Parasitol Res (2015) 114:1551–1562
DOI 10.1007/s00436-015-4339-9

ORIGINAL PAPER

Tackling the growing threat of dengue: *Phyllanthus niruri*-mediated synthesis of silver nanoparticles and their mosquitocidal properties against the dengue vector *Aedes aegypti* (Diptera: Culicidae)

Udaiyan Suresh · Kadarkarai Murugan · Giovanni Benelli · Marcello Nicoletti · Donald R. Barnard · Chellasamy Panneerselvam · Palanisamy Mahesh Kumar · Jayapal Subramaniam · Devakumar Dinesh · Balamurugan Chandramohan

Received: 21 January 2015 / Accepted: 22 January 2015 / Published online: 12 February 2015

Abstract Mosquitoes are vectors of devastating pathogens and parasites, causing millions of deaths every year. Dengue is a mosquito-borne viral infection found in tropical and subtropical regions around the world. Recently, transmission has strongly increased in urban and semiurban areas, becoming a major international public health concern. *Aedes aegypti* (Diptera: Culicidae) is the primary vector of dengue. The use of synthetic insecticides to control *Aedes* mosquitoes lead to high operational costs and adverse nontarget effects. In this scenario, eco-friendly control tools are a priority. We proposed a novel method to synthesize silver nanoparticles using the aqueous leaf extract of *Phyllanthus niruri*, a cheap and nontoxic material. The UV–vis spectrum of the aqueous medium containing silver nanostructures showed a peak at 420 nm corresponding to the surface plasmon resonance band of nanoparticles. SEM analyses of the synthesized nanoparticles showed a mean size of 30–60 nm. EDX spectrum showed the chemical composition of the synthesized nanoparticles. XRD highlighted that the nanoparticles are crystalline in nature with face-centered cubic geometry. Fourier transform infrared spectroscopy (FTIR) of nanoparticles exhibited prominent peaks 3,327.63, 2,125.87, 1,637.89, 644.35, 597.41, and 554.63 cm^{-1}. In laboratory assays, the aqueous extract of *P. niruri* was toxic against larval instars (I–IV) and pupae of *A. aegypti*. LC_{50} was 158.24 ppm (I), 183.20 ppm (II), 210.53 ppm (III), 210.53 ppm (IV), and 358.08 ppm (pupae). *P. niruri*-synthesized nanoparticles were highly effective against *A. aegypti*, with LC_{50} of 3.90 ppm (I), 5.01 ppm (II), 6.2 ppm (III), 8.9 ppm (IV), and 13.04 ppm (pupae). In the field, the application of silver nanoparticles ($10 \times LC_{50}$) lead to *A. aegypti* larval reduction of 47.6 %, 76.7 % and 100 %, after 24, 48, and 72 h, while the *P. niruri* extract lead to 39.9 %, 69.2 % and 100 % of reduction, respectively. In adulticidal experiments, *P. niruri* extract and nanoparticles showed LC_{50} and LC_{90} of 174.14 and 6.68 ppm and 422.29 and 23.58 ppm, respectively. Overall, this study highlights that the possibility

Electronic supplementary material The online version of this article (doi:10.1007/s00436-015-4339-9) contains supplementary material, which is available to authorized users.

U. Suresh · K. Murugan · C. Panneerselvam · P. M. Kumar · J. Subramaniam · D. Dinesh · B. Chandramohan
Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore 641046, Tamil Nadu, India

K. Murugan
e-mail: kmvvkg@gmail.com

G. Benelli (✉)
Insect Behavior Group, Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy
e-mail: g.benelli@sssup.it

G. Benelli
e-mail: benelli.giovanni@gmail.com

M. Nicoletti
Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

D. R. Barnard
Center for Medical, Agricultural, and Veterinary Entomology, USDA-ARS, 1600 SW 23rd Drive, Gainesville, FL 32608, USA

to employ *P. niruri* leaf extract and green-synthesized silver nanoparticles in mosquito control programs is concrete, since both are effective at lower doses if compared to synthetic products currently marketed, thus they could be an advantageous alternative to build newer and safer tools against dengue vectors.

Keywords Fourier transform infrared spectroscopy · Green synthesis · Mosquitocidal nanoparticles · Mosquito-borne diseases · Nanobiotechnologies · Phyllanthaceae · UV–vis spectroscopy · X-ray diffraction

Introduction

Mosquitoes are vectors of devastating pathogens and parasites, causing millions of deaths every year. Dengue is a mosquito-borne viral infection found in tropical and subtropical regions around the world (WHO 2012). Recently, dengue transmission has strongly increased in urban and semiurban areas, becoming a major international public health concern. In recent years, there has been increasing incidence of many vector-borne pathogens in neglected geographical regions. Over 2.5 billion people are now at risk from dengue. The World Health Organization estimates that there may be 50–100 millions of dengue infections worldwide every year. The mosquito *Aedes aegypti* (Diptera: Culicidae) is the primary vector of dengue (WHO 2012). The use of synthetic insecticides to control *Aedes* mosquitoes lead to high operational costs and adverse nontarget effects. In this scenario, eco-friendly control tools against mosquitoes are a priority (Murugan et al. 2003; Amer and Mehlhorn 2006a, b; Govindarajan and Sivakumar 2012; Benelli et al. 2013a, b, 2015a, b, c).

Nanotechnology research opens newer avenues for a wide array of applications in the fields of biomedical, sensors, antimicrobials, catalysts, electronics, optical fibers, agricultural and bio labeling (Salam et al. 2012). Recently, it has been pointed out that the plant-mediated biosynthesis of nanoparticles is advantageous over chemical and physical methods because it is cheap and environment-friendly, does not require high pressure, energy, temperature, or the use of highly toxic chemicals (Goodsell 2004). Several plants have been successfully used for efficient and rapid extracellular synthesis of silver, copper, and gold nanoparticles (Veerakumar et al. 2014a; Dinesh et al. 2015). Good examples include neem, *Azadirachta indica* (Shankar et al. 2004; Poopathi et al. 2014), *Feronia elephantum* (Veerakumar et al. 2014b), *Catharanthus roseus* (Ponarulselvam et al. 2012) and *Pongamia pinnata* (Rajesh et al. 2010). Nanoparticles possess peculiar toxicity mechanisms due to surface modification (Oberdorster et al. 2005). Silver nanoparticles have antibacterial, antifungal, antiplasmodial and mosquitocidal properties (Saxena et al. 2010; Elumalai et al. 2010). Silver and gold nanoparticles synthesized using *Chrysosporium tropicum* have been proved as larvicides against the *A. aegypti* (Soni and Prakash 2012; but see also Salunkhe et al. 2011). Recently, the adulticidal activity of silver nanoparticles synthesized using *Feronia elephantum* plant leaf extract was proved against *Anopheles stephensi*, *A. aegypti*, and *Culex quinquefasciatus* (Veerakumar et al. 2014a). The larvicidal activity of silver nanoparticles synthesized using *Pergularia daemia* latex has been screened against the *A. aegypti*, *A. stephensi*, and the nontarget fish *Poecillia reticulata* (Patil et al. 2012). Furthermore, silver nanoparticles synthesized using marine fluorescent pseudomonads were reported as toxic against human pathogenic bacteria and fungal pathogens of plants (Vellasamy et al. 2014).

The *Phyllanthus* genus (Phyllantaceae) contains over 600 species distributed throughout the tropical and subtropical regions of the world. The aerial parts of the herb *Phyllanthus niruri* have been widely used in Asian traditional medicine for the treatment of a number of diseases and disorders, such as jaundice, constipation, diarrhea, kidney ailments, ringworm, ulcers, malaria, genito-urinary infections, hemorrhoids and gonorrhea (Unander et al. 1991). Furthermore, the *P. niruri* plant extracts possess antiviral property against hepatitis B virus (HBV; Thyagarajan et al. 1988; Yeh et al. 1993). The mentioned properties seem related to the content of a great number of biologically active compounds (Rizk 1987) including alkaloids, astragalin, brevifolin, carboxylic acids, corilagin, cymene, ellagicacid, ellagitannins, gallocatechins, geraniin, hypophyllanthin, phyllanthin, lignans, lintetralins, lupeols, methyl salicylate, phyllanthine, phyllanthenol, phyllochrysine, phyltetralin, repandusinic acids, quercetin, quercetol, quercitrin, rutin, saponins, triacontanal and tricontanol (Khanna et al. 2002).

In this research, we reported a novel method to synthesize silver nanoparticles using the aqueous leaf extract of *P. niruri*, a cheap, nontoxic and eco-friendly material, that worked as reducing and stabilizing agent during the biosynthesis. Silver nanoparticles were characterized by UV–vis spectrum, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and energy-dispersive X-ray analysis (EDX). The aqueous extract of *P. niruri* and silver nanoparticles were tested in laboratory and field conditions against larvae of the dengue vector *A. aegypti*. Adulticidal assays were conducted in laboratory conditions. We also evaluated the smoke toxicity of herbal coils prepared using different plant parts of *P. niruri* against *A. aegypti*.

Materials and methods

Plant materials

Plants of *P. niruri* were collected from garden of the Bharathiar University (Coimbatore, India). Plants were identified by an expert taxonomist at the Department of Botany (Bharathiar University, Coimbatore). Voucher specimens were stored in our laboratory.

A. aegypti rearing

Eggs of *A. aegypti* were provided by the National Centre for Disease Control (NCDC) field station of Mettupalayam (Tamil Nadu, India). Eggs were transferred to laboratory conditions [27±2 °C, 75–85 % R.H., 14:10 (L:D) photoperiod] and placed in 18×13×4 cm plastic containers containing 500 ml of tap water, waiting for hatching. Larvae were fed daily with a mixture of dog biscuits and hydrolyzed yeast (3:1 ratio). Pupae were collected and transferred to plastic containers with 500 ml of water. Each container was placed inside a cubic chiffon cage (90×90×90 cm) to wait for adult emergence. Adults were fed ad libitum on 10 % (v:v) sucrose solution. Five days after emergence, mosquitoes were allowed to feed on a rabbit host. The shaved dorsal side of the rabbit was positioned on the top of the mosquito cage in contact with the cage screen overnight. Petri dishes (diameter 60 mm) lined with filter paper and containing 50 ml of water were placed inside each cage, allowing oviposition by *A. aegypti* females.

P. niruri-mediated synthesis of silver nanoparticles

The *P. niruri* aqueous leaf extract was prepared adding 10 g of washed and finely cutted leaves in a 300-ml Erlenmeyer flask filled with 100 ml of sterilized double distilled water and then boiling the mixture for 5 min, before finally decanting it. The extract was filtered using Whatman filter paper no. 1, stored at −4 °C and tested within 5 days. The filtrate was treated with aqueous 1 mM $AgNO_3$ solution in an Erlenmeyer flask and incubated at room temperature (Dinesh et al. 2015). A brown yellow solution indicated the formation of silver nanoparticles, since aqueous silver ions were reduced by the plant extract generating stable silver nanoparticles in water. Silver nitrate was purchased from the Precision Scientific (Coimbatore, India).

Characterization of green-synthesized silver nanoparticles

Synthesis of silver nanoparticles was confirmed by sampling the reaction mixture at regular intervals, and the absorption maxima was scanned by UV–vis spectra, at the wavelength of 200–700 nm in UV-3600 Shimadzu spectrophotometer at 1-nm resolution. The reaction mixture was subjected to centrifugation at 15,000 rpm for 20 min, resulting pellet was dissolved in distilled water and filtered through Millipore filter (0.45 μm). An aliquot of this filtrate containing silver nanoparticles was used for scanning electron microscopy (SEM), energy-dispersive spectroscopy (EDS), Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) analyses, and energy-dispersive X-ray (EDX) spectroscopy (Dinesh et al. 2015).

The structure and composition of freeze-dried purified silver particles was analyzed by using a 10 kV ultra high resolution scanning electron microscope with 25 μl of sample sputter-coated on copper stub, and the images of nanoparticles were studied using a FEI QUANTA-200 SEM. The surface groups of the nanoparticles were qualitatively confirmed by FTIR spectroscopy (Stuart 2002), with spectra recorded by a Perkin–Elmer Spectrum 2000 FTIR spectrophotometer. EDX assays confirmed the presence of metals in analyzed samples.

Larvicidal and pupicidal toxicity in laboratory conditions

Following the methods reported in Dinesh et al. (2015), 25 *A. aegypti* larvae (I, II, III, or IV instar) or pupae were placed were placed for 24 h in a 500-mL glass beaker filled with 250-mL of dechlorinated water plus *P. niruri* extract (75, 150, 225, 300, and 375 ppm) or *P. niruri*-synthesized silver nanoparticles (2, 4, 8, 16 and 32 ppm). Larval food (0.5 mg) was provided for each tested concentration. Each concentration was replicated five times against all instars. In control treatments, 25 larvae or pupae were transferred in 250 ml of dechlorinated water. Percentage mortality was calculated as follows: percentage mortality = (number of dead individuals/number of treated individuals)×100.

Larvicidal assays in the field

P. niruri aqueous leaf extract and *P. niruri*-synthesized silver nanoparticles were applied in six external water storage reservoirs at the National Institute of Communicable Disease Centre (Coimbatore, India), using a knapsack sprayer (Private Limited 2008, Ignition Products, India; Dinesh et al. 2015). Pre-treatment larval density was monitored. Post-treatment observations were conducted at 24, 48, and 72 h using a larval dipper. Toxicity was assessed against third- and fourth-instar larvae. Larvae were counted and identified to specific level. More than 95 % of all surveyed larvae belong to *A. aegypti*. Six trials were conducted for each test site with similar weather conditions (28±2 °C; 80 % R.H.). The required quantity of mosquitocidal was calculated on the basis of the total surface area and volume (0.25 m^3 and 250 l); the required concentration was prepared using $10\times LC_{50}$ values (Murugan et al. 2003). Percentage reduction of the larval density was calculated using the formula: percentage reduction = $(C - T)/C\times 100$, where C is the total number of mosquitoes in

the control, and T is the total number of mosquitoes in the treatment (Dinesh et al. 2015).

Adulticidal toxicity

Adulticidal bioassay was performed by World Health Organization method (WHO 1981). The *P. niruri* aqueous extract was tested at 75, 150, 225, 300, and 375 ppm, and silver nanoparticles were tested at 2, 4, 8, 16, and 32 ppm. *P. niruri* aqueous crude extract and silver nanoparticles were applied on Whatman no. 1 filter paper (size 12× 15 cm) lining a glass holding tube (diameter 30 mm, length 60 mm). Control filter paper was treated with distilled water and silver nitrate, respectively. In each test, 20 *A. aegypti* females were gently transferred into another glass holding tube. The mosquitoes were allowed to acclimatize in the tube for 1 h and then exposed to test tube lined with treated or control paper for 1 h. At the end of exposure period, the mosquitoes were transferred back to the original holding tube and kept for a 24 h recovery period. A pad of cotton soaked with 10 % (w:v) glucose solution was placed on the mesh screen at the top of the holding tube.

P. niruri-based smoke toxicity assays

Leaves, stems, and roots of *P. niruri* were used to prepare herbal coils for smoke toxicity assays against *A. aegypti*. Coils were prepared following the method of Saini et al. (1986), using 4 g of powdered plant parts (leaves, stems or roots), 2 g of sawdust (binding material), and 2 g of coconut shell charcoal powder (burning material). The three materials were mixed with distilled water forming a semisolid paste. Mosquito coils (0.6 cm thickness) were prepared from the semisolid paste and then dried in the shade (Electronic Supplementary Material Figure Fig. S1). Negative control coils were prepared following the same method, without adding *P. niruri* plant parts. Positive control was a commercial pyrethrin-based coil.

Experiments were conducted in a glass chamber measuring 140×120×60 cm. A door measuring 60×30 cm was situated at the front of the chamber. In each test, 100 blood-starved adult female mosquitoes (age 3–4 days old) were released into the chamber and were provided with a 10 % (w:v) sucrose solution. An immobilized pigeon with shaven belly was tied inside the tightly closed chamber. The experiment was repeated five times on five separate days for each treatment (i.e., leaves-based coil; stem-based coil; root-based coil, positive and negative controls). All mosquitoes were exposed to the vapor of burning coils for 1 h. After each experiment, the number of fed and unfed (alive and dead) mosquitoes were counted. The protection provided by the smoke from the plant samples against biting *A. aegypti* was calculated in terms of percentage of unfed mosquitoes due to treatment:

[(Number of unfed mosquitoes in treatment−Number of unfed mosquito in negative control)/No. of mosquitoes treated]×100

Data analysis

SPSS software package 16.0 version was used for all analyses. Mosquito mortality data were analyzed by probit analysis, calculating LC_{50} and LC_{90} following the method by Finney (1971). All mortality data were transformed into arcsine√proportion values then analyzed using a two-way ANOVA with two factors (i.e., dosage and mosquito instar for larvicidal assays in laboratory; treatment and dosage for adulticidal assays). Means were separated using Duncan's multiple range test by Alder and Rossler (1977). $P<0.05$ was used for the significance of differences between means. Mosquito larval density data from field assays were analyzed using a two-way ANOVA with two factors (i.e., the treatment and the elapsed time from treatment). Means were separated using Duncan's multiple range test. $P<0.05$ was used for the significance of differences between means.

In herbal coil toxicity experiment, the number of fed, unfed, and dead mosquitoes were analyzed by one-way ANOVA, where the factor was the treatment (i.e., leaves-based coil, stem-based coil, root-based coil, negative control, and positive control) Means were separated using Duncan's multiple range test. $P<0.05$ was used for the significance of differences between means.

Results and discussion

UV–vis spectrum of silver nanoparticles

Green-synthesized silver nanoparticles were characterized by UV–vis spectroscopy. The silver nanoparticles exhibited yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Fig. 1a–b; Shankar et al. 2004; Dinesh et al. 2015). The observed surface plasmon peak confirmed the influence of *P. niruri* leaf extract in reducing Ag^{+} ions to silver nanoparticles. UV–vis spectroscopy (420 nm) evidenced that it steadily increased with reaction time and saturated at 120 min (Fig. 1c), indicating complete reduction of the silver nitrate. The absorption peak varied as the function of reaction time and concentration of silver nitrate. As the size of ultrafine particles decreases, the energy gap is widened, hence the

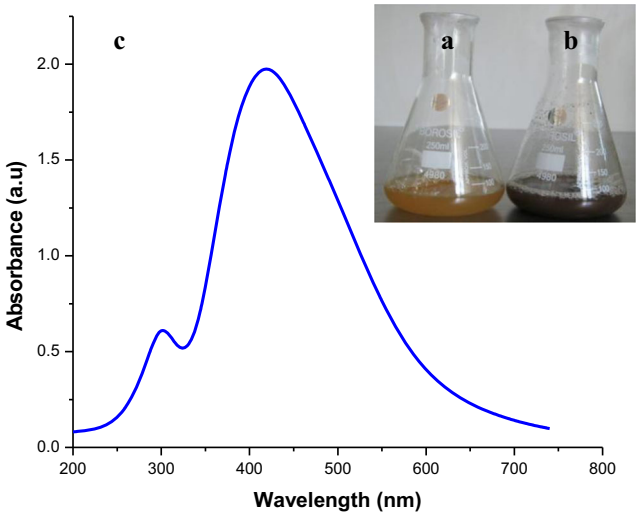

Fig. 1 Chromatic variations of the leaf extract of *Phyllanthus niruri* before (**a**) and after (**b**) the process of reduction of Ag^{+} to Ag nanoparticles. **(c)** UV visualization at 120 min (95 °C) of the absorption spectrum of silver nanoparticles synthesized using *P. niruri* leaf extract plus an aqueous solution $AgNO_3$ (1 mM)

absorption peaks shifted toward higher energy. Rai et al. (2006) reported an increase of reduction rate with an increased reaction temperature for gold nanotriangles synthesized using lemongrass extract. The strong resonance centered at 420 nm was clearly observed and increased in intensity with time. The presence of a broad resonance indicated aggregated structure of silver nanoparticles in the solution.

X-ray diffraction (XRD) studies

Nanoparticles in XRD patterns exhibited different size-dependent features leading to anomalous peak position height and width. XRD analysis was helpful to shed light on the crystalline nature of the silver nanoparticles. Bragg reflections corresponding to the (111), (200), (220), (311), and (222) sets of lattice planes were observed. The XRD pattern showed that the silver nanoparticles formed by the reduction of $AgNO_3$ by *C. ambrosioides* leaves extract were crystalline in nature (Fig. 2). Result showed that the Ag^{+} of silver nitrate had reduced to Ag^{0} by *P. niruri*. Sharp Bragg peaks may be due to the capping agent stabilizing of the nanoparticle. Our findings are in agreement with previous research conducted on silver nanoparticles synthesized using leaf extract of *Acalypha indica* (Krishnaraj et al. 2010). The XRD pattern of pure silver ions was known to display peaks at 2θ=7.9 °, 11.4 °, 17.8 °, 30.38 °, and 44 ° (Gong et al. 2007). Dubey et al. (2009) reported the size of silver nanocrystals as estimated from the full width at half maximum of the (111) peak of silver using the Scherrer formula was 20–60 nm. The XRD patterns of Ag/extract indicated that the structure of silver nanoparticles is face-centered cubic (Shameli et al. 2010). Overall, from the XRD pattern, it can be noted that silver nanoparticles synthesized using *P. niruri* leaf extract were essentially crystalline.

Fourier transformed infrared (FTIR) spectroscopy

FTIR spectroscopy was carried out to identify the possible biomolecules in *P. niruri*, which may be responsible for synthesis and stabilization of silver nanoparticles. Figure 3 shows that the FTIR spectra of aqueous silver nanoparticles prepared from the *P. niruri* leaf extract at transmittance peaks 3,327.63, 2,125.87, 1, 637.89, 644.35, 597.41, and 554.63 cm^{-1}. These compounds may be responsible for production of silver nanoparticles from leaves of *P. niruri*. The peaks indicate that the carbonyl group formed amino acid residues and that these residues "capped" the silver nanoparticles to prevent agglomeration and thereby stabilized the medium (Sathyavathi et al. 2010; see also Dinesh et al. 2015). The peaks at 1,027–1,092 cm^{-1} correspond to the C–N stretching vibration of aliphatic amines or to alcohols/phenols, representing the presence of polyphenols (Song et al. 2009). This suggests that the biological molecules could possibly perform dual functions of reduction and stabilization of silver nanoparticles in the aqueous medium, possibly by in situ oxidation of hydroxyl groups and by the intrinsic carbonyl groups, as well as those produced by oxidation with air.

Scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) analysis

Scanning electron micrographs enabled visualization of the size and shape of the silver nanoparticles (Fig. 4). SEM micrographs of the synthesized silver nanoparticles showed spherical shapes, mostly aggregated and having an average size of 30–60 nm. The shape of nanoparticles was mostly spherical (Dinesh et al. 2015) with exception of neem (*Az. indica*), which yielded polydisperse particles both with spherical and flat plate-like morphology 5–35 nm in size (Shankar et al. 2004). In agreement with our research, Ankanna et al. (2010) reported SEM micrographs of the silver nanoparticles indicating that they were well-dispersed and ranged in size 30–40 nm. Also silver nanoparticles produced using *Emblica officinalis* were predominantly spherical with an average size of 16.8 nm ranging from 7.5 to 25 nm (Ankamwar et al. 2005). Spot energy-dispersive X-ray spectroscopy (spot EDX) provides information on the composition at specific locations. Figure 5, which is a representative profile of the spot EDX analysis, showed a strong signal in the silver region confirming the formation of silver nanoparticles; a distinct

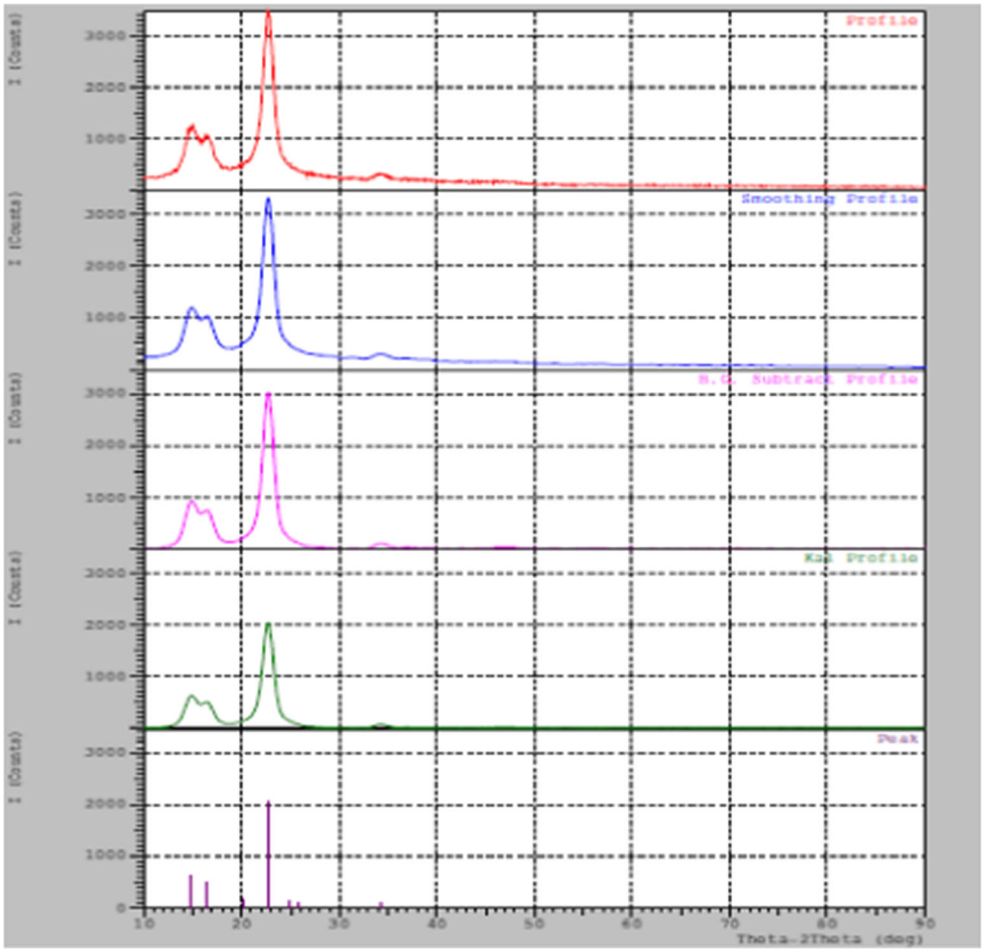

Fig. 2 X-ray diffraction (XRD) pattern of green-synthesized silver nanoparticles using the aqueous leaf extract of *Phyllanthus niruri*

signal and high atomic percent values for silver were obtained. Our results are in agreement also with an earlier report on silver nanoparticle synthesis using the fungus *Trichoderma viridae* (Fayaz et al. 2010).

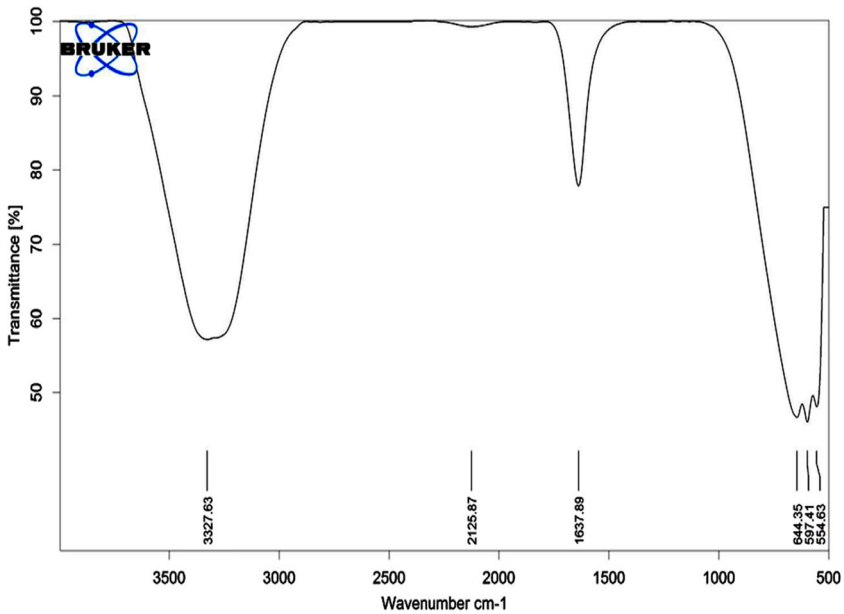

Fig. 3 Fourier transform infrared (FTIR) spectrum of vacuum-dried powder of synthesized silver nanoparticles using the extract of *Phyllanthus niruri* leaves

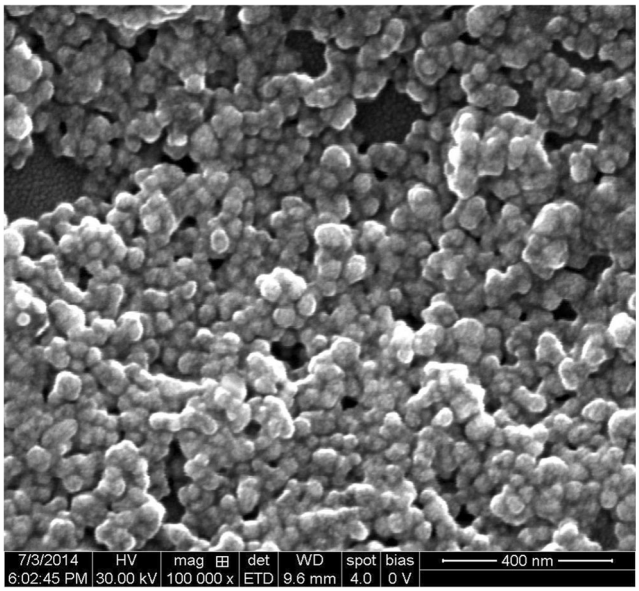

Fig. 4 Scanning electron microscopy (SEM) micrograph showing the morphological characteristics of silver nanoparticles synthesized using the *Phyllanthus niruri* leaf extract

Larvicidal and pupicidal toxicity against *A. aegypti* in laboratory

In laboratory assays, the aqueous extract of *P. niruri* was highly toxic against larval instars (I–IV) and pupae of *A. aegypti*, even at low doses. LC_{50} values were 158.24 ppm (I instar), 183.20 ppm (II), 210.53 ppm (III), 210.53 ppm (IV), and 358.08 ppm (pupae) (Table 1). Within each tested concentration, significant differences were found as a function of the targeted mosquito instar ($F_{4,120}$=746.76; P<0.01). Results of larvicidal activity also indicated that the percentage of mortality was proportional to the concentration of the *P. niruri* extract. This has been reported for a wide number of mosquitocidal botanical products (Amer and Mehlhorn 2006a; Benelli et al. 2015a, b, c; Dinesh et al. 2015). For instance, the toxicity of *Pedilanthus tithymaloides* aqueous leaf extract against young instars of *A. aegypti* was dose-dependent (Sundaravadivelan et al. 2013). Also Roni et al. (2013) reported that aqueous leaf extract of *Nerium oleander* exhibited dose-dependent larval toxicity against *A. stephensi*.

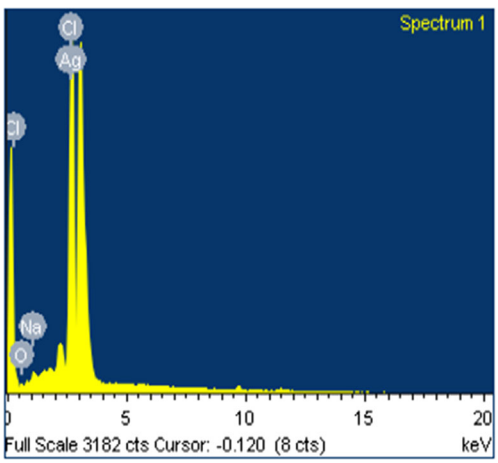

Fig. 5 Energy-dispersive X-ray (EDX) spectrum of green-synthesized silver nanoparticles using the leaf extract of *Phyllanthus niruri*

Green-synthesized nanoparticles were highly effective in laboratory experiments conducted against *A. aegypti* larvae, with LC_{50} of 3.90 ppm (I), 5.01 ppm (II), 6.2 ppm (III), 8.9 ppm (IV), and 13.04 ppm (pupae) (Table 2). Within each tested concentration, significant differences were found as a function of the targeted mosquito instar ($F_{4,120}$=771.22; P<0.01). Our results are in agreement with recent researches (see Dinesh et al. 2015 for a recent synthesis). For instance, Rajakumar and Rahuman (2011) studied the larvicidal activity of silver nanoparticles synthesized using an aqueous extract from *Eclipta prostrata* against *C. quinquefasciatus* (LC_{50}= 27.49 and 4.56 mg/l; LC_{90}=70.38 and 13.14 mg/l) and *Anopheles subpictus* (LC_{50}=27.85 and 5.14 mg/l; LC_{90}= 71.45 and 25.68 mg/l). Green-synthesized silver nanoparticles using *Euphorbia hirta* leaf extract were tested against the malarial vector *A. stephensi*, with LC_{50} values of 10.14 ppm (I instar), 16.82 ppm (II), 21.51 ppm (III), 27.89 ppm (IV), and 34.52 ppm (pupa), respectively (Priyadarshini et al. 2012). Recently, the larvicidal activity of silver nanoparticles synthesized using the aqueous bark extract of *Ficus racemosa* was tested against fourth instar larvae of the filariasis vector *C. quinquefasciatus* and the Japanese encephalitis vectors *Culex gelidus* (LC_{50}=12.00 and 11.21 mg/l, respectively; Velayutham et al. 2013).

Larvicidal and pupicidal toxicity against *A. aegypti* in the field

In the field, the application of silver nanoparticles ($10\times LC_{50}$) leads to *A. aegypti* larval reduction of 47.6 %, 76.7 %, and 100 %, while the *P. niruri* extract leads to 39.9 %, 69.2 %, and 100 % of larval reduction after 24, 48, and 72 h, respectively (Table 3). Mosquito larval density in water reservoirs was affected by the time elapsed from the treatment ($F_{3,732}$= 162.96; P<0.001). These results are in agreement with previous research by Murugan (2006) analyzing the field efficacy of biopesticides in tsunami-affected areas of India, which reported strong reduction, or even eradication, of larval populations of several mosquito vectors. More recently, the mosquitocidal efficacy of the leaf extract of *E. hirta* was investigated in a field condition against *A. stephensi*, and larval density was reduced by 13.17 %, 37.64 % and 84.00 % after 24, 48, and 72 h, respectively (Panneerselvam et al. 2013). Different mechanisms of action have been proposed to explain the efficacy of plant-borne molecules against mosquito larvae. The thin film of oily substances from plant extracts on the water surface cuts of oxygen

Table 1 Larval and pupal toxicity of *Phyllanthus niruri* aqueous leaf extract against the dengue vector *Aedes aegypti*

Targeted instar	Larval and pupal mortality (%)					LC_{50} (LC_{90})	95 % Confidence limit		Regression equation	χ^2
	Concentration (ppm)						LC_{50} (LC_{90})			
	75	150	225	300	375		*LCL*	*UCL*		
I	40.2±1.30[a]	48.2±0.83[a]	57.2±1.48[a]	67.4±1.14[a]	83.0±1.58[a]	158.24 (496.56)	116.14 (427.92)	189.44 (617.35)	*X*=+0.004, *Y*=−0.599	1.84 n.s.
II	36.2±1.71[b]	44.0±1.87[ab]	54.4±1.51[ab]	65.6±1.29[ab]	78.6±0.96[b]	183.20 (523.58)	146.23 (450.15)	213.40 (653.08)	*X*=+0.004, *Y*=−0–690	0.69n.s.
III	33.2±1.64[bc]	39.8±0.83[b]	52.0±1.58[bc]	61.6±1.14[bc]	72.8±1.52[c]	210.53 (575.40)	175.19 (487.86)	243.19 (736.70)	*X*=+0.004, *Y*=−0.739	0.30 n.s.
IV	30.4±1.08[cd]	37.8±1.44[bc]	48.4±2.07[c]	58.0±1.69[c]	66.0±1.27[d]	241.07 (645.46)	204.95 (535.10)	281.01 (846.29)	*X*=+0.003, *Y*=−0.764	0.06 n.s.
Pupa	28.2±1.92[d]	33.8±1.25[c]	37.4±1.94[d]	43.0±1.22[d]	49.2±1.52[e]	358.08 (944.57)	296.98 (706.08)	499.76 (1,632.21)	*X*=+0.001, *Y*=−0.782	1.51 n.s.

Mortality rates are means ± SD of five replicates. No mortality was observed in the control. Within each column means followed by the same letter(s) are not significantly different ($P<0.05$).

LC_{50} lethal concentration that kills 50 % of the exposed organisms, *LC_{90}* lethal concentration that kills 90 % of the exposed organisms, *LCL* lower confidence limit, *UCL* upper confidence limit, χ^2 Chi-square test, *NS* not significant

Table 2 Larval and pupal toxicity of silver nanoparticles synthesized using *Phyllanthus niruri* aqueous leaf extract against the dengue vector *Aedes aegypti*

Targeted instar	Larval and pupal mortality (%)					LC_{50} (LC_{90})	95 % Confidence limit		Regression equation	$\chi 2$
	Concentration (ppm)						LC_{50} (LC_{90})			
	2	4	8	16	32		*LCL*	*UCL*		
I	43.2±1.48[a]	50.2±1.44[a]	65.6±0.96[a]	83.0±1.58[a]	100±0.00[a]	3.985 (18.395)	2.093 (15.875)	5.483 (22.271)	*X*=0.089, *Y*=−0.354	1.37 n.s.
II	41.4±1.08[a]	47.8±1.03[ab]	61.2±1.35[ab]	73.4±2.07[b]	98.4±1.14[a]	5.015 (23.124)	2.854 (19.990)	6.792 (27.854)	*X*=0.071, *Y*=−0.355	2.20 n.s.
III	38.2±1.30[ab]	45.4±1.14[bc]	57.8±1.82[b]	69.2±1.64[c]	93.8±1.75[b]	6.216 (28.085)	3.775 (24.192)	8.720 (34.016)	*X*=0.059, *Y*=−0.364	1.04 n.s.
IV	33.2±1.71[bc]	41.2±1.35[cd]	53.6±1.15[bc]	63.6±1.51[d]	82.4±0.96[c]	8.999 (38.604)	6.023 (32.445)	11.646 (48.806)	*X*=0.043, *Y*=−0.390	2.47 n.s.
Pupa	30.0±1.36[c]	38.4±1.19[d]	47.8±1.44[d]	55.8±1.52[e]	72.6±0.74[d]	13.043 (50.226)	9.692 (40.791)	16.608 (67.827)	*X*=0.034, *Y*=−0.449	2.36 n.s.

Mortality rates are means ± SD of five replicates. No mortality was observed in the control. Within each column means followed by the same letter(s) are not significantly different ($P<0.05$).

LC_{50} lethal concentration that kills 50 % of the exposed organisms, *LC_{90}* lethal concentration that kills 90 % of the exposed organisms, *LCL* lower confidence limit, *UCL* upper confidence limit, χ^2 Chi-square test, *NS* not significant

Table 3 Field treatment of storage water tanks with aqueous leaf extract of *Phyllanthus niruri* and green-synthesized silver nanoparticles against the dengue vector *Aedes aegypti*

	Phyllanthus niruri extract ($10\times LD_{50}$)				Green-synthesized silver nanoparticles ($10\times LD_{50}$)			
	Before treatment	24 h	48 h	72 h	Before treatment	24 h	48 h	72 h
Larval density	122.67±16.82[a]	73.67±9.27[b]	37.67±7.81[c]	0±0[d]	112.17±21.68[a]	58.67±12.87[b]	26.50±9.09[c]	0±0[d]

Means ± SD followed by different letter(s) are significantly different ($P<0.05$)

supply to mosquito larvae. In addition, a number of plant-borne polar compounds dissolve into the water and penetrate the larvae through the respiratory tube, killing them by suffocation and/or by poisoning.

Adulticidal toxicity against *A. aegypti* in laboratory conditions

In adulticidal experiments conducted in the laboratory, *P. niruri* extract and nanoparticles showed LC_{50} and LC_{90} of 174.14 and 6.68 ppm and 422.29 and 23.58 ppm, respectively (Table 4). At the highest concentrations tested, the adults showed restless movements for some times with abnormal wagging and then died. Naresh Kumar et al. (2012) reported a reduction in adult longevity (4.2 in male and 11.7 in female at 10 ppm) in *A. stephensi* after treatment with silver nanoparticles produced using *Annona squamosa* extract. Furthermore, also a number of botanical products are able to exert high mortality rates against mosquito adults. For instance, the ethanol extract of *Citrus sinensis* showed LC_{50} and LC_{90} values 320.38 and 524.57 ppm against *A. aegypti* (Murugan et al. 2012), and this is often related to the content in limonoids, as elucidated by Nathan et al. (2005). Plant extracts may also have an inhibitory influence on neurosecretory cells and/or may negatively act on epidermal cells which are responsible for the production of enzymes routing the cuticular oxidation process (Murugan et al. 1996; Jeyabalan and Murugan 1999).

Smoke toxicity of *P. niruri*-based coils against *A. aegypti*

Table 5 summarizes the results of smoke toxicity experiments testing the efficacy of *P. niruri*-based coils against the biting activity of *A. aegypti*. After the treatment with the leaf-, stem-, and root-based coils, mean percentages of unfed mosquitoes were 58 %, 40 %, and 61 %, respectively. Among them, the plant root made coil showed the highest mortality. Mortality was slightly higher in the positive control. However, botanical-based coils can be valuable alternatives to permethrin-based ones. Murugan et al. (2007) studied the smoke toxicity of *Albizzia amara* and *Ocimum basilicum*,

Table 4 Adulticidal activity of aqueous leaf extract of *Phyllanthus niruri* and green-synthesized silver nanoparticles against dengue vector *Aedes aegypti*

Treatment	Dosage (ppm)	Mortality (%)	LC_{50} (*LCL-UCL*)	LC_{90} (*LCL-UCL*)	χ^2
Phyllanthus niruri leaf extract	Control	0.0±0.0[a]	174.14 (146.99–197.12)	422.29 (379.02–487.43)	0.64 n.s.
	75	32.67±1.55[b]			
	150	43.22±1.11[c]			
	225	58.35±0.22[d]			
	300	74.45±1.05[e]			
	375	86.14±1.89[f]			
Green-synthesized silver nanoparticles	Control	0.0±0.0[a]	6.68 (0.91–10.90)	23.58 (17.51–40.41)	7.20*
	2	29.14±1.22[b]			
	4	40.54±1.25[c]			
	8	62.20±1.47[d]			
	16	79.25±1.32[e]			
	32	95.12±1.36[g]			

Mortality rates are means ± SD of five replicates. No mortality was observed in the control. Within each column means followed by the same letter(s) are not significantly different ($P<0.05$). Chi-square value followed by an asterisk is significant (heterogeneity factor used in calculation of confidence limits) ($P<0.05$)

LC_{50} lethal concentration that kills 50 % of the exposed organisms, *LC_{90}* lethal concentration that kills 90 % of the exposed organisms, *LCL* lower confidence limit, *UCL* upper confidence limit, χ^2 Chi-square test, *NS* not significant

Table 5 Smoke toxicity assays conducted with different plant parts of *Phyllanthus niruri* against the biting activity of *Aedes aegypti*

Phyllanthus niruri plant part	Fed mosquitoes	Unfed mosquitoes		Total unfed
		Alive	Dead	
Leaf-based coil	17.20±1.58[a]	47.71±1.22[c]	36.50±1.87[c]	83.50±2.12[c]
Stem-based coil	35.41±1.22[b]	46.51±2.23[c]	19.03±2.91[b]	65.01±3.16[b]
Root-based coil	14.37±1.87[a]	36.06±1.00[b]	50.12±2.54[d]	86.04±2.12[c]
Negative control	76.43±3.08[c]	25.03±2.9[a]	0.00±0.00[a]	25.16±2.91[a]
Positive control	12.33±0.70[a]	44.08±3.3[c]	44.06±2.54[d]	88.09±2.91[c]

Values are means ± SD of five replicates. Negative control was a blank coil without plant material. Positive control was conducted using a pyrethrin-based commercial coil. Within a column means followed by the same letter(s) are not significantly different ($P<0.05$)

reporting that *A. amara* coils were more effective over *O. basilicum* ones against *A. aegypti*. Later on, Aarthi and Murugan (2010) highlighted the smoke-repellent properties of *Spathodea campanulata* against the malarial vector *A. stephensi*.

Conclusions

Overall, our results showed that the possibility to employ *P. niruri* leaf extract and green-synthesized silver nanoparticles in mosquito control programs is concrete, since both are effective against *A. aegypti* at lower doses if compared to synthetic products currently marketed. We believe that they could be an advantageous alternative to build newer and safer tools against dengue vectors.

Acknowledgments Kadarkarai Murugan is grateful to the Department of Science and Technology (New Delhi, India), Project No. DST/SB/EMEQ-335/2013, for providing financial support. Giovanni Benelli is supported by a Mis. 124 MODOLIVI Grant. Funds were also provided by the Italian Ministry of Education, University and Research (MIUR). Funds were also provided by the Italian Ministry of Education, University and Research (MIUR). Funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Aarthi N, Murugan K (2010) Larvicidal and smoke repellent activities of *Spathodea campanulata* P. Beauv. against the malarial vector *Anopheles stephensi* lis (Diptera: Culicidae). J Phytol 2:61–69

Alder HL, Rossler EB (1977) Introduction to probability and statistics, 6th edn. Freeman, San Francisco, p 246

Amer A, Mehlhorn H (2006a) Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera, Culicidae). Parasitol Res 99:466–472

Amer A, Mehlhorn H (2006b) Repellency effect of forty-one essential oils against Aedes, *Anopheles* and *Culex* mosquitoes. Parasitol Res 99:478–490

Ankamwar B, Chaudhary M, Sastry M (2005) Gold nanotriangles biologically synthesized using tamarind leaf extract and potential application in vapor sensing. Synth React Inorg Metal-Org Nano-Metal Chem 35:19–26

Ankanna S, Prasad TNVKV, Elumalai EK, Savithramma N (2010) Production of biogenic silver nanoparticles using *Boswellia ovalifoliolata* stem Bark. Dig J Nanomater Biostruct 5:369–372

Benelli G, Flamini G, Fiore G, Cioni PL, Conti B (2013a) Larvicidal and repellent activity of the essential oil of *Coriandrum sativum* L. (Apiaceae) fruits against the filariasis vector *Aedes albopictus* Skuse (Diptera: Culicidae). Parasitol Res 112:1155–1161

Benelli G, Canale A, Conti B (2013b) Eco-friendly control strategies against the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae): repellency and toxic activity of plant essential oils and extracts. Pharmacol Online 47:44–51

Benelli G, Bedini S, Cosci F, Toniolo C, Conti B, Nicoletti M (2015a) Larvicidal and ovideterrent properties of neem oil and fractions against the filariasis vector *Aedes albopictus* (Diptera: Culicidae): a bioactivity survey across production sites. Parasitol Res. doi:10.1007/s00436-014-4183-3

Benelli G, Bedini S, Flamini G, Cosci F, Cioni PL, Amira S, Benchikh F, Laouer H, Di Giuseppe G, Conti B (2015b) Mediterranean essential oils as effective weapons against the West Nile vector *Culex pipiens* and the *Echinostoma* intermediate host *Physella acuta*: what happens around? An acute toxicity survey on non-target mayflies. Parasitol Res. doi:10.1007/s00436-014-4267-0

Benelli G, Murugan K, Panneerselvam C, Madhiyazhagan P, Conti B, Nicoletti M (2015c) Old ingredients for a new recipe? Neem cake, a low-cost botanical by-product in the fight against mosquito-borne diseases. Parasitol Res. doi:10.1007/s00436-014-4286-x

Dinesh D, Murugan K, Madhiyazhagan P, Panneerselvam C, Nicoletti M, Jiang W, Benelli G, Chandramohan B, Suresh U (2015) Mosquitocidal and antibacterial activity of green-synthesized silver nanoparticles from *Aloe vera* extracts: towards an effective tool against the malaria vector *Anopheles stephensi*? Parasitol Res. doi:10.1007/s00436-015-4336-z

Dubey M, Bhadauria S, Kushwah BS (2009) Green synthesis of nanosilver particles from extract of *Eucalyptus Hybrida* (Safeda) leaf. Dig J Nanomater Biostruct 4:537–543

Elumalai EK, Prasad TNVKV, Hemachandran J, Therasa SV, Thirumalai T, David E (2010) Extracellular synthesis of silver nanoparticles using leaves of *Euphorbia hirta* and their antibacterial activities. J Pharmacol Sci Res 2:549–554

Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R (2010) Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against Gram-positive and Gram-negative bacteria. Nanomedicine Nanotechnol Biol Med 6:e103–e109

Finney DJ (1971) Probit analysis. Cambridge University, London, pp 68–78

Gong P, Li H, He X, Wang K, Hu J, Tan W, Zhang S, Yang X (2007) Preparation and antibacterial activity of Fe_3O_4@Ag nanoparticles. Nanotechnology 18:285604

Goodsell DS (2004) Bionanotechnology: lessons from nature. Wiley, Hoboken

Govindarajan M, Sivakumar R (2012) Adulticidal and repellent properties of indigenous plant extracts against Culex quinquefasciatus and Aedes aegypti (Diptera: Culicidae). Parasitol Res 110:1607–1620

Jeyabalan D, Murugan K (1999) Effect of certain plant extracts against the mosquito *Anopheles stephensi* Liston. Curr Sci 76:631–633

Khanna AK, Rizvi F, Chander R (2002) Lipid lowering activity of *Phyllanthus niruri* in hyperlipidemic rats. J Ethnopharmacol 82: 19–22

Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N (2010) Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B: Biointerfaces 76:50–56

Murugan K (2006) Tsunami relief work—biopesticide spray operations—a case study. In: Nadim F, Pöttler R, Einstein H, Klapperich H, Kramer S (eds) Geohazards. ECI Symposium Series, vol. P7. http://services.bepress.com/eci/geohazards/39

Murugan K, Babu R, Jeyabalan D, Senthil Kumar N, Sivaramakrishnan S (1996) Antipupational effect of neem oil and neem seed kernel extract against mosquito larvae of *Anopheles stephensi* (Liston). J Ent Res 20:137–139

Murugan K, Vahitha R, Baruah I, Das SC (2003) Integration of botanicals and microbial pesticides for the control of filarial vector. *Culex quinquefasciatus*. Ann Med Entomol 12:11–23

Murugan K, Murugan P, Noortheen A (2007) Larvicidal and repellent potential of *Albizzia amara* Boivin and *Ocimum basilicum* Linn against dengue vector, *Aedes aegypti* Insecta: Diptera: Culicidae). Bioresour Technol 98:198–201

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

Naresh Kumar A, Murugan K, Baruah I, Madhiyazhagan P, Nataraj T (2012) Larvicidal potentiality, longevity and fecundity inhibitory activities of *Bacillus sphaericus* (Bs G3-IV) on vector mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*. J Entomol Acarol Res 44:79–84

Nathan SS, Kalaivani K, Murugan K (2005) Effects of neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Acta Trop 96:47–55

Oberdorster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick (2005) Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol 2:8–43

Panneerselvam C, Murugan K, Kovendan K, Mahesh Kumar P, Subramaniam J (2013) Mosquito larvicidal and pupicidal activity of *Euphorbia hirta* Linn. (Family: Euphorbiaceae) and *Bacillus sphaericus* against *Anopheles stephensi* Liston. (Diptera: Culicidae). (Diptera: Culicidae). Asian Pac J Trop Med 6:102–109

Patil CD, Patil SV, Borase HP, Salunke BK, Salunkhe RB (2012) Larvicidal activity of silver nanoparticles synthesized using *Plumeria rubra* plant latex against *Aedes aegypti* and *Anopheles stephensi*. Parasitol Res 110:1815–1822

Ponarulselvam S, Panneerselvam C, Murugan K, Aarthi N, Kalimuthu K, Thangamani S (2012) Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* Linn. G. Don and their antiplasmodial activities. Asian Pac J Trop Biomed 2:574–80

Poopathi S, De Britto LJ, Praba VL, Mani C, Praveen M (2014) Synthesis of silver nanoparticles from *Azadirachta indica*—a most effective method for mosquito control. Environ Sci Pollut Res. doi:10.1007/s11356-014-3560-x

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

Rai A, Singh A, Ahmad M, Sastry (2006) Role of halide ions and temperature on the morphology of biologically synthesized gold nanotriangles. Langmuir 22:736–741

Rajakumar G, Rahuman A (2011) Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrata* leaf extract against filariasis and malaria vectors. Acta Trop 118:196–203

Rajesh W, Niranjan S, Jaya R, Vijay D, Sahebrao B, Kashid (2010) Extracellular synthesis of silver nanoparticles using dried leaves of *Pongamia pinnata* (L) pierre. Nano-Micro Lett 2:2106–2113

Rizk AFM (1987) The chemical constituents and economic plants of Euphorbiaceae. Bot J Linn Soc 94:293–326

Roni M, Murugan K, Panneerselvam C, Subramaniam J, Hwang JS (2013) Evaluation of leaf aqueous extract and synthesized silver nanoparticles using *Nerium oleander* against *Anopheles stephensi* (Diptera: Culicidae). Parasitol Res 112:981–990

Saini HK, Sharma RM, Bami HL, Sidhu KS (1986) Preliminary study on constituents of mosquito coil smoke. Pesticides 20:15–18

Salam HA, Rajiv P, Kamaraj M, Jagadeeswaran P, Gunalan S, Sivaraj R (2012) Plants: green route for nanoparticles synthesis. Int Res J Biol Sci 1:85–90

Salunkhe RB, Patil SV, Patil CD, Salunke BK (2011) Larvicidal potential of silver nanoparticles synthesized using fungus *Cochliobolus lunatus* against *Aedes aegypti* (Linnaeus, 1762) and *Anopheles stephensi* Liston (Diptera; Culicidae). Parasitol Res 109:823–831

Sathyavathi R, Balamurali Krishna M, Venugopal Rao S, Saritha R, Narayana Rao D (2010) Biosynthesis of silver nanoparticles using *Coriandrum sativum* leaf extract and their application in nonlinear optics. Adv Sci Lett 3:1–6

Saxena A, Tripathi RM, Singh RP (2010) Biological synthesis of silver nanoparticles using onion (*Allium cepa*) extract and their antibacterial activity. Dig J Nanomater Biostruct 5:427–432

Shameli K, Ahmad MB, Yunus WMZW, Ibrahim NA (2010) Synthesis and characterization of silver/talc nanocomposites using the wet chemical reduction method. Int J Nanomedicine 5:743–751

Shankar SS, Rai A, Ahmad A, Sastry M (2004) Biosynthesis of silver and gold nanoparticles from extracts of different parts of the *Geranium* plant. Appl Nanotechnols 1:69–77

Song YJ, Jang HK, Kim SB (2009) Biological synthesis of gold nanoparticles using *Magnolia kobus* and *Diopyros kaki* leaf extract. Process Biochem 44:1133–1138

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

Stuart BH (2002) Polymer analysis. Wiley, United Kingdom

Sundaravadivelan C, Nalini Padmanabhan M, Sivaprasath P, Kishmu L (2013) Biosynthesized silver nanoparticles from *Pedilanthus tithymaloides* leaf extract with anti-developmental activity against larval instars of *Aedes aegypti* L. (Diptera, Culicidae). Parasitol Res 112:303–311

Thyagarajan SP, Subramanian S, Thirunalasundari T, Venkateswaran PS, Blumberg BS (1988) Effect of *Phyllanthus amarus* on chronic carriers of hepatitis B virus. Lancet 2:764–766

Unander DW, Webster GL, Blumberg BS (1991) Uses and bioassays in *Phyllanthus* (Euphorbiaceae): a compilation, II. The subgenus Phyllanthus. J Ethnopharmacol 34:97–133

Veerakumar K, Govindarajan M, Hot SL (2014a) Evaluation of plant-mediated synthesized silver nanoparticles against vector mosquitoes. Parasitol Res 113:4567–4577

Veerakumar K, Govindarajan M, Rajeswary M (2014b) Low-cost and ecofriendly green synthesis of silver nanoparticles using *Feronia elephantum* (Rutaceae) against *Culex quinquefasciatus*, *Anopheles stephensi*, and *Aedes aegypti* (Diptera: Culicidae). Parasitol Res 113: 1775–1785

Velayutham K, Rahuman AA, Rajakumar G, Roopan SM, Elango G, Kamaraj C, Marimuthu S, Santhoshkumar T, Iyappan M, Siva C (2013) Larvicidal activity of green synthesized silver nanoparticles using bark aqueous extract of *Ficus racemosa* against *Culex quinquefasciatus* and *Culex gelidus*. Asian Pac J Trop Med 6:95–101

Vellasamy S, Hariharan H, Venkatesh TS, Jayaprakashvel M (2014) Larvicidal and antimicrobial activities of silver nanoparticles synthesized using marine fluorescent pseudomonads. BMC Infect Dis 14:25

WHO (1981) Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides: diagnostic test. WHO/VBC/81–807. WHO, Geneva

WHO (2012) Handbook for integrated vector management. World Health Organization, Geneva

Yeh SF, Hong CY, Huang YL, Liu TY, Choo KB, Chou CK (1993) Effect of an extract from *Phyllanthus amarus* on hepatitis B surface antigen gene expression in human hepatoma cells. Antivir Res 20:185–192