

# Chapter 7

## Green Synthesized Silver Nanoparticles: A Potential New Insecticide for Mosquito Control

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### 7.1 Introduction

#### 7.1.1 *Mosquitos and Diseases*

Mosquitoes (Family: Culicidae) are by far the most thoroughly researched insects. They probably have a much greater influence on human health and well-being throughout the world than any other arthropod, mainly because of their involvement in both transmitting a number of dreadful diseases (such as malaria, filariasis, dengue, Japanese encephalitis, Rift valley fever, Chikungunya and West Nile virus) and creating nuisance of great public health importance. Consequently, among the many species of blood-sucking insects, mosquitoes belonging to genera *Culex*, *Aedes*, and *Anopheles* are the most important arthropods from medical standpoint as they are responsible for nearly 10 % of all the sickness of human. Although, there are more than 3500 species all over the world. However, only fewer than 100 species under eight genera only are vectors of diseases. These vectors are organized in two of the three subfamilies, Anophelinae and Culicinae, while the third subfamily Toxorhynchitinae has members with mouthparts suited to feed on plant sap only. Mosquitoes are cosmopolitan in distribution and are found in all climatic zones and zoogeographical regions (Govindarajan et al. 2008a).

*Culex tritaeniorhynchus* (Giles) (Diptera: Culicidae) has emerged as an important vector of Japanese encephalitis (JE) virus in east, southeast, and south Asia (Govindarajan et al. 2008b). While *C. tritaeniorhynchus* is present throughout India, JE is endemic only in seven states (Andhra Pradesh, Assam, Bihar, Karnataka, Tamilnadu, Uttar Pradesh, and West Bengal). The change in the pattern of JE virus

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transmission in some parts of India from epidemic to endemic is partly correlated with the establishment of *C. tritaeniorhynchus* in these areas. The ability of *C. tritaeniorhynchus* to transmit and spread JE across India has become a topic of concern. Understanding the role of *C. tritaeniorhynchus* as a zoonotic and epizootic vector of JE is of primary importance in the understanding the JE epidemiology. Differences in transmission and vector competence of *C. tritaeniorhynchus* for JE provide reasons for investigation of variations in the populations of this species. However, in spite of its epidemiological importance in JE transmission, few studies have been done on *C. tritaeniorhynchus*. Neither the evolutionary history nor the population dynamics of this species is well understood (Govindarajan et al. 2011a, b, c, d).

Malaria is one of the most common vector-borne diseases widespread in tropical and subtropical regions, including parts of the America, Asia, and Africa (WHO 2007). Worldwide, there were about 247 million malaria cases with 0.881 million deaths reported in 2006 (WHO 2008). Malaria is the world's most dreadful tropical disease. As reported recently, 406 million Indians were at risk of stable *Plasmodium falciparum* transmission in 2007 with an uncertainty point estimate of 101.5 million clinical cases (95 % CI 31.0–187.0 million cases) (Hay et al. 2010; Govindarajan et al. 2008c). In India, Malaria is still the most important cause of morbidity and mortality with approximately two to three million new cases arising every year (Govindarajan et al. 2008d). Rapid increases in population, limited funds, and know-how together with environmental change and an increase in the resistance of vectors and pathogens to insecticides and drugs and a shift in vector-control operations from long-term preventive measures to on-the-spot responses have led to an increase in vector transmitted diseases (Gubler 1998). Malaria causes 1.3 % loss in economic growth in Africa per year, and the long-term impact over a 15-year period is estimated at a 20 % loss in the gross national product (Zaim and Guillet 2002). *Anopheles subpictus* is known to transmit malaria in an isolated study of multiple host-feeding in field populations, and its specific role in transmitting malaria in Sri Lanka revealed that multiple blood feeding within the same gonotrophic cycle was attributed to a local “frequent feeding strategy” in this primarily zoophagic and endophilic malaria vector (Govindarajan 2009).

*Aedes albopictus*, the Asian tiger mosquito, is a vector of dengue haemorrhagic fever (DHF), and is capable of breeding in a wide range of container types and water holding habitats. In Thailand, *A. albopictus* has been found in forested habitats ranging in elevation from 450 to 1,800 m as well as in a variety of other habitats in rural and suburban areas (Scanlon and Esah 1965; Thavara et al. 1996). Ubiquitous breeding sites, such as tree holes, coconut shells, fruit peels, water jars, unused and discarded tires and boats holding water have been found to contain *A. albopictus* larvae. Because of the diverse breeding sites of *A. albopictus*, especially in the forested areas, they may be hard to reach to monitor larval populations. Detection and measuring mosquito abundance through their egg-laying activities using ovitraps is the most common surveillance or sampling method for this and some other *Aedes* mosquitoes (Service 1992; Govindarajan et al. 2008e). Yap et al. (1995) pointed out the importance of oviposition site preferences in planning vector control programs against *Aedes* mosquitoes. However, information on oviposition attractants for *A.*

*albopictus* is rather limited at the present time. Sucharit et al. (1980) studied the oviposition behavior of *A. aegypti* and *A. albopictus* to be influenced by their own larval holding water or that of other species. They found that larval holding water of *A. albopictus* significantly increased oviposition by *A. aegypti*, but there was no oviposition attractancy for *A. albopictus*. Thavara et al. (1989) demonstrated that *A. albopictus* (*A. aegypti* absent) prefer to lay eggs in the field in containers with conditioned water that was left outside for a long period and with a stable flora together with the immature stages of this species.

### 7.1.2 Vector Control

The medical importance of mosquitoes as vectors for the transmission of serious diseases that cause morbidity, mortality, economic loss and social disruption such as malaria, lymphatic filariasis and viral diseases is well documented. Rapid increases in population, limited funds, and know-how together with environmental change and an increase in the resistance of vectors and pathogens to insecticides and drugs and a shift in vector-control operations from long-term preventive measures to on-the-spot responses have led to an increase in vector transmitted diseases (Govindarajan 2010a). The mosquito borne diseases remains endemic in more than 100 developing tropical countries and its control is a major goal for improved worldwide health. In the Indian scenario, almost the entire country is endemic to the mosquito-borne diseases due to favorable ecological conditions. Vector control is a global problem. It may be directed against the immature or adult stages of mosquitoes. Thus, one of the approaches for control of these mosquito-borne diseases is the interruption of disease transmission by killing or preventing mosquitoes from biting human beings. It is known that larvicides play a vital role in controlling mosquitoes in their breeding sites. However, it is undoubtedly the best method of protecting the community against the diseases. In recent years, it has been realized that personal protection from biting mosquitoes and other haematophagous arthropods is the first line of defence against the infectious disease. Mosquito control has been mainly affected by use of conventional insecticides, but these have caused their own problems, such as adverse effects on the environment and the encouragement of pesticide resistance in some mosquitoes (Govindarajan 2011a, b).

### 7.1.3 Disadvantage of Chemical Control

The current mosquito control approach is based on synthetic insecticides. Even though they are effective, they created many problems like insecticide resistance (Govindarajan and Karuppannan 2011). Chemical control using synthetic insecticides had been favorable so far because of their speedy action and easy application.

The control of mosquito larvae worldwide depends primarily on continued applications of organophosphates, such as temephos and fenthion, and insect growth regulators, such as diflubenzuron and methoprene (Yang et al. 2002). Although effective, continued use of synthetic insecticides for mosquito control has disrupted natural biological control systems and also resulted in lower efficacy of such insecticides and development of resistance in the mosquito population, which are undesirable effects on nontarget organisms (Govindarajan et al. 2005). Mosquito control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides which have an adverse impact on the environment and disturb ecological balance (Govindarajan 2010a). Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. The increased use of these insecticides may enter into the food chain, and thereby, the liver, kidney, etc., may be irreversibly damaged. They even result in mutation of genes and these changes become prominent only after a few generations (Ghosh and Chandra 2006). 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 larvivorous 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. There is an urgent need to develop new insecticides which are more environmentally safe and also biodegradable and target specific against mosquitoes (Govindarajan 2011c).

#### ***7.1.4 Advantage of Botanical Insecticides***

Biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans and other advantages (Liu et al. 2012; Govindarajan 2011d). 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 (Govindarajan 2010b). 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 behavioural 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. 2006a, b).

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 nematocidal 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 et al. 2007).

### ***7.1.5 Nanoparticles***

Nanomaterials are defined as materials that have at least one dimension  $\leq 100$  nm ( $1 \text{ nm} = 10^{-9} \text{ m}$ ) and they can be divided into two large groups: ultrafine nanosized particles not intentionally produced and engineered nanoparticles produced in a controlled, engineered way (Oberdorster et al. 2005). 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 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 (Lu et al. 2007). Among them, silver nanoparticles (Ag-NPs or nanosilver) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts (Sharma et al. 2009). Ag-NPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and non linear optical behavior (Krutyakov et al. 2008). These properties make them of potential value in inks, microelectronics, and medical imaging. Besides, Ag-NPs exhibit broad spectrum bactericidal and fungicidal activity (Ahamed et al. 2010) that has made them extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food and textiles, increasing their market value. To date,

nanosilver technologies have appeared in a variety of manufacturing processes and end products. Nanosilver can be used in a liquid form, such as a colloid (coating and spray) or contained within a shampoo (liquid) and can also appear embedded in a solid such as a polymer master batch or be suspended in a bar of soap (solid). Nanosilver can also be utilized either in the textile industry by incorporating it into the fiber (spun) or employed in filtration membranes of water purification systems. In many of these applications, the technological idea is to store silver ions and incorporate a time-release mechanism. This usually involves some form of moisture layer that the silver ions are transported through to create a long-term protective barrier against bacterial/fungal pathogens (Dallas et al. 2011).

## **7.1.6 Synthesis of Nanoparticle**

### **7.1.6.1 Chemical Synthesis**

Chemical synthesis of Ag NPs requires three components: precursor for Ag NPs usually silver nitrate ( $\text{AgNO}_3$ ), reducing agents such as sodium borohydride ( $\text{NaBH}_4$ ) and capping agent like polyvinylpyrrolidone ( $\text{C}_6\text{H}_9\text{NO}$ )<sub>n</sub>. In chemical synthesis, there is single reducing and capping agent which allow synthesis of nanoparticles with defined shape and size which is major advantage of this method but use of hazardous chemicals, harsh reaction parameters such as high temperature, pressure and toxic by-product creates environmental concern (Brichkin et al. 2008). There are chances of adherence of toxic chemical residues on surface of nanoparticles. This restricts use of chemically synthesized Ag NPs in medicine and healthcare. Moreover, the Ag NPs produced by chemical methods tend to agglomerate or become insoluble in aqueous system therefore, their application in living system which is aqueous too raise concern about stability and safety (Mafune et al. 2001).

Currently, many methods have been reported for the synthesis of Ag NPs by using chemical, physical, 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. Monodisperse samples of silver nanocubes were synthesized in large quantities by reducing silver nitrate with ethylene glycol in the presence of polyvinylpyrrolidone (PVP) (Sun and Xia 2002), the so-called polyol process. In this case, ethylene glycol served as both reductant and solvent. It showed that the presence of PVP and its molar ratio relative to silver nitrate both played important roles in determining the geometric shape and size of the product. It suggested that it is possible to tune the size of silver nanocubes by controlling the experimental conditions. Spherical Ag NPs with a controllable size and high monodispersity were synthesized by using the polyol process and a modified precursor injection technique (Kim et al. 2006).

In the precursor injection method, the injection rate and reaction temperature were important factors for producing uniform-sized Ag NPs with a reduced size. Ag NPs with a size of  $17 \pm 2$  nm were obtained at an injection rate of  $2.5 \text{ ml s}^{-1}$  and a reaction temperature of  $100 \text{ }^\circ\text{C}$ . The injection of the precursor solution into a hot solution is an effective means to induce rapid nucleation in a short period of time, ensuring the fabrication of Ag NPs with a smaller size and a narrower size distribution. Nearly monodisperse Ag NPs have been prepared in a simple oleylamine-liquid paraffin system (Chen et al. 2007). It was shown that the formation process of Ag NPs could be divided into three stages: growth, incubation and Ostwald ripening stages. In this method, only three chemicals, including silver nitrate, oleylamine and liquid paraffin, are employed throughout the whole process. The higher boiling point of  $300 \text{ }^\circ\text{C}$  of paraffin affords a broader range of reaction temperature and makes it possible to effectively control the size of Ag NPs by varying the heating temperature alone without changing the solvent. Otherwise, the size of the colloidal Ag-NPs could be regulated not only by changing the heating temperature, or the ripening time, but also by adjusting the ratio of oleylamine to the silver precursor.

### 7.1.6.2 Physical Synthesis

For a physical approach, the metallic NPs can be generally synthesized by evaporation–condensation, which could be carried out by using a tube furnace at atmospheric pressure. However, in the case of using a tube furnace at atmospheric pressure there are several drawbacks such as a large space of tube furnace, great consumption energy for raising the environmental temperature around the source material and a lot of time for achieving thermal stability. Therefore, various methods of synthesis of Ag NPs based on the physical approach have been developed. A thermal-decomposition method was developed to synthesize Ag NPs in powder form (Lee and Kang 2004). The Ag NPs were formed by decomposition of a  $\text{AgI}^+$ -oleate complex, which was prepared by a reaction with  $\text{AgNO}_3$  and sodium oleate in a water solution, at high temperature of  $290 \text{ }^\circ\text{C}$ . Average particle size of the Ag NPs was obtained of about 9.5 nm with a standard deviation of 0.7 nm. This indicates that the Ag NPs have a very narrow size distribution. 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. Tien et al. (2008) used the arc discharge method to fabricate Ag NPs suspension in deionized water with no added

surfactants. In this synthesis, silver wires (Gredmann, 99.99 %, 1 mm in diameter) were submerged in deionized water and used as electrodes. The experimental results show that Ag NPs suspension fabricated by means of arc discharge method with no added surfactants contains metallic Ag NPs and ionic silver. With a silver rod consumption rate of  $100 \text{ mg min}^{-1}$ , yielding metallic Ag NPs of 10 nm in size and ionic silver obtained at concentrations of approximately 11 ppm and 19 ppm, respectively.

### 7.1.6.3 Biological Synthesis

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). The formation of small, spherical, nearly monodispersed Ag NPs in the size range from 2 to 11 nm (average size of  $4 \pm 1.5 \text{ nm}$ ) 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. In another study, the use of the fungus *Trichoderma viride* (*T. viride*) for the extracellular biosynthesis of Ag NPs from silver nitrate solution was reported (Fayaz et al. 2010). In this regard *T. viride* proves to be an important biological component for extracellular biosynthesis of stable Ag NPs. The morphology of Ag NPs is highly variable, with spherical and occasionally rod-like NPs observed on micrographs. The obtained diameter of Ag NPs was in the range from 5 to 40 nm. In another study, stable Ag NPs of 5–15 nm in size were synthesized by using an air borne bacteria (*Bacillus* sp.) and silver nitrate (Pugazhenthiran et al. 2009). The biogenic NPs were observed in the periplasmic space of the bacterial cells, which is between the outer and inner cell membranes. Also, the Ag NPs were produced by using the *Lactobacillus* spp. as reducing and capping agent. Sintubin et al. (2009) were carried with different *Lactobacillus* species to accumulate and subsequently reduce  $\text{Ag}^+$ . The result showed that only the lactic acid bacterial were confirmed to have the ability to produce  $\text{Ag}^0$ . In addition, both particle localization and distribution inside the cell were dependent on *Lactobacillus* species. The mean diameter of the biogenic Ag NPs produced by this method varied with the *Lactobacillus* spp. used. The smallest NPs were produced by *L. fermentum* and had a diameter of 11.2 nm. The recovery of silver and the reduction rate were pH dependent. On the other hand, Naik et al. (2002) have demonstrated the biosynthesis of biogenic Ag NPs using peptides selected by their ability to bind to the surface of silver particles. By the nature of peptide selection against metal particles, a ‘memory effect’ has been imparted to the selected peptides. The silver-binding clones were incubated in an aqueous solution of 0.1 mM silver nitrate for 24–48 h at room temperature. The silver particles synthesized by the silver-binding peptides showed the presence of silver particles 60–150 nm in size. In summary, the biological method provides a



wide range of resources for the synthesis of Ag NPs, and this method can be considered as an environmentally friendly approach and also as a low cost technique. The rate of reduction of metal ions using biological agents is found to be much faster and also at ambient temperature and pressure conditions. In biological synthesis, the cell wall of the microorganisms plays a major role in the intracellular synthesis of NPs. The negatively charged cell wall interacts electrostatically with the positively charged metal ions and bioreduces the metal ions to NPs (Thakkar et al. 2010). When microorganisms are incubated with silver ions, extracellular Ag NPs can be generated as an intrinsic defense mechanism against the metal's toxicity. Other green syntheses of Ag NPs using plant extracts as reducing agents have been performed. This defense mechanism can be exploited as a method of NPs synthesis and has advantages over conventional chemical routes of synthesis. However, it is not easy to have a large quantity of Ag NPs by using biological synthesis.

#### **7.1.6.4 Mosquitocidal Properties of Ag NPs**

Nanotechnology is an exciting and powerful discipline of science; the altered properties of which have offered many new and profitable products and applications. Agriculture, food and medicine sector industries have been investing more in nanotechnology research. Plants or their extracts provide a biological synthesis route of several metallic nanoparticles which is more eco-friendly and allows a controlled synthesis with well-defined size and shape. The rapid drug delivery in the presence of a carrier is a recent development to treat patients with nanoparticles of certain metals. The engineered nanoparticles are more useful in increasing the crop production, although this issue is still in infancy. This is simply due to the unprecedented and unforeseen health hazard and environmental concern. The well-known metal ions such as zinc, iron and copper are essential constituents of several enzymes found in the human system even though the indiscriminate use of similar other metal nanoparticle in food and medicine without clinical trial is not advisable. These attempts to develop novel materials as mosquito larvicides are still necessary. With the progress of nano-technology, many laboratories around the world have investigated silver nanoparticle (AgNPs) production as the nanoparticle possesses more surface atoms than a microparticle, which greatly improves the particle's physical and chemical characteristics. Some physical or chemical methods that are currently available for silver nanoparticle production include mechanical smashing, a solid-phase reaction, freeze-drying, spread drying, and precipitation (co- and homo-precipitation). In general, these methods consume a lot of energy in order to maintain the high pressures and temperatures that are needed for them to work. In contrast, many bioprocesses occur under normal air pressure and temperature, resulting in vast energy savings. As a consequence, this type of procedure attracted the attention of microbiologists and chemists (Chen et al. 2003).

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. Ag NPs 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). Elumalai et al. (2010) have reported that the aqueous extract of shade dried leaves of *Euphorbia hirta* was used for the synthesis of Ag NPs and their antibacterial activities. The larvicidal activity of synthesized Ag NPs utilizing aqueous extract from *Eclipta prostrata*, a member of the Asteraceae, has been investigated against fourth instar larvae of filariasis vector, *C. quinquefasciatus* and malaria vector, *A. subpictus* (Rajakumar and Abdul Rahuman 2011). The larvicidal activities of mycosynthesized Ag NPs against vectors *A. aegypti* and *A. stephensi* responsible for diseases of public health importance have been evaluated (Salunkhe et al. 2011).

The pediculocidal and larvicidal activities of synthesized silver nanoparticles using the aqueous leaf extract of *Tinospora cordifolia* have been reported against the human capitis and fourth-instar larvae of *A. subpictus* and *C. quinquefasciatus* (Jayaseelan et al. 2011). The larvicidal activity of silver nanoparticles synthesized using *Pergularia daemia* plant latex has been screened against *A. aegypti*, *A. stephensi*, and nontarget fish *Poecilia reticulata* (Patil et al. 2012b). 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 *A. 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 *C. 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 *C. quinquefasciatus* (Kamaraj and Rahuman 2010). The extracts of *Coccoloba mollis*, *Guettarda grazielae*, *Merremia aegyptia*, *Rourea doniana*, *Spermacoce verticillata*, and *Triplaris americana* were tested for larvicidal activity against *A. aegypti* (Oliveira et al. 2010). The early fourth instar larvae

of *C. quinquefasciatus*, reared in the laboratory, were used for larvicidal assay with water, hot water, acetone, chloroform, and methanol leaf, stem bark, and flower extracts of *Acacia arabica*, *Cedrus deodara*, *Hibiscus rosasinensis*, *Mangifera indica*, *Nerium indicum*, *Nicotiana tabacum*, *Pongamia pinnata*, and *Solanum nigrum* (Rahuman et al. 2009). The chloroform–methanol extract of the mature leaves of *Solanum villosum* was investigated to establish its biocontrol potentiality under laboratory condition against the larval forms of *A. subpictus* (Chowdhury et al. 2009).

Murugan et al. (2003) studied the interactive effect of botanicals (Neem, *Pongamia*) and *Leucas aspera*, *Bacillus sphaericus* against the larvae of *C. quinquefasciatus*. The aqueous extracts of seed kernels of *Pongamia glabra*, *Adenanthera pavonina*, and *Sapindus emarginatus* were found to exhibit effective ovicidal, larvicidal, and pupicidal activity on *A. aegypti* (Koodalingam et al. 2009). The ethanolic extracts of the orange peel (*C. sinensis*) was tested for the toxicity effect on the larvae of the yellow fever mosquito *A. aegypti* (Amusan et al. 2005); the petroleum ether extract showed larvicidal activity against the *A. aegypti*, *C. quinquefasciatus*, *A. dirus* and *Mansonia uniformis* (Komalamisra et al. 2005). Larvicidal activity of ethyl acetate, butanol, and petroleum ether extracts of five species of Euphorbiaceae plants, *Jatropha curcas*, *Pedilanthus tithymaloides*, *Phyllanthus amarus*, *Euphorbia hirta*, and *Euphorbia tirucalli*, were tested against the early fourth instar larvae of *A. aegypti* and *C. quinquefasciatus* (Rahman et al. 2007). Hexane extract obtained from leaves of *Eucalyptus citriodora* was tested against larvae of *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti* to assess its toxicity and growth-inhibiting activity (Singh et al. 2007).

Aqueous extracts of nine medicinal plants were bioassayed against larvae of *C. quinquefasciatus* and *A. aegypti* among these plants, the long pepper, *Piper retrofractum* Vahl (Piperaceae), showed the highest level of activity against mosquito larvae. Larvicidal activity of *P. retrofractum*, fresh fruits of this plant were extracted in water and the extracts made into powder and bioassayed against 3rd and 4th instar larvae of *C. quinquefasciatus* and *A. aegypti* in the laboratory (Chansang et al. 2005). Larvicidal potential of petroleum ether (Pee), carbon tetrachloride (Cte) and methanol extract (Mee) of *Artemisia annua*, *Chenopodium album* and *Sonchus oleraceus* was observed against malaria vector, *A. stephensi* (Sharma et al. 2006). Nanotechnology provides the cutting edge to engineer these properties of nanomaterials for need-based application in bioscience such as biomedicine, biosensor, etc. (Thevenot et al. 2001). Nanosilica was reported to have potential as a drug delivery vehicle for medical and veterinary treatments and as pesticides in agriculture, but information on the effect on mosquitoes is not available. The reported mode of action for insecticidal activity of nanosilica is through desiccation of insect cuticle by physicosorption of lipid and is also expected to cause damage in the cell membrane resulting in cell lysis and death of the insects (Tiwari and Behari 2009).

Plants and microbes are currently used for nanoparticle synthesis. 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; Kundu et al. 2004), antimicrobial materials (Duran et al. 2005), antiviral, and anti-HIV studies (Elechiguerra et al. 2005). The silver and gold nanoparticles synthesized with *Chryso sporium tropicum* have been tested as a larvicide against the *A. 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 *A. subpictus*, *C. quinquefasciatus*, and *Rhipicephalus microplus* (Marimuthu et al. 2010).

The larvicidal and repellent properties of essential oils is from various parts of four plant species *C. citratus*, *C. zeylanicum*, *Rosmarinus officinalis*, and *Z. officinale* against *C. tritaeniorhynchus* and *A. subpictus* (Govindarajan 2011c). 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 *C. quinquefasciatus*, *A. aegypti*, and *A. stephensi* (Govindarajan 2010c). 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 *A. stephensi* (Govindarajan et al. 2008c). Nanoparticles, generally considered as particles with sizes of up to 100 nm, exhibit completely new or improved properties compared to the larger particles of the bulk material that they are composed of, based on specific characteristics such as size, distribution, and morphology (Willem and van den Wildenberg 2005). Anti-fungal, anti-inflammatory, and antiviral activities of silver nanoparticles were reported (Kim et al. 2009; Nadworny et al. 2008). However, the silica nanoparticles have been tested against the larvae and pupae of *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti* (Barik et al. 2012). Mosquitocidal properties of the Ag-NPs are summarized in Table 7.1. As far as our literature survey could ascertain, no information was available on the larvicidal activity of the experimental plant species given here against *A. subpictus*, *A. albopictus* and *C. tritaeniorhynchus*. Therefore, the aim of this study was to investigate the mosquito larvicidal activity of aqueous crude extract and Ag NPs from *Feronia elephantum*, *Heliotropium indicum* and *Sida acuta*. This is the first report on the mosquito larvicidal activity of selected plant against the target mosquitoes.

**Table 7.1** Mosquitocidal efficacy of silver nanoparticles

Plant name	Family	Particle size (nm)	Shape	Target species	Reference
<i>Annona squamosa</i>	Annonaceae	20	Spherical	<i>A. aegypti</i> , <i>A. stephensi</i> , <i>C. quinquefasciatus</i>	Arjunan et al. (2012)
<i>Parthenium hysterophorus</i>	Asteraceae	50	Spherical	<i>C. quinquefasciatus</i>	Mondal et al. (2014)
<i>Nerium oleander</i>	Apocynaceae	20–35	Spherical	<i>A. stephensi</i>	Roni et al. (2013)
<i>Leucas aspera</i>	Lamiaceae	0.5	Clustered and irregular	<i>A. aegypti</i>	Suganya et al. (2014)
<i>Rhizophora mucronata</i>	Rhizophoraceae	420	Spherical	<i>C. quinquefasciatus</i>	Gnanadesigan et al. (2011)
<i>Cadaba indica</i>	Capparaceae	30–60	Spherical	<i>A. stephensi</i> , <i>C. quinquefasciatus</i>	Kalimuthu et al. (2013)
<i>Murraya koenigii</i>	Rutaceae	20–35	Spherical	<i>A. stephensi</i> , <i>A. aegypti</i>	Suganya et al. (2013)
<i>Pedilanthus tithymalooides</i>	Euphorbiaceae	15–30	Spherical and crystalline nature	<i>A. aegypti</i>	Sundaravadevelan et al. (2013)
<i>Trichoderma harzianum</i>	Hypocreaceae	10–20	Crystalline nature	<i>A. aegypti</i>	Sundaravadevelan and Nalini Padmanabhan (2014)
<i>Anthocepholus cadamba</i>	Rubiaceae	20–50	Crystalline nature	<i>C. quinquefasciatus</i>	Naresh kumar et al. (2013)
<i>Eclipta prostrata</i>	Asteraceae	35–60	triangles, pentagons, and hexagons	<i>C. quinquefasciatus</i> , <i>A. subpictus</i>	Rajakumar and Abdul Rahuman (2011)
<i>Solanum nigrum</i>	Solanaceae	50–100	Spherical to polyhedral in shape	<i>C. quinquefasciatus</i> , <i>A. stephensi</i>	Rawani et al. (2013)
<i>Ficus racemosa</i>	Moraceae	250–60	Cylindrical, uniform, Rod shaped	<i>C. quinquefasciatus</i> , <i>C. gelidus</i>	Velayutham et al. (2013)

(continued)

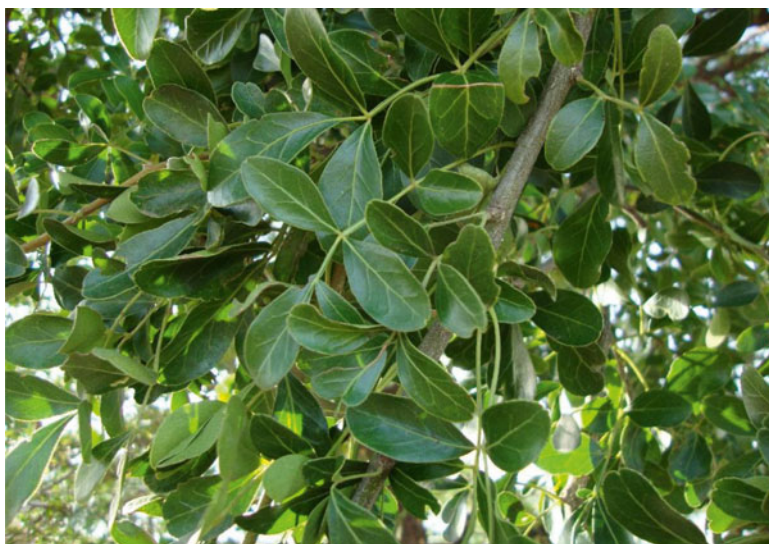
Table 7.1 (continued)

Plant name	Family	Particle size (nm)	Shape	Target species	Reference
<i>Pithecellobium dulce</i>	Fabaceae	300–800	Crystallite	<i>C. quinquefasciatus</i>	Raman et al. (2012)
<i>Nelumbo nucifera</i>	Nelumbonaceae	25–80	Roughly circular	<i>A. subpictus</i> , <i>C. quinquefasciatus</i>	Santhoshkuma et al. (2011)
<i>Mimosa pudica</i>	Mimosaceae	25–50	Cubic	<i>A. subpictus</i> , <i>C. quinquefasciatus</i>	Marimuthu et al. (2011)
<i>Tinospora cordifolia</i>	Menispermaceae	50–100	Spherical	<i>A. subpictus</i> , <i>C. quinquefasciatus</i>	Jayaseelan et al. (2011)
<i>Plumeria rubra</i>	Apocynaceae	32–220	Spherical	<i>A. aegypti</i> , <i>A. stephensi</i>	Chandrashekhar et al. (2012)
<i>Euphorbia hirta</i>	Euphorbiaceae	30–60	Cubic	<i>A. stephensi</i>	Priyadarshini et al. (2012)
<i>Pedilanthus tithymalooides</i>	Euphorbiaceae	15–30	Spherical	<i>A. aegypti</i>	Sundaravadivelan et al. (2013)
<i>Vinca rosea</i>	Apocynaceae	25–47	Spherical	<i>A. stephensi</i> , <i>C. quinquefasciatus</i>	Subarani et al. (2013)
<i>Feronia elephantum</i>	Rutaceae	20–60	Spherical	<i>A. stephensi</i> , <i>A. aegypti</i> , <i>C. quinquefasciatus</i>	Veerakumar et al. (2014a)
<i>Feronia elephantum</i>	Rutaceae	20–60	Spherical	<i>A. stephensi</i> , <i>A. aegypti</i> , <i>C. quinquefasciatus</i>	Veerakumar and Govindarajan (2014)
<i>Heliotropium indicum</i>	Boraginaceae	20–60	Spherical	<i>A. stephensi</i> , <i>A. aegypti</i> , <i>C. quinquefasciatus</i>	Veerakumar et al. (2014b)
<i>Sida acuta</i>	Malvaceae	20–60	Spherical	<i>C. quinquefasciatus</i> , <i>A. stephensi</i> , <i>A. aegypti</i>	Veerakumar et al. (2013)

## 7.2 Materials and Methods

### 7.2.1 Collection of Materials

Fresh leaves of *F. elephantum*, *H. Indicum* and *S. acuta*, (Figs. 7.1, 7.2, and 7.3) were collected from 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 specimens were numbered and kept in our research laboratory for further reference. Silver nitrate was obtained from Qualigens Fine Chemicals, Mumbai, India.



**Fig. 7.1** *Feronia elephantum* Correa (Family: Rutaceae). Common names: Wood Apple, Elephant Apple, Monkey Fruit, Curd Fruit; Plant: Erect slow growing tree; Leaves: Deciduous, alternate, 7.5–12.5 cm long, dark-green, leathery, dotted with oil glands and slightly lemon-scented when crushed; Flower: Dull-red or greenish flowers 1.25 cm wide, borne in small, loose, terminal or lateral panicles. Usually bisexual; Fruit: Round to oval, 5–12.5 cm wide, with a hard, woody, grayish-white, scurfy rind about 6 mm thick; The pulp is brown, mealy, odorous, resinous, astringent, acid or sweetish, with numerous small, white seeds scattered through it. The wood-apple is native and common in the wild in dry plains of India. The rind must be cracked with a hammer. The fruit shell is fashioned into snuffboxes and other small containers. The fruit is much used in India as a liver and cardiac tonic, and, when unripe, as an astringent means of halting diarrhea and dysentery and effective treatment for hiccough, sore throat and diseases of the gums. The pulp is poulticed onto bites and stings of venomous insects, as is the powdered rind. Juice of young leaves is mixed with milk and sugar candy and given as a remedy for biliousness and intestinal troubles of children. Oil derived from the crushed leaves is applied on itch and the leaf decoction is given to children as an aid to digestion. Leaves, bark, roots and fruit pulp are all used against snakebite



**Fig. 7.2** *Heliotropium indicum* L. (Boraginaceae). *Heliotropium indicum*, one of the largest heliotropes found in Texas, is introduced, and is one of the few annuals within this genus (in Texas). India heliotrope grows upright (2–3 ft in height) and is very leafy, when compared to other heliotropes. The leaves are dark green, alternate, entire, and hispid (hairy). The stems are also hispid. Flowers are blue or violet (rarely white), and like all heliotropes, the younger flowers are located towards the tip of the inflorescence (flower cluster), while mature seed are lower on the flower stalk. There are approximately 14 species of *Heliotropium* in Texas. Most are upland species found in the western portions of the state. Six are commonly found in wetlands. Most have white flowers, although blue or violet is not uncommon. Vegetatively, most heliotropes have smallish and narrow leaves and the growth habit is prostrate, or generally so. The seed head, and the way that the flowers are restricted to the tips, is very characteristic of the entire genus. *Heliotropium* from helios (sun) and trope (turn) flowers turn toward the sun. Some species are considered poisonous, while others are considered fair browse for sheep and goats. Although apparently not preferred by waterfowl, some incidental use has been documented

### 7.2.2 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. *A. albopictus* feeding was done from 12 noon to 4:00 p.m. and *A. subpictus* and *C. tritaeniorhynchus* were fed during 6:00–10:00 p.m. A membrane feeder with the





**Fig. 7.3** *Sida acuta* Burm.f. (Family: Malvaceae). Common names: Common *Sida*, Prickly *Sida*, Broomweeds, Wireweed, Cheeseweed; Plant: 1 m; Shrub with slender branches and fibrous stems; Leaves: Slightly concave oval or elongated leaves with a shiny surface and toothed margins; Flower: 10 mm across. The flowers are yellow, conspicuous and borne on short stalks in the leaf axils along the branches; Fruit: The fruit is a dark brown capsule; it splits into 6–10 single-seeded segments when ripe. Native to pantropical regions, it can be found throughout the warm regions of the world. *Sida* is a weed of tropical pastures. Leaves and roots are used in traditional medicine

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  $28 \pm 2$  °C, 70–85 % relative humidity, with a photoperiod of 12-h light and 12-h dark.

### 7.2.3 Preparation of Plant Extracts

The leaves (*F. elephantum*, *H. Indicum* and *S. acuta*) 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.

### 7.2.4 *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  $-15\text{ }^{\circ}\text{C}$  and could be used within 1 week. The filtrate was treated with aqueous 1 mM  $\text{AgNO}_3$  (21.2 mg of  $\text{AgNO}_3$  powder in 125 mL Milli-Q water) solution in an Erlenmeyer flask and incubated at room temperature. Eighty-eight-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).

### 7.2.5 *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 300–800-nm range operated at a resolution of 1 nm. Further, the reaction mixture was subjected to centrifugation at  $60,000\times g$  for 40 min; the resulting pellet was dissolved in deionized water and filtered through Millipore filter (0.45  $\mu\text{m}$ ). An aliquot of this filtrate containing silver nanoparticles was used for Fourier transform infrared (FTIR). For electron microscopic studies, 25  $\mu\text{L}$  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  $4\text{ cm}^{-1}$  in KBr pellets. An aliquot of this filtrate containing silver nanoparticles was used for X-ray diffraction (XRD) analysis.

### 7.2.6 *Larvicidal Activity*

Larvicidal activity of the aqueous crude extract and Ag NPs from of *S. acuta*, *H. Indicum*, *F. elephantum* 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 30–300  $\mu\text{g/mL}$  concentrations and Ag NPs was tested at range of 8–60  $\mu\text{g/mL}$  concentrations. Twenty numbers of late third instar larvae were introduced into a 500-mL glass beaker containing 249 mL of dechlorinated water and 1 mL 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).

### 7.2.7 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.

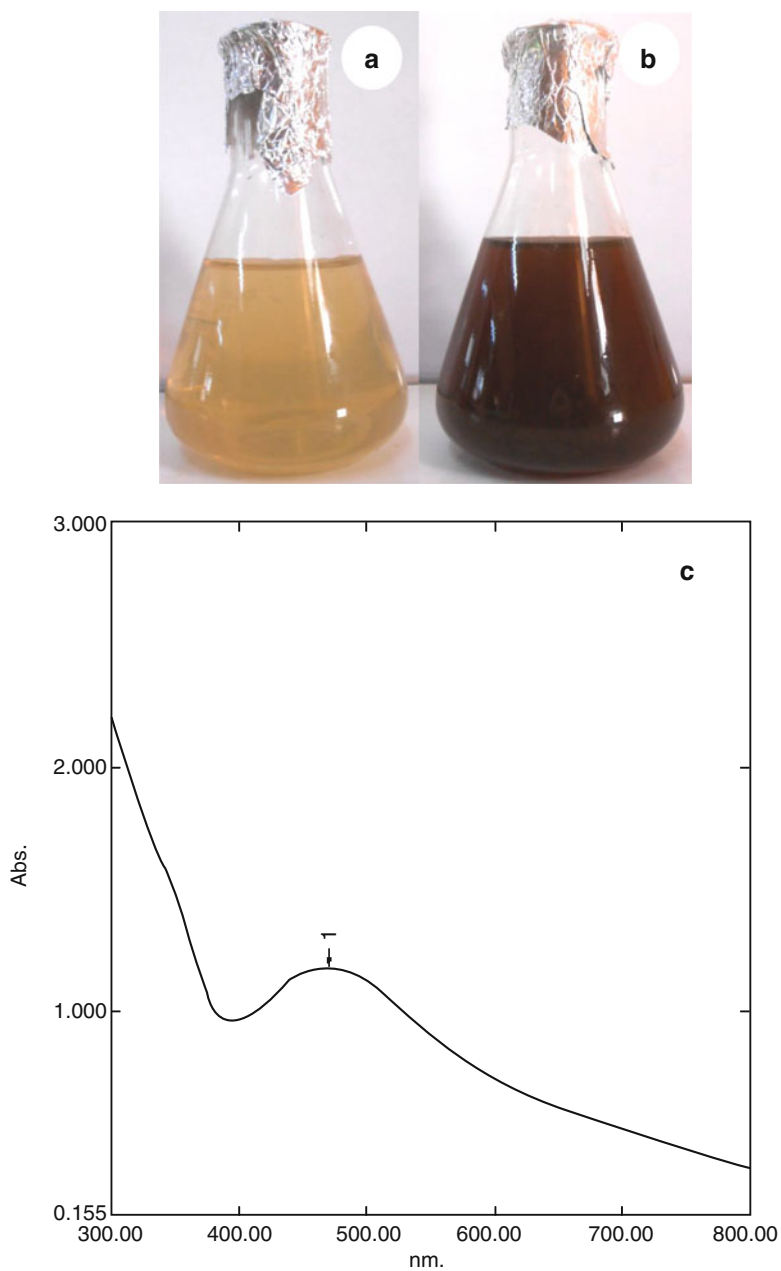
## 7.3 Results

### 7.3.1 UV-Vis Analysis of Ag NPs

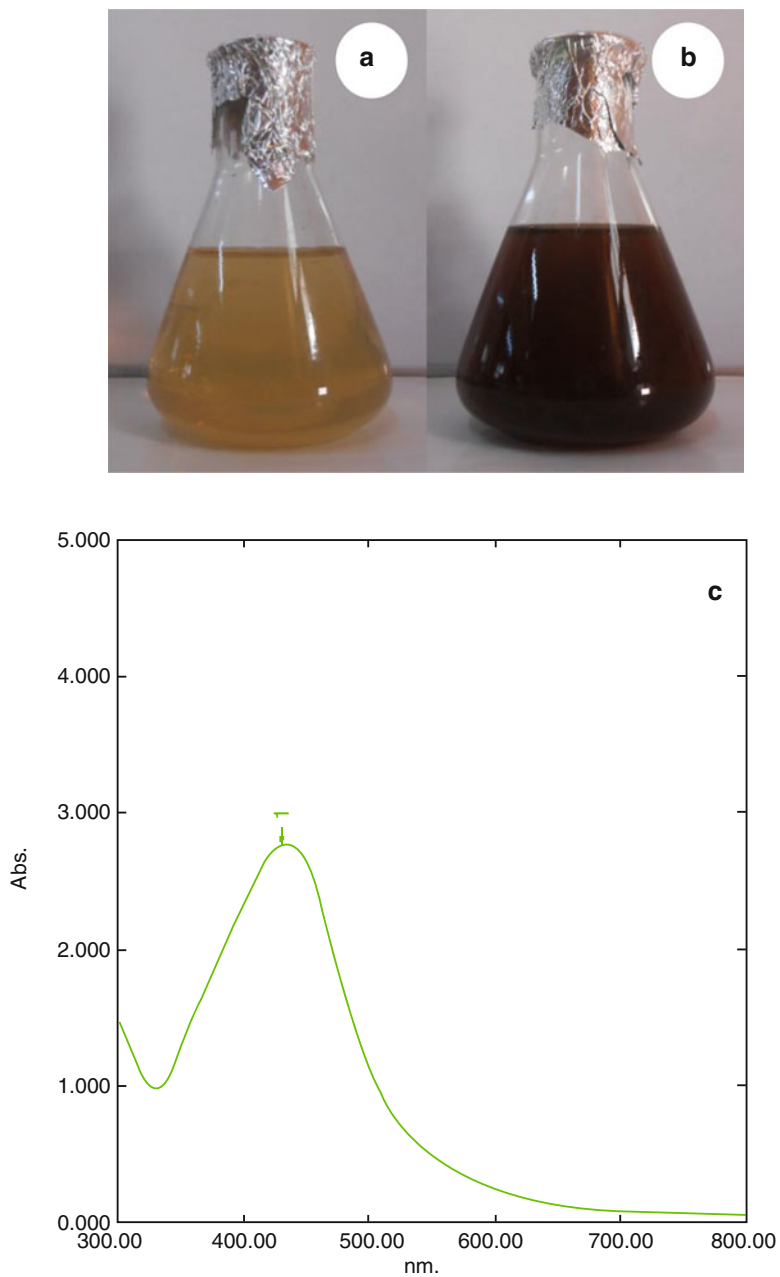
Leaves extracts from all three plants under study (*F. elephantum*, *H. Indicum* and *S. acuta*) showed rapid conversion of silver nitrate into silver nanoparticles indicated by color changes from colorless to red brown within few minutes of extract addition in 100 ppm  $AgNO_3$  solution (Figs. 7.4a, b, 7.5a, b, and 7.6a, b). A representative scheme of biosynthesis and UV-vis spectrum is given in Figs. 7.4c, 7.5c and 7.6c. Synthesized silver nanoparticles primarily characterized by UV-visible spectroscopy. Ag NPs give typical spectrum having maximum absorption in range of 420–450 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.

### 7.3.2 FT-IR Analysis of Ag NPs

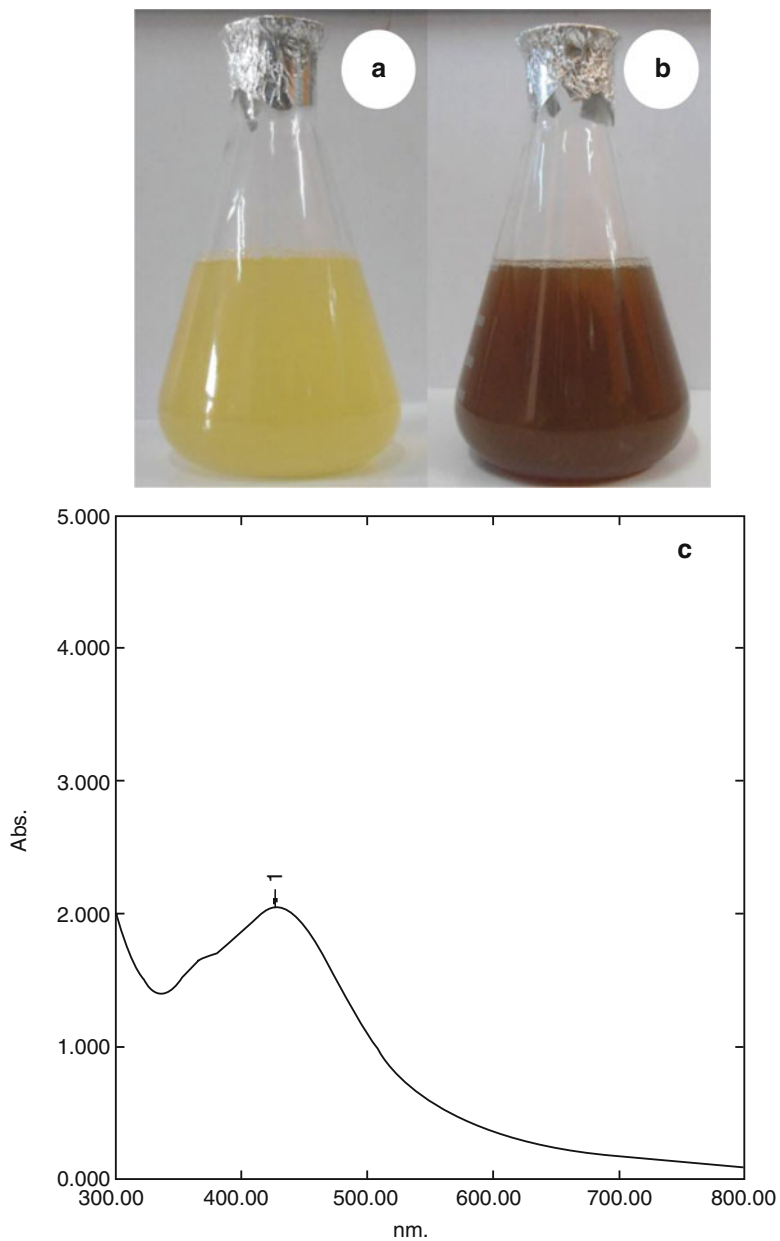
Typical IR spectrum of lyophilized powder of *F. elephantum* leaves extract showed presence of C–H bending ( $671.81$ ,  $762.21$ , and  $822.47\text{ cm}^{-1}$ ), C–O stretch ( $1016.97$  and  $1,120.07$ ), –C–H bending ( $1,384.10$ ), C=C bending ( $1,617.90$ ), C–H stretch ( $2,849.59$ ), and N–H stretch ( $3,422.14$ ) (Fig. 7.7). FTIR analysis of the purified nanoparticles of *H. Indicum* showed the presence of bands due to O–H group C=H bending ( $824.98$ ), C=O stretch ( $1,094.71$ ), N=H bending ( $1,603.81$ ), –C=O stretch



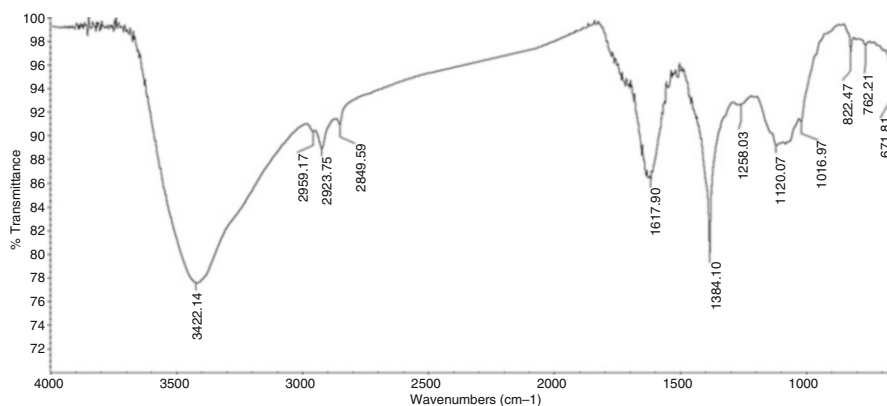
**Fig. 7.4** (a) Photograph showing change in color after adding  $\text{AgNO}_3$  before reaction. (b) After reaction time of 6 h. (c) UV-Vis spectra of aqueous silver nitrate with *F. elephantum* leaf extract



**Fig. 7.5** (a) Photographs showing change in color after adding  $\text{AgNO}_3$  before reaction and (b) After reaction time of 6 h. (c) UV-Vis spectra of aqueous silver nitrate with *H.indicum* leaf extract



**Fig. 7.6** (a) Photographs showing change in color after adding  $\text{AgNO}_3$  before reaction. (b) After reaction time of (6 h). (c) UV-Vis spectra of aqueous silver nitrate with *S.acuta* leaf extract



**Fig. 7.7** FT-IR spectrum of synthesized AgNPs using *F. elephantum* leaf extract

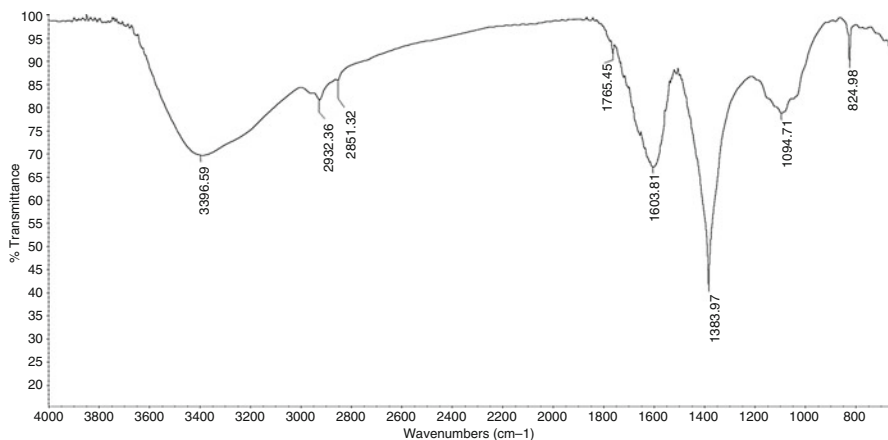
(1,765.45), C–H stretch (2,851.32), C–H stretch (2,932.36), and O–H stretch (3,396.59) (Fig. 7.8). FT-IR analysis of *S.acuta* Ag NPs showed the presence of bands due to O–H group (1,269.92  $\text{cm}^{-1}$ ), C=N stretch (1,486.57), –NH<sub>2</sub>(1636.98), =NH (2,332.25), –H stretch (2,358.27), and O–H stretch (3,345.57) (Fig. 7.9).

### 7.3.3 SEM, EDX and TEM Analysis of Ag NPs

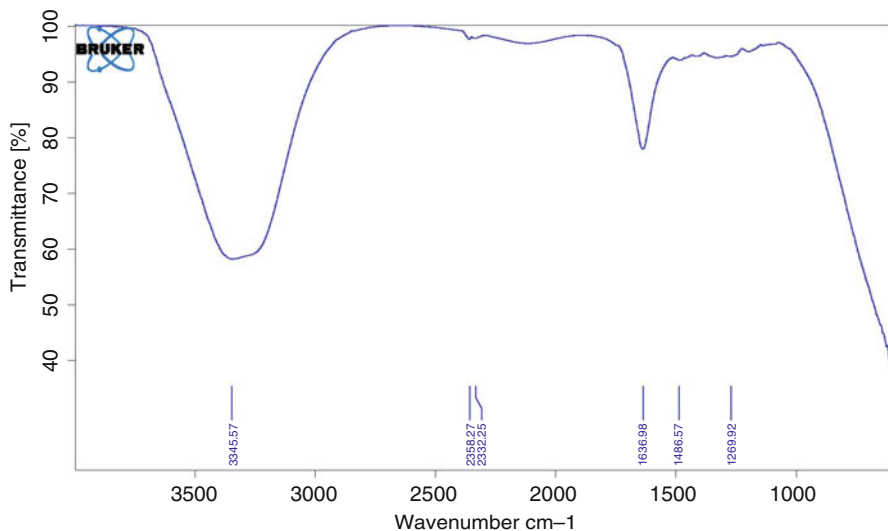
SEM micrographs of the synthesized Ag NPs of *F. elephantum*, *H. indicum* and *S. acuta* magnified at  $\times 500$ ,  $\times 3,000$  and  $\times 5,000$  and measured at 20–60 nm, respectively are shown in Figs. 7.10a, 7.11a, and 7.12a. The triangular, pentagonal, and hexagonal structures are clear. Energy-dispersive X-ray spectroscopy (EDX) proves the chemical purity of the synthesized Ag NPs (Figs. 7.10b, 7.11b, and 7.12b). Transmission electron microscopy has been employed to characterize the size, shape and morphology of synthesized silver nanoparticles. The TEM image of silver nanoparticles is shown in Figs. 7.13a, 7.14a, and 7.15a. 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 22 nm. Figures 7.13b, 7.14b, and 7.15b shows the histogram of size distribution of silver nanoparticles.

### 7.3.4 XRD Analysis of Ag NPs

After reaction, the diffraction peaks formed facets of the face-centered cubic crystal 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



**Fig. 7.8** FT-IR spectrum of synthesized AgNPs using *H.indicum* leaf extract

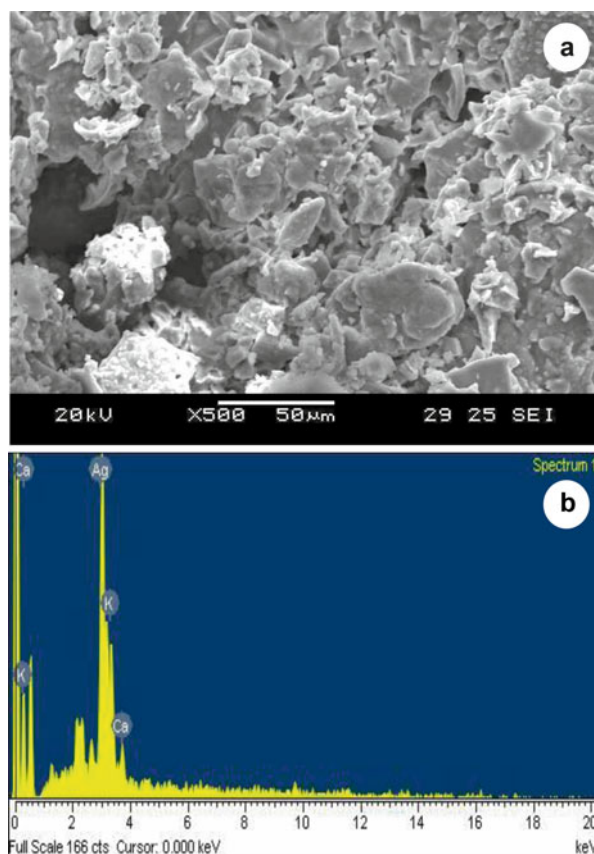


**Fig. 7.9** FT-IR spectrum of synthesized AgNPs using *S.acuta* leaf extract

stabilizing the nanoparticles. Figures 7.16, 7.17, and 7.18 depicts the X-ray diffraction (XRD) pattern of *F. elephantum*, *H. Indicum* and *S. acuta* -powdered silver nanoparticles in the  $2\theta$  range. It exhibits a broad peak at  $38.4^\circ$ ,  $44.5^\circ$ , and  $64.2^\circ$  and  $78.4^\circ$ . The broadening of the peaks clearly indicates that the particles are in the nanoregime. Apart from these, many unidentified peaks at  $28^\circ$ ,  $29^\circ$ ,  $30^\circ$ ,  $32^\circ$ ,  $35^\circ$ ,  $43^\circ$ ,  $45^\circ$  and  $52^\circ$  arise, possibly due to other chemical reactions or organic impurities present in the sample.



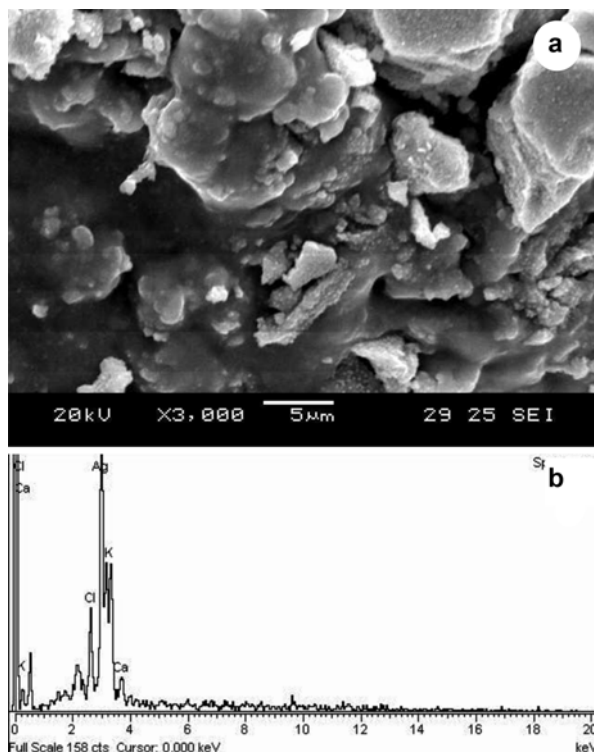
**Fig. 7.10** Scanning electron micrographs of AgNPs synthesized with *F. elephantum* leaf extract and 1.0 mM AgNO<sub>3</sub> solution and incubated at 60 °C for 6 h at pH 7.0. (a) Magnified ×500; inset bar represents 50 μm. (b) EDX image showing chemical composition



### 7.3.5 Larvicidal Efficacy of Aqueous Extract and Synthesized Ag NPs

The results of larvicidal activity of *S. acuta*, *H. Indicum* and *F. elephantum* aqueous leaf extract and Ag NPs against late third instar *A. subpictus*, *A. albopictus* and *C. tritaeniorhynchus* was noted and presented in Tables 7.2, 7.3, 7.4, 7.5, 7.6 and 7.7 (Figs. 7.19, 7.20, and 7.21). From the three plant aqueous leaf extract and Ag NPs tested against late third instar *A. subpictus*, *A. albopictus* and *C. tritaeniorhynchus*, the highest larvicidal activity was observed in *F. elephantum*, moderate larvicidal activity was observed in *H. Indicum* and lowest larvicidal activity was observed in *S. acuta*. All three plant aqueous leaf extract and synthesized Ag NPs showed the larvicidal efficacy within 24 h 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 *F. elephantum* were highly effective against third instar larvae of *A. subpictus*, *A. albopictus* and *C. tritaeniorhynchus*

**Fig. 7.11** Scanning electron micrographs of AgNPs synthesized with *H.indicum* leaf extract and 1.0 mM AgNO<sub>3</sub> solution and incubated at 60 °C for 6 h at pH 7.0. (a) Magnified  $\times 3000$ ; inset bar represents 5  $\mu\text{m}$ . (b) EDX image showing chemical composition

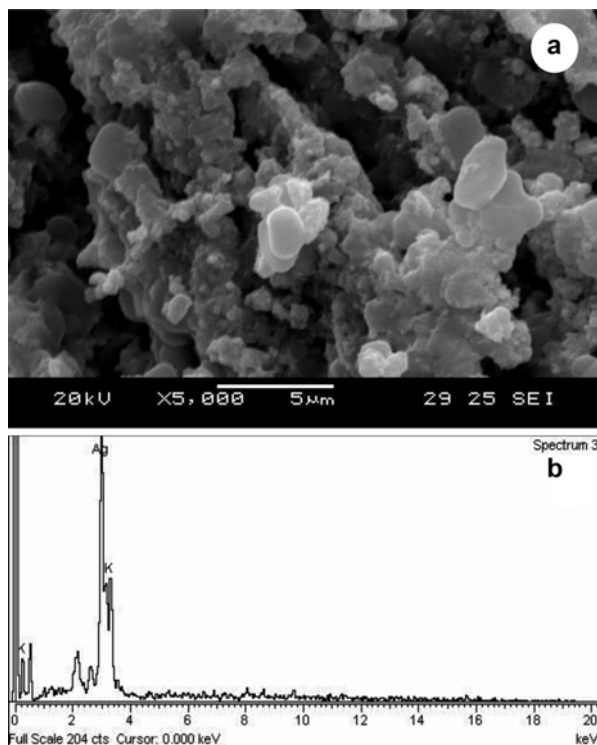


with the LC<sub>50</sub> and LC<sub>90</sub> values were 20.01, 21.59, 24.04  $\mu\text{g}/\text{mL}$  and 34.76, 37.06, 40.86  $\mu\text{g}/\text{mL}$ , respectively. The control showed nil mortality in the concurrent assay.  $\chi^2$  value was significant at  $p \leq 0.05$  level. High larvicidal activity of *F. elephantum* 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 *F. elephantum* > *H. Indicum* > *S. acuta* against third instars of *A. subpictus* followed by *A. albopictus* and *C. tritaeniorhynchus*. The larvae of *A. subpictus* were found highly susceptible to the synthesized Ag NPs than the larvae of *A. albopictus* and *C. tritaeniorhynchus*.

## 7.4 Discussion

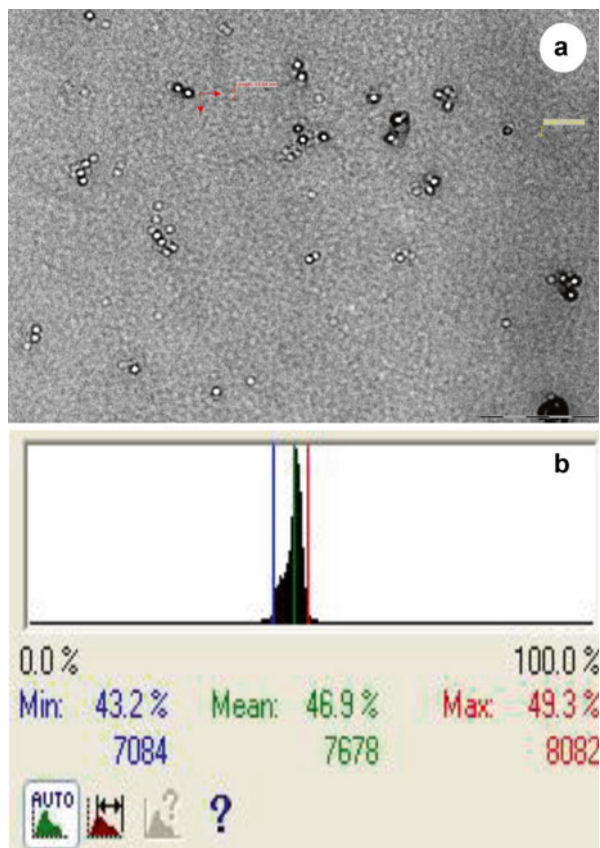
Mosquito-borne diseases are one of the most public health problems in the developing countries. Many approaches have been developed to control mosquito menace. One such approach to prevent mosquito-borne disease is by killing mosquito at larval stage. Management of this disease vector using synthetic chemicals has failed because of insecticide resistance, vector resurgence, and environmental pollution

**Fig. 7.12** Scanning electron micrographs of AgNPs synthesized with *S.acuta* leaf extract and 1.0 mM AgNO<sub>3</sub> solution and incubated at 60 °C for 6 h at pH 7.0. (a) Magnified  $\times 5000$ ; inset bar represents 5  $\mu\text{m}$ . (b) EDX image showing chemical composition

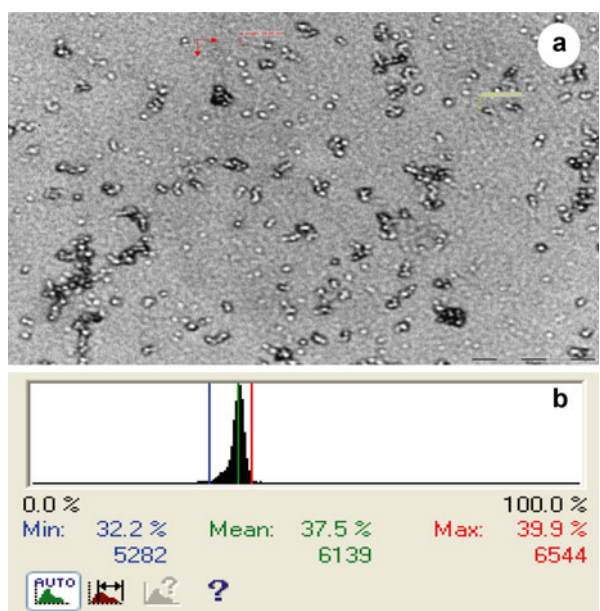


(Wondji et al. 2009). In recent years, silver nanoparticles play a major role in anti-bacterial, antifungal, and insect control programs. The development of novel technology was in the field of insect control particularly mosquito control due to the resistance behavior of the mosquitoes. Plant materials which synthesized silver nanoparticles also used for the mosquito control (Borase et al. 2013) are more popular. Petroleum ether, acetone, ethyl acetate, aqueous extract, methanol and ethanol fractionate of *Eichhornia crassipes* Solms was tested for their larvicidal efficacy against the different instars (I, II, III and IV) and pupae of *C. quinquefasciatus*. The larval mortality was observed after 24 h of the treatment. Ethanol fractionate of *E. crassipes* showed the highest larvicidal and pupicidal activity against *C. quinquefasciatus* compared to other solvent extracts and fractionates with LC<sub>50</sub> 71.43, 94.68, 120.42, 152.15 and 173.35 ppm for I, II, III, IV and pupae, respectively (Jayanthi et al. 2012). Vinayachandra et al. (2011) reported that the effect of aril and kernel extracts of *Knema attenuata* on larvae of *A. albopictus* and *A. stephensi* under laboratory conditions. The aril was extracted with chloroform and ethanol; the kernel was extracted with ethanol and hexane. All the graded concentrations (100, 200, 300, 400 and 500 ppm) showed significant larval mortality after 24 h of observation. Chloroform extracts of aril showed 100 % mortality against both larval forms of *A. albopictus* and *A. stephensi* at the concentration of 500 ppm. Among the extracts tested, chloroform extracts of aril and ethanol extracts of kernel exhibited

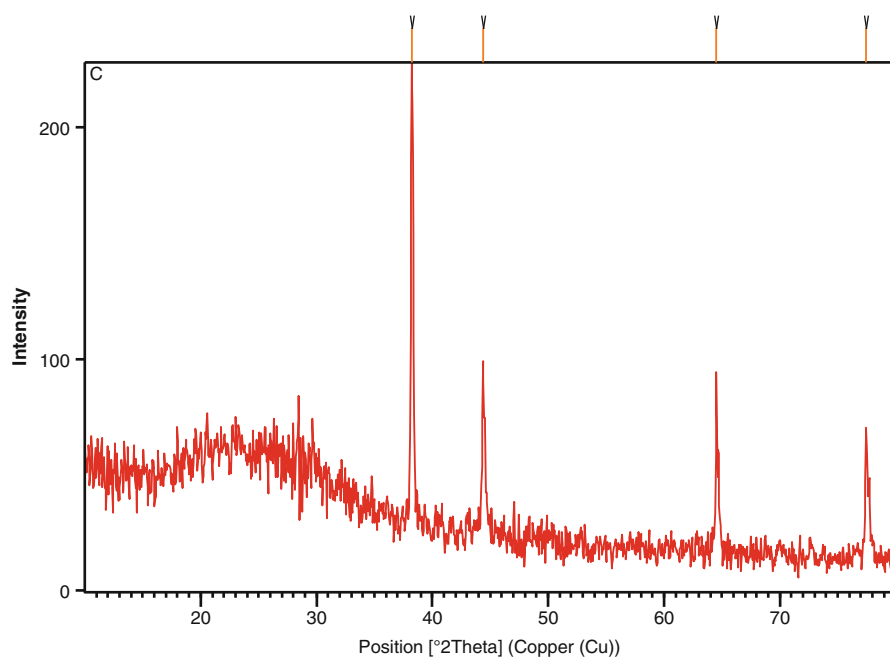
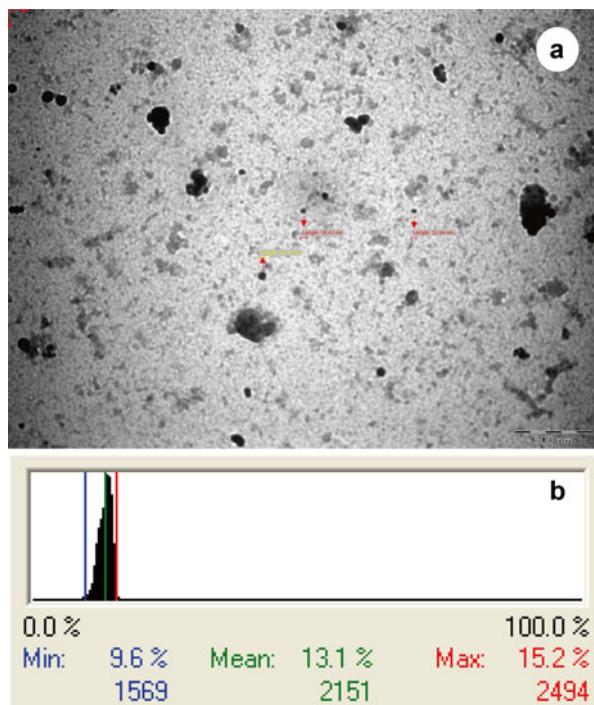
**Fig. 7.13** Transmission electron microscopic image (a) and histogram (b) showing synthesized AgNPs from *F. elephantum*



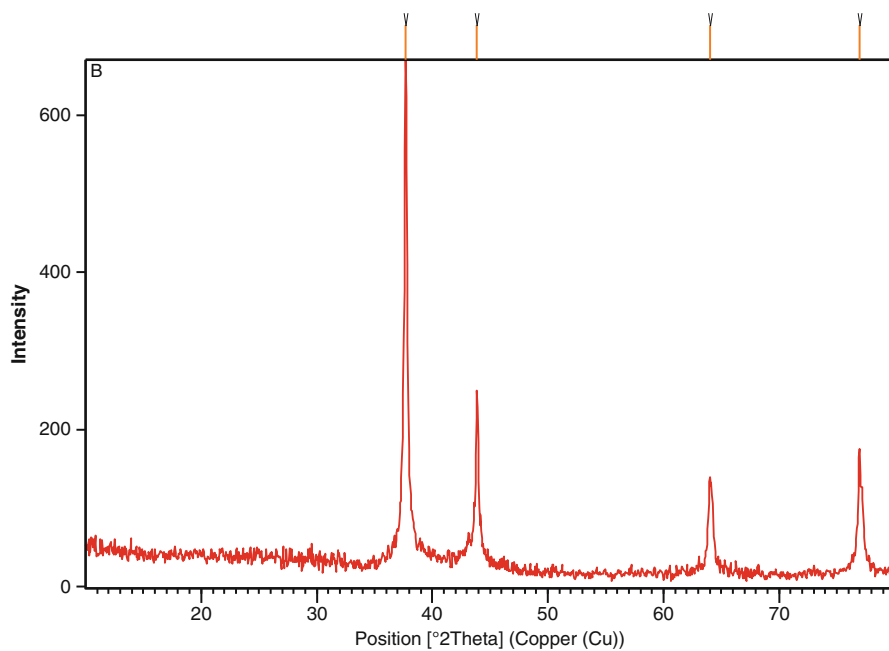
**Fig. 7.14** Transmission electron microscopic image (a) and histogram (b) showing synthesized AgNPs from *H.indicum*



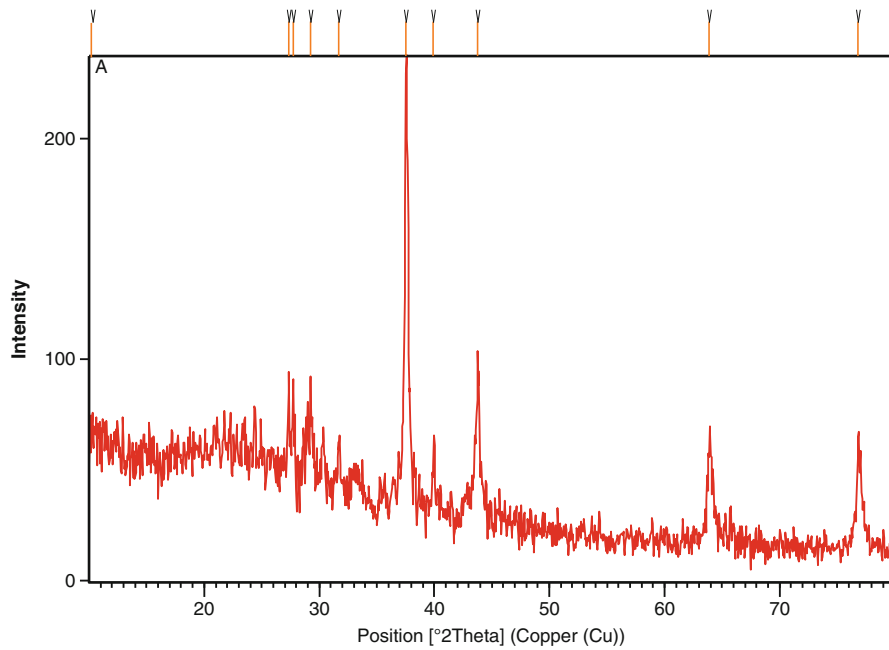
**Fig. 7.15** Transmission electron microscopic image (a) and histogram (b) showing synthesized AgNPs from *S.acuta*



**Fig. 7.16** X-Ray diffraction showing synthesized AgNPs from *F. elephantum*



**Fig. 7.17** X-Ray diffraction showing synthesized AgNPs from *H.indicum*



**Fig. 7.18** X-Ray diffraction showing synthesized AgNPs from *S.acuta*

**Table 7.2** Larvicidal activity of *Sida acuta* aqueous leaf extract against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquitoes	Concentration	24 h mortality (%) $\pm$ SD <sup>a</sup>	LC <sub>50</sub> ( $\mu$ g/mL) (LCL-UCL)	LC <sub>90</sub> ( $\mu$ g/mL) (LCL-UCL)	$\chi^2$
<i>A. subpictus</i>	Control	0.0 $\pm$ 0.0	131.73	241.94	20.320*
	60	29.3 $\pm$ 0.8	(92.13–	(198.89–	
	120	48.5 $\pm$ 1.6	168.41)	331.55)	
	180	70.4 $\pm$ 1.2			
	240	82.6 $\pm$ 0.4			
	300	100.0 $\pm$ 0.0			
<i>A. albopictus</i>	Control	0.0 $\pm$ 0.0	144.61	257.66	9.737*
	60	23.8 $\pm$ 1.2	(119.08–	(224.59–	
	120	41.6 $\pm$ 2.0	169.59)	311.47)	
	180	64.9 $\pm$ 0.6			
	240	83.2 $\pm$ 0.4			
	300	96.4 $\pm$ 1.3			
<i>C. tritaeniorhynchus</i>	Control	0.0 $\pm$ 0.0	161.44	280.33	9.807*
	60	20.1 $\pm$ 2.0	(135.52–	(244.35–	
	120	36.4 $\pm$ 1.5	188.06)	340.34)	
	180	52.5 $\pm$ 0.8			
	240	78.9 $\pm$ 1.6			
	300	94.3 $\pm$ 0.2			

SD standard deviation, LCL lower confidence limits, UCL upper confidence limits,  $\chi^2$  Chi-square test \*p < 0.05, level of significance

<sup>a</sup>Values are mean  $\pm$  SD of five replicates

**Table 7.3** Larvicidal activity of silver nanoparticles synthesized using *Sida acuta* against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquitoes	Concentration	24 h mortality (%) $\pm$ SD <sup>a</sup>	LC <sub>50</sub> ( $\mu$ g/mL) (LCL-UCL)	LC <sub>90</sub> ( $\mu$ g/mL) (LCL-UCL)	$\chi^2$
<i>A. subpictus</i>	Control	0.0 $\pm$ 0.0	25.68	48.29	30.914*
	12	30.8 $\pm$ 1.0	(15.69–	(37.42–	
	24	41.6 $\pm$ 0.8	36.11)	79.62)	
	36	62.5 $\pm$ 1.6			
	48	80.4 $\pm$ 1.2			
	60	100.0 $\pm$ 0.0			
<i>A. albopictus</i>	Control	0.0 $\pm$ 0.0	28.10	51.71	24.070*
	12	25.2 $\pm$ 0.3	(19.61–	(41.29–	
	24	38.8 $\pm$ 1.2	37.36)	77.14)	
	36	57.1 $\pm$ 2.0			
	48	76.4 $\pm$ 0.5			
	60	98.3 $\pm$ 0.2			
<i>C. tritaeniorhynchus</i>	Control	0.0 $\pm$ 0.0	30.19	56.26	21.219*
	12	21.9 $\pm$ 1.6	(22.79–	(45.43–	
	24	34.2 $\pm$ 1.2	40.24)	81.46)	
	36	52.1 $\pm$ 0.3			
	48	70.8 $\pm$ 0.8			
	60	95.4 $\pm$ 1.4			

SD standard deviation, LCL lower confidence limits, UCL upper confidence limits,  $\chi^2$  Chi-square test \*p < 0.05, level of significance

<sup>a</sup>Values are mean  $\pm$  SD of five replicates

**Table 7.4** Larvicidal activity of *Heliotropium indicum* aqueous leaf extract against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquitoes	Concentration	24 h mortality (%)±SD <sup>a</sup>	LC <sub>50</sub> (µg/mL) (LCL-UCL)	LC <sub>90</sub> (µg/mL) (LCL-UCL)	χ <sup>2</sup>
<i>A.subpictus</i>	Control	0.0±0.0	93.16	169.67	22.281*
	40	28.6±0.8	(65.00–	(138.44–	
	80	45.6±1.6	120.07)	238.76)	
	120	62.4±1.2			
	160	80.3±0.4			
	200	100.0±0.0			
<i>A. albopictus</i>	Control	0.0±0.0	100.83	182.13	12.491*
	40	24.5±1.2	(80.42–	(155.54–	
	80	41.2±2.0	121.13)	229.81)	
	120	59.6±0.6			
	160	78.3±0.4			
	200	95.4±1.3			
<i>C.tritaeniorhynchus</i>	Control	0.0±0.0	109.14	193.40	9.662*
	40	19.2±2.0	(91.26–	(167.97–	
	80	39.5±1.5	127.58)	236.39)	
	120	54.3±0.8			
	160	74.6±1.6			
	200	92.1±0.2			

SD standard deviation, LCL lower confidence limits, UCL upper confidence limits, χ<sup>2</sup> Chi-square test \*p<0.05, level of significance

<sup>a</sup>Values are mean±SD of five replicates

**Table 7.5** Larvicidal activity of silver nanoparticles synthesized using *Heliotropium indicum* against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquitoes	Concentration	24 h mortality (%)±SD <sup>a</sup>	LC <sub>50</sub> (µg/mL) (LCL-UCL)	LC <sub>90</sub> (µg/mL) (LCL-UCL)	χ <sup>2</sup>
<i>A.subpictus</i>	Control	0.0±0.0	23.44	43.04	24.601*
	10	29.5±1.0	(15.80–	(34.74–	
	20	45.2±0.8	30.72)	62.53)	
	30	62.3±1.6			
	40	78.4±1.2			
	50	100.0±0.0			
<i>A. albopictus</i>	Control	0.0±0.0	25.84	46.37	15.220*
	10	23.9±0.3	(20.13–	(39.04–	
	20	40.1±1.2	31.62)	60.58)	
	30	58.5±2.0			
	40	74.2±0.5			
	50	96.3±0.2			
<i>C.tritaeniorhynchus</i>	Control	0.0±0.0	28.54	50.76	12.184*
	10	20.7±1.6	(23.31–	(43.20–	
	20	35.3±1.2	34.15)	64.89)	
	30	52.4±0.3			
	40	68.2±0.8			
	50	91.5±1.4			

SD standard deviation, LCL lower confidence limits, UCL upper confidence limits, χ<sup>2</sup> Chi-square test \*p<0.05, level of significance

<sup>a</sup>Values are mean±SD of five replicates



**Table 7.6** Larvicidal activity of *Feronia elephantum* aqueous leaf extract against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquitoes	Concentration	24 h mortality (%)±SD <sup>a</sup>	LC <sub>50</sub> (µg/mL) (LCL-UCL)	LC <sub>90</sub> (µg/mL) (LCL-UCL)	χ <sup>2</sup>
<i>A.subpictus</i>	Control	0.0±0.0	74.14	126.48	14.933*
	30	22.3±1.0	(58.90–	(107.78–	
	60	36.2±1.5	89.38)	160.92)	
	90	61.4±2.0			
	120	82.5±1.8			
	150	100.0±0.0			
<i>A. albopictus</i>	Control	0.0±0.0	80.19	136.53	12.224*
	30	20.5±1.5	(66.09–	(117.81–	
	60	31.6±1.3	94.70)	169.43)	
	90	56.2±2.0			
	120	77.4±1.6			
	150	97.3±1.2			
<i>C.tritaeniorhynchus</i>	Control	0.0±0.0	87.11	145.38	10.035*
	30	17.2±1.8	(74.29–	(127.03–	
	60	26.5±1.2	100.78)	176.38)	
	90	50.3±0.8			
	120	72.4±1.6			
	150	94.6±2.0			

SD standard deviation, LCL lower confidence limits, UCL upper confidence limits, χ<sup>2</sup> Chi-square test \*p < 0.05, level of significance

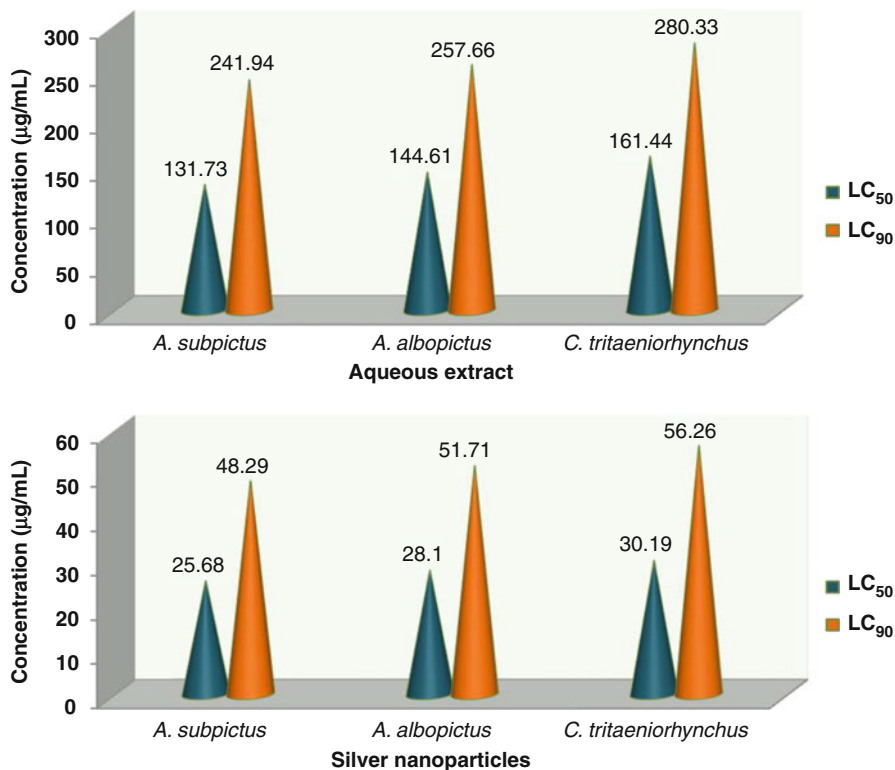
<sup>a</sup>Values are mean ± SD of five replicates

**Table 7.7** Larvicidal activity of silver nanoparticles *Feronia elephantum* against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquitoes	Concentration	24 h mortality (%) ± SD <sup>a</sup>	LC <sub>50</sub> (µg/mL) (LCL-UCL)	LC <sub>90</sub> (µg/mL) (LCL-UCL)	χ <sup>2</sup>
<i>A.subpictus</i>	Control	0.0±0.0	20.01	34.76	18.464*
	8	23.2±2.0	(15.33–	(29.06–	
	16	38.4±1.8	24.85)	46.29)	
	24	57.5±0.4			
	32	79.6±1.6			
	40	100.0±0.0			
<i>A. albopictus</i>	Control	0.0±0.0	21.59	37.06	9.846*
	8	20.4±1.6	(18.19–	(32.36–	
	16	32.5±2.0	25.09)	44.86)	
	24	54.3±1.9			
	32	78.6±1.4			
	40	95.2±1.6			
<i>C.tritaeniorhynchus</i>	Control	0.0±0.0	24.04	40.86	11.549*
	8	18.4±1.3	(20.17–	(35.15–	
	16	26.3±1.2	28.33)	51.25)	
	24	48.2±0.2			
	32	67.5±1.8			
	40	92.1±1.9			

SD standard deviation, LCL lower confidence limits, UCL upper confidence limits, χ<sup>2</sup> Chi-square test \*p < 0.05, level of significance

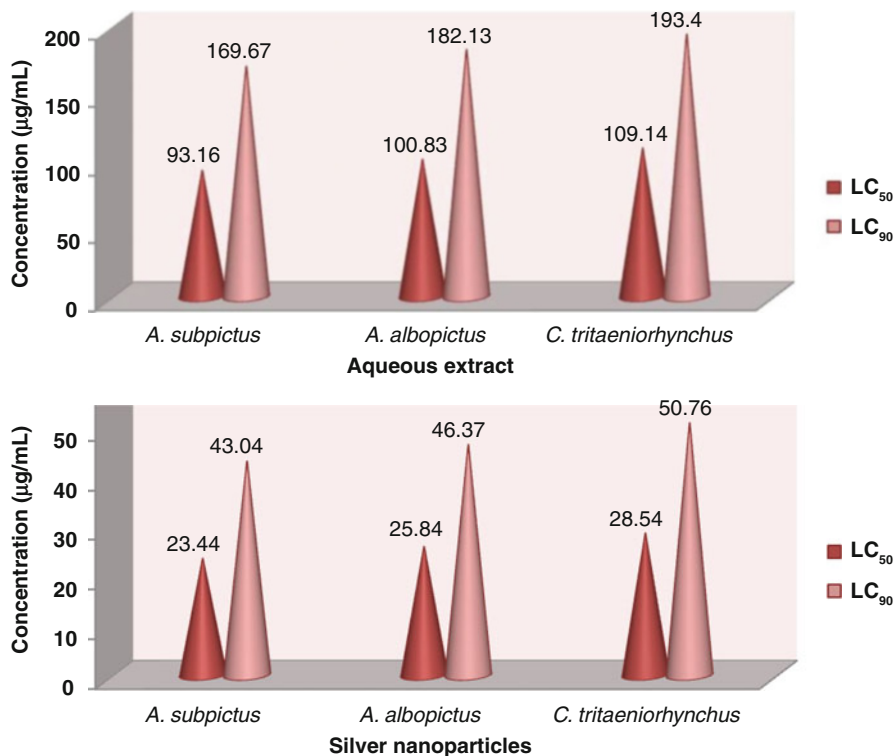
<sup>a</sup>Values are mean ± SD of five replicates



**Fig. 7.19** Graph showing the LC<sub>50</sub> and LC<sub>90</sub> values of larvicidal activity of *S.acuta* aqueous leaf extract and silver nanoparticles against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

higher toxicity against both *A. albopictus* (LC<sub>50</sub>, 141 and 159 ppm; LC<sub>90</sub>, 290 and 342 ppm) and *A. stephensi* (LC<sub>50</sub>, 160 and 162 ppm; LC<sub>90</sub>, 445 and 458 ppm). Hexane extracts of kernel exhibited least toxicity against *A. albopictus* (LC<sub>50</sub>, 239 ppm; LC<sub>90</sub>, 484 ppm), whereas ethanol extracts of aril showed the least toxicity against *A. stephensi* (LC<sub>50</sub>, 290; LC<sub>90</sub>, 498).

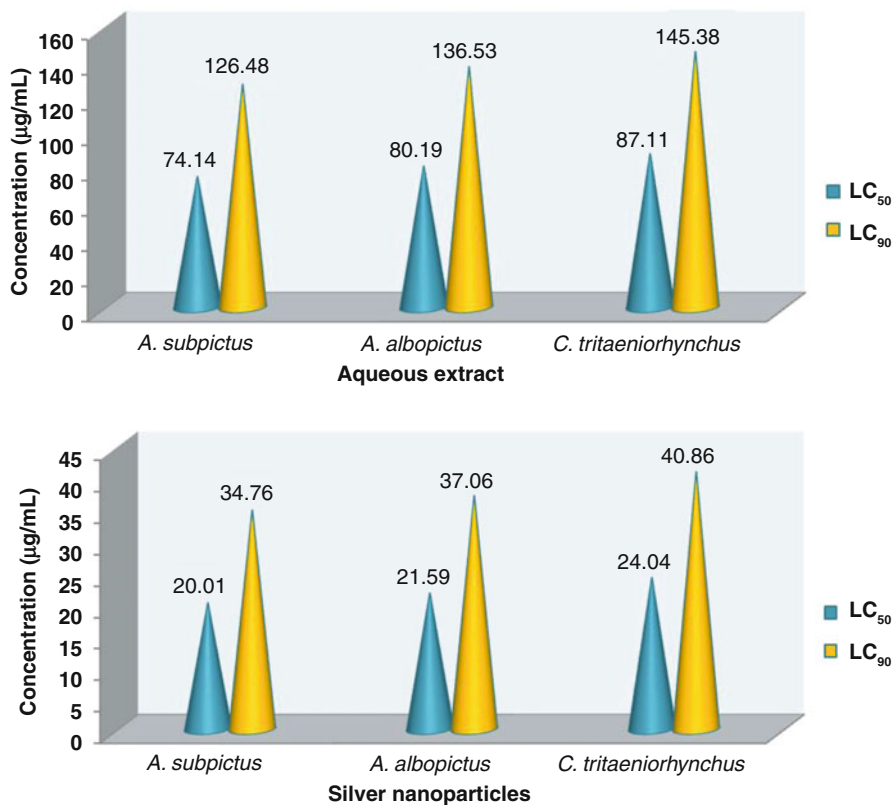
Mahesh Kumar et al. (2012) have reported that the LC<sub>50</sub> value of first to fourth instar larvae and pupae was 155.29, 198.32, 271.12, 377.44 and 448.41 ppm, respectively. The LC<sub>90</sub> value of first to fourth instar larvae and pupae was 687.14, 913.10, 1,011.89, 1,058.85 and 1141.65 ppm, respectively. Patil et al. (2011) evaluated larvicidal activity of extracts of medicinal plants *Plumbago zeylanica* and *Cestrum nocturnum* against *A. aegypti*; the LC<sub>50</sub> values of both the plants were less than 50 ppm. The larvicidal stability of the extracts at five constant temperatures (19, 22, 25, 28 and 31 °C) evaluated against fourth instars larvae revealed that toxicity of both plant extracts increases with increase in temperature. Prophiro et al. (2012) reported that the susceptibility of the larvae was determined under three different temperatures of 15 °C, 20 °C, and 30 °C with lethal concentrations for *Copaifera* sp.



**Fig. 7.20** Graph showing the LC<sub>50</sub> and LC<sub>90</sub> values of larvicidal activity of *H.indicum* aqueous leaf extract and silver nanoparticles against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

ranged from LC<sub>50</sub>=47 to LC<sub>90</sub>=91 (milligrams per liter), and for *Carapa guianensis*, they were LC<sub>50</sub>=136 to LC<sub>90</sub>=551 (milligrams per liter), respectively. 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 *C. pipiens* pallens. The larval mortality was observed after 24 h of exposure. Among three solvent extracts from *P. leptostachya*, the petroleum ether extract exhibited the best larvicidal activity. The corresponding LC<sub>50</sub> values of petroleum ether, ethyl acetate, and methanol extracts were 3.23, 5.23, and 61.86 ppm against the early fourth instar larvae of *C. pipiens* (Xiao et al. 2012). The bio-efficacy of *Aloe vera* leaf extract and bacterial insecticide, *Bacillus sphaericus* larvicidal activity was assessed against the first to fourth instars larvae of *A. aegypti*, under the laboratory conditions. The LC<sub>50</sub> of *A. vera* against the first to fourth instars larvae were 162.74, 201.43, 253.30 and 300.05 ppm and the LC<sub>90</sub> 442.98, 518.86, 563.18 and 612.96 ppm, respectively (Subramaniam et al. 2012).

The hexane extract of *M. koenigii* was found to be the most effective providing 100 % mortality at 750 ppm against the larvae of *A. stephensi* at 48 h followed by



**Fig. 7.21** Graph showing the LC<sub>50</sub> and LC<sub>90</sub> values of larvicidal activity of *F. elephantum* aqueous leaf extract and silver nanoparticles against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

*C. quinquefasciatus* at 1,000 ppm at 48 h. Hexane extract showed the least LC<sub>50</sub> value of 418.74 and 466.09 ppm against *A. stephensi* and *C. quinquefasciatus*, but it was diethyl ether in the case of *A. aegypti* with an LC<sub>50</sub> value of 511.12 ppm (Arivoli and Samuel 2011). Essential oils extracted by steam distillation from rhizome of *Z. officinalis* and leaf and stem of *Achyranthes aspera* were evaluated for larvicidal, attractant/repellent, and oviposition attractant/ deterrent activity against two mosquito species viz., *A. aegypti* and *C. quinquefasciatus*. The highest larvicidal activity, i.e., LC<sub>50</sub>=154 ppm and LC<sub>50</sub>=197 ppm for *A. aegypti* and *C. quinquefasciatus*, respectively was shown by *Z. officinalis*. This oil also offers 5-h protection at the concentration of 0.5 mg/cm<sup>2</sup> from both mosquito species (Khandagle et al. 2011). Khanna et al. (2011) have reported that the larvicidal crude leaf extract of *Gymnema sylvestri* showed the highest mortality in the concentration of 1,000 ppm against the larvae of *A. subpictus* (LC<sub>50</sub>=166.28 ppm) and against the larvae of *C. quinquefasciatus* (LC<sub>50</sub>=186.55 ppm), and the maximum efficacy was observed in gymnemagenol compound isolated from petroleum ether leaf extract of *G. sylvestri* with LC<sub>50</sub>

values against the larvae of *A. subpictus* at 22.99 ppm and against *C. quinquefasciatus* at 15.92 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., *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in benzene extract of *E. coronaria* against the larvae of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* with the LC<sub>50</sub> and LC<sub>90</sub> values were 79.08, 89.59, and 96.15 ppm and 150.47, 166.04, and 174.10 ppm, respectively (Govindarajan et al. 2011b). Mathew et al. (2009) reported that leaf chloroform extracts of *Nyctanthes arbortristis* showed lethal values (LC<sub>50</sub>=526.3 and 780.6 ppm (24 h) and LC<sub>50</sub>=303.2 and 518.2 ppm (48 h)) against *A. aegypti* and *A. stephensi*, respectively. Elimam et al. (2009) to investigate the larvicidal, adult emergence inhibition and oviposition deterrent activity of aqueous leaves extract of *Calotropis procera* against *A. arabiensis* and *C. quinquefasciatus* as natural mosquito larvicide. LC<sub>50</sub> and LC<sub>90</sub> 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 *A. 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 *C. quinquefasciatus*.

Mathivanan et al. (2010) determine that the LC<sub>50</sub> and LC<sub>90</sub> values of crude methanol extract of leaves of *Ervatamia coronaria* on *C. quinquefasciatus*, *A. aegypti*, and *A. stephensi* larvae in 24 h were 72.41, 65.67, and 62.08 and 136.55, 127.24, and 120.86 mg/L, respectively. The ethanolic leaf extract of *Cassia obtusifolia* was investigated for their larvicidal and oviposition deterrence effects against *A. stephensi*. Concentrations ranging from 25 to 125 mg/l were assessed at 24 h post-treatment against late third instar larvae. The leaf extract had significant larvicidal effect with LC<sub>50</sub> and LC<sub>90</sub> values were 52.2 and 108.7 mg/l, respectively. In oviposition behaviour study, four different concentrations ranging from 100 to 400 mg/l were studied against gravid female mosquitoes. Essential oil from *Tagetes filifolia* showed the strongest larvicidal activity against the third instar larvae of *A. aegypti* with the LC<sub>50</sub> value of 47.7 ppm (Ruiz et al. 2011). Conti et al. (2010) studied *Foeniculum vulgare* essential oil for larvicidal activity against fourth instar larvae of *A. albopictus* and the oil showed larvicidal activity with an IC<sub>50</sub> value of 142.9 ppm. In *Calotropis procera* against *A. stephensi*, showed 99 % mortality at 64 ppm for *A. stephensi*, only 44 % mortality against *C. quinquefasciatus*, and a maximum of 67 % in 256 ppm, respectively (Shahi et al. 2010). *Clitoria ternatea* leaf methanol extract showed dose-dependent larvicidal activity against *A. stephensi* with LC<sub>50</sub> values of 555.6 (24 h) and 867.3 (48 h) ppm, also the root extracts with LC<sub>50</sub> value of 340 ppm (48 h). Seed extract showed larvicidal activity (LC<sub>50</sub>=116.8, 195 ppm) after 24 h and (LC<sub>50</sub>=65.2, 154.5 ppm) after 48 h treatment against *A. stephensi* and *A. aegypti*, respectively. Larvicidal activity of flower methanol extract showed LC<sub>50</sub> values 233 and 302.5 ppm against *A. stephensi* and *A. aegypti*, respectively, after 48 h treatment. Methanol extract showed 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/

cm<sup>2</sup>, respectively) which indicates highest toxicity or insecticidal activity (Ashraful Alam et al. 2009).

Kamalakaran et al. (2011) determined the biological activities of methanol extracts of *Acalypha indica* and *Achyranthes aspera* leaves individually and in combination as botanical insecticides against *A. aegypti*. Based on LC<sub>50</sub> values for 4th instar *A. aegypti*, the combined extracts showed the strongest larvicidal activity (277 ppm). *Achyranthes aspera* and *Acalypha indica* extracts individually gave similar results (409 and 420 ppm, respectively). Crude extracts of fruits and leaves of *Centratherum anthelminticum* in different solvents were tested for larvicidal activity against *A. stephensi*, the vector of malaria. The petroleum ether crude extract of both fruits and leaves exhibited significant larvicidal activity against III instar larvae with LC<sub>50</sub> values of 162.60 ppm and 522.94 ppm, respectively after 24 h. The petroleum ether extract of fruit was 11.66, 2.15 and 1.32 times more toxic than that of leaf extract after 24, 48 and 72 h, respectively at LC<sub>90</sub> level. However, at LC<sub>50</sub> level the corresponding values were 3.22, 1.83 and 1.19, respectively (Srivastava et al. 2008). Gleiser and Zygadlo (2007) reported that the essential oils of *Lippia turbinata* and *L. polystachya* exhibit LC<sub>50</sub> values of 74.9 and 121 mg/l, respectively against *C. 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 *A. 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<sub>50</sub> values were less than 100 µg/mL (4.1–89.4 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<sub>50</sub> and LC<sub>90</sub> 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 (Promisiri et al. 2006).

Cypermethrin and crude extracts of *Solanum xanthocarpum* were both observed for their larvicidal activity against *C. quinquefasciatus*. Petroleum ether extract with lethal concentration LC<sub>50</sub> and LC<sub>90</sub> of 41.28 and 111.16 ppm after 24 h and LC<sub>50</sub> 38.48 and LC<sub>90</sub> 80.83 ppm after 48 h, respectively, was found to be the most effective, followed by carbon tetrachloride and methanol extracts (Mohan et al. 2006). Sakthivadivel and Daniel (2008) showed the crude petroleum ether leaf extract of *Jatropha curcas* to have larvicidal activity with the LC<sub>50</sub> of <100 ppm on the early fourth instar larvae of vector mosquitoes including *C. quinquefasciatus*, *A. stephensi*, and *A. aegypti*. Investigations were made to test the larval toxicity and smoke repellent potential of *Albizia amara* and *Ocimum basilicum* at different concentration (2, 4, 6, 8 and 10 %) against the different instar (I, II, III and IV) larvae and pupae of *A. aegypti*. The LC<sub>50</sub> values of *A. amara* and *O. basilicum* for I instar larvae was 5.412 and 3.734, II instar 6.480 and 4.154, III instar 7.106 and 4.664, IV instar 7.515 and 5.124, respectively. The LC<sub>50</sub> and LC<sub>90</sub> values of pupae were 6.792, 5.449 % and 16.925, 15.474 %. The smoke toxicity of *A. amara* was more effective against *A. aegypti* than the *O. basilicum* (Murugan et al. 2007).

The insecticidal activities of extracts and oils of 17 medicinal plants of Brazil have been determined using an *A. aegypti* larvicidal bioassay. Oils from *Anacardium occidentale*, *Copaifera langsdorffii*, *Carapa guianensis*, *Cymbopogon winterianus* and *Ageratum conyzoides* showed high activities with LC<sub>50</sub> values of 14.5, 41, 57, 98 and 148 µg/l, respectively. The most active ethanolic extract tested was that from the stem of *Annona glabra* which presented an LC<sub>50</sub> value of 27 µg/l (Mendonca et al. 2005). Hidayatulfathi et al. (2005) using the hexane fraction showed the highest larvicidal effect on *A. aegypti* 4th instar larvae with LC<sub>50</sub> value of 1.88 ppm and the LC<sub>90</sub> value of 10.76 ppm respectively. Mosquito larvicidal activity of crude carbon-tetra-chloride, methanol and petroleum ether extracts of *Solanum xanthocarpum* fruits was examined against *A. stephensi* and *C. quinquefasciatus*. Among the extracts tested, carbon-tetra-chloride extract was the most effective with LC<sub>50</sub> values of 5.11 ppm after 24 h and 1.27 ppm after 48 h of treatment against *A. stephensi*. In the case of *C. quinquefasciatus* the petroleum ether extract was observed as most toxic with LC<sub>50</sub> values of 62.62 ppm after 24 h and 59.45 ppm after 48 h of exposure period, respectively (Mohan et al. 2005).

Sivagnaname and Kalyanasundaram (2004) reported that the methanolic extracts of the leaves of *Atlantia monophylla* were evaluated for mosquitocidal activity against immature stages of three mosquito species, *C. quinquefasciatus*, *A. stephensi*, and *A. aegypti* in the laboratory. Larvae of *C. quinquefasciatus* and pupae of *A. stephensi* were found more susceptible, with LC<sub>50</sub> values of 0.14 mg/l and 0.05 mg/l, respectively. Insect growth regulating activity of this extract was more pronounced against *A. aegypti*, with EI<sub>50</sub> value 0.002 mg/l. Larvicidal efficacies of extracts of five species of Cucurbitaceous plants, *Momordica charantia*, *Trichosanthes anguina*, *Luffa acutangula*, *Benincasa cerifera* and *Citrullus vulgaris* were tested against the late third larval age group of *C. quinquefasciatus*. The larval mortality was observed after 24 h exposure. The LC<sub>50</sub> values of *M. charantia*, *T. anguina*, *L. acutangula*, *B. cerifera* and *C. vulgaris* were 465.85, 567.81, 839.81, 1189.30 and 1636.04 ppm, respectively (Prabakar and Jebanesan 2004). Larvicidal activity of methanol extracts of 22 Australian and 12 Mexican plants against early 4th-instar larvae of *A. aegypti* and *C. pipiens* pallens was examined. At 200 ppm, 100 % mortality in larvae of *A. aegypti* and *C. pipiens* pallens was obtained in extracts of *Kigelia pinnata* and *Ruta chalepensis*. The extract of *K. pinnata* gave 76.3 and 80.3 % mortalities in larvae of *A. aegypti* and *C. pipiens* pallens at 100 ppm but, at 50 ppm, 23.7 and 42.1 % mortality against larvae of *A. aegypti* and *C. pipiens* pallens, respectively. The extract of *R. chalepensis* gave 81.2 and 87.9 % mortality in larvae of *A. aegypti* and *C. pipiens* pallens at 100 ppm but 23.4 and 53.3 % mortality against larvae of *A. aegypti* and *C. pipiens* pallens at 50 ppm, respectively. Larvicidal activities of *K. pinnata* and *R. chalepensis* extracts were significantly reduced when used at 25 ppm (Kim et al. 2002).

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 *C. pipiens*. The essential oils of *I. verum* and *P. anisum* demonstrated high larvicidal activity with a LC<sub>50</sub> <18 mgL<sup>-1</sup>. The other two essential oils of *D. caryophyllus* and *L. sativum* revealed

moderate larvicidal activity, displaying a  $LC_{50}$  value above  $50 \text{ mgL}^{-1}$ . Among the pure components, the most toxic were eugenol, (E)-anethole, and  $\alpha$ -terpinyl acetate, with  $LC_{50}$  values 18.28, 16.56, and  $23.03 \text{ mgL}^{-1}$ , respectively. Eucalyptol (1,8 cineole) and  $\beta$ -caryophyllene were inactive at concentrations even as high as  $100 \text{ mgL}^{-1}$ , 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 *C. quinquefasciatus* and *A. 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). Sagnou et al. (2012) reported that the larvicidal activity of isolated curcuminoid compound curcumin from commercially available turmeric extract exhibited  $LC_{50}$  and  $LC_{90}$  values of 19.07 and 61.63 ppm, respectively, against the fourth instar larvae of *C. pipiens*.

Two coumarins compounds, imperatorin and osthole, from the fruit of *Cnidium monnieri* were effective against the third instar larvae of insecticide-susceptible *C. pipiens pallens* and *A. aegypti* and wild *C. pipiens pallens* with  $LC_{50}$  values of 3.14, 2.88, 4.60 ppm and 13.11, 13.14, 15.26 ppm, respectively (Wang et al. 2012); Two natural furocoumarins, 5-methoxypsoralen, and 8-methoxypsoralen, isolated from the milky sap of *Ficus carica* exhibited  $LC_{50}$  values of 9.4 and 56.3 ppm, respectively, against the early fourth stage larvae of *A. aegypti* (Chung et al. 2011). A new  $C_{15}$  acetogenin isolated from the petroleum ether extract of *Laurencia papillosa* exhibited  $LC_{50}$  values of 30.7, 36.9, and 41.8 ppm on the second, third, and fourth instar larvae of *C. pipiens*, respectively (Abou-Elnaga et al. 2011). A sesquiterpene compound,  $1\alpha$ ,  $3\alpha$ ,  $4\beta$ -trihydroxy-9-cadinen-8-one, isolated from the chloroform extract of the roots of black galingale (*Kaempferia parviflora*) exhibited  $LC_{50}$  and  $LC_{90}$  values of 0.7 and  $3.8 \mu\text{M}$ , respectively, against the early fourth stage larvae of *A. aegypti* (Moon et al. 2011). Three alkaloids, namely evodiamine, rutaecarpine, wuchuyamide I, and two limonoids comprising evodol and limonin, derived from the chloroform extract of *Evodia rutaecarpa* unripe fruits showed  $LC_{50}$  values of 12.51, 17.02, 26.16, 52.22, and 32.43 ppm against the early fourth instar larvae of *A. albopictus* (Liu et al. 2012).

Isolated compounds such as prenylated flavonoids derived from *Dodonaea viscosa* showed larvicidal activity against *A. albopictus* and *C. pipiens* (Niu et al. 2010). Jang et al. (2005) also found that b-thujaplicin from *Chamaecyparis obtusa* leaves was effective against fourth-instar larvae of *A. aegypti*, *Ochlerotatus togoi*, and *C. pipiens* with  $LC_{50}$  values of 2.91, 2.60, and 1.33 ppm, respectively. Cheng et al. (2004) reported that the cinnamaldehyde, cinnamyl acetate, and eugenol all had excellent larvicidal effect against *A. aegypti* larvae in 24 h with  $LC_{50}$  values of 29, 33, and 33  $\mu\text{g/ml}$ , respectively. Neotenone and neorautanone isoflavonoids isolated from *Neorautanenia mitis* display activity against adult *A. gambiae* mosquitoes with  $LD_{50}$  values of 80 and 90 ppm, respectively (Joseph et al. 2004).

The petroleum ether extract of dried ground whole fruits of *Piper nigrum* afforded 20 compounds (1–20) including two new insecticidal amides named as pipnoohine (1), and pipyahyine (2), seven reported for the first time from this plant (12, 13, 15–17, 19, 20), and eleven known compounds (3–11, 14, 18). The structure of 1 has been elucidated as (2E,4E,12Z)-N-(4-methylpentyl)octadeca-2,4,12-



trienamide and that of 2 as (2E,4E,11E)-12-(benzo[1,3]dioxol-5-yl)-N-(3-methylbutyl)dodeca-2,4,11-trien-amide through extensive ID-, 2D-NMR spectral studies and chemical reactions. 1 and 2 exhibited toxicity at 35.0 and 30.0 ppm respectively against fourth instar larvae of *A. aegypti* (Siddiqui et al. 2004). Ho et al. (2003) reported that the isolated compounds such as meliternatin (3, 5-dimethoxy-3', 4', 6, 7- bismethylendioxyflavone) (6) and six other minor polyoxygenated flavones derived from *Melicope subunifoliolata* showed larvicidal activity against *A. aegypti*. They reported that methanol and ethanol flower extracts exhibited 96 and 100 % larval mortality against the third instar; 88 and 100 % against the fourth instar of *A. aegypti*, respectively, at 0.75 and 1.00-mg/ml concentrations. Yang et al. (2003) reported a similar result, that emodin had strong larvicidal effects against the larvae of *A. aegypti*, *O. togoi* and *C. pipiens* pallens, showing LC<sub>50</sub> values of approximately 1.4, 1.9, and 2.2 mg/l, respectively. Redwane et al. (2002) reported that gal- lotannins isolated from *Quercus lusitania* infectoria galls had the LC<sub>50</sub> value of 373 ppm against *C. pipiens*. The active components dymalol, nymania-3 and triterpenes isolated from the extract of *Dysoxylum malabaricum* act as an oviposition repellent and/or deterrent to *A. stephensi* (Govindachari et al. 1999). The mosquitocidal compound ar-turmerone which isolated from rhizomes of *Curcuma longa* seems 100 % mortality of *A. aegypti* larvae at 50 mg/l concentration (Roth et al. 1998). Seven mosquitocidal compounds isolated from *Magnolia salicifolia* show 100 % mortality at the concentration of 15–100 mg/l (Kelm et al. 1997). They further reported that the higher activity was due to the presence of flavonoid (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one). Pereira and Gurudutt (1990) who observed that (–)-3-epicaryoptin isolated from *C. inerme* inhibited the growth of *C. quinquefasciatus*.

Ag NPs also induced chromosomal aberrations and aneuploidy, which showed that silver nanoparticles were cytotoxic and genotoxic to fish cells (Wise et al. 2010). Earthworms (*Eisenia fetida*) were exposed to AgNO<sub>3</sub>, (94.21 mg kg<sup>-1</sup>) and Ag NPs (727.6 mg kg<sup>-1</sup>) with similar size ranges coated with either polyvinylpyrrolidone (hydrophilic) or oleic acid (amphiphilic) 773.3 mg kg<sup>-1</sup> during a standard sub-chronic reproduction toxicity test (Shoults-Wilson et al. 2010). The use of nanoparticulate silver, copper, and their oxides will be considered in relation to their effects on bacterial populations. Silver nanoparticles formed exhibited good antibiotic activity against both Gram-positive and Gram-negative pathogens and *Candida albicans*, suggesting their broad-spectrum antimicrobial activity (Kumar et al. 2010). The toxicity of Ag NPs to natural aquatic bacterial assemblages appears to be concentration dependent for concentrations between 0 and 5 μM (Dasari and Hwang 2010). Mechanisms of toxicity are still poorly understood although it seems clear that in some cases, nanoscale-specific properties may cause bio-uptake and toxicity over and above that caused by the dissolved Ag ion (Fabrega et al. 2011). Larvicidal studies were carried out against *C. quinquefasciatus*, and results were compared with bulk permethrin. The LC<sub>50</sub> of nanopermethrin and bulk permethrin to *C. 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 repellent activity compared to

the nanoemulsion with small droplet size. The synthesized zinc oxide nanoparticles showed the  $LC_{50}$  and  $\chi^2$  values against *R. microplus* (13.41 mg/l; 0.982), *Pediculus humanus capitis* (11.80; 0.966 mg/l), and the larvae of *A. subpictus* (3.19; 0.945 mg/l) and *C. quinquefasciatus* (4.87; 0.970 mg/l), respectively (Kirthi et al. 2011). The highest mortality was found in methanol, aqueous, and synthesized AgNPs, which used *N. nucifera* plant extract against the larvae of *A. subpictus* ( $LC_{50}$ =8.89, 11.82, and 0.69 ppm;  $LC_{90}$ =28.65, 36.06, and 2.15 ppm) and against the larvae of *C. quinquefasciatus* ( $LC_{50}$ =9.51, 13.65, and 1.10 ppm;  $LC_{90}$ =28.13, 35.83, and 3.59 ppm) (Santhoshkumar et al. 2011). Larvicidal activity of synthesized Ag NPs utilizing an aqueous extract from *Eclipta prostrata*, was observed in crude aqueous, and synthesized Ag NPs against *C. quinquefasciatus* ( $LC_{50}$ =27.49 and 4.56 mg/l;  $LC_{90}$ =70.38 and 13.14 mg/l) and against *A. subpictus* ( $LC_{50}$ =27.85 and 5.14 mg/l;  $LC_{90}$ =71.45 and 25.68 mg/l), respectively (Rajakumar and Abdul Rahuman 2011). The synthesized Ag NPs of *Musa paradisiaca* showed the  $LC_{50}$  values against *H. bispinosa* (1.87 mg/l), *H. maculata* (2.02 mg/l), and larvae of *A. stephensi* (1.39 mg/l), against *C. tritaeniorhynchus* (1.63 mg/l). Synthesized Ag NPs using *T. cordifolia* extract tested against the larvae of *A. subpictus* ( $LC_{50}$ =6.43 mg/l) and against the larvae of *C. quinquefasciatus* ( $LC_{50}$ =6.96 mg/l) (Jayaseelan et al. 2011).

Microbes and plants are currently used for nanoparticle synthesis. The use of plants for the fabrication of nanoparticles is a rapid, low-cost, eco-friendly, and a single-step method for biosynthesis process (Huang et al. 2007). The usage of plants can also be suitably scaled-up for large-scale synthesis of nanoparticles in a controlled manner according to their size, shape, and dispersity. Moreover, the use of plants in the process of nanoparticle synthesis is more beneficial than other processes since the nanoparticles are produced extracellularly. Recently, syntheses of silver nanoparticles by using plant extracts are getting more popular (Li et al. 2007; Song and Kim 2009). Chandran et al. (2006) synthesized silver nanoparticles by using the *Aloe vera* extract at 24 h of incubation. Potential antiplasmodial activity of synthesized silver nanoparticle using *Andrographis paniculata* with the inhibitory concentration ( $IC_{50}$ ) values were  $26 \pm 0.2$  % at 25  $\mu$ g/ml,  $83 \pm 0.5$  % at 100  $\mu$ g/ml (Panneerselvam et al. 2011). Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* and their antiplasmodial activities against *P. falciparum* have been reported by Ponarulselvam et al. (2012). The particle shape of plant-mediated Ag NPs was mostly spherical with the 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 Ag NPs 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-Oglycosides 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 *B. thuringiensis* subsp. *varisraelensis* (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). Levels of

effect of larvicidal activity varied with plant extract depending on the species. In contrast, larvicidal action of aqueous leaf extract of *Cassia obtusifolia* (Rajkumar and Jebanesan 2009) and *Ocimum canum*, *Ocimum sanctum*, and *R. nasutus* (Kamaraj et al. 2008) exhibited their lethal effect against larvae of *A. stephensi* and *A. aegypti*. An effective larval control of neem seed extract against *A. gambiae* was reported by Gianotti et al. (2008). The ethanolic aerial and root extract of *Phyllanthus amarus* showed high insecticidal activity against stored grain pest *Tribolium castaneus* (Khanna et al. 2003). Anti-ecdysteroid and growth inhibition effect of biomolecule Azadirachtin was documented (Zebit 1984), and the isolated compounds, fatty acids, and ricinine from leaf extracts of *R. communis* showed excellent insecticidal activity against the leaf-cutting ant *Acta sexdens rubropilosa* (Bigi et al. 2010). FT-IR studies and XRD analysis showed the presence of bioorganic components which acted as a probable stabilizer for the synthesized Ag NPs (Prathna et al. 2011). FT-IR peaks that were corresponding to aromatic rings, geminal methyls, and ether linkages indicate the presence of flavones and terpenoids responsible for the stabilization of the Ag NPs synthesized by the *Sesuvium portulacastrum* leaf extract (Nabikhan et al. 2010). Kumar et al. (2010) have synthesized Ag NPs using *Syzygium cumini* leaf and seed extract as reducing and stabilizing agent. *B. thuringiensis* subsp. var *israelensis* known for its crystal toxin being used as commercial larvicide is known to control filarial vector *C. quinquefasciatus*, *C. tritaeniorhynchus*, *C. sitiens*, malarial vector *A. stephensi*, and dengue vector *A. aegypti*. The LC<sub>50</sub> values for culicines (57.8–300.96 ng/mL), anophelines (740.47–790.61 ng/mL), and *A. aegypti* (514.34–600.03 ng/mL) have been reported (Manonmani and Balaraman 2001).

The maximum efficacy was observed in crude aqueous and synthesized Ag NPs against *C. quinquefasciatus* (LC<sub>50</sub> 27.49 and 4.56 mg L<sup>-1</sup>; LC<sub>90</sub> 70.38 and 13.14 mg L<sup>-1</sup>) and against *A. subpictus* (LC<sub>50</sub> 27.85 and 5.14 mg L<sup>-1</sup>; LC<sub>90</sub> 71.45 and 25.68 mg L<sup>-1</sup>), respectively. A biological method has been used to synthesize stable silver nanoparticles that were tested as mosquito larvicides against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* (Arjunan et al. 2012). The median LC<sub>50</sub> of silver nanoparticles that killed fourth instars of *A. aegypti*, *C. quinquefasciatus*, and *A. stephensi* were 0.30, 0.41, and 2.12 ppm, respectively. The higher mortality rates at lower doses are comparable with earlier reports of Ag NPs produced by plant *N. nucifera* leaf extracts (LC<sub>50</sub>=0.69 ppm, LC<sub>90</sub>=2.15 ppm) against *A. subpictus* and *C. quinquefasciatus* (LC<sub>50</sub>=1.10 ppm, LC<sub>90</sub>=3.59 ppm) (Thirunavukkarasu et al. 2010). The ethyl acetate extract of *E. prostrata* showed an LC<sub>50</sub> value of 78.28 and LC<sub>90</sub> value of 360.75 ppm against *A. subpictus* and LC<sub>50</sub> 119.89 and LC<sub>90</sub> 564.85 ppm against *C. tritaeniorhynchus*. *Eclipta paniculata* were the most active with a LC<sub>90</sub> of 17.2 mg L<sup>-1</sup> and LC<sub>50</sub> of 3.3 mg L<sup>-1</sup> against the larvae of *Aedes fluviatilis* (Macedo et al. 1997).

Ag NPs synthesized using *Euphorbia hirta* plant leaf extract against malarial vector *A. stephensi* was determined; the highest larval mortality was found in synthesized Ag NPs against the first to fourth instar larvae and pupae with the following values: LC<sub>50</sub> (10.14, 16.82, 21.51, and 27.89 ppm, respectively), LC<sub>90</sub> (31.98, 50.38, 60.09, and 69.94 ppm, respectively), and LC<sub>50</sub> and LC<sub>90</sub> of pupae (34.52 and 79.76 ppm, respectively) (Priyadarshini et al. 2012). The mortality effect of silver

nanoparticles on mosquito larvae may be enabled by the small size of the particles, which allows passage through the insect cuticle and into individual cells where they interfere with molting and other physiological processes. The mosquito larvicidal activity of UV irradiation-induced Ag NPs were found to decrease the survival of fourth instar larvae of *A. aegypti* by 88 % after 24 h of exposure at 1-ppm concentration (Sap-Iam et al. 2010). Silver NPs were synthesized using leaf extract of *Acalypha indica*; from the SEM image, the size of the control silver nitrate obtained was greater than 1,000 nm, whereas synthesized silver NPs measured 20–30 nm in size (Krishnaraj et al. 2010). The silver nanoparticles formed were predominantly cubical with uniform shape. It is known that the shape of metal nanoparticles considerably changes their optical and electronic properties (Xu and Käll 2002). The synthesized Ag NPs using *Aloe vera* only after 24 h of reaction and in the presence of ammonia, which in the study mentioned enhances the formation of a soluble silver complex. The sharpening of the peaks clearly indicates that the particles were in the nanoregime (Chandran et al. 2006). The filter paper contact bioassay study showed pronounced pediculicidal activity in the flower bud hexane extract of *Syzygium aromaticum* and the percent mortality were 40, 82, and 100 at 5, 10, and 20 min, and the median lethal time ( $LT_{50}$ ) value was 5.83 ( $0.5 \text{ mg cm}^{-2}$ ); 28, 82, and 100 at 5, 10, and 30 min ( $LT_{50}=6.54; 0.25 \text{ mg cm}^{-2}$ ); and 13, 22, 42, 80, and 100 at 5, 10, 20, 40, and 80 min ( $LT_{50}=18.68; 0.125 \text{ mg cm}^{-2}$ ), respectively against *P. humanus capitis* (Bagavan et al. 2011). In conclusion, green synthesis shows that the environmentally benign and renewable source of *S. acuta*, *H. Indicum*, *F. elephantum* 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 *A. subpictus*, *A. albopictus* and *C. tritaeniorhynchus*.

## 7.5 Conclusion

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 screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive and imported products, and stimulate local efforts to enhance the public health system. 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. Nanotechnology has the potential to revolutionize the existing technologies used in various sectors including agriculture. Nanotechnology may have concrete solutions against many agriculture-related problems like insect pest management using traditional methods, adverse effects of chemical pesticides, development of improved crop varieties, etc. nanomaterials in different forms can be used for efficient management of insect pests and formulations of potential insecticides and pesticides. Nanoparticle-mediated gene transfer would be useful for the development of new insect resistant varieties. Therefore, it can also conclude that nanotechnology can provide green and eco-friendly alternatives for insect pest management without harming the nature.

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