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Original Research Article

One-pot and eco-friendly synthesis of silver nanocrystals using *Adiantum raddianum*: Toxicity against mosquito vectors of medical and veterinary importance

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ABSTRACT

Mosquitoes (Diptera: Culicidae) represent a key threat for millions of humans and animals worldwide, since they act as vectors for devastating parasites and pathogens. Eco-friendly control tools are a priority. Plant-mediated biosynthesis of nanoparticles is rapid and cost-effective. Here we biosynthesized poly-dispersed silver nanocrystals (AgNPs) using a cheap aqueous leaf extract of *Adiantum raddianum*. AgNPs were characterized by UV–vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive spectroscopy (EDX) and X-ray diffraction analysis (XRD). The acute toxicity of *A. raddianum* extract and biosynthesized AgNPs was evaluated against larvae of the malaria vector *Anopheles stephensi*, the dengue vector *Aedes aegypti* and the filariasis vector *Culex quinquefasciatus*. Compared to the leaf aqueous extract, AgNPs showed higher toxicity against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* with LC_{50} values of 10.33, 11.23 and 12.19 μg/ml, respectively. Biosynthesized AgNPs were found safer to non-target organisms *Diplonychus indicus*, *Anisops bouvieri* and *Gambusia affinis*, with respective LC_{50} values ranging from 517.86 to 635.98 μg/ml. Overall, this study firstly shed light on the potential of *A. raddianum* as a potential bio-resource for rapid, cheap and effective nanosynthesis of novel mosquitocides.

Introduction

Arthropods represent a key threat for millions of humans and animals worldwide (Benelli et al., 2016a,b; Mehlhorn et al., 2012). *Anopheles* mosquitoes act as malaria vectors in tropical and sub-tropical areas worldwide, while *Aedes* species are of pivotal importance as dengue, chikungunya and Zika virus vectors (Benelli, 2015a; Benelli and Mehlhorn, 2016). Besides this, large populations of Culicidae can cause irritation and extensive blood loss to livestock. This could lead to reduced productivity and sometimes death. Furthermore, mosquitoes act as vectors of many different parasites and pathogens that infect livestock and pets, with special reference to horses, dogs and pigs. Good examples are the Eastern Equine Encephalomyelitis virus, the Western Equine Encephalomyelitis virus, the Venezuelan Equine Encephalomyelitis complex and the dog heartworm, *Dirofilaria immitis* (Benelli, 2015a; Mehlhorn et al., 2012). Therefore, Culicidae vector control is a critical requirement in epidemic disease situations, as is an urgent need to develop new and improved mosquito control methods that are economical and effective yet safe for non-target organisms and the environment (Benelli, 2015b; Govindarajan, 2011; Govindarajan et al., 2005).

Nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology (Benelli, 2016a,b). New

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applications of nanoparticles and nanomaterials are emerging rapidly (Ivanov et al., 2009). Nanocrystalline silver particles have found important applications in the field of high sensitivity biomolecular detection and diagnostics (Kelly et al., 2003), antimicrobials and therapeutics (Savithramma et al., 2011), parasitology and entomology (Benelli, 2016b; Govindarajan et al., 2016a,b,c,d; Veerakumar et al., 2014). However, there is still need for economic, commercially viable as well eco-friendly routes to synthesize effective and stable mosquitocidal silver nanoparticles (Benelli, 2016c).

Eco-friendly nanosynthesis processes include the employ of plant parts (e.g. leaves, bark, rhizomes and roots, see Benelli (2016a) and Muthukumaran et al. (2015) for a review), bacteria and fungi (Saxena et al., 2010). Indeed, chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large-scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals (Benelli, 2016a,c).

Adiantum raddianum (Adiantaceae) is a plant widely distributed in several Asian countries (Chopra et al., 1956). It is employed in traditional medicine as diuretic, expectorant, emollient, used for coughs, urinary disorders, alopecia and menstrual difficulties (Chopra et al., 1956; Meenakshi et al., 2008). To the best of our knowledge, the mosquitocidal potential of *A. raddianum* is currently unknown. In this study, we proposed a cheap and rapid method of biosynthesis of AgNPs using the aqueous leaf extract of *A. raddianum*. Bio-reduced AgNPs were characterized by UV–vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive spectroscopy (EDX) and X-ray diffraction analysis (XRD). The acute toxicity of *A. raddianum* leaf extract and green-synthesized AgNPs was evaluated against larvae of the malaria vector *Anopheles stephensi*, the dengue, yellow fever and Zika virus vector *Aedes aegypti* and the filariasis vector *Culex quinquefasciatus*. Furthermore, we evaluated the biotoxicity of *A. raddianum* aqueous extract and biosynthesized AgNPs on two non-target aquatic organisms sharing the same ecological niche of mosquito young instars, *Diplonychus indicus* and the biocontrol agent *Gambusia affinis*.

Materials and methods

Materials

Silver nitrate was procured from Merck, India. The glassware was acid-washed thoroughly and then rinsed with Millipore Milli-Q water. Healthy and fresh leaves of *A. raddianum* were collected from Nilgiris, Western Ghats (11° 10′ N to 11° 45′ N latitude and 76° 14′ E to 77° 2′ E longitude), Tamil Nadu State, India. The identity was confirmed at the Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu. Voucher specimens were numbered (ID: AUDZ-642) and kept in our laboratory and are available upon request.

Preparation of plant leaf extracts

The leaves of *A. raddianum* were dried in the shade and ground to fine powder in an electric grinder. Aqueous extract was prepared by mixing 50 g of dried leaf powder with 500 ml of water (boiled and cooled distilled water) with constant stirring on a magnetic stirrer. The suspension of dried leaf powder in water was left for 3 h and filtered through Whatman n. 1 filter paper and the filtrate were stored in an amber-colored airtight bottle at 10 °C until testing.

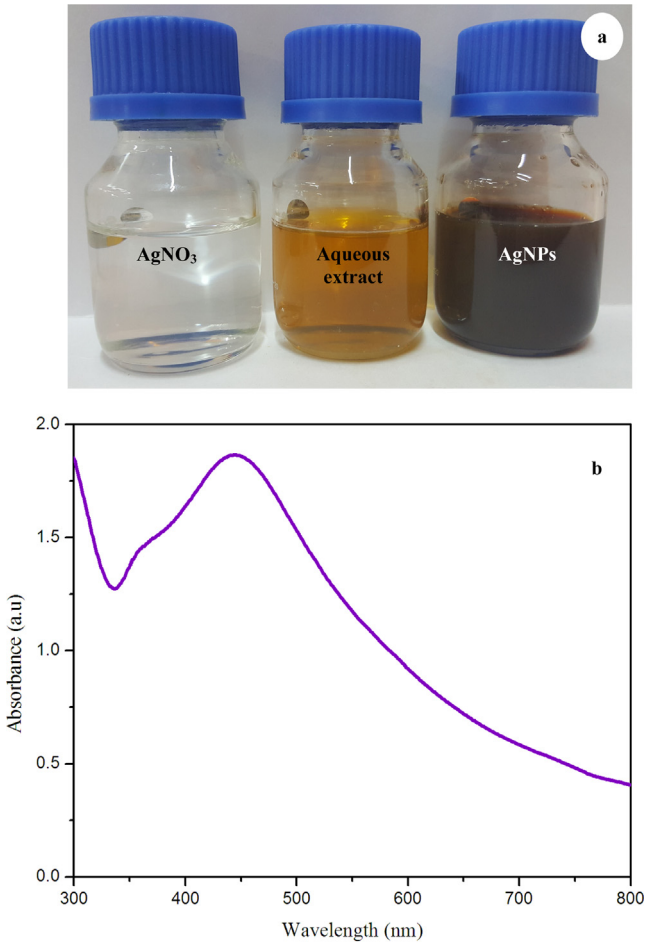

Fig. 1. (a) Color intensity of *Adiantum raddianum* aqueous extract before and after the reduction of silver nitrate (1 mM). The color change indicates Ag^+ reduction to elemental nanosilver. (b) UV–vis spectrum of silver nanoparticles after 180 min from the reaction.

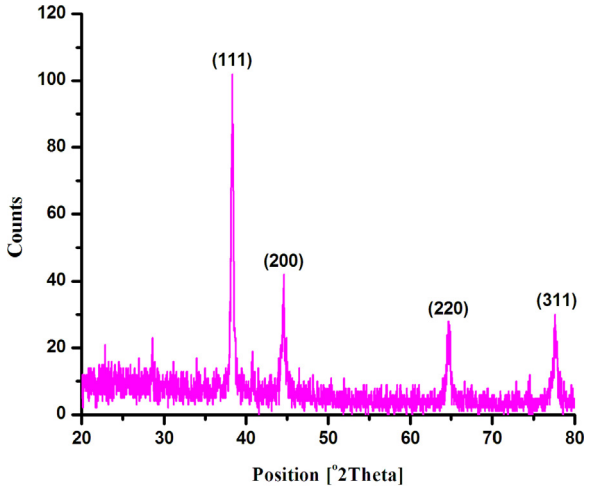

Fig. 2. XRD pattern of silver nanoparticles green synthesized using the *Adiantum raddianum* aqueous extract.

Synthesis of silver nanoparticles

The broth solution of fresh leaves was prepared by taking 10 g of thoroughly washed and finely cut leaves in a 300-ml Erlenmeyer flask along with 100 ml of sterilized double-distilled water and then boiling the mixture for 5 min before finally decanting it. The extract was filtered with Whatman filter paper n. 1, stored at −15 °C and tested within a week. The filtrate was treated with aqueous 1 mM $AgNO_3$ (21.2 mg of $AgNO_3$ powder in 125 ml Milli-Q water) solution in an Erlenmeyer flask and incubated at room temperature. Eighty-eight milliliters of an aqueous solution of 1 mM silver nitrate was reduced using 12 ml of leaf extract at room temperature for 10 min, resulting in a brown–yellow solution indicating the formation of AgNPs.

Characterization of synthesized silver nanoparticles

The bioreduction of Ag^+ ions was monitored using UV–vis spectrophotometer (UV-160v, Shimadzu, Japan) analysis on size, morphology and composition of AgNPs were performed by scanning electron microscopy (Hitachi S3000 H SEM), transmission electron microscopy (TEM Technite 10 Philips) and energy dispersive X-ray spectrum (EDX). The purified AgNPs were examined for the presence of biomolecules using FT-IR spectrum (Thermo Scientific Nicolet 380 FT-IR Spectrometer) KBr pellets and crystalline AgNPs were determined by XRD analysis.

Mosquito rearing

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

Acute toxicity against mosquito larvae

Larvicidal activity of the aqueous crude extract and AgNPs from *A. raddianum* was evaluated according to WHO protocol (2005). Based on the wide range and narrow range tests, aqueous crude extract was tested at 70, 140, 210, 280 and 350 μm ml concentrations and Ag NP was tested at 5, 10, 15, 20, and 25 μm ml concentrations. Twenty late III 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 or AgNPs was added. For each concentration, five replicates were performed. 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.

Biotoxicity on non-target organisms

The AgNP toxicity on non-target organisms was assessed following the method by Sivagnaname and Kalyanasundaram (2004). The effect of aqueous extract and AgNPs of the potential

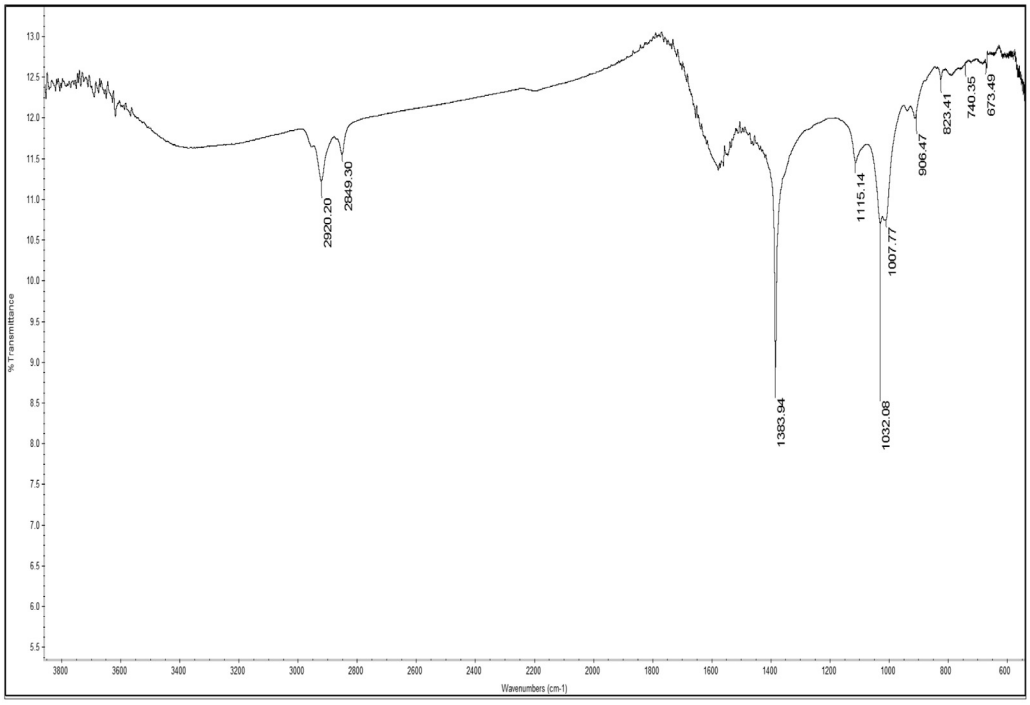

Fig. 3. FTIR spectrum of silver nanoparticles green synthesized using the *Adiantum raddianum* aqueous leaf extract.

plant was tested against non-target organisms *D. indicus* and *G. affinis*. The species were field collected and separately maintained in cement tanks (85 cm diameter and 30 cm depth) containing water at 27 ± 3 °C.

The *A. raddianum* aqueous extract and AgNPs were tested at concentrations of even 50 times higher the doses tested on mosquito larvae. Ten replicates will be performed for each concentration along with four replicates of untreated controls. The non-target organisms were observed for mortality and other abnormalities such as sluggishness and reduced swimming activity after 48 h exposure. The exposed non-target organisms were also observed continuously for ten days to understand the post treatment effect of this extract on survival and swimming activity.

Data analysis

Mortality data were subjected to probit analysis. LC_{50} and LC_{90} were calculated using the method by Finney (1971). In experiments evaluating biotoxicity on non-target organisms, the Suitability Index (SI) was calculated for each non-target species using the following formula (Deo et al., 1988).

$$SI = \frac{LC_{50}\text{of non} - \text{target organisms}}{LC_{50}\text{of target vector species}}$$

All data were analyzed using the SPSS Statistical Software Package version 16.0. A probability level of $P < 0.05$ was used for the significance of differences between values.

Results

Biosynthesis and characterization of silver nanoparticles

In our experiments, when the plant extract was added to silver nitrate solution, the formation of AgNPs occurred and color changed rapidly to dark brown (Fig. 1a). AgNPs were characterized using UV–vis spectroscopy, where an intense, broad absorption peak was observed at 447 nm (Fig. 1b). In XRD analysis, four diffraction peaks were observed at 37.67, 43.84, 64.03 and 76.96 represent the (111), (200), (220) and (311) (Fig. 2), while main peaks linked to the presence of reducing and stabilizing biomolecules are shown in the FTIR spectrum (Fig. 3). SEM of AgNPs showed that nanoparticles were mostly spherical or with

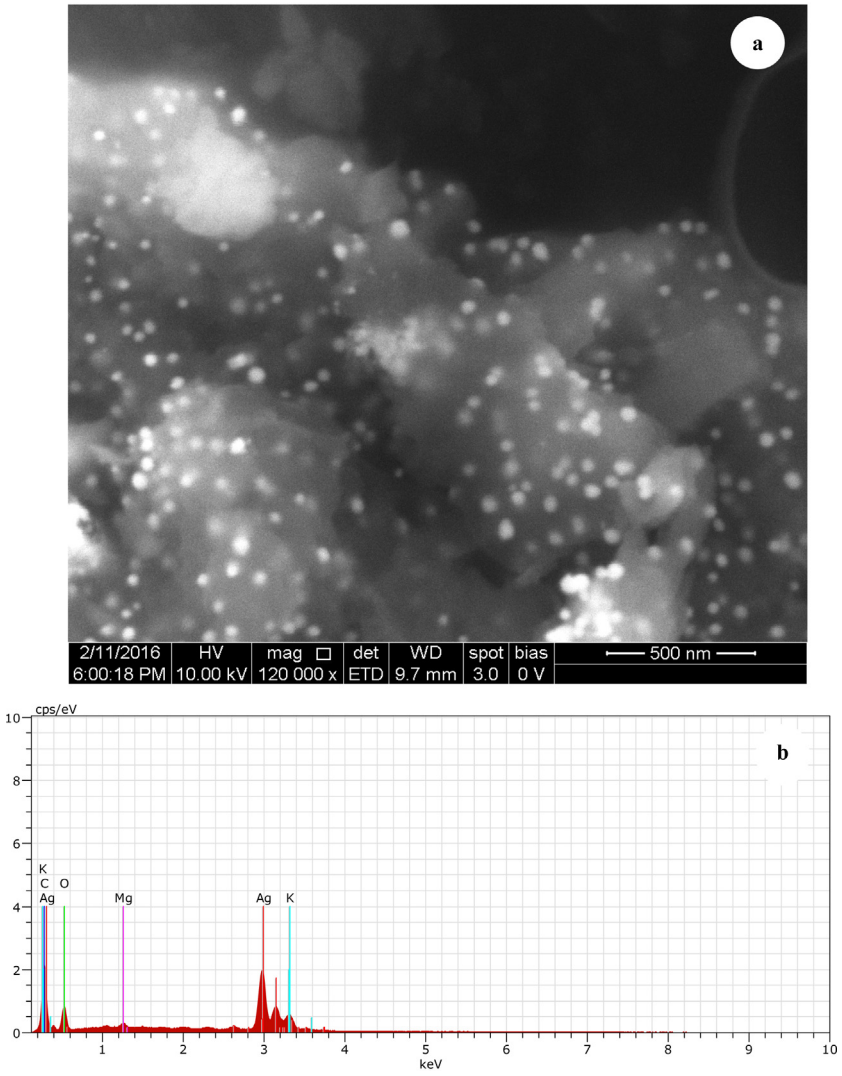

Fig. 4. Silver nanoparticles biofabricated using the *Adiantum raddianum* leaf extract: (a) SEM (1,20,000×); (b) EDX spectrum showing the chemical composition.

cubic structures (Fig. 4a). Fig. 4b shows spot EDX analysis, confirming the presence of silver in the sample. Fig. 5 shows the TEM of AgNPs synthesized using *A. raddianum* leaf extract. Among shapes, spheres, truncated triangles, and decahedral morphologies dominate, ranging from 9.69 to 13.9 nm with an average size of 10.9 nm.

Acute toxicity against mosquito larvae

In laboratory conditions, both the *A. raddianum* leaf extract and AgNPs showed dose-dependent larvicidal effect against all tested mosquito species (Tables 1 and 2). Compared to the leaf aqueous extract, green-synthesized AgNPs showed higher toxicity against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* with LC_{50} values of 10.33, 11.23 and 12.19 μg/mL, respectively (Table 2).

Biotoxicity on non-target organisms

Both toxicity treatments achieved negligible toxicity against *D. indicus* and *G. affinis*, with LC_{50} values ranging from 517.86 to 8430.41 μg/mL (Tables 3 and 4). Focal observations highlighted that longevity and swimming activity of the study species were not altered for a week after testing. SI indicated that *A. raddianum*-fabricated AgNPs were less toxic to the non-target organism tested if compared to the targeted mosquitoes (Table 5).

Discussion

In UV–vis spectroscopy, an intense broad absorption peak was observed at 447 nm after 180 min from the reaction, and it was probably due to surface plasmon resonance (SPR). The SPR peak is very sensitive to the size and shape of the nanoparticles, amount of extract, silver nitrate concentration and the type of biomolecules present in the leaf extract (Govindarajan and Benelli, 2016a,b). Our UV–vis results are in agreement with previous research (Singh et al., 2010; Zargar et al., 2011), where the AgNPs were observed as stable in solution and also showed little aggregation. Besides, the plasmon bands were broadened with an absorption tail in longer wavelengths, and this may be related to the size distribution of nanoparticles (Ahmad et al., 2003).

The XRD pattern confirmed the crystalline nature of green-synthesized silver nanoparticles (Benelli, 2016a). Four diffraction peaks were observed at 38.22, 44.37, 64.54 and 77.47 represent the (111), (200), (220) and (311), reflections and the face-centered cubic structure of metallic silver, respectively. FTIR spectroscopy shed light on the identity of the biomolecules responsible for reduction and efficient stabilization of the AgNPs. The peak at 2920 cm^{-1} indicates carboxylic acid (Li et al., 2007). 2849 cm^{-1} may indicate the presence of methylene group (CH_2) next to OH or NH_2 functional group. The band at 1384 cm^{-1} may corresponds to C—C stretching of aromatic amines. The bands at 1115 cm^{-1}, 1032 cm^{-1} and 1007 cm^{-1} might indicate the presence of C—O stretching alcohols, carboxylic acids, esters and ethers. The peak obtained at 823 cm^{-1} could be due to the bending vibrations of C—H groups of phenyl rings. 673 cm^{-1} probably corresponds to C—H stretching strong vinyl di-substituted alkenes (Prakash et al., 2013).

SEM and TEM showed that the green-synthesized nanoparticles were mostly spherical, but also sub-triangular and decahedral morphologies were detected, with an average size of 14.96 nm. As a general trend, the shape of plant-synthesized Ag NP was spherical, with exception of some neem-synthesized AgNPs. They are poly-disperse, with spherical or flat, plate-like, morphology, and mean size range of 5–35 nm in size (Shankar et al., 2004). For example, AgNPs fabricated using *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). Most of the AgNPs was roughly circular in shape with smooth edges. In agreement with these findings, AgNPs from *Annona squamosa* leaf extract were spherical in shape with an average size ranging from 20 to 100 nm (Vivek et al., 2012) while Thirunavokkarasu et al. (2013) reported spherical nanoparticles with size ranging from 8 to 90 nm in *Desmodium gangeticum*. The present TEM analysis also showed that the surfaces of the AgNPs were surrounded by a black thin layer of some material, which might be due to the capping organic constituents of the plant broth, as also highlighted by Rafiuddin (2013).

In laboratory conditions, the *A. raddianum* aqueous leaf extract showed larvicidal activity against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*; with LC_{50} values ranging from 143.22 to 169.94 μg/mL. In latest years, a growing number of evidences have been provided about the larvicidal efficacy of plant-borne larvicides (Veerekumar et al., 2013; Benelli, 2015a,b). Furthermore, the *A. raddianum*-synthesized AgNPs were highly toxic against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* larvae, with LC_{50} ranging from 10.33 to 12.19 μg/mL. In latest years, a growing number of plant-synthesized AgNPs have been studied for their excellent larvicidal activity against important mosquito vectors (Benelli, 2016a,c). Combination of metal nanoparticles with bioactive principles bestows improved efficiency. The present study implicated that the percentage of mosquito larvicidal mortality increased by many folds with the addition of bio-stabilized AgNPs. For instance, Muthukumaran et al. (2015) studied the potential of *Gmelina asiatica*-mediated synthesis of AgNPs against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*, obtaining higher LC_{50} values (i.e., 22.44, 25.77, and 27.83 μg/mL, respectively). A further example is the larvicidal activity of *Leucas aspera*-synthesized AgNPs against *A. aegypti* and *A. stephensi,* with LC_{50} ranging from 13.06 to 25.54 ppm for *A. aegypti,* and from 12.45 ppm to 22.26 ppm for *A. stephensi* (Sivapriyajothi et al., 2014). Similarly, low doses of AgNPs biosynthesized using *Euphorbia hirta* leaf extract have been reported as toxic against *A. stephensi*, with LC_{50} values ranging from 10.14 ppm (I instar larvae), to 34.52 ppm (pupae) (Priyadarshini et al., 2012).

The observed larvicidal activity may be attributed to the interaction between silver nanoparticles and the extracellular

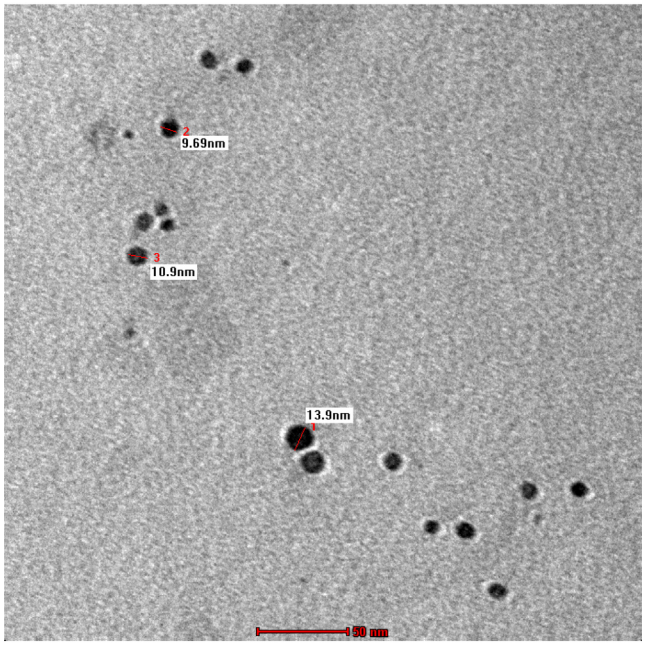

Fig. 5. TEM of silver nanoparticles green synthesized using the *Adiantum raddianum* aqueous extract.

Table 1
Larvicidal activity of *Adiantum raddianum* aqueous leaf extract against the mosquito vectors *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*.

Mosquito species	Concentration (μg/ml)	Mortality (%) ± SD[a]	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
An. stephensi	70	28.0 ± 1.2	143.22	285.74	3.46	$y = 12.03 + 0.259x$	3.351 (4)
	140	49.2 ± 0.8	(126.35–158.04)	(264.80–313.31)			n.s.
	210	67.5 ± 0.8					
	280	88.3 ± 0.6					
	350	99.1 ± 0.4					
Ae. aegypti	70	25.6 ± 0.8	155.96	306.19	3.14	$y = 7.97 + 0.262x$	2.170 (4)
	140	44.2 ± 0.6	(139.17–171.02)	(283.71–335.98)			n.s.
	210	63.8 ± 0.6					
	280	84.5 ± 0.4					
	350	97.3 ± 0.6					
Cx. quinquefasciatus	70	21.9 ± 0.6	169.94	321.63	2.65	$y = 3.1 + 0.269x$	2.573 (4)
	140	40.6 ± 0.4	(153.84–184.80)	(298.31–352.54)			n.s.
	210	59.3 ± 0.4					
	280	80.2 ± 0.6					
	350	95.4 ± 0.8					

No mortality was observed in the control.
SD = standard deviation.
LC_{50} = lethal concentration that kills 50% of the exposed organisms.
LC_{90} = lethal concentration that kills 90% of the exposed organisms.
UCL = 95% upper confidence limit.
LCL = 95% lower confidence limit.
χ^2 = chi square.
d.f. = degrees of freedom.
n.s. = not significant ($\alpha = 0.05$).
[a] Values are mean ± SD of five replicates.

Table 2
Larvicidal activity of silver nanocrystals synthesized using the *Adiantum raddianum* leaf extract against the mosquito vectors *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*.

Mosquito species	Concentration (μg/ml)	Mortality (%) ± SD[a]	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
An. stephensi	5	27.5 ± 0.8	10.33	20.51	3.35	$y = 11.42 + 3.644x$	3.667 (4)
	10	49.2 ± 0.8	(9.14–11.39)	(19.01–22.48)			n.s.
	15	66.3 ± 0.6					
	20	88.4 ± 0.4					
	25	99.0 ± 0.6					
Ae. aegypti	5	24.2 ± 0.6	11.23	21.81	2.99	$y = 7.17 + 3.714x$	2.496 (4)
	10	45.6 ± 0.4	(10.05–12.29)	(20.23–23.91)			n.s.
	15	62.1 ± 0.4					
	20	85.3 ± 0.6					
	25	97.2 ± 0.8					
Cx. quinquefasciatus	5	20.5 ± 0.4	12.19	22.86	2.56	$y = 2.39 + 3.814x$	2.082 (4)
	10	41.3 ± 0.4	(11.05–13.24)	(21.23–25.03)			n.s.
	15	59.4 ± 0.6					
	20	80.6 ± 0.6					
	25	96.2 ± 0.4					

No mortality was observed in the control.
SD = standard deviation.
LC_{50} = lethal concentration that kills 50% of the exposed organisms.
LC_{90} = lethal concentration that kills 90% of the exposed organisms.
UCL = 95% upper confidence limit.
LCL = 95% lower confidence limit.
χ^2 = chi square.
d.f. = degrees of freedom.
n.s. = not significant ($\alpha = 0.05$).
[a] Values are mean ± SD of five replicates.

lipoprotein matrix. This interaction increases the permeability of the plasma membrane of cells. Additionally, an interaction between AgNPs and the sulfur-containing proteins or phosphorous containing compound leads to denaturation of organelles and enzymes and reduces ATP synthesis, which finally causes the loss of the cellular function and cell death (Sap-Iam et al., 2010; Sondi and Salopek-Sondi, 2004).

In our experiments, the biotoxicity of *A. raddianum* aqueous extract and green-synthesized AgNPs on non-target organisms *D. indicus* and *G. affinis*, with LC_{50} ranging from 517.86 to 8430.41 μm/ml. In addition, the SI pointed out that *A. raddianum*-fabricated AgNPs were less toxic to the non-target organism tested if compared to the targeted mosquito larvae. Currently, limited knowledge is available about the acute toxicity of mosquitocidal nanoparticles towards non-target aquatic species (Benelli, 2016a). *Pergularia rubra*- and *Pergularia daemia*-synthesized AgNPs did not exhibit any evident toxicity effect against *Poecilia reticulata* fishes, after 48 h of exposure to LC_{50} and LC_{90} values calculated on IV instar larvae of *A. aegypti* and *A. stephensi* (Patil et al., 2012). Subarani et al. (2013) did not reported toxicity effects of *Vinca rosea*-synthesized AgNPs against *P. reticulata*, after 72 h of exposure to dosages toxic against *A. stephensi* and *C. quinquefasciatus*. Similarly, Haldar et al. (2013) did not detected toxicity of AgNPs produced using dried green fruits of *D. roxburghii* against

Table 3
Biotoxicity of *Adiantum raddianum* aqueous leaf extract against two non-target organisms sharing the same ecological niche of *Anopheles, Aedes* and *Culex* mosquito vectors.

Non-target organism	Concentration (μg/ml)	Mortality (%) ± SD[a]	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
D. indicus	3000	26.3 ± 1.2	6329.54	12263.35	2.94	$y = 9.58 + 0.006x$	5.691 (4)
	6000	48.2 ± 0.8	(5641.24–6943.39)	(11383.45–13417.63)			n.s.
	9000	65.8 ± 0.6					
	12000	87.2 ± 1.2					
	15000	100.0 ± 0.0					
G. affinis	4000	27.8 ± 0.8	8430.41	17154.12	3.98	$y = 11.6 + 0.004x$	1.096 (4)
	8000	46.2 ± 0.6	(7417.96–9318.29)	(15868.05–18862.34)			n.s.
	12000	68.3 ± 0.6					
	16000	85.6 ± 0.4					
	20000	97.1 ± 0.6					

No mortality was observed in the control.
SD = standard deviation.
LC_{50} = lethal concentration that kills 50% of the exposed organisms.
LC_{90} = lethal concentration that kills 90% of the exposed organisms.
UCL = 95% upper confidence limit.
LCL = 95% lower confidence limit.
χ^2 = chi square.
d.f. = degrees of freedom.
n.s. = not significant ($\alpha = 0.05$).
[a] Values are mean ± SD of five replicates.

Table 4
Acute toxicity of silver nanocrystals synthesized using the *Adiantum raddianum* leaf extract against two non-target organisms sharing the same ecological niche of *Anopheles, Aedes* and *Culex* mosquito vectors.

Non-target organism	Concentration (μg/ml)	Mortality (%) ± SD[a]	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
D. indicus	250	28.7 ± 1.2	517.86	1006.83	2.99	$y = 10.42 + 0.074x$	6.167 (4)
	500	46.2 ± 1.2	(460.60–568.80)	(934.37–1101.83)			n.s.
	750	66.5 ± 0.4					
	1000	89.4 ± 0.4					
	1250	100.0 ± 0.0					
G. affinis	300	26.4 ± 0.8	635.98	1261.72	3.34	$y = 10.34 + 0.061x$	2.656 (4)
	600	48.3 ± 0.8	(563.63–700.02)	(1169.29–1383.67)			n.s.
	900	65.2 ± 1.2					
	1200	87.3 ± 1.2					
	1500	98.1 ± 0.4					

No mortality was observed in the control.
SD = standard deviation.
LC_{50} = lethal concentration that kills 50% of the exposed organisms.
LC_{90} = lethal concentration that kills 90% of the exposed organisms.
UCL = 95% upper confidence limit.
LCL = 95% lower confidence limit.
χ^2 = chi square.
d.f. = degrees of freedom.
n.s. = not significant ($\alpha = 0.05$).
[a] Values are mean ± SD of five replicates.

Table 5
Suitability index of two non-target organisms over young instars of *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* exposed to *Adiantum raddianum* aqueous leaf extract and green-synthesized silver nanocrystals.

Treatment	Non-target organism	*An. stephensi*	*Ae. aegypti*	*Cx. quinquefasciatus*
Aqueous leaf extract	*Diplonychus indicus*	44.19	40.58	37.24
	Gambusia affinis	58.86	54.05	49.60
Silver nanoparticles	*Diplonychus indicus*	50.13	46.11	42.48
	Gambusia affinis	61.56	56.63	52.17

P. reticulata, after 48 h-exposure to LC_{50} of IV instar larvae of *A. stephensi* and *C. quinquefasciatus*. Rawani et al. (2013) showed that mosquitocidal AgNPs synthesized using *Solanum nigrum* berry extracts were not toxic against two mosquito predators, *Toxorhynchites* larvae and *Diplonychus annulatum*, and *Chironomus circumdatus* larvae, exposed to lethal concentrations of dry nanoparticles calculated on *A. stephensi* and *C. quinquefasciatus* larvae. AgNPs synthesized using the 2,7.bis [2-[diethylamino]-ethoxy] fluorence isolate from the *Melia azedarach* leaves did not show acute toxicity against *Mesocyclops pehpeiensis* copepods (Ramanibai and Velayutham, 2015).

Later on, Govindarajan et al. (2016e) assessed the biotoxicity of *C. spinarum*-synthesized silver nanoparticles on the non-target aquatic organisms *Anisops bouvieri*, *D. indicus* and *G. affinis*. Toxicity testing revealed minimal toxicity, obtaining LC_{50} values in the range of 424 to 6402 μm/ml. Similarly, Govindarajan et al. (2016f) reported that the *Malva sylvestris*-synthesized silver nanoparticles exhibited minimal biotoxicity against nontarget organisms

D. indicus and *G. affinis*, as with LC_{50} values ranging from 813 to 10,459 μg/ml. *B. cristata*-fabricated silver nanoparticles tested on the non-target organisms *A. bouvieri*, *D. indicus* and *G. affinis*, showed LC_{50} values ranging from 633 to 8595 μg/ml (Govindarajan and Benelli, 2016a). Moreover, the aqueous extract and biosynthesized AgNPs of *Quisqualis indica* had a moderate biotoxic effect on two mosquito predators *A. bouvieri* (LC_{50} 653 μg/ml) and *G. affinis* (LC_{50} 2183 μg/ml) (Govindarajan et al., 2016g). In addition, the exposure to extremely low doses (e.g. 1 ppm) of green-synthesized metal nanoparticles did not negatively impact the predation rates of different mosquito predators (Murugan et al., 2015). It has been formulated that very low doses of plant-synthesized metal nanoparticles may reduce the motility of mosquito larvae, enhancing predation of mosquito fishes, tadpoles, odonate nymphs and other mosquito natural enemies (Benelli, 2016a,b).

Conclusions

Overall, we synthesized silver nanoparticles using a cheap aqueous extract of *A. raddianum* leaves as reducing and stabilizing agent. Our AgNPs were mostly spherical in shape, crystalline in nature, with face-centered cubic geometry, and the mean size was 9–13 nm. This research highlighted that *A. raddianum*-synthesized AgNPs are easy to produce, stable over time, and can be employed at low dosages to strongly reduce populations of vectors mosquitoes of medical and veterinary importance without detrimental effects on predation rates of non-target aquatic organisms, such as *D. indicus* and *G. affinis*.

Conflicts of interest

The authors declare no conflicts of interest.

Compliance with ethical standards

All applicable international and national guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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References

Ahmad, A., Mukherjee, M., Mandal, D., Senapati, S., Khan, M.I., Kumar, R., Sastry, M., 2003. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids Surf. B: Biointerfaces 28, 313–318.
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.
Benelli, G., 2015a. Research in mosquito control: current challenges for a brighter future. Parasitol. Res. 114, 2801–2805.
Benelli, G., 2015b. Plant-borne ovicides in the fight against mosquito vectors of medical and veterinary importance: a systematic review. Parasitol. Res. 114, 3201–3212.
Benelli, G., 2016a. Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review. Parasitol. Res. 115, 23–34.
Benelli, G., 2016b. Plant-mediated synthesis of nanoparticles: a newer and safer tool against mosquito-borne diseases? Asia Pac. J. Trop. Biomed. 6, 353–354.
Benelli, G., 2016c. Green synthesized nanoparticles in the fight against mosquito-borne diseases and cancer – a brief review. Enzyme Microb. Technol. 95, 58–68.
Benelli, G., Mehlhorn, H., 2016. Declining malaria, rising dengue and Zika virus: insights for mosquito vector control. Parasitol. Res. 115, 1747–1754.
Benelli, G., Pavela, R., Canale, A., Mehlhorn, H., 2016a. Tick repellents and acaricides of botanical origin: a green roadmap to control tick-borne diseases? Parasitol. Res. 115, 2545–2560.
Benelli, G., Lo Iacono, A., Canale, A., Mehlhorn, H., 2016b. Mosquito vectors and the spread of cancer: an overlooked connection? Parasitol. Res. 115, 2131–2137.
Chopra, R.N., Nayar, S.L., Chopra, I.C., 1956. Glossary of Indian Medicinal Plants. CSIR, New Delhi.
Deo, P.G., Hasan, S.B., Majumdar, S.K., 1988. Toxicity and suitability of some insecticides for household use. Int. Pest Control 30, 118–129.
Finney, D.J., 1971. Probit Analysis. Cambridge University Press, London, pp. 68–72.
Govindarajan, M., Benelli, G., 2016a. Facile biosynthesis of silver nanoparticles using *Barleria cristata*: mosquitocidal potential and biotoxicity on three non-target aquatic organisms. Parasitol. Res. 115, 925–935.
Govindarajan, M., Benelli, G., 2016b. One-pot green synthesis of silver nanocrystals using *Hymenodictyon orixense*: a cheap and effective tool against malaria, chikungunya and Japanese encephalitis mosquito vectors? RSC Adv. 6, 59021–59029.
Govindarajan, M., Jebanesan, A., Reetha, D., 2005. Larvicidal effect of extracellular secondary metabolites of different fungi against the mosquito, *Culex quinquefasciatus*. Trop. Biomed. 22, 1–3.
Govindarajan, M., Rajeswary, M., Veerakumar, K., Hoti, S.L., Mehlhorn, H., Barnard, D. R., Benelli, G., 2016a. Novel synthesis of silver nanoparticles using *Bauhinia variegata*: a recent eco-friendly approach for mosquito control. Parasitol. Res. 115, 723–733.
Govindarajan, M., Rajeswary, M., Veerakumar, K., Muthukumaran, U., Hoti, S.L., Benelli, G., 2016b. Green synthesis and characterization of silver nanoparticles fabricated using *Anisomeles indica*: mosquitocidal potential against malaria, dengue and Japanese encephalitis vectors. Exp. Parasitol. 161, 40–47.
Govindarajan, M., Rajeswary, M., Muthukumaran, U., Hoti, S.L., Khater, H.F., Benelli, G., 2016c. Single-step biosynthesis and characterization of silver nanoparticles using *Zornia diphylla* leaves: a potent eco-friendly tool against malaria and arbovirus vectors. J. Photochem. Photobiol. B 161, 482–489.
Govindarajan, M., Khater, H.F., Panneerselvam, C., Benelli, G., 2016d. One-pot fabrication of silver nanocrystals using *Nicandra physalodes*: a novel route for mosquito vector control with moderate toxicity on non-target water bugs. Res. Vet. Sci. 107, 95–101.
Govindarajan, M., Nicoletti, M., Benelli, G., 2016e. Bio-physical characterization of poly-dispersed silver nanocrystals fabricated using *Carissa spinarum*: a potent tool against mosquito vectors. J. Cluster Sci. 27, 745–761.
Govindarajan, M., Hoti, S.L., Rajeswary, M., Benelli, G., 2016f. One-step synthesis of polydispersed silver nanocrystals using *Malva sylvestris*: an eco-friendly mosquito larvicide with negligible impact on non-target aquatic organisms. Parasitol. Res. 115, 2685–2695.
Govindarajan, M., Vijayan, P., Shine, K., Alharbi, N.S., Benelli, G., 2016g. One-pot biogenic fabrication of silver nanocrystals using *Quisqualis indica*: effectiveness on malaria and Zika virus mosquito vectors, and impact on non-target aquatic organisms. J. Photochem. Photobiol. B 162, 646–655.
Govindarajan, M., 2011. Evaluation of indigenous plant extracts against the malarial vector, *Anopheles stephensi* (Liston) (Diptera: Culicidae). Parasitol. Res. 109, 93–103.
Haldar, K.M., Haldar, B., Chandra, G., 2013. Fabrication, characterization and mosquito larvicidal bioassay of silver nanoparticles synthesized from aqueous fruit extract of putranjiva, *Drypetes roxburghii* (Wall.). Parasitol. Res. 112, 1451–1459.
Ivanov, V.K., Shaporev, A.S., Sharikov, F.Y., Baranchikov, A.Y., 2009. Hydrothermal and microwave-assisted synthesis of nanocrystalline ZnO photocatalysts. Superlattices Microstruct. 45 (4–5), 421–424.
Kelly, K.L., Coronado, E., Zhao, L.L., Schatz, G.C., 2003. The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment. J. Phys. Chem. B 107 (3), 668–677.
Li, S., Shen, Y., Xie, A., Yu, X., Qiu, L., Zhang, L., Zhang, Q., 2007. Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract. Green Chem. 9, 852–858.
Meenakshi, S., Sing, N., Khare, P.B., Rawat, A.K.S., 2008. Antimicrobial activity of some important *Adiantum* species used traditionally in indigenous systems of medicine. J. Ethnopharmacol. 115, 327–329.
Mehlhorn, H., Al-Rasheid, K.A., Al-Quraishy, S., Abdel-Ghaffar, F., 2012. Research and increase of expertise in arachno-entomology are urgently needed. Parasitol. Res. 110, 259–265.
Murugan, K., Benelli, G., Panneerselvam, C., Subramaniam, J., Jeyalalitha, T., Dinesh, D., Nicoletti, M., Hwang, J.S., Suresh, U., Madhiyazhagan, P., 2015. *Cymbopogon citratus*-synthesized gold nanoparticles boost the predation efficiency of copepod *Mesocyclops aspericornis* against malaria and dengue mosquitoes. Exp. Parasitol. 153, 129–138.
Muthukumaran, U., Govindarajan, M., Rajeswary, M., 2015. Synthesis and characterization of silver nanoparticles using *Gmelina asiatica* leaf extract against filariasis, dengue, and malaria vector mosquitoes. Parasitol. Res. 114, 1817–1827.
Patil, C.D., Borase, H.P., Patil, S.V., Salunkhe, R.B., Salunke, B.K., 2012. Larvicidal activity of silver nanoparticles synthesized using *Pergularia daemia* plant latex against *Aedes aegypti* and *Anopheles stephensi* and nontarget fish *Poecillia reticulata*. Parasitol. Res. 111, 555–562.
Prakash, P., Gnanaprakasama, P., Emmanuel, R., Arokiyaraj, S., Saravanan, M., 2013. Green synthesis of silver nanoparticles from leaf extract of *Mimusops elengi*,

Linn. for enhanced antibacterial activity against multi drug resistant clinical isolates. Colloids Surf. B: Biointerfaces 108, 255–259.
Priyadarshini, A., 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.
Rafiuddin, Z.Z., 2013. Bio-conjugated silver nanoparticles from *Ocimum sanctum* and role of cetyltrimethyl ammonium bromide. Colloids Surf. B: Biointerfaces 108, 90–94.
Ramanibai, R., Velayutham, K., 2015. Bioactive compound synthesis of Ag nanoparticles from leaves of *Melia azedarach* and its control for mosquito larvae. Res. Vet. Sci. 98, 82–88.
Rawani, A., Ghosh, A., Chandra, G., 2013. Mosquito larvicidal and anti- microbial activity of synthesized nano-crystalline silver particles using leaves and green berry extract of *Solanum nigrum* L. (Solanaceae: Solanales). Acta. Trop. 128, 613–622.
Sap-Iam, N., Homklinchan, C., Larpudomlert, R., Warisnoicharoen, W., Sereemaspun, A., Dubas, S., 2010. UV irradiation-induced silver nanoparticles as mosquito larvicides. J. Appl. Sci. (Faisalabad) 10, 3132–3136.
Savithramma, N., Linga, R.M., Rukmini, K., Suvarnalatha, D.P., 2011. Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. Int. J. Chemtech Res. 3 (3), 1394–1402.
Saxena, A., Tripathi, R.M., Singh, R.P., 2010. Biological synthesis of silver nanoparticles by using onion *Allium cepa* extract and their antibacterial activity. Digest J. Nanomater. Biostruct. 5, 427–432.
Shankar, S.S., Rai, A., Ahmad, A., Sastry, M., 2004. Biosynthesis of silver and gold nanoparticles from extracts of different parts of the *Geranium* plant. Appl. Nanotechnol. 1, 69–77.
Singh, B.P., Hatton, B.J., Singh, B., Cowie, A.L., Kathuria, A., 2010. Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrastings oils. J. Environ. Qual. 39, 1–12.
Sivagnaname, N., Kalyanasundaram, M., 2004. Laboratory evaluation of methanolic extract of *Atlantia monophylla* (Family: Rutaceae) against immature stages of mosquitoes and non-target organisms. Mem. Inst. Oswaldo Cruz 99, 115–118.
Sivapriyajothi, S., Mahesh Kumar, P., Kovendan, K., Subramaniam, J., Murugan, K., 2014. Larvicidal and pupicidal activity of synthesized silver nanoparticles using *Leucas aspera* leaf extract against mosquito vectors, *Aedes aegypti* and *Anopheles stephensi*. J. Entomol. Acarol. Res. 46, 1787.
Sondi, I., Salopek-Sondi, B., 2004. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. J. Colloid Interface Sci. 275, 177–182.
Subarani, S., Sabhanayakam, S., Kamaraj, C., 2013. Studies on the impact of biosynthesized silver nanoparticles (Ag NPs) in relation to malaria and filariasis vector control against *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol. Res. 112, 487–499.
Thirunavokkarasu, M., Balaji, U., Behera, S., Panda, P.K., Mishra, B.K., 2013. Biosynthesis of silver nanoparticles from extract of *Desmodium gangeticum* (L.) DC. and its biomedical potential. Spectrochim. Acta Mol. Biomol. Spectrosc. 116, 424–427.
Veerakumar, K., Govindarajan, M., Rajeswary, M., Muthukumaran, U., 2014. Low-cost and eco-friendly green synthesis of silver nanoparticles using *Feronia elephantum* (Rutaceae) against *Culex quinquefasciatus*, *Anopheles stephensi*, and *Aedes aegypti* (Diptera: Culicidae). Parasitol. Res. 113, 1775–1785.
Veerekumar, K., Govindarajan, M., Rajeswary, M., 2013. Green synthesis of silver nanoparticles using *Sida acuta* (Malvaceae) leaf extract against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). Parasitol. Res. 112, 4073–4085.
Vivek, R., Thangam, R., Muthuchelian, K., Gunasekaran, P., Kaveri, K., Kannan, S., 2012. Green biosynthesis of silver nanoparticles from *Annona squamosa* leaf extract and its in vitro cytotoxic effect on MCF-7 cells. Process Biochem. 47, 2405–2410.
World Health Organization, 2005. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. Communicable Disease Control, Prevention and Eradication, WHO Pesticide Evaluation Scheme. WHO, Geneva WHO/CDS/WHOPES/GCDPP/1.3.
Zargar, M., Hamid, A.A., Bakar, F.A., Shamsudin, M.N., Shameli, K., Jahanshiri, F., Farahani, F., 2011. Green synthesis and antibacterial effect of silver nanoparticles using *Vitex negundo* L. Molecules 6, 6667–6676.