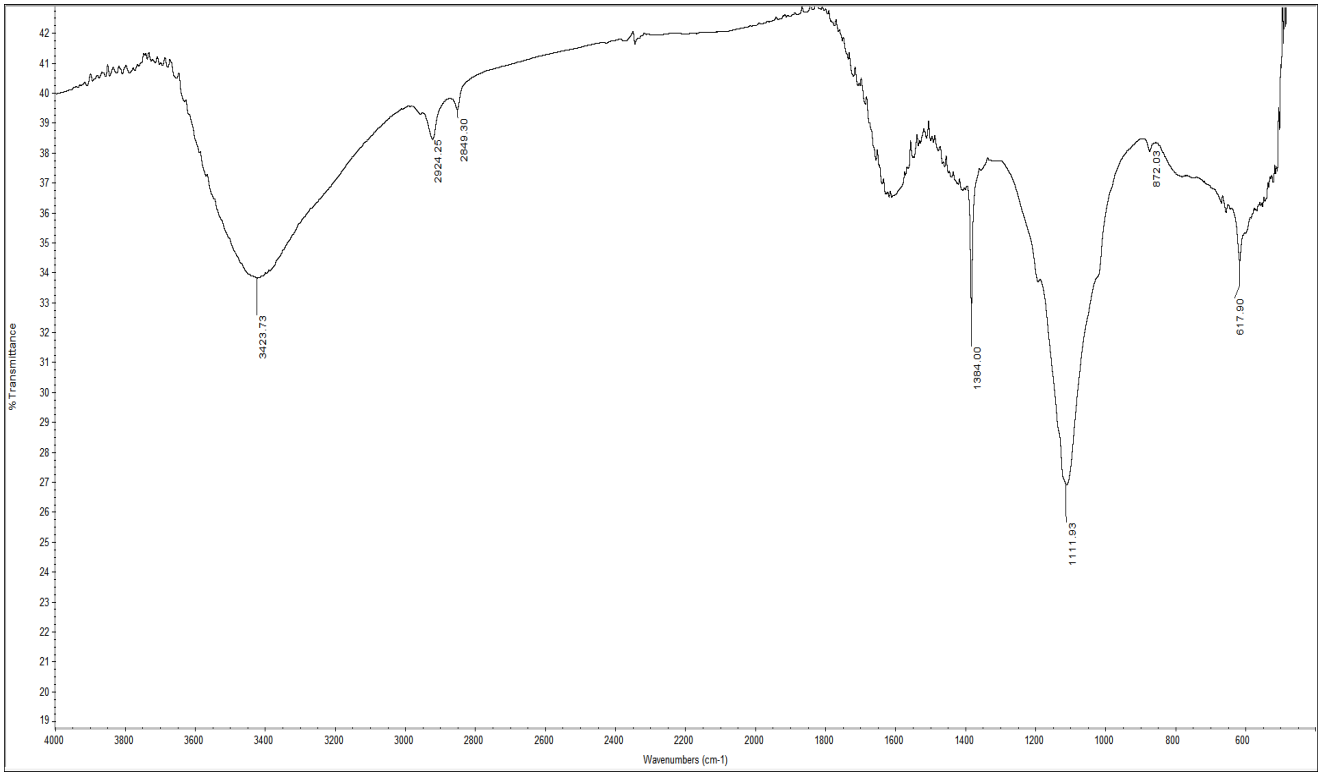

Figure 3. FT-IR spectrum of silver nanoparticles green-synthesized using *C. asiatica* aqueous leaf extract.

Table 2. Adulticidal activity of silver nanoparticles against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus.*

Mosquitoes	Concentration (µg/mL)	24 h mortality[a] (%) ± SD	LD_{50}(µg/mL) (LCL-UCL)	LD_{90}(µg/mL) (LCL-UCL)	χ^2
An.stephensi	Control	0.0±0.0	26.60	48.34	18.46*
	12	29.2±0.8	(19.27-33.51)	(40.11-64.77)	
	24	45.8±1.2			
	36	67.8±1.6			
	48	84.5±1.8			
	60	100.0±0.0			
Ae. aegypti	Control	0.0±0.0	29.16	52.84	18.86*
	12	26.2±0.4	(21.47-36.68)	(43.75-71.72)	
	24	44.8±1.2			
	36	59.1±1.6			
	48	78.6±1.8			
	60	98.5±1.2			
C.quinquefasciatus	Control	0.0±0.0	32.23	58.24	19.08*
	12	21.4±1.2	(24.16-40.65)	(47.95-80.81)	
	24	43.2±0.8			
	36	55.1±1.6			
	48	68.6±1.4			
	60	95.2±1.2			

SD standard deviation, LCL lower confidence limits, UCL upper confidence limits, χ^2 Chi-square test.

*$p<0.05$, level of significance. [a] Values are mean ± SD of five replicates.

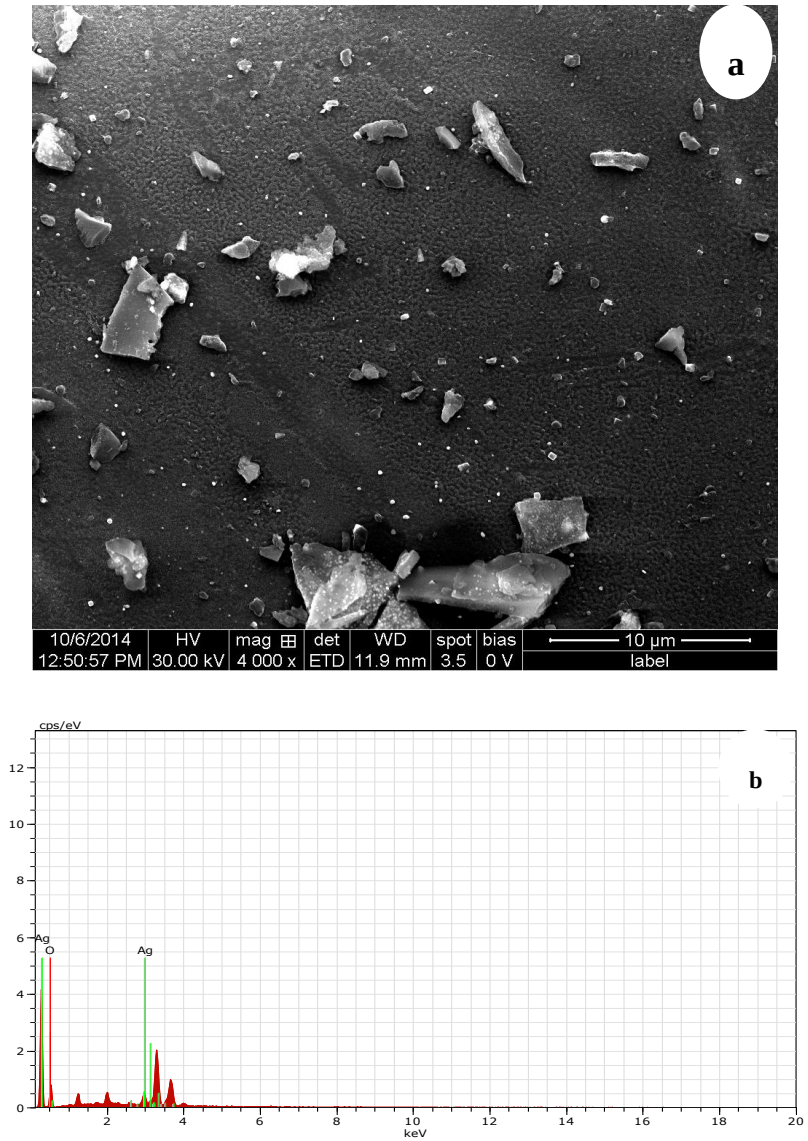

Figure 4. Scanning electron micrographs of AgNPs synthesized with *C. asiatica* leaf extract and 1.0 mM $AgNO_3$ solution and incubated at 60 °C for 6 h at pH 7.0. **a** Magnified ×4,000; inset bar represents10 µm; **b** EDX image showing chemical composition.

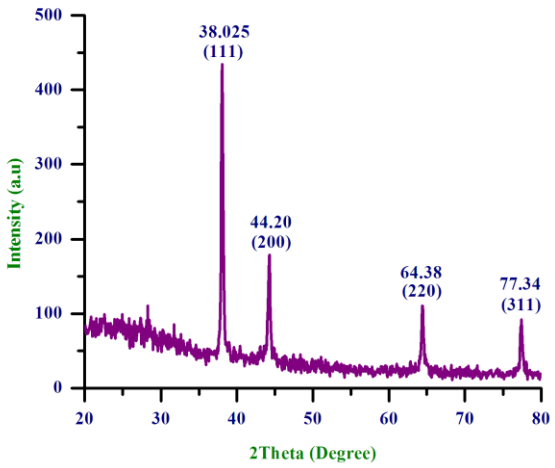

Figure 5. XRD pattern of silver nanoparticles green-synthesized using *C.asiatica* leaf extract.

DISCUSSION

Our results showed that synthesized AgNPs of *C. asiatica* have significant adulticidal activity against *An.stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. This result is also comparable to earlier reports of Veerakumar *et al.,* (2014) who reported that synthesized AgNPs from *Heliotropium indicum* against the adult of *An. stephensi* (LD_{50}=26.712 µg/mL; LD_{90}= 49.061 µg/mL), *Ae. aegypti* (LD_{50}=29.626 µg/mL; LD_{90}= 54.269 µg/mL), and *Cx. quinquefasciatus* (LD_{50}=32.077 µg/ mL; LD_{90}=58.426 µg/mL) respectively. Silver nanoparticles synthesized using *F. elephantum* leaf extract were toxic against adults of *An. stephensi*, *Ae. aegypti*, and *Cx. Quinquefasciatus*. *An. stephensi* LD_{50} and LD_{90} were 18.041 and 32.575 µg/ml. *Ae. aegypti* LD_{50} and LD_{90} were 20.399 and 37.534 µg/ml. *Cx. quinquefasciatus* LD_{50} and LD_{90} were 21.798 and 39.596 µg/ml (Veerakumar and Govindarajan 2014). The adult mortality was found in ethanol extract of *Citrus sinensis* with the LC_{50} and LC_{90} values of 272.19 and 457.14 ppm, *An. stephensi* of 289.62 and 494.88 ppm, and *Ae. aegypti* of 320.38 and 524.57 ppm, respectively (Murugan *et al*.,2012). Biosurfactant surfacing, produced by *Bacillus subtilis* subsp. *subtilis*, is a potential bio adulticide for ULV spray against malaria-transmitting *An. stephensi* mosquitoes (Geetha *et al.*, 2011).

The effects of the tested extract, adult emergence, and adulticidal activity of the mosquitoes are remarkably greater than those reported for other plant extracts in the literature. For example, at the highest concentration, 50 % inhibition of the emergence of the adult mosquitoes was observed by the use of the ethyl acetate fractions of *Calophyllum inophyllum* seed and leaf, *Solanum suratense*, and *Samadera indica* leaf extracts and the petroleum ether fraction of *Rhinocanthus nasutus* leaf extract on *Cx. quinquefasciatus*, *An. stephensi*, and *Ae. aegypti* (Muthukrishnan and Puspalatha 2001). Similarly 88 % of the adult mortality was observed by the use of *Pelargonium citrosa* leaf extracts at 2 % concentration against *An. stephensi* (Jeyabalan *et al*., 2003). A similar result was obtained in the root extract of *Valeriana jatamansi* which exhibited adulticidal activity of 90 % lethal concentration against adult *An. stephensi*, *An. culicifacies*, *Ae. aegypti*, *An. albopictus*, and *Cx. quinquefasciatus* and were 0.14, 0.16, 0.09, 0.08, and 0.17 and 0.24, 0.34, 0.25, 0.21, and 0.28 mg/cm^2, respectively (Dua *et al*., 2008). The larvicidal and adulticidal activities of ethanolic and water mixture (50:50) of plant extracts *Eucalyptus globulus*, *Cymbopogon citratus*, *Artemisia annua*, *Justicia gendarussa*, *Myristica fragrans*, *A. squamosa*, and *Centella asiatica* were tested against *An. stephensi*, and the most effective between 80 and 100 % was observed in all extracts (Senthilkumar *et al*., 2009). Mathivanan *et al*., (2010) determine that the LC_{50} and LC_{90} values of crude methanol extract of leaves of *Ervatamia coronaria* on *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* larvae in 24 h were 72.41, 65.67, and 62.08 mg/L and 136.55, 127.24, and 120.86 mg/L, respectively. Komalamisra *et al*., (2005) have reported that the petroleum ether and methanol (MeOH) extracts of *Rhinacanthus nasutus* and *Derris elliptica* exhibited larvicidal effects against *An. aegypti*, *Cx. quinquefasciatus*, *A. dirus*, and *Mansonia uniformis* with LC_{50} values between 3.9 and 11.5 mg/L, while the MeOH extract gave LC_{50} values of between 8.1 and 14.7 mg/L. *D. elliptica* petroleum ether extract showed LC_{50} values of between 11.2 and 18.84 mg/L, and the MeOH extract exhibited LC_{50} values between 13.2 and 45.2 mg/L. Khanna *et al*., (2011) have reported that the larvicidal crude leaf extract of *Gymnema sylvestre* showed the highest mortality in the concentration of 1,000 ppm against the larvae of *An. subpictus* (LC_{50}=166.28 ppm) and against the larvae of *Cx. quinquefasciatus* (LC_{50}=186.55 ppm).

The maximum efficacy was observed in gymnemagenin compound isolated from petroleum ether leaf extract of *G. sylvestre* with LC_{50} values against the larvae of *An. subpictus* at 22.99 ppm and against *Cx. quinquefasciatus* at 15.92 ppm. Elango and Rahuman (2011) reported that the highest larval mortality was found in leaf ethyl acetate extract of *A. malabarica*, acetone extract of *Eclipta prostrata*, and methanol extract of *A. lineata*, *Chrysanthemum indium*, and *Sesbania grandiflora* after 24 h against *An. subpictus* (LC_{50}=2.53, 2.82, 2.31, 2.56, and 2.08 mg/mL, and LC_{90}= 6.40, 8.06, 7.45, 6.98, and 6.20 mg/mL, respectively), the hexane extract of *A. lineata* and *Datura metel*, methanol extract of *Aristolochia bracteolata* and *E. prostrata* showed larval mortality after 48 h against *An. subpictus* (LC_{50}=3.05, 2.11, 3.00, and 2.18 mg/mL, and LC_{90}=9.06, 6.22, 8.23, and 5.77 mg/mL, respectively). Zahir *et al*., (2009) reported that the maximum efficacy was observed in the leaf ethyl acetate extract *of Achyranthes aspera*, leaf chloroform extract of *A. malabarica*, flower methanol of *G. superba*, and leaf methanol extract of *R. communis* against the larvae of *An. subpictus* (LC_{50}=48.83, 135.36, 106.77, and 102.71 ppm, and LC_{90}= 225.36, 527.24, 471.90, and 483.04 ppm) respectively. Yadav *et al*., (2002) have reported the methanol, chloroform and ether extracts of *Euphorbia tirucalli* latex and stem bark were evaluated for larvicidal activity against laboratory-reared larvae of *C. quinquefasciatus*. Sharma *et al*., (2005) reported that the acetone extract of *Nerium indicum* and *Thuja oriertelis* has been studied with LC_{50} values of 200.87, 127.53, 209.00 and 155.97 ppm against III instar larvae of *An. stephensi* and *Cx. quinquefasciatus*, respectively. *Clitoria ternatea* leaf methanol extract showed dose-dependent larvicidal activity against *An. stephensi* with LC_{50} values of 555.6 (24 h) and 867.3 (48 h) ppm, also the root extracts with LC_{50} value of 340 ppm (48 h). Seed extract showed larvicidal activity (LC_{50}=116.8, 195 ppm) after 24 h and (LC_{50}=65.2, 154.5 ppm) after 48 h treatment against *An. stephensi* and *Ae. aegypti*, respectively. Larvicidal activity of flower methanol extract showed LC_{50} values 233 and 302.5 ppm against *An. stephensi* and *Ae. aegypti*, respectively, after 48 h treatment. Methanol extract showed lowest LD values against several instar of larvae and 50 adult (121.59, 142.73, 146.84, 202.98, 290.65, 358.42 and 300.03 µg/cm^2, respectively) which indicates highest toxicity or insecticidal activity (Ashraful Alam *et al*., 2009). *Sphaeranthus indicus* LC_{50} values were 544.93, 377.86 and 274.79 ppm and LC_{90} values were 1,325.32, 1,572.55 and 1,081.29 ppm at 24 h;

Cleistanthus collinus LC_{50} values were 375.34, 318.29 and 226.10 ppm, and LC_{90} values were 699.65, 1,577.62 and 1,024.92 ppm at 24 h; and *Murraya koenigii* LC_{50} values were 963.53, 924.85 and 857.62 ppm and LC_{90} values were 1,665.12, 1,624.68 and 1,564.37 ppm at 24 h, respectively.

However, the highest larval mortality was observed in *C. collinus* followed by *S. indicus* and *M. koenigii* of various concentrations at 24, 48 and 72 h against *Cx. quinquefasciatus* (Kovendan *et al*., 2012). Bagavan *et al*., (2009) observed that the highest mortality was found in leaf hexane extract of *Annona squamosa*, methanol extracts of *G. superba* and *Phyllanthus emblica* against *H. bispinosa* with LC_{50} values of 145.39, 225.57, and 256.08 ppm, respectively; and the acetone and ethyl acetate extracts of *A. squamosa*, methanol extract of *Centella asiatica*, acetone extracts of *G. superba*, and ethyl acetate, hexane, and methanol extracts of *Pergularia daemia* against *An. subpictus* showed the LC_{50} values of 17.48, 18.60, 26.62, 18.43, 34.06, 13.63, and 50.39 ppm, respectively. Singhi *et al*., (2006) have reported that the latex of *Calotropis procera* has shown larvicidal efficacy against all three important vector species: *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* in India. Patil *et al*., (2011) evaluated the larvicidal activity of extracts of medicinal plants *Plumbago zeylanica* and *Cestrum nocturnum* against *Ae. aegypti*; the LC_{50} values of both the plants were less than 50 ppm. The larvicidal stability of the extracts at five constant temperatures (19°C, 22°C, 25°C, 28°C, and 31°C) evaluated against fourth-instar larvae revealed that toxicity of both plant extracts increases with increase in temperature. Prophiro *et al*., (2012) reported that the susceptibility of larvae was determined under three different temperatures, 15°C, 20°C, and 30°C, with lethal concentrations for *Copaifera* sp. ranging from LC_{50} 47 mg/L to LC_{90} 91 mg/L and for *Carapa guianensis* LC_{50} 136 mg/L to LC_{90} 551 mg/L.

The synthesized zinc oxide nanoparticles against *Rhipicephalus microplus* and *Pediculus humanus* capitis and the larvae *of An. subpictus* and *Cx. quinquefasciatus* showed LC_{50} values of 29.14, 11.80, 11.14, and 12.39 mg/L, respectively (Kirthi *et al*., 2011). Soni and Prakash (2012) have reported the synthesized AgNPs using *Chrysosporium tropicum* against the third instar larvae of *An. aegypti* with LC_{50}=4 ppm, LC_{90}=8.91 ppm, and LC_{99}=13.18 ppm. AgNPs 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 *Ae. aegypti* (LC_{50} 1.29, 1.48, and 1.58; LC_{90} 3.08, 3.33, and 3.41 ppm) and against *An. stephensi* (LC_{50} 1.17, 1.30, and 1.41; LC_{90} 2.99, 3.13, and 3.29 ppm) (Salunkhe *et al*., 2011). Synthesis of silver nanoparticles was carried out using leaves of *Catharanthus roseus* and their antiplasmodial activities against *P. falciparum* (Ponarulselvam *et al*., 2012). In the present results, synthesized AgNPs against first to fourth instar larvae and pupae of *Ae.aegypti* had LC_{50} values of 13.34, 17.19, 22.03, 27.57, and 34.84 ppm and LC_{90} values of 36.98, 47.67, 55.95, 67.36, and 77.72 ppm, respectively. AgNPs synthesized using *Euphorbia hirta* plant leaf extract against malarial vector *An. stephensi* was determined; the highest larval mortality was found in the synthesized AgNPs against the first to fourth instar larvae and pupae with LC_{50} values of 10.14, 16.82, 21.51, and 27.89 ppm, respectively, LC_{90} values of 31.98, 50.38, 60.09, and 69.94 ppm, respectively, and LC_{50} and LC_{90}values of pupae of 34.52 and 79.76 ppm, respectively (Agalya Priyadarshini *et al*., 2012). In the present results, synthesized AgNPs 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. The larvicidal effect of aqueous crude leaf extracts, silver nitrate, and synthesized silver nanoparticles of *Mimosa pudica* showed that the highest mortality was found in synthesized AgNPs against the larvae of *An. subpictus* (LC_{50}=8.89, 11.82, and 0.69 ppm) and against the larvae of *Cx. quinquefasciatus* (LC_{50}= 9.51, 13.65, and 1.10 ppm) (Marimuthu *et al*., 2011).

CONCLUSION

In conclusion, green synthesis shows that the environmentally benign and renewable source of *C. asiatica* is used as an effective reducing agent for the synthesis of AgNPs. This biological reduction of silver nanoparticles would be a boon for the development of clean, nontoxic, and environmentally acceptable green approach to produce AgNPs involving organisms even ranging to higher plants. The formed AgNPs are highly stable and have significant mosquito adulticidal activity of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. This is the first report on the mosquito adulticidal activity of synthesized nanoparticles from *C. asiatica*.

ACKNOWLEDGMENTS

The authors would like to thank 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

REFERENCES

Agalya Priyadarshini, K., Murugan, K., Panneerselvam, C., Ponarulselvam, S., Hwang J-S. and Nicoletti, M., 2012. Biolarvicidal and pupicidal potential of silver nanoparticles synthesized using *Euphorbia hirta* against *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitol. Res.*, 111: 997-1006.

Ansari, A.A., Alhoshan, M., Alsalhi, M.S. and Aldwayyan, A.S., 2010. Prospects of nanotechnology in clinical immunodiagnostics. *Sensors.*, 10: 6535–6581.

Ashraful Alam, M., Rowshanul, H.M., Nikkon, F., Khalequzzaman, M. and Rezaul Karim, M., 2009. Insecticidal Activity of Root Bark of *Calotropis gigantea* L. against *Tribolium castaneum* (Herbst). *World J. Zool.*, 4(2): 90-95.

Bagavan, A., Kamaraj, C., Elango, G., Zahir, A.A. and Rahuman, A.A., 2009. Adulticidal and larvicidal efficacy of some medicinal plant extracts against tick, fluke and mosquitoes. *Vet. Parasitol.,* 166:286-292.

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 Magnusson, P., 2003. Management of patients with lymphoedema caused by filariasis in North-eastern Tanzania: alternative approaches. *Physiotherapy*, 89: 743-749.

Dua, V.K., Alam, M.F., Pandey, A.C., Rai, S., Chopra, A.K. and Kaul, V.K., 2008. Insecticidal activity of *Valeriana jatamansi* (Verbenaceae) against mosquitoes. *J. Am. Mosq. Control. Assoc.,* 24: 315-318.

Elango, G. and Rahuman, A.A., 2011. Evaluation of medicinal plant extracts against ticks and fluke. *Parasitol. Res.,* 108(3): 513-519.

Elango, G., Rahuman, A.A., Bagavan, A., Kamaraj, C., Zahir, A.A., Rajakumar, G., Marimuthu S. and Santhoshkumar, T., 2010. Studies on effects of indigenous plant extracts on malarial vector, *Anopheles subpictus* Grassi (Diptera: Culicidae). *Trop Biomed.,* 27(2): 143-154.

Finney. D.J., 1971. Probit analysis. Cambridge Univ. Press, Cambridge, 333 pp.

Geetha, I., Paily, K.P. and Manonmani, A.M., 2011. Mosquito adulticidal activity of a biosurfactant produced by *Bacillus subtilis* subsp. *subtilis. Pest Manag. Sci.,* 68: 1447-1450.

Govindarajan, M., 2016. Green Synthesized Silver Nanoparticles: A Potential New Insecticide for Mosquito Control. Springer International Publishing Switzerland, H. Mehlhorn (ed.), Nanoparticles in the Fight Against Parasites. *Parasitol. Res. Mon*. Chapter 7, doi: 10.1007/978-3-319-25292-6_7 (ISSN: 2192-3671).

Govindarajan, M. and Benelli, G., 2016. Facile biosynthesis of silver nanoparticles using *Barleria cristata*: mosquitocidal potential and biotoxicity on three non-target aquatic organisms. *Parasitol Res.* doi: 10.1007/s00436-015-4817-0.

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 Pac. Entomol.,* 19 51–58.

Govindarajan, M., Rajeswary, M., Veerakumar, K., Muthukumaran, U., Hoti, S.L. and Benellic, G., 2016b. Green synthesis and characterization of silver nanoparticles fabricated using *Anisomeles indica*: Mosquitocidal potential against malaria, dengue and Japanese encephalitis vectors. *Exp. Parasitol.,* 161: 40-47.

Govindarajan, M., Rajeswary, M., Veerakumar, K., Muthukumaran, U., Hoti S.L., Mehlhorn, H., Donald, R., Barnard and Benelli, G., 2016c. Novel synthesis of silver nanoparticles using *Bauhinia variegata*: a recent eco-friendly approach for mosquito control. *Parasitol. Res.,* 115: 723-733.

Govindarajan, M., Sivakumar, R., Rajeswary, M. and Yogalakshmi, K., 2012. Chemical composition and larvicidal activity of essential oil from *Mentha spicata* (Linn.) against three mosquito species. *Parasitol Res.,* 110: 2023-2032.

Govindarajan, M., Sivakumar, R., Rajeswary, M. and Yogalakshmi, K., 2013. Chemical composition and larvicidal activity of essential oil from *Ocimum basilicum* (L.) against *Culex tritaeniorhynchus, Aedes albopictus* and *Anopheles subpictus* (Diptera: Culicidae). *Exp. Parasitol.,* 134: 7-11.

Gubler, D.J., 2002. Epidemic dengue/dengue haemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol.,* 10: 1-4.

Guzman, M.G. and Kouri, G., 2002. Dengue: an update. *Lancet Infect. Dis.,* 2: 33-42.

Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X., Wang, H., Wang, Y., Shao, W., He, N., Hong, J. and Chen, C., 2007. Biosynthesis of silver and gold nanoparticles by novel sun dried *Cinnamomum camphora* leaf. *Nanotech.,* 18(10): 105104-105114.

Isman, M.B., 1997. Neem and other botanical insecticides: barriers to commercialization. *Phytoparasitica.,* 25: 339-344.

Jeyabalan, D., Arul, N. and Thangamathi, P., 2003. Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector *Anopheles stephensi* Liston. *Biores Technol.*, 89: 185-189.

Khanna, V.G., Kannabiran, K., Rajakumar, G., Rahuman, A.A. and Santhoshkumar, T., 2011. Biolarvicidal compound gymnemagenol isolated from leaf extract of miracle fruit plant, *Gymnema sylvestre* (Retz) Schult against malaria and filariasis vectors. *Parasitol Res.,* doi:10.1007/ s00436-011-2384-6.

Kirthi, A.V., Rahuman, A.A., Rajakumar, G., Marimuthu, S., Santhoshkumar, T., Jayaseelan, C. and Velayutham, K., 2011. Acaricidal, pediculocidal and larvicidal activity of synthesized ZnO nanoparticles using wet chemical route against blood feeding parasites. *Parasitol Res.,* 109: 461–472.

Komalamisra, N., Trongtokit, Y., Rongsriyam, Y. and Apiwathnasorn, C., 2005. Screening for larvicidal activity in some Thai plants against four mosquito vector species. *Southeast Asian J. Trop. Med. Public Health,* 36(6): 1412-1422.

Kovendan, K., Arivoli, S., Maheshwaran, R., Baskar, K. and Vincent, S., 2012. Larvicidal efficacy of *Sphaeranthus indicus*, *Cleistanthus collinus* and *Murraya koenigii* leaf extracts against filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitol Res.,* doi:10.1007/s00436-012-2927-5.

Kumar, V. and Yadav, S.K., 2009. Plant-mediated synthesis of silver and gold nanoparticles and their applications. *J. Chem. Technol. Biotechnol.,* 84: 151-157.

Marimuthu, S., Rahuman, A.A., Rajakumar, G., Santhoshkumar, T., Kirthi, A.V., Jayaseelan, C., Bagavan, A., Zahir, A.A., Elango, G. and Kamaraj, C., 2011. Evaluation of green synthesized silver nanoparticles against parasites. *Parasitol Res.,* 10: 2212-2224.

Mathivanan, T., Govindarajan, M., Elumalai, K., Krishnappa, K. and Ananthan, A., 2010. Mosquito larvicidal and phytochemical properties of *Ervatamia coronaria* Stapf. (Family: Apocynaceae*). J. Vector Borne Dis.,* 47: 178-180.

Mehlhorn, H., Schmahl, G. and Schmidt, J., 2005. Extract of the seeds of the plant *Vitex agnus castus* proven to be highly efficacious as a repellent against ticks, fleas, mosquitoes and biting flies. *Parasitol. Res.,* 95: 363-365.

Mittal, P.K., Adak, T. and Subbarao, S.K., 2005. Inheritance of resistance to *Bacillus sphaericus* toxins in a laboratory selected strain of *An. stephensi* (Diptera: Culicidae) and its response to *Bacillus thuringiensis* var. *israelensis*. *Curr Sci*., 89: 442-443.

Mohanpuria, P., Rana, N.K. and Yadav, S.K., 2008. Biosynthesis of nanoparticles: technological concepts and future applications. *J. Nanopart. Res*., 10: 507-517.

Murugan, K., Mahesh, Kumar, P., Kovendan, K., Amerasan, D. and Subramaniam, J., 2012. Larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* orange peel extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol. Res.*, 111(4): 1757-1769.

Muthukrishnan, J. and Puspalatha, E., 2001. Effects of plant extracts on fecundity and fertility of mosquitoes. *J. Appl. Entomol.,* 125: 31-35.

Muthukumaran, U., Govindarajan, M. and Rajeswary, M., 2015a. Mosquito larvicidal potential of silver nanoparticles synthesized using *Chomelia asiatica* (Rubiaceae) against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res.,* 114 (3): 989-999.

Muthukumaran, U., Govindarajan, M. and Rajeswary, M., 2015b. Synthesis and characterization of silver nanoparticles using *Gmelina asiatica* leaf extract against filariasis, dengue, and malaria vector mosquitoes. *Parasitol Res.*, 114: 1817-27.

Patil, C.D., Patil, S.V., Salunke, B.K. and Salunkhe, R.B., 2011. Bioefficacy of *Plumbago zeylanica* (Plumbaginaceae) and *Cestrum nocturnum* (Solanaceae) plant extracts against *Aedes aegypti* (Diptera: Culicide) and nontarget fish *Poecili areticulata*. *Parasitol Res.,* 108(5): 1253-263.

Ponarulselvam, S., Panneerselvam, C., Murugan, K., Aarthi, N., Kalimuthu, K. and Thangamani, S., 2012. Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* Linn G. Don and their antiplasmodial activities. *Asian Pac. J. Trop. Biomed*., 2(7): 574-580.

Prophiro, J.S., Silva, M.A.N., Kanis, L.A., Rocha, L.C.B.P., Duque-Luna, J.E. and Silva, O.S., 2012. First report on susceptibility of wild *Aedes aegypti* (Diptera: Culicidae) using *Carapa guianensis* (Meliaceae) and *Copaifera* sp. (Leguminosae). *Parasitol Res.,* 110: 699-705.

Roni, M., Murugan, K., Panneerselvam, C., Subramaniam, J., Nicoletti, M., Madhiyazhagan, P., Dinesh, D., Suresh, U., Khater, H.F., Wei, H., Canale, A., Alarfaj A.A., Munusamy, M.A., Higuchi, A. and Benelli, G., 2015. Characterization and biotoxicity of *Hypnea musciformis*-synthesized silver nanoparticles as potential eco-friendly control tool against *Aedes aegypti* and *Plutella xylostella*. *Ecotoxicol. Environ. Saf.,* 121: 31-38.

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. doi: 10.1007/s00436-011-2328-1.

Senthilkumar, N., Varma, P. and Gurusubramanian, G., 2009. Larvicidal and adulticidal activities of some medicinal plants against the malarial vector, *Anopheles stephensi* (Liston). *Parasitol Res.,* 104: 237-244.

Shaalan, E.A.S., Canyonb, D., Younesc, M.W.F., Abdel-Wahaba, H. and Mansoura, A.H., 2005. A review of botanical phytochemicals with mosquitocidal potential. *Environ. Int.,* 3: 1149-1166.

Shahi, M., Hanafi-Bojd, A.A., Iranshahi, M., Vatandoost, H. and Hanafi-Bojd, M.Y., 2010. Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales: Asclepiadaceae) against *C. quinquefasciatus* and *An. stephensi* (Diptera: Culicidae). J. *Vector Bor. Dis.,* 47: 185-188.

Sharma, P., Mohan, L. and Srivastava, C.N., 2005. Larvicidal potential of *Nerium indicum* and *Thuja oriertelis* extracts against malaria and Japanese encephalitis vector. *J. Environ. Biol.,* 26(4): 657-660.

Sharma, A.H., Parasher, O.P. and Singh, A.T., 2009. Species B of *Anopheles culicifacies* (Diptera:

Culicidae) is reproductively less fit than species A and C of the complex. *Acta Trop.*, 112: 316-319.

Singhi, M., Joshi, V. and Dam, P.K., 2006. Studies on *Calotropis procera* as larvicidal and repellent plant against vectors of dengue and DHF in Rajasthan, India. Annual report 2005-06. *Desert Medicine Research Center, Jodhpur.*, pp 24-28.

Song, J.Y. and Kim, B.S., 2009. Rapid biological synthesis of silver nanoparticles susing plant leaf extracts. *Bioprocess Biosyst. Eng.*, 32: 79-84.

Soni, N. and Prakash, S., 2012. Efficacy of fungus mediated silver and gold nanoparticles against *Aedes aegypti* larvae. *Parasitol. Res.*, 110: 175-184.

Subramaniam, J., Murugan, K., Panneerselvam, C., Kovendan, K., Madhiyazhagan, P., Mahesh Kumar, P., Dinesh, D., Chandramohan, B., Suresh, U., Nicoletti, M., Higuchi, A., Hwang, J.S., Kumar, S., Alarfaj, A.A., Munusamy, M.A., Messing, R.H. and Benelli, G., 2015. Eco-friendly control of malaria and arbovirus vectors using the mosquito fish *Gambusia affinis* and ultra-low dosages of *Mimusops elengi* synthesized silver nanoparticles: towards an integrative approach?. *Environ. Sci. Pollut. Res.*, 22: 20067-20083.

Suresh, U., Murugan, K., Benelli, G., Nicoletti, M., Barnard, D.R., Panneerselvam, C., Mahesh Kumar, P., Subramaniam, J., Dinesh, D. and Chandramohan, B., 2015. Tackling the growing threat of dengue: *Phyllanthus niruri*-mediated synthesis of silver nanoparticles and their mosquitocidal properties against the dengue vector *Aedes aegypti* (Diptera: Culicidae). *Parasitol Res.*, 114: 1551-1562.

Templeton, A.C., Wuelfing, W.P. and Murray, R.W., 2000. Monolayerprotected cluster molecules. *Acc. Chem. Res.*, 33(1): 27-36.

Trpis, M. and Hausermann, W., 1978. Genetics of house entering behaviour in East African populations of *Aedes aegypti* (L) (Diptera: Culicidae) and its relevance to speciation. *Bull. Entomol. Res.*, 8: 521-32.

Veerakumar, K. and Govindarajan, M., 2014. Adulticidal properties of synthesized silver nanoparticles using leaf extracts of *Feronia elephantum* (Rutaceae) against filariasis, malaria, and dengue vector mosquitoes. *Parasitol Res.*, 113: 4085–4096.

Veerakumar, K., Govindarajan, M. and Hoti, S.L., 2014. Evaluation of plant-mediated synthesized silver nanoparticles against vector mosquitoes. *Parasitol Res.*, 113: 4567-4577.

Veerakumar, K., Govindarajan, M. and Rajeswary, M., 2013. Green synthesis of silver nanoparticles using *Sida acuta* (Malvaceae) leaf extract against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Parasitol Res.*, 112: 4073-4085.

WHO, 1992. Lymphatic filariasis: the disease and its control. *5th report. Who Expert Committee on Filariasis. Tech. Rep. Ser.*, 821: 1-71.

WHO, 1999. Prevention and control of dengue and DHF. *WHOSEARO Regional Publication*, 29.

World Health Organization, 2007. Global plan to combat neglected tropical diseases 2008-2015. *WHO/CDS/NTD/*2007.40.

Yadav, R., Srivastava, V.K., Chandra, R. and Singh, A., 2002. Larvicidal activity of latex and stem bark of *Euphorbia tirucalli* plant on the mosquito *Culex quinquefasciatus*. *J. Commun. Dis.*, 34(4): 264-269.

Zahir, A.A., Rahuman, A.A., Kamaraj, C., Bagavan, A., Elango, G., Sangaran, A. and Kumar, B.S., 2009. Laboratory determination of efficacy of indigenous plant extracts for parasites control. *Parasitol. Res.*, 105 (2): 453-461.