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Bio- synthesized silver nanoparticles: A potent novel bio-pesticide for vector mosquitoes

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ABSTRACT

Mosquito transmit serious human diseases like malaria, dengue, chikungunya, yellow fever, filariasis, encephalitis accounted for global mortality and morbidity with increased resistance to common insecticides. Plants may be alternative sources of mosquito control agents. In the present study silver nanoparticles (Ag NPs) were synthesized from aqueous leaf extracts of two plant species (*Chomelia asiatica* and *Gmelina asiatica*) and there effects on third instar larvae of *Anopheles subpictus, Aedes albopictus* and *Culex tritaeniorhynchus* were evaluated. Synthesized Ag NPs were characterized by UV-Vis spectroscopy, fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and X-ray diffraction (XRD) analysis. Range of concentrations of synthesized Ag NPs (10 to 60 µg/mL) and aqueous leaf extract (50 to 300 µg/mL) were tested against the larvae of *An. subpictus, Ae. albopictus* and *Cx. tritaeniorhynchus*. The Ag NPs of *C. asiatica* were highly effective against third instar larvae of *An. subpictus*, *Ae. albopictus* and *Cx. tritaeniorhynchus* with LC_{50} and LC_{90} values were 23.13, 25.83, 27.50 µg/mL and 42.21, 45.94, 47.91 µg/mL, respectively. The control showed nil mortality in the concurrent assay. Results obtained from this study present biosynthesized silver nanoparticles as novel biolarvicidal agent and can be used along with traditional insecticides as approach of Integrated Pest Management (IPM).

Keywords: *Chomelia asiatica, Gmelina asiatica, Anopheles subpictus, Aedes albopictus, Culex tritaeniorhynchus, Ag NPs.*

INTRODUCTION

Mosquitoes and the diseases they spread have been responsible for killing more people than all the wars in history. Even today, mosquitoes transmitting malaria kill 2 million to 3 million people and infect another 200 million or more every year. Tens of millions more are killed and debilitated by a host of other mosquito-borne diseases, including filariasis, yellow fever, dengue and encephalitis. Vector and vector-borne diseases have become a challenging problem to public health in these days as it has social and economical impact especially in subtropical and tropical countries. Mosquitoes are the most important arthropod disease vectors, transmitting nine dreadful human diseases in over 100 countries, causing mortality of nearly two million people every year. They are the living dipteran creature is the important vector of many of the vector borne diseases having the potentiality to kill more than a million victims annually around the world. Mosquitoes are vectors of a variety of pathogens and parasites. They are regarded as pests by humans because they interfere directly or indirectly with their health and socio-economic well-being.

Mosquitoes can be found all over the world from the Tropics to the Arctic. Some mosquitoes can be found 200 miles from their birthplace. Of all the harmful creatures on earth, this little “vampire” probably poses the greatest threat to mankind. There are more than 3,500 species in the culicid, or mosquito family, worldwide and mosquito-borne

diseases infect about 700 million people each year and kill 3 million according to the Centers for Disease Control. On average, one person dies every 10 seconds as a result of a little mosquito "bite". Comprising approximately 3500 species, mosquitoes are found beyond the tropical and subtropical regions of the world with which they are classically associated. Particularly true for the chief genera which vector human disease-causing pathogens—*Anopheles* (malaria, filariasis), *Aedes* (yellow fever, dengue, chikungunya), and *Culex* (West Nile, Japanese encephalitis, filariasis)—mosquitoes are distributed globally, even in the Arctic.

Mosquito control

Mosquito control is of serious concern in developing countries like India due to the lack of general awareness, development of resistance, and socioeconomic reasons. Every year, a large part of the population is affected by one or more mosquito-borne diseases. Mosquito control, which includes both anti-larval and anti-adult measures, constitutes an important aspect of any mosquito control programs. Control either by biological or chemical means is the basic requirement for planning an effective vector control strategy. Mosquitoes are capable of transmitting potential pathogens to human beings, and they are responsible for several infectious diseases like malaria, filariasis, Japanese encephalitis, yellow fever, dengue, and chikungunya. Therefore, become a challenging problem to public health worldwide, and it has a serious social and economical impact especially in tropical and subtropical countries. Mosquito-borne diseases are endemic over 100 countries, causing mortality of nearly two million people every year, and at least one million children die of such diseases each year, leaving as many as 2100 million people at risk around the world. In India, various species *of Aedes, Anopheles*, and *Culex* mosquitoes are important insect vectors of human diseases. Rapid increase in human population, allocation of limited funds for mosquito control program, and lack of awareness among people together with environmental change and adaptability of vector mosquitoes resulted in an increase in mosquito-transmitted diseases. Thus, the effort towards mosquito control continues to be an important strategy in preventing the mosquito- borne diseases. Limited knowledge of the disease, its focal distribution and the fact that it affects mainly poor rural communities contribute to low reporting of cases. Steady progress is being made now to develop tools for diagnosis, to understand exactly how infection is transmitted and to develop treatment and prevention, and these offer the prospect of better disease control.

Chemical insecticides

The approach to combat these diseases largely relies on interruption of the disease transmission cycle by either destruction of the aquatic stages or by killing the adult mosquitoes using chemical insecticides. The drastic effects of chemical insecticide-based intervention measures for the control of disease vectors have received wide public apprehension and have caused many problems like insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans, and other non-target organisms. To alleviate these problems, major emphasis has been on the use of natural plant-based products as larvicides which can provide an alternate to synthetic insecticides. Continued applications of organophosphates such as temephos and fenthion and insect growth regulators such as diflubenzuron and methoprene are most common and widely used for mosquito control. But, continued use of synthetic chemical insecticide based measures for vector control has resulted in lower efficacy of such insecticides and appearance of resistance in mosquito population, had undesirable effects on non-target organisms, and produce damages to environment and human health (Govindarajan, 2011). Chemical insecticides are very costly. In larval mosquito control, application of insecticides in ponds, wells, and other water bodies may cause health hazards to human and larvivorus fishes.

Nowadays, mosquito coils containing synthetic pyrethroids and other organophosphorus compounds because so many side effects, such as breathing problem, eye irritation, headache, asthma, itching, and sneezing to the users. With the use mosquito repellent, people complained of ill health effect and sometimes required medical

treatment. In addition, pests were becoming resistant to chemical treatments. Indoor residual spraying of insecticides stains the walls and leaves a long lasting unpleasant odor. These problems have highlighted the need for the development of new strategies for selective mosquito control. Synthetic pesticide exposure among human has been linked to immune dysfunction along with various forms of cancer and birth defects (Nigam and Venkatakrishna, 2001). In this situation, the change of insecticides has hampered the program with increased costs. The cost of spraying with malathion and deltamethrin is 2.5-folds than the costs of spraying of DDT. Thus, the future of vector control mainly relies on the strategies for the management of existing insecticide resistance in malarial vectors and to limit its further spread. The most important aspect of the management of resistance is to either avoid or delay the onset of resistance by effectively manipulating or influencing the factors responsible for the development of resistance. One of the possible ways of avoiding development of insecticide resistance in field is using nonchemical control method, i.e., biopesticides (Amer and Mehlhorn, 2006a). Therefore, it is the hour to launch extensive search to explore eco-friendly biological materials for control.

Biopesticides

Biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans, and other advantages (Liu et al., 2000). Recently there has been a concerted effort to promote the use of botanical pesticides (as possible alternative to synthetic chemical insecticides), which provide a pest specific, cost effective, easy to use, readily biodegradable and environment friendly method (Shaalana, 2005). Therefore, an effort should be made to find alternative insecticides. Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest-managing agents. A number of plants and microbes have been reported as selective with little or no harmful effect on non-target organisms and the environment (Govindarajan and Sivakumar, 2011).

One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Further, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which act concertedly on both behavioral and physiological processes. Thus there is very little chance of pests developing resistance to such substances. Identifying bio-insecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative product to fight against mosquito borne diseases. These well known drawbacks with synthetic insecticides shifted the mosquito control programme to use of eco-friendly, biodegradable and microbial plant compounds with mosquitocidal property (Govindarajan et al., 2008a).

Natural products of plant origin are generally preferred because of their less harmful nature to nontarget organisms and their innate biodegradability. Medicinal plants may be an alternative source of mosquito control agent because they have been reported to show several bioactivities such as insecticidal, antifungal, and nematicidal activities. It has been shown that the use of botanicals as mosquito control agents can be effectual in minimizing these adverse impacts due to their eco-safety, target specificity, negligible resistance, reduced number of applications, higher acceptability, and suitability for rural areas. Many researchers have reported that extracts from various plants can be used as effective and advantageous alternatives to synthetic insecticides or along with other insecticides under integrated vector control programs for the control of mosquitoes (Govindarajan, 2010a; Niraimathi et al., 2010 Kovendan et al., 2012).

Nanoparticles

Nanoparticles are particles with at least one dimension smaller than 1 micron and potentially as small as atomic and molecular length scales (~0.2 nm). Nanoparticles can have amorphous or crystalline form and their surfaces can act as carriers for liquid droplets or gases. To some degree, nanoparticulate matter should be considered a distinct state of matter, in addition to the solid, liquid, gaseous, and plasma states, due to its distinct properties (large surface area and quantum size effects). Examples of materials in crystalline nanoparticle form are fullerenes and carbon nanotubes, while traditional crystalline solid forms are graphite and diamond. Many authors limit the size of nanomaterials to 50 nm or 100 nm, the choice of this upper limit being justified by the fact that some physical properties of nanoparticles approach those of bulk when their size reaches these values. However, this size threshold varies with material type and cannot be the basis for such a classification. A legitimate definition extends this upper size limit to 1 micron, the sub-micron range being classified as nano. Nanoparticulate matter – refers to a collection of nanoparticles, emphasizing their collective behavior. Nanotechnology can be defined as the design, synthesis, and application of materials and devices whose size and shape have been engineered at the nanoscale. It exploits unique chemical, physical, electrical, and mechanical properties that emerge when matter is structured at the nanoscale. Nanotoxicology was proposed as a new branch of toxicology to address the adverse health effects caused by nanoparticles. Despite suggestions that nanotoxicology should only address the toxic effects of engineered nanoparticles and structures we recommend that nanotoxicology should also encompass the toxic effects of atmospheric particles, as well as the fundamentals of virology and bacteriology. While significant differences exist between the health effects of nonbiological particles and viruses and bacteria, there are significant common aspects of intrusion and translocation.

Synthesis of silver nanoparticles

Chemical approach

Many methods have been reported for the synthesis of Ag-NPs by using chemical, physical, photochemical and biological routes. Each method has advantages and disadvantages with common problems being costs, scalability, particle sizes and size distribution. Among the existing methods, the chemical methods have been mostly used for production of Ag-NPs. Chemical methods provide an easy way to synthesize Ag-NPs in solution. Generally, the chemical synthesis process of the Ag-NPs in solution usually employs the following three main components: (i) metal precursors, (ii) reducing agents and (iii) stabilizing/capping agents. The formation of colloidal solutions from the reduction of silver salts involves two stages of nucleation and subsequent growth. It is also revealed that the size and the shape of synthesized Ag-NPs are strongly dependent on these stages. Furthermore, for the synthesis of monodispered Ag-NPs with uniform size distribution, all nuclei are required to form at the same time. In this case, all the nuclei are likely to have the same or similar size, and then they will have the same subsequent growth. The initial nucleation and the subsequent growth of initial nuclei can be controlled by adjusting the reaction parameters such as reaction temperature, pH, precursors, reduction agents (i.e. NaBH4, ethylene glycol, glucose) and stabilizing agents (i.e. PVA, PVP, sodium oleate) (Chen and Zhang, 2012).

The nanoparticles formed in a solution of high surfactant concentration are smaller than those formed in a solution of low surfactant concentration (Mafune et al., 2001). Chemical approach Chemical reduction is the most frequently applied method for the preparation of AgNPs as stable, colloidal dispersions in water or organic solvents. Commonly used reductants are borohydride, citrate, ascorbate and elemental hydrogen. The reduction of silver ions (Ag+) in aqueous solution generally yields colloidal silver with particle diameters of several nanometers. Also, AgNPs can be prepared inside microemulsion. The synthesis of AgNPs in two-phase aqueous organic systems is based on the initial spatial separation of reactants (metal

precursor and reducing agent) in two immiscible phases. The rate of subsequent interaction between the metal precursor and the reducing agent is controlled by the interface between the two liquids and by the intensity of interphase transport between the aqueous and organic phases, which is mediated by a quaternary alkylammonium salt. Metal clusters formed at the interface are stabilized, due to their surface being coated with stabilizer molecules occurring in the nonpolar aqueous medium, and transferred to the organic medium by the interphase transporter (Krutyakov et al., 2010). Recently, biosynthetic methods employing naturally occurring reducing agents such as polysaccharides, biological microorganism such as bacteria and fungus or plants extract, i.e. green chemistry, have emerged as a simple and viable alternative to more complex chemical synthetic procedures to obtain AgNPs.

Physical approach

In physical processes, metal nanoparticles are generally synthesized by evaporation–condensation, which could be carried out using a tube furnace at atmospheric pressure. The source material within a boat centered at the furnace is vaporized into a carrier gas. Nanoparticles of various materials, such as Ag, Au, PbS and fullerene, have previously been produced using the evaporation/condensation technique (Gurav et al., 1994). In another work Jung et al (2006) reported an attempt to synthesize metal NPs via a small ceramic heater that has a local heating area. The small ceramic heater was used to evaporate source materials. The results showed that the geometric mean diameter, the geometric standard deviation and the total number concentration of NPs increase with heater surface temperature. The particle generation was very stable, because the temperature of the heater surface does not fluctuate with time. Spherical NPs without agglomeration were observed, even at high concentration with high heater surface temperature. The generated Ag-NPs were pure silver, when air was used as a carrier gas. The geometric mean diameter and the geometric standard deviation of Ag-NPs were in the range of 6.2–21.5 nm and 1.23-1.88 nm, respectively.

However, the generation of silver nanoparticles (AgNPs) using a tube furnace has several drawbacks, because a tube furnace occupies a large space, consumes a great deal of energy while raising the environmental temperature around the source material, and requires a lot of time to achieve thermal stability. A typical tube furnace requires power consumption of more than several kilowatts and a preheating time of several tens of minutes to attain a stable operating temperature. Moreover, nanoparticles can be modified in size and shape due to their further interaction with the laser light passing through (Link et al., 2000; Mahfouz et al., 2008). Also, the formation of nanoparticles by laser ablation is terminated by the surfactant coating.

Biological approach

As mentioned above, when Ag-NPs are produced by chemical synthesis, three main components are needed: a silver salt (usually $AgNO_3$), a reducing agent (i.e. ethylene glycol) and a stabilizer or aping agent (i.e. PVP) to control the growth of the NPs and prevent them from aggregating. In case of the biological synthesis of Ag-NPs, the reducing agent and the stabilizer are replaced by molecules produced by living organisms. These reducing and/or stabilizing compounds can be utilized from bacteria, fungi, yeasts, algae or plants (Sintubin et al., 2012). A facile biosynthesis using the metal-reducing bacterium, *Shewanella oneidensis*, seeded with a silver nitrate solution, was reported (Suresh et al., 2010). The formation of small, spherical, nearly monodispersed Ag-NPs in the size range from 2 to 11 nm (average size of 4±1.5 nm) was observed. The Ag-NPs exhibit useful properties such as being hydrophilic, stable, and having a large surface area. This bacterially based method of synthesis is economical, simple, reproducible, and requires less energy when compared to chemical synthesis routes. Biological processes almost invariably take place at the nanoscale level, across membranes and at interfaces. Transport and retention of inorganic elements in the environment is mediated by nanophases. Such transport plays a major role in both desirable (nutrient transport and availability) and undesirable (pollutant transport) processes critical to agriculture and the

environment. The role of biological processes in changing the inorganic surface of this planet is just being fully recognized.

Mosquitocidal properties of Ag NPs

Nanotechnology is rapidly growing by producing nanoproducts and nanoparticles (NPs) that can have novel and size-related physico-chemical properties differing significantly from larger matter (Ju-Nam Y and Lead, 2008). The novel properties of NPs have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices (De et al., 2008; Ghosh Chaudhuri and Paria, 2012). 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). Among the various known synthesis methods, plant-mediated nanoparticles synthesis is preferred as it is cost-effective, environmentally friendly, and safe for human therapeutic use (Kumar and Yadav, 2009). 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). Biosynthesized AgNPs are used in label-free colorimetric assay to detect enzymatic reactions, (Wei et al., 2008), antimicrobial materials (Duran et al., 2005), anti-viral, and anti-HIV studies (Elechiguerra et al., 2005). The larvicidal activity of silver nanoparticles synthesized using *Pergularia daemia* plant latex has been screened against *Ae. aegypti, An. stephensi*, and nontarget fish *Poecilia reticulata* (Patil et al., 2012). Synthesis of silver nanoparticles was carried out using leaves of *Catharanthus roseus* and their antiplasmodial activities against *P. falciparum* (Ponarulselvam et al., 2012). Biolarvicidal and pupicidal potential of silver nanoparticles synthesized with *Euphorbia hirta* has been screened against the larvae of *An. stephensi* (Priyadarshini et al., 2012). The larvicidal activity of crude petroleum ether, ethyl acetate, and methanol extracts of the whole plants of *Phryma leptostachya* was assayed for its toxicity against the early fourth instar larvae of *Cx. pipiens* pallens. The larval mortality was observed after 24 h of exposure (Xiao et al. ,2012). The hexane, ethyl acetate, and methanol extracts of *Aristolochia indica, Cassia angustifolia, Diospyros melanoxylon, Dolichos biflorus ,Gymnema sylvestre Schult, Justicia procumbens, Mimosa pudica,* and *Zingiber zerumbet* were tested for the adulticidal, repellent, and larvicidal activity against *C. gelidus* and *Cx. quinquefasciatus* (Kamaraj et al., 2010).

The use of plants for synthesis of nanoparticles is rapid, low-cost, eco-friendly, and a single-step method for biosynthesis process (Huang et al., 2007). Among the various known synthesis methods, plant-mediated nanoparticles synthesis is preferred as it is cost-effective, environmentally friendly, and safe for human therapeutic use (Kumar and Yadav, 2009). 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). Biosynthesized Ag NPs are used in label-free colorimetric assay to detect enzymatic reactions (Wei et al., 2008), surface plasmon resonance studies (Turney et al., 2004), antimicrobial materials (Duran et al., 2005), anti-viral, and anti-HIV studies (Elechiguerra et al., 2005). The silver and gold nanoparticles synthesized with *Chrysosporium tropicum* have been tested as a larvicide against the *Ae. aegypti* larvae (Soni and Prakash, 2012). They found that the silver nanoparticles were more effective against the mosquito larval stages than the gold nanoparticles. The silver nanoparticles synthesized with *Nelumbo nucifera* leaf extract have been tested against the malaria and filariasis vectors (Santhoshkumar et al., 2011). 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., 2011). Green Ag NPs have been synthesized using various natural products like *Azadirachta indica* (Tripathi et al., 2009), Glycine max (Vivekanandhan et al., 2009), *Cinnamon zeylanicum* (Sathishkumar et al., 2009), and *Camellia sinensis* (Begum et al., 2009). Such studies could prove to have an enormous impact in the

immediate future if plant tissue culture and downstream processing procedures were applied in order to synthesize metallic as well as oxide nanoparticles on industrial scale. Currently, there is limited knowledge about the possible adverse effects that Ag nanotechnologies can exert to aquatic organisms, but there could be a potential for increased exposure to both ionic Ag and Ag NPs because of the rapid development of commercialized nanoproducts. As far as our literature survey could ascertain, no information was available on the larvicidal activity of the experimental plant species given here against *An. subpictus, Ae. albopictus* and *Cx. tritaeniorhynchus*. Therefore, the aim of this study was to investigate the mosquito larvicidal activity of aqueous crude extract and Ag NPs from *C.asiatica* and *G.asiatica*. This is the first report on the mosquito larvicidal activity of selected plant against the target mosquitoes.

MATERIALS AND METHODS

Collection of materials

Fresh leaves of *Chomelia asiatica* and *Gmelina asiatica* (Figures 1 and 2) were collected from in and around kodiyakarai, Tamil Nadu, India and the taxonomic identification was made by botanist from the 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.

Figure 2. *Gmelina asiatica* plant.

Mosquitoes

The laboratory-bred pathogen-free strains of mosquitoes were reared in the vector control laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. 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. *An. subpictus* feeding was done from 12 noon to 4:00 p.m. and *Ae. albopictus* and *Cx. tritaeniorhynchus* were fed during 6:00 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 was 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 photoperiod of 12-h light and 12-h dark.

Preparation of plant extracts

The leaves (*C. asiatica* and *G. 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 (Veerakumar

et al., 2013). 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 till use.

Synthesis of silver nanoparticles

The broth solution of fresh plant 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 and could be used within 1 week. The filtrate was treated with aqueous 1mM $AgNO_3$ (21.2 mg of $AgNO_3$ powder in 125 mLMilli-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 leaves extract at room temperature for 10 min, resulting in a brown-yellow solution indicating the formation of Ag NPs (Veerakumar and Govindarajan, 2014).

Characterization of the synthesized nanoparticles

Synthesis of AgNP 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 300–700-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 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), and TEM (JEOL, model 1200EX) measurements were operated at an accelerating voltage of 120 kV and later with an XDL 3000 powder. FTIR spectra of the samples were measured using PerkinElmer Spectrum One instrument in the diffuse reflectance mode at a resolution of 4 cm−1 in KBr pellets. An aliquot of this filtrate containing silver nanoparticles was used for X-ray diffraction (XRD) analysis.

Larvicidal activity

Larvicidal activity of the aqueous crude extract and Ag NPs from of *C. asiatica* and *G. asiatica* was evaluated according to WHO protocol (2005). Based on the wide range and narrow range tests, aqueous crude extract was tested at the range of 50 to 300 µg/mL concentrations and Ag NPs was tested at range of 10 to 60 µg/mL concentrations. Twenty numbers of late third instar larvae were introduced into a 500-mL glass beaker containing 249 mL of dechlorinated water and 1mL of desired concentrations of leaf extract and silver nanoparticles was added. For each concentration, five replicates were performed, for a total of 100 larvae. Larval mortality was recorded at 24 h after exposure, during which no food was given to the larvae. Each test included a set control groups (silver nitrate and distilled water) with five replicates for each individual concentration. The lethal concentrations (LC_{50} and LC_{90}) were calculated by probit analysis (Finney, 1971).

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{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 AND DISCUSSIONS

UV-Vis analysis of Ag NPs

Leaves extracts from two plants under present study (*C. asiatica* and *G. asiatica*) showed rapid conversion of silver nitrate into silver nanoparticles indicated by color changes from colorless to red brown within few minutes of extract addition in 100 ppm $AgNO_3$ solution (Figures 3 a, b; 4 a, b).

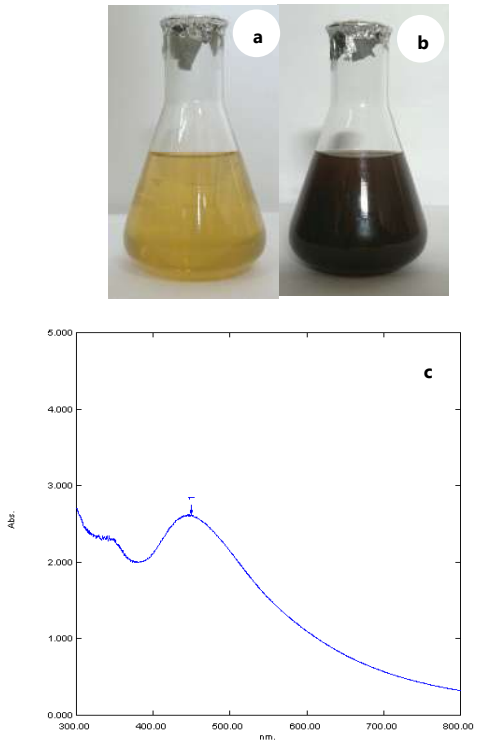

Figure 3. Photographs showing change in color after adding $AgNO_3$ **a** before reaction and **b** 6 h after the reaction. **c** UV–Vis spectra of aqueous silver nitrate with *C.asiatica* leaf extract.

A representative scheme of biosynthesis and UV-Vis spectrum is given in figures 3c and 4c. Synthesized silver nanoparticles primarily characterized by UV-visible spectroscopy. Ag NPs give typical spectrum having maximum absorption in range of 300-800 nm. This absorption is unique property of metal nanoparticles called SPR (Surface Plasmon Resonance) arises due to conduction of electrons on surface of AgNPs. After adding leaves extract in $AgNO_3$ solution, the biomolecules are stabilized in medium, interact with each other, and with silver salt, after initial interaction silver salt are consumed and the process of nucleation, reduction and capping starts leading nanoparticles synthesis.

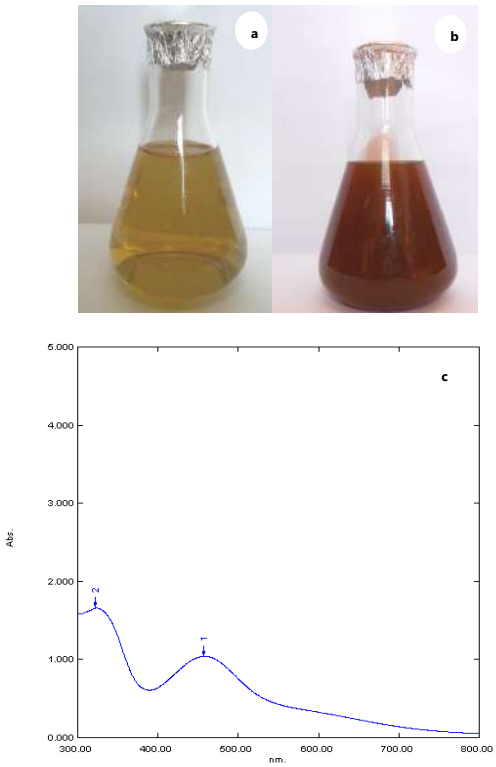

Figure 4. Photographs showing the change in color after adding $AgNO_3$. **a** Before the reaction, **b** 6 h after the reaction. **c** UV–vis spectra of aqueous silver nitrate with *G. asiatica* leaf extract.

FT-IR analysis of Ag NPs

Typical IR spectrum of lyophilized powder of *C. asiatica* leaves extract showed presence of bands due to O–H group C=H bending (824.98), C=O stretch (1094.71), N=H bending (1603.81), –C=O stretch (1765.45), C –H stretch (2851.32), C –H stretch (2932.36), and O–H stretch (3396.59) (Figure 5). FTIR analysis of the AgNPs from *G. asiatica* showed the presence of bands due to O–H group C=H bending (824.98), C=O stretch (1094.71), N=H bending (1603.81), –C=O stretch (1765.45), C–H stretch (2851.32), C–H stretch (2932.36), and O–H stretch (3396.59) (Figure 6).

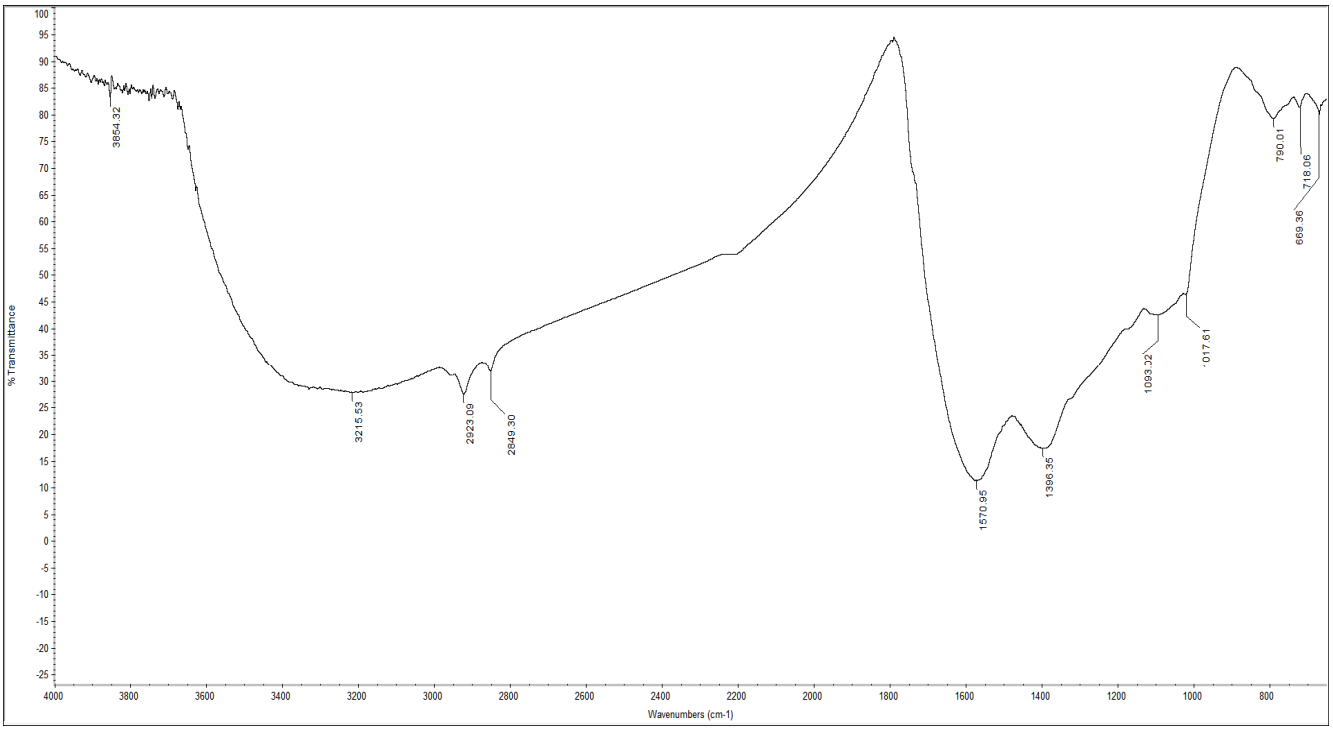

Figure 5. FT-IR spectrum of synthesized AgNPs using *C.asiatica* leaf extract.

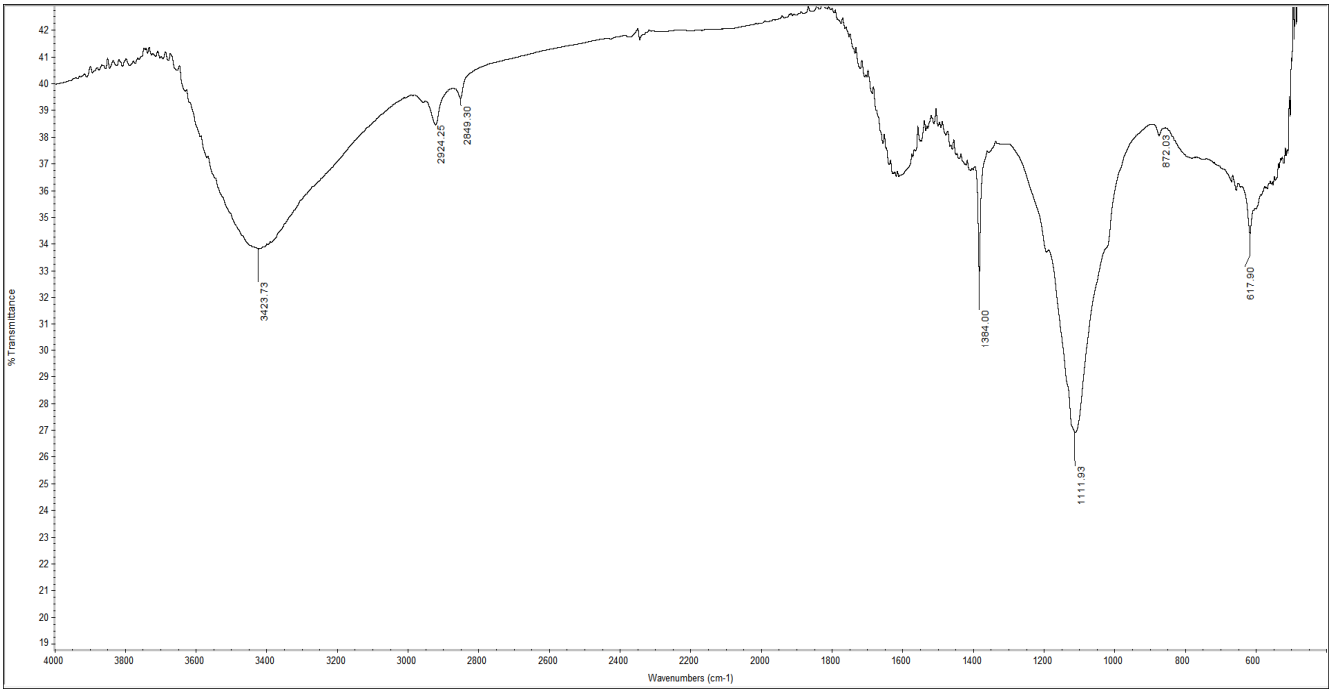

Figure 6. FTIR spectrum of synthesized AgNPs using *G. asiatica* leaf extract.

SEM, EDX and TEM analysis of Ag NPs

SEM micrographs of the synthesized Ag NPs of *C. asiatica* and *G. asiatica* magnified at ×15000 and measured at 20 to 60 nm, respectively are shown in Figures 7a, and 8a. The triangular, pentagonal, and hexagonal structures are clear. Energy-dispersive X-ray spectroscopy (EDX) proves the chemical purity of the synthesized Ag NPs (Figures 7b, and 8b). Transmission electron microscopy has been employed to characterize the size, shape and morphology of synthesized silver nanoparticles.

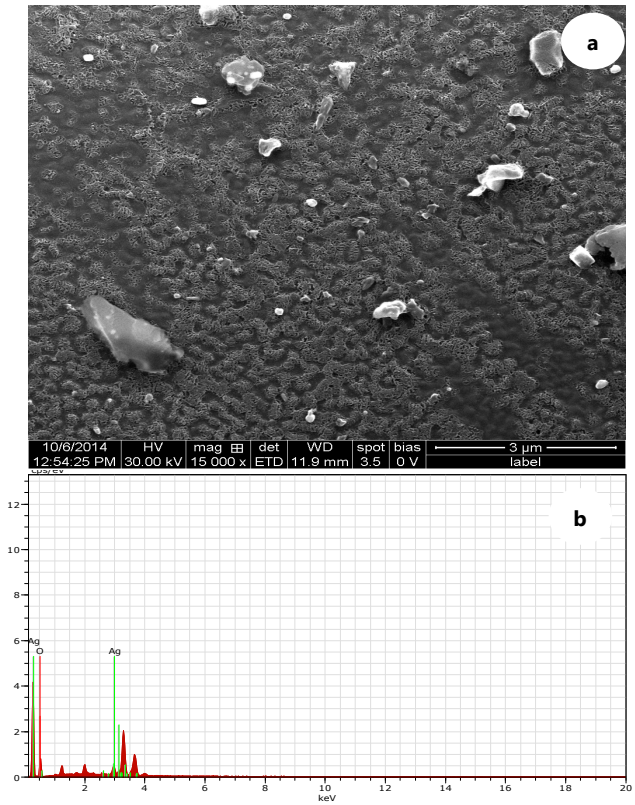

Figure 7. Scanning electron micrographs of AgNPs synthesized with *C.asiatica* leaf extract and 1.0 mM $AgNO_3$ solution and incubated at 60 °C for 6 h at pH 7.0; **a** magnified X15000, inset bar 3µm; **b** EDX image showing chemical composition.

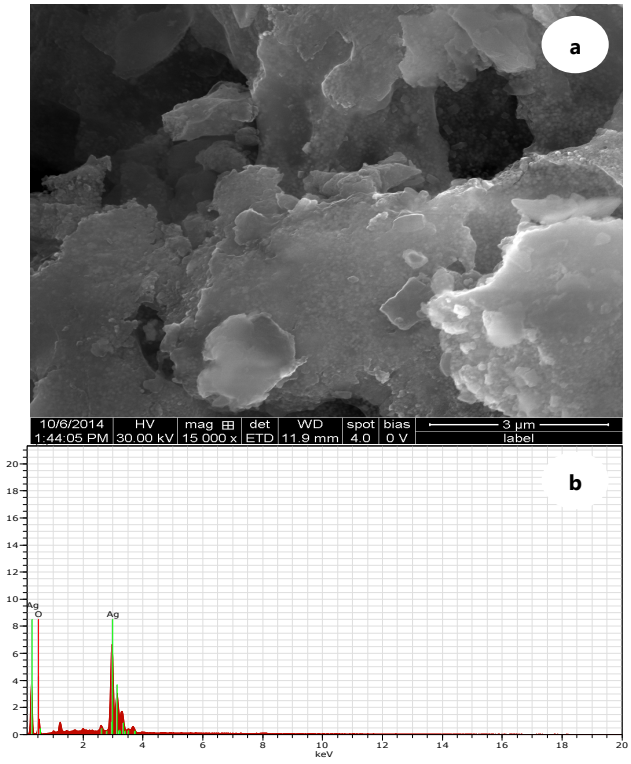

Figure 8. Scanning electron micrographs of AgNPs synthesized with *G. asiatica* leaf extract and 1.0 mM $AgNO_3$ solution and incubated at 60 °C for 6 h at pH 7.0. a Magnified ×15,000; inset bar represents 3 µm; b EDX image showing chemical composition.

The TEM image of silver nanoparticles is shown in figures 9a, and 10a. 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. The average particles size measured from the TEM image is 57.85nm. Figure 9b, and 10b shows the histogram of size distribution of silver nanoparticles.

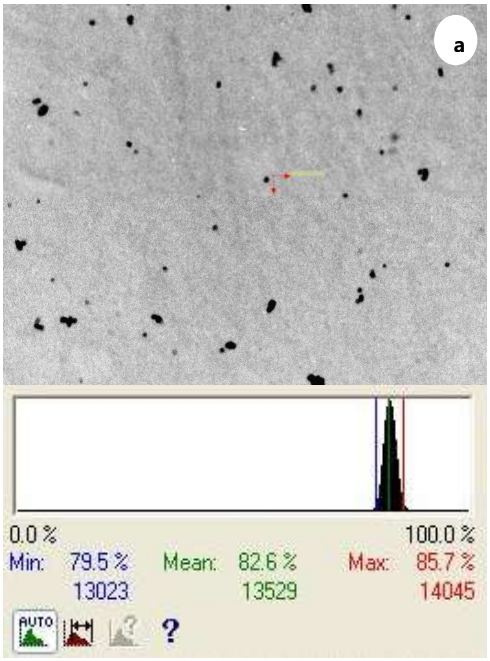

Figure 9. Transmission electron microscopic image and histogram showing synthesized AgNPs from *C.asiatica*.

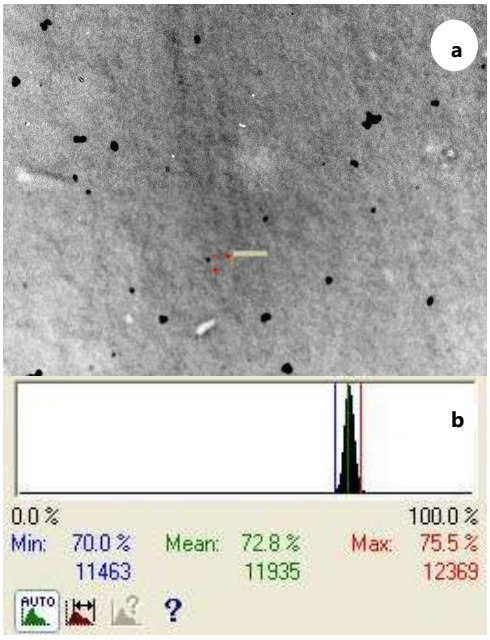

Figure 10. Transmission electron microscopic image and histogram showing synthesized AgNPs from G. asiatica.

XRD analysis of Ag NPs

After reaction, the diffraction peaks formed facets of the face- centered cubic crystral structure. A few unassigned peaks were also noticed in the vicinity of the characteristic peaks. These sharp Bragg peaks might have resulted due to the capping agent stabilizing the nanoparticles.

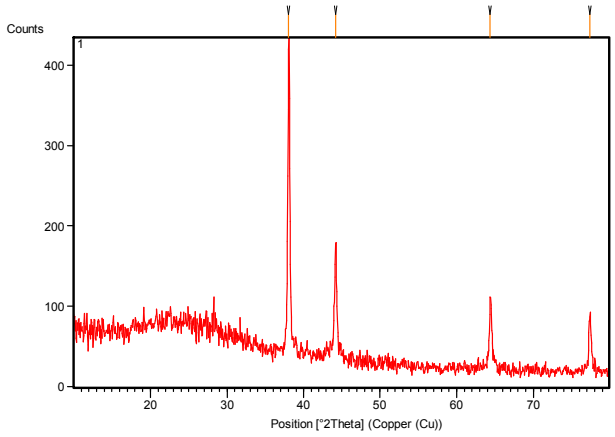

Figure 11. X-Ray diffraction showing synthesized AgNPs from *C.asiatica*.

Figures 11 and 12 depicts the X-ray diffraction (XRD) pattern of *C. asiatica* and *G. asiatica* -powdered silver nanoparticles in the 2θ range. It exhibits a broad peak at 38.0°, 44.2°, and 64.8° and 77.3°. The broadening of the peaks clearly indicates that the particles are in the nanoregime. Apart from these, many unidentified peaks at 38.001°, 44.19°, 64.34°, and 77.28° arise, possibly due to other chemical reactions or organic impurities present in the sample.

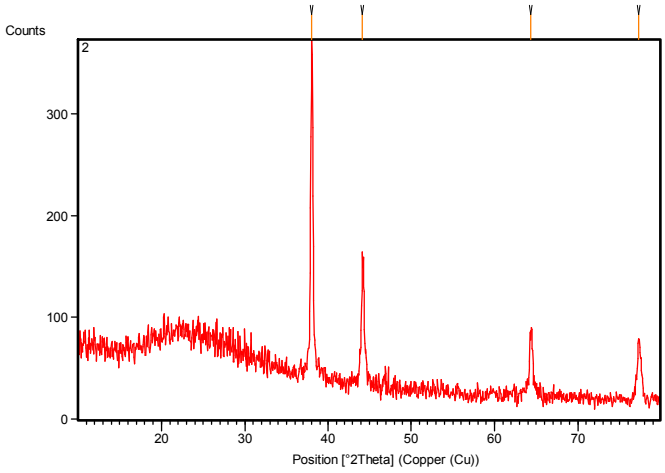

Figure 12. X-Ray diffraction showing synthesized AgNPs from *G. asiatica*.

Larvicidal efficacy of aqueous extract and synthesized Ag NPs

The results of larvicidal activity of *C. asiatica* and *G. asiatica* aqueous leaf extract and Ag NPs against late third instar *An. subpictus*, *Ae. albopictus* and *Cx. tritaeniorhynchus* was noted and presented in table 1,2,3, and 4 (Figures 13 and 14).

Table 1. Larvicidal activity of *Chomelia asiatica* aqueous leaf extract *Anopheles subpictus, Aedes albopictus,* and *Culex tritaeniorhynchus.*

Mosquitoes	Concentration	24 h mortality (%) ± SD[a]	LC_{50} (µg/mL) (LCL-UCL)	LC_{90} (µg/mL) (LCL-UCL)	χ^2
An. subpictus	Control	0.0±0.0	112.68	202.21	18.655*
	50	27.2±1.2	(82.40-141.52)	(167.97-270.98)	
	100	48.6±1.8			
	150	62.4±2.0			
	200	86.3±1.6			
	250	100.0±0.0			
Ae. albopictus	Control	0.0±0.0	122.52	218.00	13.892*
	50	24.8±0.8	(96.48-148.23)	(185.41-277.50)	
	100	42.1±1.3			
	150	58.2±0.2			
	200	82.6±1.6			
	250	97.5±1.4			
Cx.tritaeniorhynchus	Control	0.0±0.0	133.26	234.34	12.587*
	50	21.6±1.3	(108.12-159.12)	(200.51-295.55)	
	100	38.4±0.2			
	150	52.3±1.4			
	200	77.1±1.8			
	250	95.2±1.5			

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

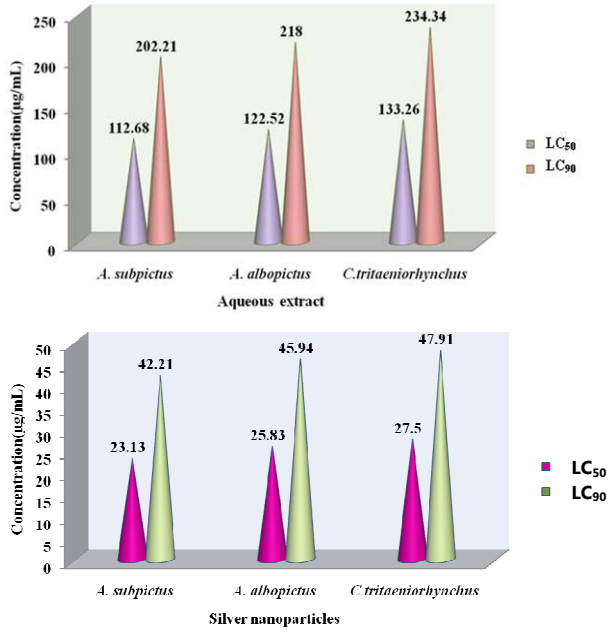

Figure 13. Graph showing the LC_{50} and LC_{90} values of larvicidal activity of *Chomelia asiatica* aqueous leaf extract and silver nanoparticles against *Anopheles subpictus, Aedes albopictus*, and *Culex tritaeniorhynchus*.

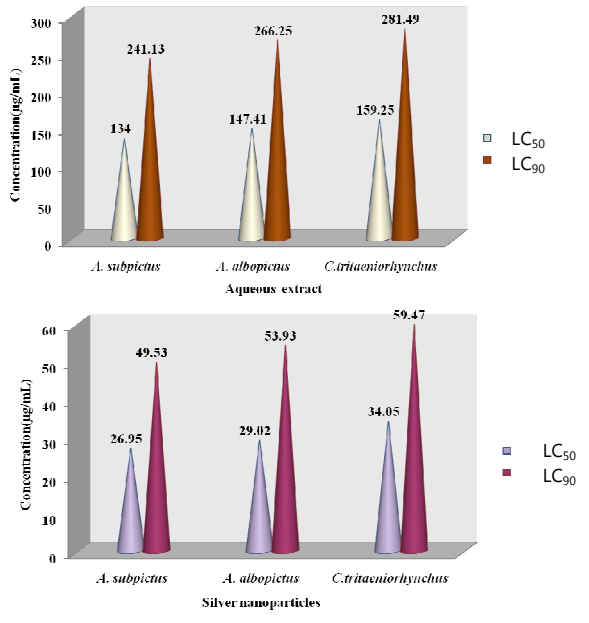

Figure 14. Graph showing the LC_{50} and LC_{90} values of larvicidal activity of *Gmelina asiatica* aqueous leaf extract and silver nanoparticles against *Anopheles subpictus, Aedes albopictus,* and *Culex tritaeniorhynchus.*

Table 2. Larvicidal activity of AgNPs from *Chomelia asiatica* against *Anopheles subpictus, Aedes albopictus*, and *Culex tritaeniorhynchus*.

Mosquitoes	Concentration	24 h mortality (%) ± SD[a]	LC_{50} (µg/mL) (LCL-UCL)	LC_{90} (µg/mL) (LCL-UCL)	χ^2
An. subpictus	Control	0.0±0.0	23.13	42.21	23.048*
	10	29.5±0.6	(15.94-29.99)	(34.32-59.97)	
	20	46.2±1.2			
	30	60.4±1.3			
	40	82.1±1.4			
	50	100.0±0.0			
Ae. albopictus	Control	0.0±0.0	25.83	45.94	11.802*
	10	22.6±1.4	(20.95-30.74)	(39.46-57.36)	
	20	40.2±2.0			
	30	56.5±0.3			
	40	78.1±1.5			
	50	95.4±1.3			
Cx.tritaeniorhynchus	Control	0.0±0.0	27.50	47.91	12.522*
	10	20.3±1.5	(22.48-32.76)	(41.02-60.47)	
	20	36.5±1.2			
	30	50.2±0.8			
	40	74.6±1.6			
	50	94.8±0.2			

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 replicate

Table 3. Larvicidal activity of *Gmelina asiatica* aqueous leaf extract against *Anopheles subpictus, Aedes albopictus*, and *Culex tritaeniorhynchus*.

Mosquitoes	Concentration	24 h mortality (%) ± SD[a]	LC_{50} (µg/mL) (LCL-UCL)	LC_{90} (µg/mL) (LCL-UCL)	χ^2
An. subpictus	Control	0.0±0.0	134.00	241.13	17.732*
	60	28.6±2.0	(98.75-167.50)	(201.03-319.94)	
	120	46.4±1.4			
	180	65.3±1.5			
	240	86.2±1.3			
	300	100.0±0.0			
Ae. albopictus	Control	0.0±0.0	147.41	266.25	13.033*
	60	23.2±0.2	(116.37-177.81)	(227.03-336.85)	
	120	44.6±1.3			
	180	63.1±0.8			
	240	78.2±1.6			
	300	96.4±1.4			
Cx.tritaeniorhynchus	Control	0.0±0.0	159.25	281.49	10.889*
	60	20.3±0.5	(131.16-187.87)	(243.10-347.92)	
	120	39.8±1.6			
	180	56.4±0.8			
	240	75.2±2.0			
	300	94.6±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 replicate

Table 4. Larvicidal activity of AgNPs from *Gmelina asiatica* against *Anopheles subpictus, Aedes albopictus*, and *Culex tritaeniorhynchus*.

Mosquitoes	Concentration	24 h mortality (%) ± SD[a]	LC_{50}(µg/mL) (LCL-UCL)	LC_{90}(µg/mL) (LCL-UCL)	χ^2
An. subpictus	Control	0.0±0.0	26.95 (18.50-34.86)	49.53 (40.36-69.68)	22.225*
	12	30.4±0.2			
	24	48.2±1.3			
	36	62.8±1.5			
	48	83.7±0.8			
	60	100.0±0.0			
Ae. albopictus	Control	0.0±0.0	29.02 (21.22-36.52)	53.93 (44.67-72.89)	17.866*
	12	27.2±1.5			
	24	42.6±1.2			
	36	68.3±0.6			
	48	75.4±1.3			
	60	95.8±0.2			
Cx.tritaeniorhynchus	Control	0.0±0.0	34.05 (28.40-40.05)	59.47 (51.31-73.84)	10.713*
	12	19.4±1.2			
	24	35.2±0.3			
	36	49.6±1.4			
	48	72.1±0.8			
	60	92.5±1.6			

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 replicate

From the two plant aqueous leaf extract and Ag NPs tested against late third instar *An. subpictus*, *Ae. albopictus* and *Cx. tritaeniorhynchus*, the highest larvicidal activity was observed in *C. asiatica*, and lowest larvicidal activity was observed in *G. asiatica*. Above two plants aqueous leaf extract and synthesized Ag NPs showed the larvicidal efficacy within 24 hr of exposure. Mortality rate (Y) is positively related to the concentration of dose (X) indicating that mortality increases with the increasing dose. Among the Ag NPs tested, the Ag NPs of *C. asiatica* were highly effective against third instar larvae of *An. subpictus*, *Ae. albopictus* and *Cx. tritaeniorhynchus* with the LC_{50} and LC_{90} values were 23.13, 25.83, 27.50 µg/mL and 42.21, 45.94, 47.91 µg/mL, respectively. The control showed nil mortality in the concurrent assay. χ^2 value was significant at $p \leq 0.05$ level. High larvicidal activity of *C. asiatica* mediated Ag NPs can be correlated with its lower particle size than other Ag NPs from different plants. Smaller particle size increase surface area to volume ratio and thus increases its action against larvae. The order of effectiveness decreased from *C. asiatica* > *G. asiatica* against third instars of *An. subpictus* followed by *Ae. albopictus* and *Cx. tritaeniorhynchus*. The larvae of *An. subpictus* were found highly susceptible to the synthesized Ag NPs than the larvae of *Ae. albopictus* and *Cx. tritaeniorhynchus*.

Discussion

Today, environmental safety is considered to be of paramount importance. An insecticide does not need to cause high mortality on target organisms in order to be acceptable but should be eco- friendly in nature. Phytochemicals may serve as these are relatively safe, inexpensive and readily available in many parts of the world. Several plants are used in traditional medicines for the mosquito larvicidal activities in many parts of the world. The ethno-pharmacological approaches used in the search of new bioactive toxins from plants appear to be predictive compared to the random screening approach. The recently developed new isolation techniques and chemical characterization through different types of spectroscopy and

chromatography together with new pharmacological testing have led to an interest in plants as the source of new larvicidal compounds. Synergistic approaches such as application of mosquito predators with botanical blends and microbial pesticides will provide a better effect in reducing the vector population and the magnitude of epidemiology.

Larvicidal studies were carried out against *Cx. quinquefasciatus*, and results were compared with bulk permethrin. The LC_{50} of nanopermethrin and bulk permethrin to *Cx. quinquefasciatus* were 0.117 and 0.715 mg/l, respectively (Anjali et al., 2010). Sakulku et al. (2009) have reported the low release rate of nanoemulsion with a large droplet size that resulted in prolonged mosquito repellant activity compared to the nanoemulsion with small droplet size. AgNPs synthesized by filamentous fungus *Cochliobolus lunatus* and its larvicidal activity was 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). The larvicidal effect of aqueous crude leaf extracts, silver nitrate solution and synthesized AgNPs 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). The synthesized zinc oxide nanoparticles showed the LC_{50} and r2 values against *Rhipicephalus microplus* (13.41 mg/l; 0.982), *Pediculus humanus capitis* (11.80; 0.966 mg/l), and the larvae of *An. subpictus* (3.19; 0.945 mg/l) and *Cx. quinquefasciatus* (4.87; 0.970 mg/l), respectively (Kirthi et al., 2011). The highest mortality was found in methanol, aqueous, and synthesized AgNPs, which used *Nelumbo nucifera* plant extract against the larvae of *An. 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 *Cx. quinquefasciatus* (LC_{50}=9.51, 13.65, and 1.10 ppm; LC_{90}=28.13, 35.83, and 3.59 ppm) (Santhoshkumar et al., 2011).

Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* and their antiplasmodial activities against *Plasmodium falciparum* have been reported by Ponarulselvam et al., (2012). The particle shape of plant-mediated AgNPs was mostly spherical with exception of neem (*Azadirachta indica*) which yielded polydisperse particles both with spherical and flat plate-like morphology 5–35 nm in size (Shankar et al., 2004). SEM images of AgNPs from *Emblica officinalis* were also predominantly spherical with an average size of 16.8 nm ranging from 7.5 to 25 nm (Ankamwar et al., 2005). Tian et al., (2007) reported that the numerous flavonoids including quercetin or quercetin 3-O-glycosides were isolated from lotus leaves that were used for silver nanoparticle synthesis. Earlier studies by various authors state that the uses of plant extract, plant-derived essential oils, and bacterial agents especially different strains of *Bacillus thuringiensis* subsp. *var israelensis* (B175 and B17) are alternative available potential resources for mosquito control. The efficacy of different plant extracts and *B. thuringiensis* subsp. *var israelensis* (Bti) varies from species to species (Mohana, 2010). Varied levels of effect of larvicidal activity varied with plant extract depending on the species. In contrast, larvicidal action of aqueous leaf extract of *C. obtusifolia* (Rajkumar and Jebanesan, 2009) and *Ocimum canum*, *O. sanctum*, and *Rhinacanthus nasutus* (Kamaraj et al., 2008) exhibited their lethal effect against larvae of *An. stephensi* and *Ae. aegypti*. An effective larval control of neem seed extract against *Anopheles gambiae* was reported by Gianotti et al. (2008). The ethanolic aerial and root extract of *P. amarus* showed high insecticidal activity against stored grain pest *Triboliumcastaneus* (Khanna et al., 2003).

Similarly, the isolated piperidine alkaloid, pipernonaline compound from the fruit extract of *Piper longum*, showed high mortality rate at LC_{50} level against larvae of *Culex pipiens* (Lee, 2000), and gluanol acetate, a tetracyclic triterpenes mosquito larvicidal compound derived from *Ficus racemosa* Linn, showed excellent mortalityagainst larvae of *Ae. aegypti* at 64.99 ppm concentration level (Rahuman et al., 2008). 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), and the maximum efficacy was observed in gymnemagenol 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* at15.92 ppm. The larvicidal, ovicidal, and repellent activities of crude benzene and ethyl acetate extracts of leaf of *Ervatamia coronaria* and *Caesalpinia pulcherrima* were assayed for their toxicity against three important vector mosquitoes, *viz., An. stephensi, Ae. aegypti*, and *Cx. quinquefasciatus*. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in benzene extract of *E. coronaria* against the larvae of *An. stephensi, Ae. aegypti*, and *Cx. quinquefasciatus* with the LC_{50} and LC_{90} values were 79.08, 89.59, and 96.15 ppm and 150.47, 166.04, and 174.10 ppm, respectively (Govindarajan et al., 2011). Elimam et al. (2009) to investigate the larvicidal, adult emergence inhibition and oviposition deterrent activity of aqueous leaves extract of *Calotropis procera* against *An. arabiensis* and *Cx. quinquefasciatus* as natural mosquito larvicide. LC_{50} and LC_{90} values calculated were 273.53–783.43, 366.44–1018.59 and 454.99–1224.62 ppm for 2nd, 3rd and 4th larval instars, respectively, of *An. arabiensis* and 187.93–433.51, 218.27–538.27 and 264.85–769.13 ppm for 2nd, 3rd and 4th larval instars, respectively, of *Cx. quinquefasciatus*.

Gleiser and Zygadlo (2007) reported that the essential oils of *Lippia turbinata* and *L. polystachya* exhibit LC_{50} values of 74.9 and 121 mg/l, respectively against *Cx. quinquefasciatus*. A preliminary study was conducted to investigate the effects of the extracts of 112 medicinal plant species, collected from the southern part of Thailand, on *Ae. aegypti*. Studies on larvicidal properties of plant extracts against the fourth instar larvae revealed that extracts of 14 species showed evidence of larvicidal activity. Eight out of the 14 plant species showed 100% mosquito larvae mortality. The LC_{50} values were less than 100 µg/mL (4.1 µg/mL-89.4 µg/mL). Six plant species were comparatively more effective against the fourth instar larvae at very low concentrations. Three medicinal plants with promising larvicidal activity, having LC_{50} and LC_{90} values being 4.1 and 16.4 µg/mL for *Mammea siamensis*, 20.2 and 34.7 µg/mL for *Anethum graveolens* and 67.4 and 110.3 µg/mL for *Annona muricata*, respectively (Promsiri et al., 2006).

The larvicidal effect exhibited by essential oils and the major constituents of *Dianthus caryophyllus, Lepidium sativum, Pimpinella anisum,* and *Illicium verum* against late third to early fourth instar mosquito larvae of *Cx. pipiens*. The essential oils of *I. verum* and *P. anisum* demonstrated high larvicidal activity with a LC_{50} <18 mgL^{-1}. The other two essential oils of *D. caryophyllus* and *L. sativum* revealed moderate larvicidal activity, displaying a LC_{50} value above 50 mgL^{-1}. Among the pure components, the most toxic were eugenol, (E)-anethole, and α-terpinyl acetate, with LC_{50} values 18.28, 16.56, and 23.03 mgL^{-1}, respectively. Eucalyptol (1,8 cineole) and β- caryophyllene were inactive at concentrations even as high as 100 mgL^{-1}, showing the least significant activity against mosquito larvae (Kimbaris et al., 2012). Larvicidal activity of compound pectolinaringenin derived from the chloroform extract of *Clerodendrum phlomidis* against *Cx. quinquefasciatus* and *Ae. aegypti* was proved with LC_{50} and LC_{90} values of 0.62 and 2.87 ppm, and 0.79 and 5.31 ppm, respectively (Muthu et al., 2012).

ZnO NPs have some excellent properties like exceptional mechanical strength, antistatic, antibacterial, and UV absorption properties (Thuenemann and Ruland., 2000). The UV sharp peak may be ascribed to the monodispersed, ZnO NPs while the slope line corresponds to a UV absorbance caused by larger NP aggregates that persist in the solution (Santilli et al., 2007). XRD confirms the presence of ZnO in the synthesized material. The presence of starch in the completely washed nano ZnO indicates their strong binding nature (Yadav et al., 2006). Manusadžianas et al. (2009) reported that the lethality response of aquatic organisms (macrophytic algae cells of *Nitellopsis obtusa*, shrimps *Thamnocephalus platyurus*, and rotifer *Brachionus calyciflorus*) induced by sonicated and non-sonicated nano ZnO suspensions with various particle sizes (10 and 20–30 nm) and nano ZnO particles showed LC_{50} values of 438, 0.21, and 0.6 mg/L for 20– 30 nm, respectively. The soluble fraction of the ZnO NPs (i.e., the Zn2+ ion) the toxic actions, ZnO NPs exert

a higher toxic effect in its insoluble form compared to that of the same amount of ionic zinc. The NPs toxic action can be linked to a chemical effect and/or stress or stimuli caused by the peculiar physical characteristics of the nano state (Manzo et al., 2011). Lice are extremely intolerant of zinc because it weakens their shell. It has been reported that adhesion of NP aggregates to the exoskeleton of parasites may cause physical effects and/or loss of mobility (Baun et al., 2008). 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 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).

The larvicidal and repellent properties of essential oils is from various parts of four plant species *Cymbopogon citratus, Cinnamomum zeylanicum, Rosmarinus officinalis,* and *Zingiber officinale* against *Cx. tritaeniorhynchus* and *An. subpictus* (Govindarajan, 2011b). Govindarajan (2010b) reported that the larvicidal activity of the crude extract of *Sida acuta* against three important mosquitoes with LC_{50} values range between 38 and 48 mg/L. The crude extract had strong repellent action against three species of mosquitoes as it provided 100 % protection against *An. stephensi* for 180 min followed by *Ae. aegypti* (150 min) and *Cx. quinquefasciatus* (120 min), respectively. The larvicidal activity of AgNPs 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 µg mL−1) and aqueous leaf extract (50, 100, 150, 200, and 250 µg mL−1) were tested against the larvae of *Cx. quinquefasciatus* (LC_{50}, 26.13 and 130.30 µg mL−1), *An. stephensi* (LC_{50}, 21.92 and 109.94 µg mL−1), and *Ae. aegypti* LC_{50} (23.96 and 119.32 µg mL−1), respectively (Veerakumar et al., 2013). Methanol extract showed the lowest LD values against several instars of larvae and 50 adult (121.59, 142.73, 146.84, 202.98, 290.65, 358.42, and 300.03 µg/cm2, respectively) which indicates the highest toxicity or insecticidal activity (Ashraful Alam et al., 2009). Sharma et al. (2005) reported that the acetone extract of *Nerium indicum* and *Thuja orientelis* 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. Prophiro et al. (2012) reported that the susceptibility of larvae was determined under three different temperatures, 15, 20, and 30 °C, with lethal concentrations for *Copaifera* sp. ranging from LC_{50} of 47 mg/L to LC_{90} of 91 mg/L and for *Carapa guianensis* LC_{50} of 136 to LC_{90} of 551 mg/L.

Kovendan et al. (2012) have reported hexane, chloroform, ethyl acetate, and methanol extract of *J. curcas* with LC_{50} values of 230.32, 212.85, 192.07, and 113.23 ppm; *Hyptis suaveolens* with LC_{50} values of 213.09, 217.64, 167.59, and 86.93 ppm; *Abutilon indicum* with LC_{50} values of 204.18, 155.53, 166.32, and 111.58 ppm; and *L. aspera* with LC_{50} values of 152.18, 118.29, 111.43, and 107.73 ppm, respectively, against third instar larvae of *Cx. quinquefasciatus*. Mahesh Kumar et al. (2012) have reported that 43% mortality was noted at first instar larvae by the treatment of *S. xanthocarpum* at 50 ppm, whereas it has been increased to 92 % at 650 ppm; 21.2 % mortality was noted at 50 ppm of *S. xanthocarpum* leaf extract treatment at 24 h exposure. The LC_{50} values of first to fourth instar larvae and pupae were 155.29, 198.32, 271.12, 377.44, and 448.41 ppm, respectively. The LC_{90} values of first to fourth instar larvae and pupae were 687.14, 913.10, 1,011.89, 1,058.85, and 1,141.65 ppm, respectively. Cheng et al. (2003) examined plant essential oils against *Ae. aegypti* larvae with LC_{50} values ranging from 36.0 to 86.8 µg/mL. *Solanum xanthocarpum* fruit petroleum ether extract was observed as the most toxic with LC_{50} values of 62.62 ppm after 24 h and 59.45 ppm after 48 h of exposure period against the larvae of *Cx. quinquefasciatus* (Mohan et al., 2005). Mathew et al. (2009) reported that leaf chloroform extracts of *Nyctanthes arbortristis* showed lethal values (LC_{50} of 0526.3 and 780.6 ppm (24 h) and LC_{50} of 0303.2 and 518.2 ppm (48 h)) against *Ae. aegypti* and *An. stephensi*, respectively. Flower methanol extracts of the above plants showed lethal values (LC_{50}=679.4 and 244.4 ppm; LC_{90}=1,071.3 and

433.7 ppm) against *An. stephensi* after 24 and 48 h, respectively.

The aqueous extract of *R. nasutus* showed LC_{50} values of 5,124 and 9,681 mg/l against *Cx. quinquefasciatus* and *Ae. Aegypti*, respectively (Chansang et al., 2005). The ethanol extracts of the aerial parts from five *Labiatae* species, *Teucrium divaricatum* was the most toxic, followed by *Mentha longifolia, Melissa officinalis, Salvia sclarea,* and *Mentha pulegium* against the third and fourth instar larvae of *Cx. pipiens* with LC_{50} values of 18.6, 26.8, 39.1, 62.7, and 81.0 ppm, respectively (Cetin et al., 2006). Rahuman et al. (2000) also found that nhexadecanoic acid in *Feronia limonia* dried leaves was effective against fourth-instar larvae of *Cx. quinquefasciatus, An. stephensi*, and *Ae. aegypti* with LC_{50} values of 129.24, 79.58, and 57.23 µg ml−1, respectively. Tiwary et al. (2007) observed the larvicidal activity of linalool-rich essential of *Zanthoxylum armatum* against different mosquito species *viz., Cx. quinquefasciatus* (LC_{50} 49 ppm), *Ae. aegypti* (LC_{50} 54 ppm) and *An. stephensi* (LC_{50} 58 ppm). Mathivanan et al. (2010) to determine the LC_{50} and LC_{90} values of crude methanol extract of leaves of *E. coronaria* on *Cx. quinquefasciatus, Ae. aegypti*, and *An. stephensi* larvae in 24 h were 72.41, 65.67, 62.08 mg l−1, and 136.55, 127.24, 120.86 mg l−1, respectively. Choochote et al. (1999) tested four fractions of *Kaempferia galanga* for larvicidal activity against *Cx. quinquefasciatus* among them, the hexane fraction exhibited the highest larvicidal effect with the LC_{50} of 42.33 ppm. The essential oil from *P. graveolens* was evaluated three different concentrations 1.0, 2.5, and 5.0 mg/cm^2 exerted 100% protection up to 3.0, 4.0, and 5.30 h, respectively. The total percentage of protection of *P. graveolenswas* 49.64% at 1.0mg/cm2, 62.19% at 2.5 mg/cm2, and 74.03% at 5.0 mg/cm2 for 10 h (Pushpanathan et al., 2008). The LC_{50} values of methanol, benzene, and acetone extract of *P. acidula* against *Cx. quinquefasciatus* and *Ae. aegypti* were 10.81, 41.07, and 53.22 and 22.10, 43.99, and 57.66 ppm, respectively. One hundred percent ovicidal activities were observed at 350 and 450 ppm for *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes, respectively; 1.0, 2.5, and 5.0 mg/cm^2 concentrations of *P. acidula* gave 10% protection up to 2.30, 4.00, and 6.45 and 2.45, 4.30, and 7.0 h, respectively (Samidurai et al., 2009). The larvicidal activity of *S. acuta* was evaluated against third-instar larvae of *An. subpictus* and *Cx. tritaeniorhynchus*. The leaf extract and active compound cryptolepine showed negligible mortality against early third-instar larvae of *An. subpictus* and *Cx. tritaeniorhynchus*; the 24 h LC_{50} value was observed at 38.68 and 50.81, and 9.98 and 12.69 mg/l for crude leaf extract and active compound cryptolepine, respectively (Niraimathi et al., 2010). The LC_{50} values of benzene, hexane, ethyl acetate, methanol, and chloroform extract of *E. alba* against early third-instar larvae of *Ae. aegypti* were 151.38, 165.10, 154.88, 127.64, and 146.28 ppm, respectively (Govindarajan and Karuppannan, 2011). The methanol extracts of *Euphorbia tirucalli* latex and stem bark were evaluated for larvicidal activity against laboratory-reared larvae of *Cx. quinquefasciatus* with LC_{50} values of 177.14 and 513.387 mg/L, respectively (Yadav et al., 2002). In conclusion, green synthesis shows that the environmentally benign and renewable source of *C. asiatica and G. 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 Ag NPs involving organisms even ranging to higher plants. The formed Ag NPs are highly stable and have significant mosquito larvicidal activity of *An. subpictus, Ae. albopictus* and *Cx. tritaeniorhynchus*.

CONCLUSIONS

Plant extracts are used to make nanoparticles it will be of low cost, environment friendly and easily scaled up. Plant induced synthesis of nanoparticles is most suitable method because it does not leave any toxic contaminants. In health industry, storage of food, textile industry and many other environmental fields, AgNPs has been used as an anti-bacterial agent. The evidence of toxicity of AgNP is still not well established although it has been widely used for decades. These results

revealed that the green, biological synthesis of silver/gold nano particles have the potential to be utilized as a good, rapid, eco-friendly approach for the control of mosquito population. It is totally a new pathway but, can be effectively utilized for the efficient killing of mosquitoes. Therefore, biological control can thus provide an effective and environmental friendly approach, which can be used as an alternative to minimize the mosquito population. To understand the current research trends of nanoparticles in mosquito control, research papers on NPs synthesised using biological organisms such as plant extracts, fungi and bacteria were thoroughly analyzed and discussed in terms of the type of nanoparticles, test species, exposure medium and suitable concentration. The researches demonstrated a wide range of results even when using the same nanoparticles. This was because, the particle size, surface coating, and the test medium supposedly made difference in the results. Therefore, in future, researches can be conducted by considering the above factors also.

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Conflicts of Interest

The authors declare no conflict of interest.