Environ Sci Pollut Res (2018) 25:10228–10242
DOI 10.1007/s11356-017-8482-y

Biophysical characterization of *Acacia caesia*-fabricated silver nanoparticles: effectiveness on mosquito vectors of public health relevance and impact on non-target aquatic biocontrol agents

Giovanni Benelli[1] · Shine Kadaikunnan[2] · Naiyf S. Alharbi[2] · Marimuthu Govindarajan[3]

Received: 3 August 2016 / Accepted: 18 January 2017 / Published online: 5 February 2017

Abstract Mosquito-borne diseases lead to serious public health concerns in tropical and sub-tropical countries worldwide, due to development of mosquito resistance to synthetic pesticides, non-target effects of pesticides, and socioeconomic reasons. Currently, green nanotechnology is a promising research field, showing a wide range of potential applications in vector control programs. The employ of natural products as reducing agents to fabricate insecticidal nanocomposites is gaining research attention worldwide, due to low costs and high effectiveness. Interestingly, biophysical features of green-synthesized nanoparticles strongly differ when different botanicals are employed for nanosynthesis. In this study, a cheap *Acacia caesia* leaf extract was employed to fabricate silver nanoparticles (Ag NPs) with ovicidal, larvicidal, and adulticidal toxicity against three mosquito vectors, *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*. Ag NPs were analyzed by various biophysical methods, including spectroscopy (UV-visible spectrophotometry, XRD, FTIR, EDX) and microscopy (SEM, TEM, AFM) techniques. High acute larvicidal potential was observed against larvae of *An. subpictus* (LC_{50} = 10.33 μg/ml), *Ae. albopictus* (LC_{50} = 11.32 μg/ml), and *Cx. tritaeniorhynchus* (LC_{50} = 12.35 μg/ml). Ag NPs completely inhibited egg hatchability on three vectors at 60, 75, and 90 μg/ml, respectively. In adulticidal assays, LD_{50} values were 18.66, 20.94, and 22.63 μg/ml. If compared to mosquito larvae, Ag NPs were safer to three non-target aquatic biocontrol agents, with LC_{50} ranging from 684 to 2245 μg/ml. Overall, our study highlights the potential of *A. caesia* as an abundant and cheap bioresource to fabricate biogenic Ag NPs effective against mosquito young instars and adults, with moderate impact on non-target aquatic biocontrol agents.

Keywords Biological control · Chikungunya · Dengue · Japanese encephalitis · Mosquito control · Non-target predators

Responsible editor: Markus Hecker

✉ Giovanni Benelli
benelli.giovanni@gmail.com

✉ Marimuthu Govindarajan
drgovind1979@gmail.com

[1] Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy

[2] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

[3] Unit of Vector Control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalai Nagar, Tamil Nadu 608 002, India

Introduction

Arthropods are important vectors of pathogens and parasites of public health relevance (Benelli et al. 2016a, b). In particular, recent outbreaks of mosquito-borne diseases such as dengue, chikungunya, West Nile, Japanese encephalitis, and Zika virus underlined the key role of mosquito control strategies (Benelli and Mehlhorn 2016; Ward and Benelli 2017). However, the application of chemical insecticides leads to pesticide resistance, toxicity to non-target organisms, as well as environmental and human health concerns (Mehlhorn et al. 2012; Naqqash et al. 2016; Govindarajan et al. 2016a, b, c, d, e). Therefore, current research focuses on biopesticides and repellents formulated using plant extracts and essential oils as novel and safer alternatives for mosquito control (Maguranyi et al. 2009; Mathew et al. 2009; Semmler et al. 2009; Zhu and Tian 2011, 2013; Benelli 2015a, b; Benelli et al. 2014, 2015a,

b, c; Govindarajan 2016; Pavela and Benelli 2016a, b; Govindarajan and Benelli 2016a, b, c).

Recently, green synthesis of metal nanoparticles using cheap and abundant plant extracts and related metabolites showed a number of important advantages, ranging from reduced energy inputs to avoidance of highly toxic chemical for nanosynthesis (Bar et al. 2009; Parashar et al. 2009; Zhang et al. 2011; Vivek et al. 2012; Benelli 2016a, b, c, d; Yugandhar and Savithramma 2016). In particular, silver is well known for its antimicrobial activity, widely exploited in medicine and industrial processes (Reda et al. 2011; Zargar et al. 2011; Vijayakumar et al. 2013). Among biological methods, the use of plants for synthesis of metal nanoparticles attracts wide research attention since it is a quick, cheap, and one-step method (Kowshik et al. 2003; Madhiyazhagan et al. 2015; Murugan et al. 2015a, b, c; Roni et al. 2015; Subramaniam et al. 2015; Sujitha et al. 2015; Benelli 2016a, b, c, d; Chandramohan et al. 2016).

Green-synthesized Ag NPs have been produced using extracts from a wide number of plant species as reducing and capping agents. Good examples are *Emblica officinalis* (Ankamwar et al. 2005), *Aloe vera* (Chandran et al. 2006), *Cinnamomum camphora* (Huang et al. 2007), *Cinnamon zeylanicum* (Sathishkumar et al. 2009a), *Azadirachta indica* (Tripathi et al. 2009), *Glycine max* (Vivekanandhan et al. 2009), *Camellia sinensis* (Begum et al. 2009), *Ocimum sanctum* (Ahmad et al. 2010), *Pongamia pinnata* (Raut et al. 2010), *Allium sativum* (Rastogi and Arunachalam 2011), *Tagetes erecta* (Krishnamurthy et al. 2012), *Cocos nucifera* (Roopan et al. 2013), *Desmodium gangeticum* (Thirunavokkarasu et al. 2013), *Andrographis paniculata* (Kotakadi et al. 2014), and *Hibiscus sabdariffa* (Thovhogi et al. 2015). Effective mosquitocidal activity on *Anopheles stephensi*, *Anopheles subpictus*, *Aedes aegypti*, *Aedes albopictus*, *Culex tritaeniorhynchus*, and *Culex quinquefasciatus* has been reported for Ag NPs fabricated using extracts from *Eclipta prostrata* (Rajakumar and Abdul Rahuman 2011), *Plumeria rubra* (Patil et al. 2012a), *Pergularia daemia* (Patil et al. 2012b), *Drypetes roxburghii* (Haldar et al. 2013), *Sida acuta* (Veerakumar et al. 2013), *Pongamia pinnata* (Naik et al. 2014), *Feronia elephantum* (Veerakumar et al. 2014a), *Leucas aspera* (Suganya et al. 2014), *Heliotropium indicum* (Veerakumar et al. 2014b), *Bauhinia variegata* (Govindarajan et al. 2016f), *Clerodendrum chinense* (Govindarajan et al. 2016g), *Malva sylvestris* (Govindarajan et al. 2016h), *Mussaenda glabra* (Govindarajan et al. 2016i), and *Zornia diphylla* (Govindarajan et al. 2016c). In addition, the efficacy of green-synthesized Ag NPs has been reported as effective in reducing young instars populations of mosquito vectors even in the field (Suresh et al. 2015).

Interestingly, the biophysical features and bioactivity of green-fabricated NPs strongly differ when different botanicals

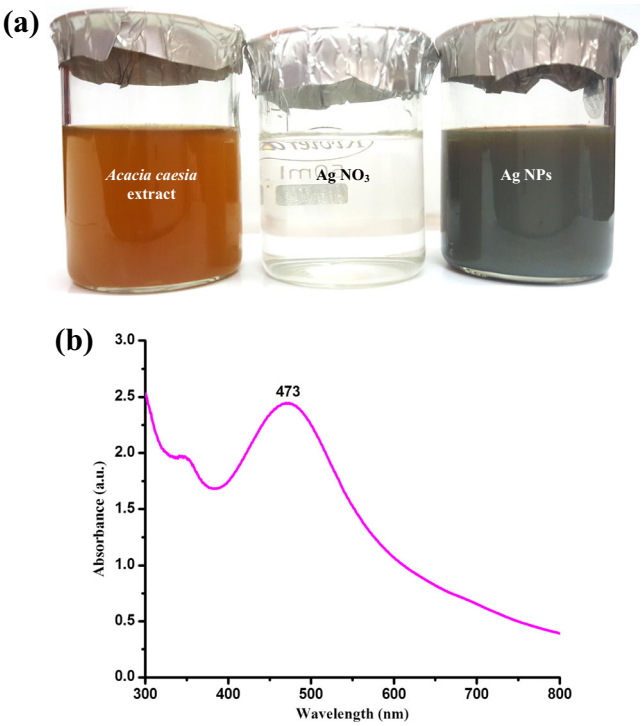

Fig. 1 **a** Color intensity of *Acacia caesia* aqueous extract before and after the reduction of silver nitrate (1 mM). **b** UV-visible spectrum of Ag nanoparticles after 180 min

are employed for nanosynthesis, highlighting the importance of screening local botanical resources for the biofabrication of nanomosquitocides (Benelli 2016a, b). *Acacia caesia* (L.) Willd. (Mimosaceae) is widely distributed in hills of the Western Ghats, around the altitude of 500 m a.s.l. Medicinal value of this plant lies in bioactive phytochemical constituents (Pullaiah 2006) including alkaloids, flavonoids, glycosides, and saponins. *A. caesia* extracts exhibited antimicrobial and antioxidant activity (Sathishkumar et al. 2009b). Leaves are used in the treatment of asthma, skin diseases (Paulsamy et al. 2010), menstrual disorder (Pullaiah 2006), and scabies (Thambiraj and Paulsamy 2010). To our best of knowledge, the mosquitocidal properties of *A. caesia* have not been investigated.

In this research, *A. caesia* leaf extract was used to synthesize Ag NPs with ovicidal, larvicidal, and adulticidal toxicities against three mosquito vectors, malaria vector *An. subpictus*, chikungunya and Zika virus vector *Ae. albopictus*, and Japanese encephalitis vector *Cx. tritaeniorhynchus*. Ag NPs were analyzed by various biophysical methods, including spectroscopy (UV-visible spectrophotometry, XRD, FTIR, EDX) and microscopy (SEM, TEM, atomic force microscopy (AFM)) analyses. Furthermore, toxicity of *A. caesia* aqueous leaf extract and *A. caesia*-fabricated Ag NPs was evaluated on three non-target biological control agents, i.e., *Anisops bouvieri*, *Diplonychus indicus*, and *Gambusia affinis*, which predate on Culicidae young instars.

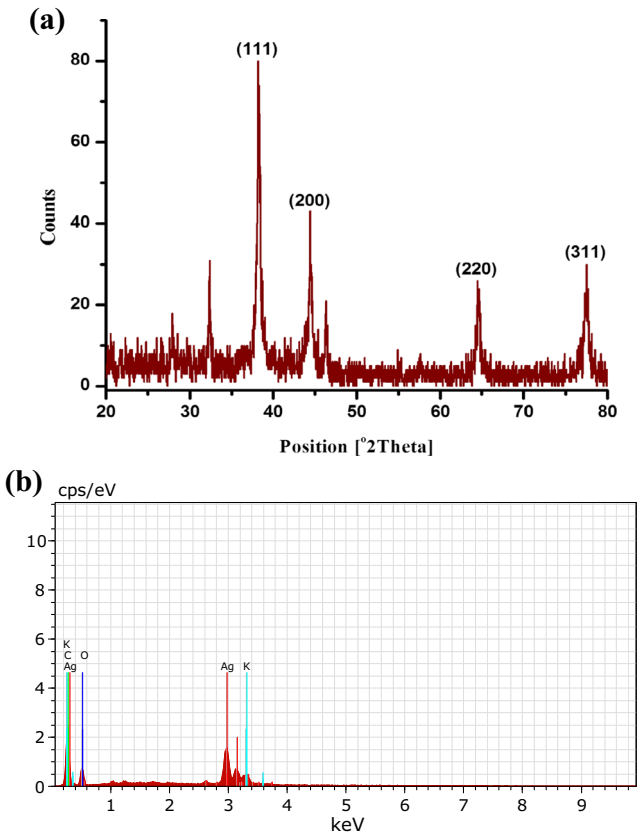

Fig. 2 **a** XRD pattern of Ag nanoparticles rapidly synthesized employing the *Acacia caesia* aqueous extract. **b** EDX spectrum of green-fabricated Ag nanoparticles

Materials and methods

Collection of materials

Analytical grade $AgNO_3$ was from Merck (India), glassware was washed with acid and rinsed afterwards with Millipore Milli-Q water. *A. caesia* leaves were collected from Western Ghats, India (10° 10′ 23.1″ N 77° 03′ 41.5″ E) in June 2016. Plant taxonomic identification was conducted by Prof. V. Venkatesalu, Annamalai University, India.

Preparation of *A. caesia* leaf extracts

Leaves were thoroughly washed with tap water, rinsed with Millipore-Milli-Q water, and left to dry out in the shade, before grinding them to fine powder. Aqueous extract was prepared by adding 50 g of the aforementioned leaf powder to 0.5 l of distilled water, under continuous stirring. After 3 h, the suspension was left for 3 h and then filtered using a Whatman no. 1 filter paper. The filtrate was stored at 10 °C until the assays.

Biogenic synthesis and characterization of silver nanoparticles

Ten milliliters of the *A. caesia* leaf extract were mixed with 90 ml of 1 mM $AgNO_3$ solution and heated in a water bath, set at 80 °C for 10 min. A color change from yellow to brown designates the formation of colloidal Ag NPs. To track formation of Ag NPs, a UV-Vis spectrophotometry (UV-160v) was used, with 200–800 nm wavelength and a 1-nm resolution

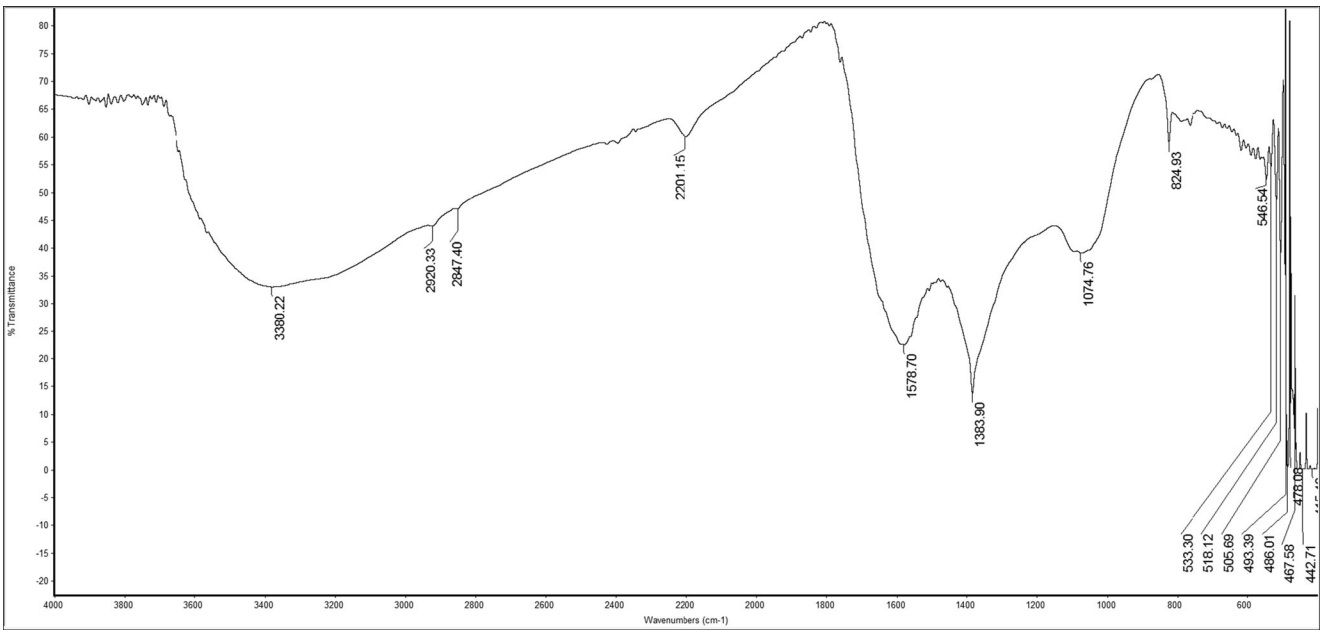

Fig. 3 FTIR spectrum of biogenic Ag nanoparticles synthesized using the *Acacia caesia* aqueous leaf extract

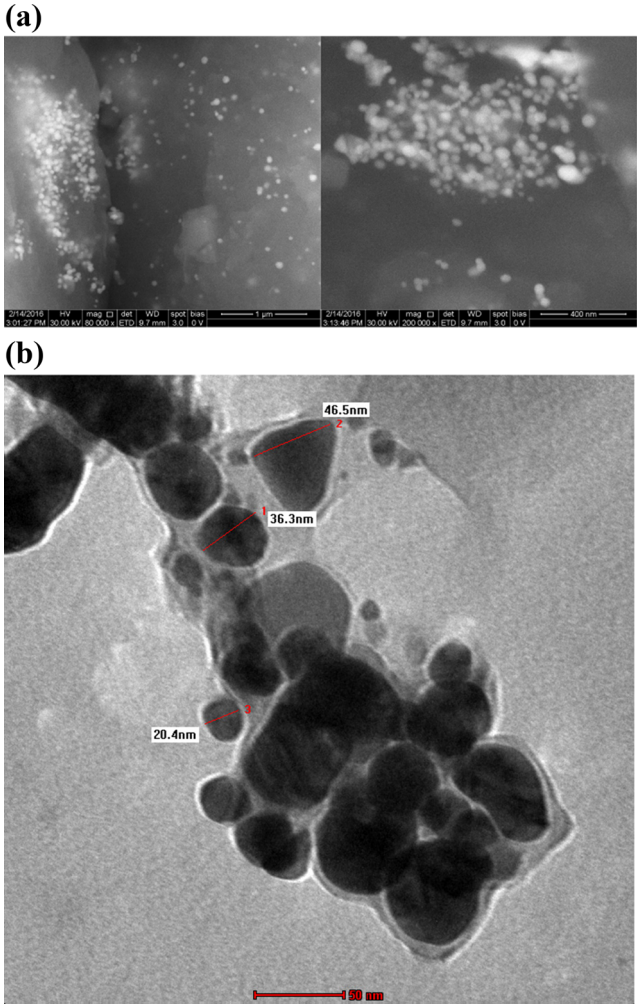

Fig. 4 SEM and TEM micrographs of *Acacia caesia*-synthesized Ag nanoparticles

(Shimadzu UV 1700, Japan). To examine size, morphology, and composition of the resulting Ag NPs, EDX, TEM (Technite 10 Philips), AFM (Agilent Technologies AFM-5500), and SEM (Hitachi S3000 H) were used. To determine whether the purified Ag NPs were capped by *Acacia* extract-borne metabolites, FTIR spectroscopy (Thermo Scientific Nicolet 380) was employed, while XRD shed light on the possible presence of Ag nanocrystals.

Mosquito rearing

Pathogen- and parasite-free species of the three mosquito species were reared continuously for several generations at Annamalai University (Govindarajan and Benelli 2016a). They were maintained at 28 ± 2 °C, 70–85% R.H., with a photoperiod of 12-h light and 12-h dark. To feed the larvae, a mixture of yeast powder and dog biscuits at a ratio of 1 to 3 was used. When adult feeding was about to take place, 3 to 4 days had passed after emergence, and the subjects were fed only on raisins and water during that time, whereas a 12-h starvation period preceded feeding. Adult feeding involved 500 mosquitoes per cage being fed on blood for 4 h, via a Parafilm-fitted feeding unit. *Ae. albopictus* were fed during the 12.00 to 16.00 h period, whereas *An. subpictus* and *Cx. tritaeniorhynchus* were from 18.00 to 22.00 h (see Govindarajan and Benelli 2016a, b).

Larvicidal activity

The protocol by World Health Organization (2005) was employed to assess whether *A. caesia* aqueous extract and biosynthesized Ag NPs exerted acute larvicidal activity on the three mosquito vectors. During the experiment, 20 late III instar larvae were added in 250 ml of dechlorinated water plus the leaf extract (five doses, ranging from 60 to 300 μg ml^{-1}, in 60 μg ml^{-1} increments) or Ag NPs (five doses, from 5 to 25 μg ml^{-1}, in 5 μg ml^{-1} increments); for each tested dose, 5 replicates were conducted. Mortality was assessed after 24 h, during which the larvae were not fed. For every test, a corresponding test of control groups was conducted, containing $AgNO_3$ and distilled water, with five repetitions (Govindarajan and Benelli 2016b).

Ovicidal activity

In agreement with Su and Mulla (1998), for each mosquito vector, 100 eggs were exposed to six concentrations of the leaf extract, ranging from 150 to 900 μg ml^{-1} in 150 μg ml^{-1} increments, and an equal number of silver nitrate concentrations, ranging from 15 to 90 μg ml^{-1} in 15 μg ml^{-1} increments. After exposure for 24 h, the eggs were counted under the microscope and then assessed for hatching after being transferred to cups containing distilled water. Six repetitions per each concentration were carried out, accompanied by untreated controls, and estimated the hatch rates 48 h after treatment by using the following equation:

$$\text{Hatchability}\ (\%) = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} \times 100$$

Adulticidal activity

To assess the adulticidal activity of extract and Ag NPs, the World Health Organization (1981) standard method was followed. Several concentrations of both the leaf extract and Ag NPs, in the range of 9–500 μg ml^{-1}, were tested, following the same process that was described previously, using 12 × 15 cm-sized Whatman no. 1 filter papers treated with 5 ml of aqueous solution (Govindarajan and Sivakumar 2011). Control papers were treated with distilled water or aqueous $AgNO_3$. Twenty female mosquitoes were selected and gently inserted them into a plastic container. Mosquitoes were let

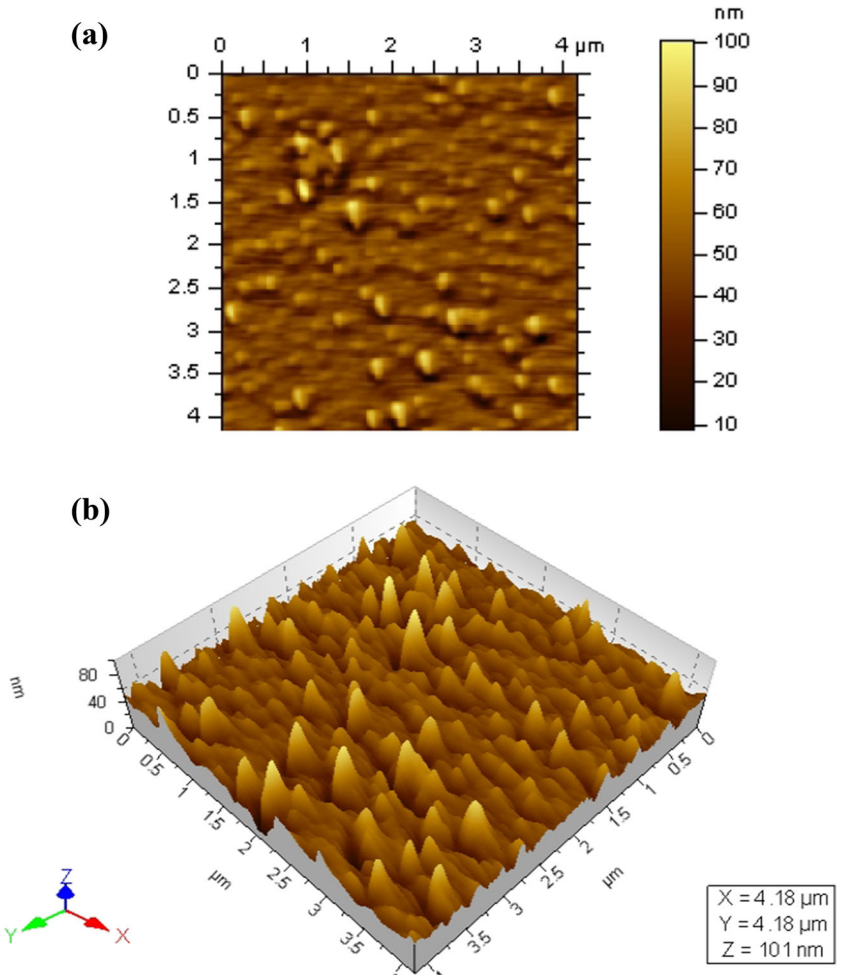

Fig. 5 AFM micrograph of Ag nanoparticles fabricated using the aqueous extract of *Acacia caesia* leaves. **a** Studies of spherical polydispersed particles (2.5-µm resolution). **b** 3D image of Ag nanoparticles analyzed by NOVA-TX software. **c** Histogram showing the particle size distribution. **d** Line graph showing the size distribution of nano-Ag

acclimatize in the container for 1 h, followed by exposure to the test paper for another hour. When the exposure period ended, we returned the test subjects to the plastic container and left them for 24 h to recover. After the recovery period, a piece of cotton soaked in a 10% glucose solution was inserted in the mesh screen, then we assessed the mosquito mortality and repeated each experiment five times.

Toxicity on non-target aquatic insects and fishes

Following Sivagnaname and Kalyanasundaram (2004) with minor changes by Govindarajan and Benelli (2016b), we evaluated the acute toxicity of aqueous leaf extract and Ag NPs on *A. bouvieri*, *D. indicus*, and *G. affinis*. We tested concentrations of leaf extract and Ag NPs up to 50 times higher than the calculated LC_{50} for the mosquito larvae, and the experiment for every test concentration was repeated 10 times, accompanied by four repetitions for the corresponding untreated controls. Then, mortality was noted and any other abnormal behavior (e.g., sluggishness or decreased swimming activity) was also observed after 48 h of exposure. After exposure, survival rates and swimming activity were monitored for ten additional days in order to investigate the incidence of any residual post-treatment effect of extract and Ag NPs.

Data analysis

Probit analysis was used to investigate the mortality data (Benelli 2017). The method by Finney (1971) was used to determine LC_{50} (LD_{50}) and LC_{90} (LD_{90}). To assess the toxicity of the extract and silver nanoparticles on non-target organisms, suitability index (SI) was estimated for every individual non-target species via the formula below (Deo et al. 1988).

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

The SPSS Statistical Software Package version 16.0 was used for data analysis.

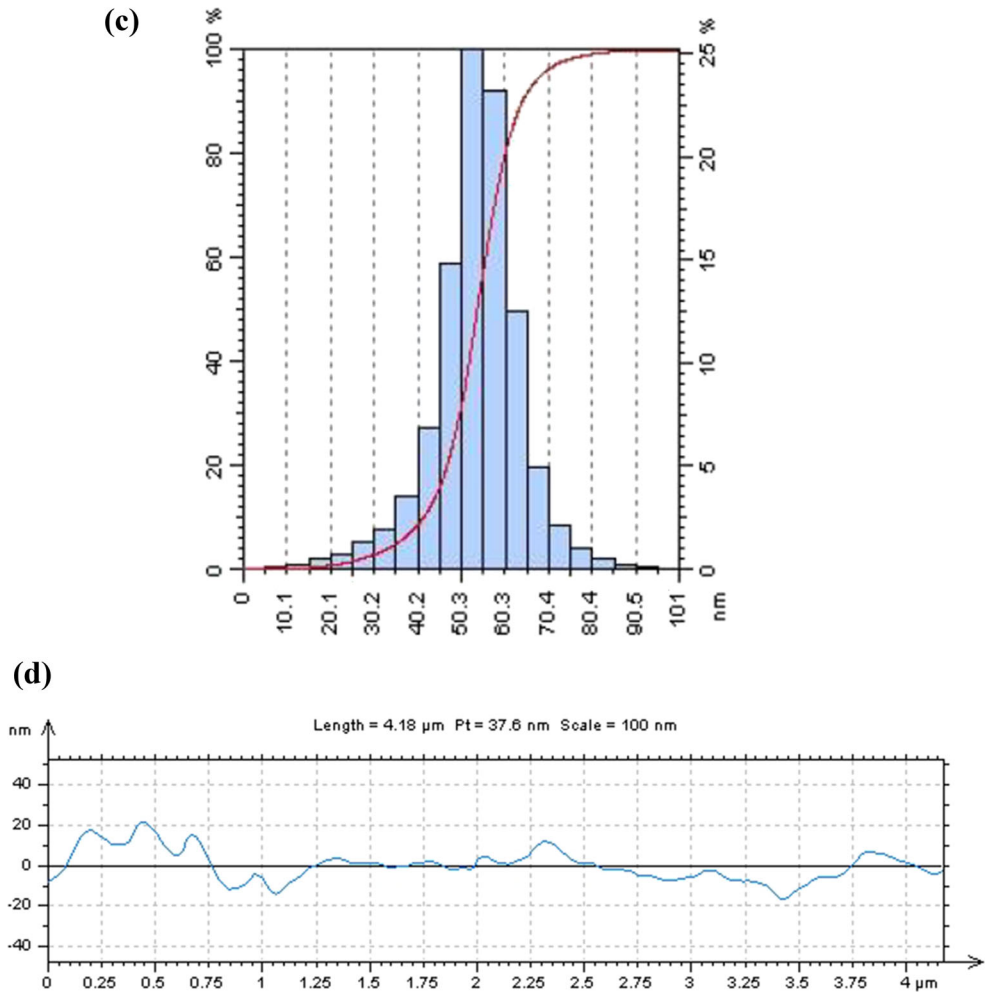

Fig. 5 (continued)

Results and discussion

Biophysical characterization of silver nanoparticles

When *A. caesia* leaf extract was mixed with aqueous solution of Ag^+ ions, the color of plant extract changed from yellow to dark brown within 180 min (Fig. 1a) (see also Sathishkumar et al. 2009a, b). Figure 1b shows the UV-Vis spectrum of Ag NP suspension, showing a main absorption peak at 473 nm. The presence of broad resonance indicated an aggregated structure of Ag NPs in the suspension (Rashmi and Preeti 2009; Jayaseelan et al. 2011). Furthermore, Ag NPs fabricated in the present investigation showed a crystalline structure, as evidenced by peaks at 2θ values of 38.52°, 43.64°, 65.37°, and 78.62° corresponding to 111, 200, 220, and 311 facets of face-centered cubic crystalline structure (Fig. 2a) (Kalishwaralal et al. 2008; Marimuthu et al. 2011; Kumar et al. 2012). The presence of elemental nanosilver in the material was confirmed by EDX analysis (Fig. 2b). Indeed, Ag NPs exhibited typical optical absorption peak at 3 keV, due to surface plasmon resonance (SPR) (Magudapatty et al. 2001; Dinesh et al. 2015).

FTIR analysis was carried out to identify the possible bioreducing molecules in the leaf extract of *A. caesia*. Figure 3 shows the FTIR spectrum of Ag NPs synthesized from the aqueous leaf extract of *A.* caesia. The intense peak at 3380.22 cm^{-1} may be ascribed to the O-H stretch of alcohols or phenols (Gopinath et al. 2013; Velayutham et al. 2013). The peak at 2920.33 and 2847.40 cm^{-1} probably indicate to CH stretching (Sanghi and Verma 2009; Ramanibai and Velayutham 2015). The band at 1578.70 cm^{-1} may correspond to carboxylic group N-H bending (Shivshankar et al. 2003). The peak at 1383.90 cm^{-1} may be assigned to C-O stretching while 824.93 cm^{-1} could indicate to C-Cl stretching alkyl halides. The peak at 546.54 cm^{-1} was probably linked to C-H stretching strong vinyl di-substituted alkenes (Angajala et al. 2014). Overall, the carbonyl and hydroxyl groups present in tannins, flavonoids, alkaloids, steroids, and glycosides are the main groups responsible for reducing Ag^+ to Ag^0 as well as capping and stabilizing the Ag NPs.

Table 1 Acute toxicity of the *Acacia caesia* aqueous leaf extract on third instar larvae of *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquito species	Concentration (μg/ml)	Mortality (%) ± SD	LC_{50} (μg/ml) (95% LCL-UCL)	LC_{90} (μg/ml) (95% LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Anopheles subpictus	60	26.4 ± 1.2	125.09 (111.45–137.25)	241.68 (224.43–264.25)	2.90	$y = 9.91 + 0.312x$	4.83 (4) n.s.
	120	48.2 ± 0.8					
	180	67.5 ± 0.6					
	240	88.1 ± 0.4					
	300	100.0 ± 0.0					
Aedes albopictus	60	22.1 ± 0.8	135.82 (122.32–148.08)	257.14 (238.98–280.93)	2.66	$y = 5.39 + 0.32x$	2.75 (4) n.s.
	120	45.6 ± 0.6					
	180	63.8 ± 1.2					
	240	84.5 ± 0.4					
	300	98.5 ± 0.8					
Culex tritaeniorhynchus	60	19.3 ± 0.4	147.35 (133.90–159.83)	274.31 (254.83–300.00)	2.49	$y = 1.7 + 0.32x$	2.04 (4) n.s.
	120	41.7 ± 0.6					
	180	59.4 ± 1.2					
	240	80.3 ± 0.4					
	300	96.1 ± 0.8					

A. caesia-synthesized Ag NPs were characterized by SEM and TEM analyses. The nanoparticles were predominantly spherical in shape (Fig. 4a). Our results on spherical shapes of green-synthesized Ag NPs are in agreement with Elavazhagan and Arunachalam (2011) as well as to Suresh et al. (2015), while a limited number of reported showed the synthesis of cubic or flat Ag NPs (recently reviewed by Benelli 2016a). In addition, TEM (Fig. 4b) confirmed that nanoparticles are spherical in shape and polydispersed without significant agglomeration; mean sizes ranged from 20 to 46 nm (Krishnamurthy et al. 2012; Kotakadi et al. 2014). AFM data analyzed by NOVA-TX software highlighted that *A. caesia*-synthesized Ag NPs showed a size ranging from 10.1 to 90.5 nm, with most of them falling within the range 40.2 to 70.4 nm (Fig. 5). At variance with our results, AFM studies on *Hymenodictyon oxirense*, *Carissa carandas*, and *M. sylvestris*-fabricated Ag NPs showed lower size ranges (i.e., mean size of 0.7–2.93, 1.65–7.44, and 3.5–13.9 nm, respectively) (Govindarjan and Benelli 2016b, c; Govindarajan et al. 2016h). This highlights the key role of screening different plant products as sources of reducing agents for nanosynthesis of mosquitocidal products.

Toxicity against mosquito vectors

The employ of natural products as reducing agents to fabricate insecticidal nanocomposites is gaining research attention worldwide, due to low costs and high effectiveness (Benelli 2016a, b). In our study, the *A. caesia* leaf extract and synthesized Ag NPs exhibited dose-dependent acute larvicidal

Table 2 Acute toxicity of silver nanoparticles fabricated using the *Acacia caesia* leaf extract on third instar larvae of *Anopheles subpictus*, *Aedes albopictus*, and t*Culex tritaeniorhynchus*

Mosquito species	Concentration (μg/ml)	Mortality (%) ± SD	LC_{50} (μg/ml) (95% LCL-UCL)	LC_{90} (μg/ml) (95% LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Anopheles subpictus	5	27.5 ± 0.4	10.33 (9.19–11.35)	20.08 (18.64–21.97)	2.98	$y = 10.51 + 3.718x$	5.64 (4) n.s.
	10	48.3 ± 1.2					
	15	66.4 ± 0.8					
	20	89.2 ± 0.6					
	25	100.0 ± 0.0					
Aedes albopictus	5	23.4 ± 0.4	11.32 (10.18–12.34)	21.50 (19.97–23.51)	2.70	$y = 5.67 + 3.806x$	2.91 (4) n.s.
	10	44.3 ± 0.8					
	15	62.8 ± 0.6					
	20	85.2 ± 1.2					
	25	98.1 ± 0.8					
Culex tritaeniorhynchus	5	19.6 ± 0.6	12.35 (11.22–13.40)	23.07 (21.42–25.25)	2.52	$y = 1.71 + 3.822x$	1.97 (4) n.s.
	10	41.3 ± 0.8					
	15	58.4 ± 12.					
	20	80.2 ± 0.4					
	25	95.7 ± 0.8					

Table 3 Ovicidal effectiveness of the *Acacia caesia* aqueous leaf extract on *Anopheles subpictus*, t*Aedes albopictus*, and t*Culex tritaeniorhynchus*

Mosquito species	Egg raft age (h)	Egg hatchability (%)							*F* value (*d.f.*)	*P* value
		Control	150 μg/ml	300 μg/ml	450 μg/ml	600 μg/ml	750 μg/ml	900 μg/ml		
Anopheles subpictus	0–6	100 ± 0.0a	29.6 ± 1.0b	17.5 ± 1.2c	NH	NH	NH	NH	165.94 (5)	<0.001
	6–12	100 ± 0.0a	37.2 ± 1.5b	20.2 ± 1.5c	NH	NH	NH	NH	157.31 (5)	<0.001
	12–18	100 ± 0.0a	48.2 ± 1.2b	31.6 ± 1.4c	16.3 ± 1.2d	NH	NH	NH	138.47 (5)	<0.001
Aedes albopictus	0–6	100 ± 0.0a	54.3 ± 1.4b	36.4 ± 1.3c	20.2 ± 1.5d	NH	NH	NH	187.35 (5)	<0.001
	6–12	100 ± 0.0a	58.6 ± 1.5b	40.2 ± 1.2c	22.5 ± 1.8d	NH	NH	NH	162.74 (5)	<0.001
	12–18	100 ± 0.0a	64.3 ± 1.6b	51.7 ± 1.4c	33.4 ± 1.6d	17.5 ± 1.2e	NH	NH	139.28 (5)	<0.001
Culex tritaeniorhynchus	0–6	100 ± 0.0a	68.5 ± 1.1b	53.8 ± 1.2c	28.6 ± 1.3d	15.4 ± 1.0e	NH	NH	158.36 (5)	<0.001
	6–12	100 ± 0.0a	77.2 ± 1.3b	58.3 ± 1.5c	34.3 ± 1.4d	18.3 ± 1.2e	NH	NH	149.79 (5)	<0.001
	12–18	100 ± 0.0a	86.5 ± 1.2b	65.7 ± 1.4c	48.6 ± 1.2d	32.2 ± 1.2e	16.2 ± 1.5f	NH	161.72 (5)	<0.001

Within each row, different letters indicate significant differences among values (ANOVA, Tukey's HSD, $P < 0.001$)

NH no hatchability

efficacy against all tested mosquito species (Tables 1 and 2, respectively). Compared to the aqueous extract, Ag NPs exhibited high effectiveness against *An. subpictus* (LC_{50} = 10.33 μg/ml) followed by *Ae. albopictus* (LC_{50} = 11.32 μg/ml), and *Cx. tritaeniorhynchus* (LC_{50} = 12.35 μg/ml) (Table 2). In ovicidal assays, egg hatchability was inversely proportional to the concentration of extract and Ag NPs and directly proportional to the eggs (Tables 3 and 4). Ag NPs completely inhibited egg hatchability on the three vectors at 60, 75, and 90 μg/ml, respectively. Control eggs showed 100% hatchability. Results of the adulticidal activity of *A.* caesia leaf extract and synthesized Ag NPs are presented in Tables 5 and 6. Highest adulticidal activity was observed for green-synthesized Ag NPs against *An. subpictus* (LD_{50} = 18.66 μg/ml), *Ae. albopictus* (LD_{50} = 20.94 μg/ml), and *Cx. tritaeniorhynchus* (LD_{50} = 22.63 μg/ml) (Tables 5 and 6).

In latest years, a growing number of green-fabricated metal nanoparticles have been produced for mosquitocidal purposes, showing that the biophysical properties of the produced nanoparticles are strongly affected by the botanicals used as reducing agents (Murugan et al. 2016a, b; Suresh et al. 2015; Panneerselvam et al. 2016; Subramaniam et al. 2016; see Benelli 2016a, b for

Table 4 Ovicidal effectiveness of silver nanoparticles fabricated using the *Acacia caesia* leaf extract on *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquito species	Egg raft age (h)	Egg hatchability (%)							*F* value (*d.f.*)	*P* value
		Control	15 μg/ml	30 μg/ml	45 μg/ml	60 μg/ml	75 μg/ml	90 μg/ml		
Anopheles subpictus	0–6	100 ± 0.0a	29.4 ± 1.2b	16.5 ± 1.3c	NH	NH	NH	NH	112.62 (5)	<0.001
	6–12	100 ± 0.0a	38.8 ± 1.5b	21.3 ± 1.4c	NH	NH	NH	NH	128.38 (5)	<0.001
	12–18	100 ± 0.0a	47.4 ± 1.8b	34.5 ± 1.5c	18.4 ± 1.7d	NH	NH	NH	135.28 (5)	<0.001
Aedes albopictus	0–6	100 ± 0.0a	51.5 ± 1.3b	35.3 ± 1.3c	17.6 ± 1.2d	NH	NH	NH	165.37 (5)	<0.001
	6–12	100 ± 0.0a	57.2 ± 1.1b	36.2 ± 0.8c	21.4 ± 1.8d	NH	NH	NH	128.76 (5)	<0.001
	12–18	100 ± 0.0a	66.3 ± 1.2b	51.4 ± 1.2c	35.7 ± 1.3d	17.5 ± 1.2e	NH	NH	162.79 (5)	<0.001
Culex tritaeniorhynchus	0–6	100 ± 0.0a	69.5 ± 1.6b	53.3 ± 1.5c	36.5 ± 1.4d	18.2 ± 1.4e	NH	NH	116.34 (5)	<0.001
	6–12	100 ± 0.0a	77.4 ± 1.4b	57.2 ± 0.2c	39.8 ± 1.5d	21.4 ± 1.3e	NH	NH	135.71 (5)	<0.001
	12–18	100 ± 0.0a	85.2 ± 1.0b	67.6 ± 0.8c	48.3 ± 1.4d	29.4 ± 1.6e	19.5 ± 1.2f	NH	128.96 (5)	<0.001

Within each row, different letters indicate significant differences among values (ANOVA, Tukey's HSD, $P < 0.001$)

NH no hatchability

Table 5 Adulticidal effectiveness of *Acacia caesia* aqueous leaf extract on *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquito species	Concentration (μg/ml)	Mortality (%) ± SD	LD_{50} (μg/ml) (95% LCL-UCL)	LD_{90} (μg/ml) (95% LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Anopheles subpictus	100	28.2 ± 1.4	209.19 (185.63–230.08)	411.56 (381.61–451.00)	3.18	$y = 10.59 + 0.184x$	5.76 (4) n.s.
	200	46.5 ± 0.5					
	300	67.1 ± 1.2					
	400	86.4 ± 1.5					
	500	100.0 ± 0.0					
Aedes albopictus	100	24.2 ± 1.5	233.03 (209.53–254.40)	449.24 (416.42–492.76)	2.87	$y = 5.49 + 0.186x$	2.84 (4) n.s.
	200	41.3 ± 1.2					
	300	62.5 ± 0.8					
	400	81.4 ± 1.2					
	500	97.2 ± 0.6					
Culex tritaeniorhynchus	100	19.4 ± 1.2	255.55 (232.75–276.93)	476.28 (441.73–522.20)	2.50	$y = 0.59 + 0.19x$	1.07 (4) n.s.
	200	38.2 ± 0.6					
	300	57.3 ± 0.8					
	400	78.5 ± 1.3					
	500	94.1 ± 1.5					

reviews). Unfortunately, knowledge on their mechanism(s) of action is patchy and still requires further research (Benelli 2016b, d). In addition, scarce information is available on the ovicidal potential of metal nanoparticles fabricated using plant-borne products. On the other hand, more knowledge has been recently reported concerning the larvicidal and adulticidal potential of nanoformulated mosquitocidals (Veerakumar et al. 2014a, b; see reviews by Benelli 2016a, d). For instance, Raman et al. (2012) reported on the larvicidal activity of Ag NPs biosynthesized using *Pithecellobium dulce*. These nanoparticles showed effective larvicidal activity against the filariasis vector *C. quinquefasciatus* (LC_{50} = 21.56 mg l^{-1}) due to high surface-to-volume ratio. Ag NPs biosynthesized using the aqueous extract of *Solanum nigrum* showed LC_{50} values of 1.33, 1.59, and 1.56 ppm for dry leaves, fresh leaves, and berries on the malaria vector *An. stephensi* (Rawani et al. 2013). Veerekumar et al. (2013) investigated the toxicity of Ag NPs synthesized from *S. acuta* leaves and their larvicidal activity on *An. stephensi* (LC_{50} = 21.92 μg ml^{-1}), *Ae. aegypti* (LC_{50} = 23.96 μg ml^{-1}), and *Cx. quinquefasciatus* (LC_{50} = 26.13 μg ml^{-1}). Later on, Kumar et al. (2014) investigated the larvicidal potential of the *Morinda tinctoria* leaf aqueous extract and biosynthesized Ag NPs against the larvae of *Cx. quinquefasciatus*. Ag NP LC_{50} was 1.442 ppm against *Cx. quinquefasciatus*. Soni and Prakash (2014) reported the synthesis of effective Ag NPs using the leaf and bark extract of neem, *A. indica*. The biosynthesized Ag NPs were tested as larvicides, pupicides, and adulticides against *An. stephensi* and *Cx. quinquefasciatus*. The larvae of *Cx. quinquefasciatus* showed a 100% mortality rate after 30 min of exposure. In pupicidal assays against *Cx. quinquefasciatus*, the LC_{50} value

Table 6 Adulticidal effectiveness of silver nanoparticles fabricated using the *Acacia caesia* leaf extract on *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*

Mosquito species	Concentration (μg/ml)	Mortality (%) ± SD	LD_{50} (μg/ml) (95% LCL-UCL)	LD_{90} (μg/ml) (95% LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Anopheles subpictus	9	29.5 ± 1.5	18.66 (16.45–20.61)	37.58 (34.78–41.29)	3.68	$y = 12.01 + 1.988x$	6.67 (4) n.s.
	18	47.4 ± 0.6					
	27	66.2 ± 1.8					
	36	85.3 ± 0.5					
	45	100.0 ± 0.0					
Aedes albopictus	9	23.5 ± 1.8	20.94 (18.89–22.81)	39.73 (36.88–43.49)	2.68	$y = 4.64 + 2.107x$	4.08 (4) n.s.
	18	42.2 ± 1.2					
	27	61.3 ± 0.6					
	36	82.4 ± 0.4					
	45	98.2 ± 1.5					
Culex tritaeniorhynchus	9	20.5 ± 0.6	22.63 (20.59–24.54)	42.22 (39.17–46.27)	2.51	$y = 1.12 + 2.113x$	2.61 (4) n.s.
	18	39.2 ± 1.5					
	27	56.4 ± 1.2					
	36	79.2 ± 0.8					
	45	95.6 ± 1.6					

Table 7 Acute toxicity of the *Acacia caesia* aqueous leaf extract on three non-target aquatic biocontrol organisms predating mosquito young instars

Non-target organism	Concentration (μg/ml)	Mortality (%) ± SD	LC_{50} (μg/ml) (95% LCL-UCL)	LC_{90} (μg/ml) (95% LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Gambusia affinis	12,000	20.4 ± 0.8	27,277.05 (18,642.73–33,820.03)	51,065.76 (42,854.16–69,001.45)	2.54	$y = 4.96 + 0.002x$	8.28 (9) n.s.
	24,000	46.9 ± 0.8					
	36,000	66.2 ± 1.0					
	48,000	80.5 ± 1.0					
	60,000	100.0 ± 0.0					
Diplonychus indicus	5000	22.8 ± 0.8	11,063.27 (9961.10–12,062.30)	20,815.73 (19,359.76–22,716.20)	2.59	$y = 6.16 + 0.004x$	5.74 (9) n.s.
	10,000	45.6 ± 1.2					
	15,000	67.4 ± 0.6					
	20,000	83.6 ± 0.6					
	25,000	100.0 ± 0.0					
Anisops bouvieri	4000	21.7 ± 0.8	9012.19 (8164.27–9789.24)	16,581.63 (15,445.22–18,058.18)	2.39	$y = 4.14 + 0.005x$	5.20 (9) n.s.
	8000	44.3 ± 1.0					
	12,000	65.6 ± 0.6					
	16,000	84.9 ± 1.0					
	20,000	100.0 ± 0.0					

was 4 μg ml^{-1}. In the case of adult mosquitoes, LC_{50} of 1.06 μL cm^{-2} was obtained after 4 h of exposure. Notably, it has been argued that, after Ag NPs reached the larvae midgut epithelial membrane, enzymes were inactivated and generated peroxides lead to cell death (see also Benelli 2016a).

Toxicity on non-target aquatic insects and fishes

Here, the acute biotoxicity of *A. caesia* aqueous extract and green-synthesized Ag NPs was investigated testing them on three aquatic predators of mosquito young instars, including the important biocontrol agent *G. affinis*. Nanoparticle-based treatment achieved moderate toxicity against *A. bouvieri*, *D. indicus*, and *G. affinis*, with LC_{50} ranging from 684 to 2245 μg/ml (Tables 7 and 8). In addition, our continuous observations (lasting 10-day post-treatment) evidenced that longevity and swimming activity of the three biocontrol agents were not influenced for at least 10 days after testing. SI indicated that *A. caesia*-fabricated Ag NPs were less toxic to the non-target organism tested if compared to the targeted larval populations of the three mosquito vectors (Table 9).

While extensive research has focused on the mosquitocidal properties of plant mediated-synthesized Ag NPs, their impact against non-target mosquito predators has been evaluated only in a limited number of studies (Muthukumaran et al. 2015; Benelli 2016a; Govindarajan and Benelli 2016a; Pavela and

Table 8 Acute toxicity of biogenic silver nanoparticles fabricated using the *Acacia caesia* leaf extract on three non-target aquatic biocontrol organisms predating mosquito young instars

Non-target organism	Concentration (μg/ml)	Mortality (%) ± SD	LC_{50} (μg/ml) (95% LCL-UCL)	LC_{90} (μg/ml) (95% LCL-UCL)	Slope	Regression equation	χ^2 (*d.f.*)
Gambusia affinis	1000	23.2 ± 1.2	2245.69 (2022.20–2448.59)	4247.03 (3946.72–4640.80)	2.65	$y = 5.9 + 0.019x$	6.78 (4) n.s.
	2000	45.3 ± 0.6					
	3000	64.7 ± 0.6					
	4000	82.5 ± 1.0					
	5000	100.0 ± 0.0					
Diplonychus indicus	400	23.7 ± 1.0	892.95 (803.54–974.03)	1691.33 (1571.55–1848.39)	2.66	$y = 6.21 + 0.048x$	6.79 (4) n.s.
	800	45.9 ± 0.8					
	1200	63.4 ± 0.6					
	1600	83.8 ± 0.8					
	2000	100.0 ± 0.0					
Anisops bouvieri	300	21.6 ± 0.6	684.96 (619.20–745.05)	1278.34 (1189.08–1395.06)	2.52	$y = 4.53 + 0.065x$	7.11 (4) n.s.
	600	44.7 ± 1.0					
	900	65.2 ± 1.2					
	1200	81.4 ± 1.2					
	1500	100.0 ± 0.0					

Table 9 Suitability index of three non-target aquatic biocontrol agents over young instars of *Anopheles subpictus*, t*Aedes albopictus*, and *Culex tritaeniorhynchus* exposed to the *Acacia caesia* aqueous leaf extract and green silver nanoparticles

Treatment	Species	*Anopheles subpictus*	*Aedes albopictus*	*Culex tritaeniorhynchus*
Acacia caesia aqueous leaf extract	*Gambusia affinis*	218.05	200.83	185.11
	Diplonychus indicus	88.44	81.45	75.08
	Anisops bouvieri	72.04	66.35	61.16
Green-fabricated Ag nanoparticles	*Gambusia affinis*	217.39	198.38	181.83
	Diplonychus indicus	86.44	78.88	72.30
	Anisops bouvieri	66.30	60.50	55.46

Govindarajan 2016; Mahesh Kumar et al. 2016). For instance, Govindarajan et al. (2016h) recently showed little biotoxicity of *M. sylvestris*-synthesized AgNPs on non-target aquatic organisms *D. indicus* (LC_{50} = 813.16 μg/ml) and *G. affinis* (LC_{50} = 1044.52 μg/ml). In addition, an effective option for effective mosquito control may be the employ of biological control agents of mosquito young instars in the presence of ultra-low quantities of nanoformulated botanicals, which boost their predation rates (Murugan et al. 2015b; Benelli and Mehlhorn 2016).

Conclusions

Overall, the employ of natural products as reducing agents to fabricate insecticidal nanocomposites is gaining research attention worldwide, due to low costs, quick synthesis routes, and high effectiveness. Interestingly, biophysical features of green-synthesized nanoparticles strongly differ when different botanicals are employed for nanosynthesis, pointing out the value of screening local botanical resources as reducing and capping agents for nanomosquitocide production. In this study, we highlighted that a cheap *A. caesia* leaf extract can be used for effective and eco-friendly fabrication of Ag NPs with larvicidal, ovicidal, and adulticidal toxicity against three important mosquito vectors of medical and veterinary relevance, including the invasive Zika virus vector *Ae. albopictus*. XRD, SEM, TEM, and AFM showed that the obtained Ag NPs were crystalline, mostly spherical, and with a mean size of 40.2–70.4 nm. Notably, if compared to mosquito larvae, *A. caesia*-fabricated Ag NPs were risk-free to three mosquito predators. Overall, our study pointed out the promising utility of *A. caesia* as an abundant and cheap bioresource to synthesize Ag NPs effective against mosquito young instars and adults, with moderate impact on non-target aquatic biocontrol agents controlling young instar populations of Culicidae vectors.

Acknowledgements The authors extend their sincere appreciations to the Deanship of Scientific Research at King Saud University for funding this Prolific Research Group (PRG-1437-36).

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

References

Ahmad N, Sharma S, Alam MK, Singh VN, Shamsi SF, Mehta BR, Fatma A (2010) Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids Surf B Biointerfaces 81:81–86

Angajala G, Ramya R, Subashini R (2014) In-vitro anti-inflammatory and mosquito larvicidal efficacy of nickel nanoparticles phytofabricated from aqueous leaf extracts of *Aegle marmelos* Correa. Acta Trop 135:19–26

Ankamwar B, Damle C, Ahmad A, Sastry M (2005) Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution. J Nanosci Nanotechnol 5:1665–1671

Bar H, Bhui DK, Sahoo GP, Sarkar P, Pyne S, Misra A (2009) Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*. Colloids Surf A Physicochem Eng Asp 348:212–216

Begum NA, Mondal S, Basu S, Laskar RA, Mandal D (2009) Biogenic synthesis of Au and Ag nanoparticles using aqueous solutions of black tea leaf extracts. Colloids Surf B Biointerfaces 71:113–118

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? Asian Pac J Trop Biomed 6:353–354

Benelli G (2016c) Spread of Zika virus: the key role of mosquito vector control. Asian Pac J Trop Biomed 6:468–471

Benelli G (2016d) Green synthesized nanoparticles in the fight against mosquito-borne diseases and cancer—a brief review. Enzym Microb Technol 95:58–68

Benelli G (2017) Commentary: Data analysis in bionanoscience – issues to watch for. J Clust Sci. doi:10.1007/s10876-016-1143-3

Benelli G, Mehlhorn H (2016) Declining malaria, rising dengue and Zika virus: insights for mosquito vector control. Parasitol Res 115:1747–1754

Benelli B, Conti B, Garreffa R, Nicoletti M (2014) Shedding light on bioactivity of botanical by-products: neem cake compounds deter oviposition of the arbovirus vector *Aedes albopictus* (Diptera: Culicidae) in the field. Parasitol Res 113:933–940

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 114:227–236

Benelli G, Murugan K, Panneerselvam C, Madhiyazhagan P, Conti B, Nicoletti M (2015b) Old ingredients for a new recipe? Neem cake, a low-cost botanical by-product in the fight against mosquito-borne diseases. Parasitol Res 114:391–397

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. doi:10.1007/s00436-016-5095-1

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

Chandramohan B, Murugan K, Panneerselvam C, Madhiyazhagan P, Chandirasekar R, Dinesh D, Mahesh Kumar P, Kovendan K, Suresh U, Subramaniam J, Rajaganesh R, Aziz AT, Syuhei B, Saleh Alsalhi M, Devanesan S, Nicoletti M, Wei H, Benelli G (2016) Characterization and mosquitocidal potential of neem cake-synthesized silver nanoparticles: genotoxicity and impact on predation efficiency of mosquito natural enemies. Parasitol Res 115:1015–1025

Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M (2006) Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. Biotechnol Prog 22:577–583

Deo PG, Hasan SB, Majumdar SK (1988) Toxicity and suitability of some insecticides for household use. Int Pest Control 30:118–129

Dinesh D, Murugan K, Madhiyazhagan P, PanneerselvamC NM, 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 114:519–1529

Elavazhagan T, Arunachalam KD (2011) *Memecylon edule* leaf extract mediated green synthesis of silver and gold nanoparticles. Int J Nanomedicine 6:1265–1278

Finney DJ (1971) Probit analysis. Cambridge University Press, London, pp 68–72

Gopinath V, Priyadarshini S, Priyadharshini NM, Pandian K, Velusamy P (2013) Biogenic synthesis of antibacterial silver chloride nanoparticles using leaf extracts of *Cissus quadrangularis* Linn. Mater Lett 91:224–227

Govindarajan M (2016) Green synthesized silver nanoparticles: a potential new insecticide for mosquito control. Springer International Publishing Switzerland, H. Mehlhorn (ed.), Nanoparticles in the fight against parasites—parasitology research monographs. P.99–153. doi: 10.1007/978-3-319-25292-6_7 (ISSN: 2192–3671)

Govindarajan M, Sivakumar R (2011) Mosquito adulticidal and repellent activities of botanical extracts against malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). Asian Pac J Trop Med 4:941–947

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, Benelli G (2016c) A facile one-pot synthesis of eco-friendly nanoparticles using *Carissa carandas*: ovicidal and larvicidal potential on malaria, dengue and filariasis mosquito vectors. J Clust Sci. doi:10.1007/s10876-016-1035-6

Govindarajan M, Hoti SL, Benelli G (2016a) Facile fabrication of eco-friendly nano-mosquitocides: biophysical characterization and effectiveness on neglected tropical mosquito vectors. Enzyme Microb Tech. doi:10.1016/j.enzmictec.2016.05.005

Govindarajan M, Rajeswary M, Veerakumar K, Muthukumaran U, Hoti SL, 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, Khater HF, 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 Clust Sci 27:745–761

Govindarajan M, Rajeswary M, Veerakumar K, Hoti SL, Mehlhorn H, Barnard DR, Benelli G (2016f) 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, Hoti SL, Murugan K, Kovendan K, Arivoli S, Benelli G (2016g) *Clerodendrum chinense*-mediated biofabrication of silver nanoparticles: mosquitocidal potential and acute toxicity against non-target aquatic organisms. J Asia Pac Entomol 19:51–58

Govindarajan M, Hoti SL, Rajeswary M, Benelli G (2016h) 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, Rajeswary M, Hoti SL, Nicoletti M, Benelli G (2016i) Facile synthesis of mosquitocidal silver nanoparticles using *Mussaenda glabra* leaf extract: characterization and impact on non-target aquatic organisms. Nat Prod Res. doi:10.1080/14786419.2016.1185721

Govindarajan M, Rajeswary M, Veerakumar K, Muthukumaran U, Hoti SL, Khater HF, 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

Haldar KM, Halder 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

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

Jayaseelan C, Rahuman AA, Rajakumar G, Vishnu Kirthi A, Santhoshkumar T, Marimuthu S (2011) Synthesis of pediculocidal and larvicidal silver nanoparticles by leaf extract from heartleaf moonseed plant, *Tinospora cordifolia* Miers. Parasitol Res 109:185–194

Kalishwaralal K, Deepak V, Ramkumarpandian S, Nellaiah H, Sangiliyandi G (2008) Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*. Mater Lett 62:4411–4413

Kotakadi VS, Gaddam SA, Rao YS, Prasad TNVKV, Reddy AV, Gopal DVRS (2014) Biofabrication of silver nanoparticles using *Andrographis paniculata*. Eur J Med Chem 73:135–140

Kowshik M, Ashataputre S, Kharrazi S, Kulkarni SK, Paknikari KM, Vogel W (2003) Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. J Urban Nanotechnol 14:95–100

Krishnamurthy NB, Nagaraj B, Malaka BL, Liny L, Dinesh R (2012) Green synthesis of gold nanoparticles using *Tagetes erecta* L. (marigold) flower extract and evaluation of their antimicrobial activities. Int J Pharm Biosci 3:212–221

Kumar KR, Nattuthurai N, Gopinath P, Mariappan T (2014) Synthesis of eco-friendly silver nanoparticles from *Morinda tinctoria* leaf extract and its larvicidal activity against *Culex quinquefasciatus*. Parasitol Res 114:411–417

Kumar R, Roopan SM, Prabhakarn A, Khanna VG, Chakroborty S (2012) Agricultural waste *Annona squamosa* peel extract: biosynthesis of silver nanoparticles. Spectrochim Acta A 90:173–176

Madhiyazhagan P, Murugan K, Naresh Kumar A, Nataraj T, Dinesh D, Panneerselvam C, Subramaniam J, Mahesh Kumar P, Suresh U, Roni M, Nicoletti M, Alarfaj AA, Higuchi A, Munusamy MA, Benelli G (2015) *Sargassum muticum*-synthesized silver nanoparticles: an effective control tool against mosquito vectors and bacterial pathogens. Parasitol Res 114:4305–4317

Magudapatty P, Gangopadhyayrans P, Panigrahi BK, Nair KGM, Dhara S (2001) Electrical transport studies of Ag nanoparticles embedded in glass matrix. Physica B 299:142–146

Maguranyi SK, Webb CE, Mansfield S, Russell RC (2009) Are commercially available essential oils from Australian native plants repellent to mosquitoes? J Am Mosq Control Assoc 25:292–300

Mahesh Kumar P, Murugan K, Madhiyazhagan P, Kovendan K, Amerasan D, Chandramohan B, Dinesh D, Suresh U, Nicoletti M, Saleh Alsalhi M, Devanesan S, Wei H, Kalimuthu K, Hwang JS, Lo Iacono A, Benelli G (2016) Biosynthesis, characterization and acute toxicity of *Berberis tinctoria* fabricated silver nanoparticles against the Asian tiger mosquito, *Aedes albopictus*, and the mosquito predators *Toxorhynchites splendens* and *Mesocyclops thermocyclopoides*. Parasitol Res 115:751–759

Marimuthu S, Rahuman AA, Govindasamy R, Thirunavukkarasu S, Arivarasan VK, Chidambaram J (2011) Evaluation of green synthesized silver nanoparticles against parasites. Parasitol Res 108:1541–1549

Mathew N, Anitha MG, Bala TSL, Sivakumar SM, Narmadha R, Kalyanasundaram M (2009) Larvicidal activity of *Saraca indica*, *Nyctanthes arbor-tristis* and *Clitoria ternatea* extracts against three mosquito vector species. Parasitol Res 104:1017–1025

Mehlhorn H, Al-Rasheid KA, 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, Suganya A, Dinesh D, Panneerselvam C, Nicoletti M, Hwang JS, Mahesh Kumar P, Subramaniam J, Suresh U (2015a) Toxicity of seaweed-synthesized silver nanoparticles against the filariasis vector *Culex quinquefasciatus* and its impact on predation efficiency of the cyclopoid crustacean *Mesocyclops longisetus*. Parasitol Res 114:2243–2253

Murugan K, Benelli G, Panneerselvam C, Subramaniam J, Jeyalalitha T, Dinesh D, Nicoletti M, Hwang JS, Suresh U, Madhiyazhagan P (2015b) *Cymbopogon citratus*-synthesized gold nanoparticles boost the predation efficiency of copepod *Mesocyclops aspericornis* against malaria and dengue mosquitoes. Exp Parasitol 153:129–138

Murugan K, Samidoss CM, Panneerselvam C, Higuchi A, Roni M, Suresh U, Chandramohan B, Subramaniam J, Madhiyazhagan P, Dinesh D, Rajaganesh R, Alarfaj AA, Nicoletti M, Kumar S, Wei H, Canale A, Mehlhorn H, Benelli G (2015c) Seaweed-synthesized silver nanoparticles: an eco-friendly tool in the fight against *Plasmodium falciparum* and its vector *Anopheles stephensi*? Parasitol Res 114:4087–4097

Murugan K, Panneerselvam C, Samidoss CM, Madhiyazhagan P, Roni M, Subramaniam J, Dinesh D, Rajaganesh R, Paulpandi M, Wei H, Aziz AT, Alsalhi MS, Devanesan S, Nicoletti M, Pavela R, Canale A, Benelli G (2016a) In vivo and in vitro effectiveness of *Azadirachta indica*-synthesized silver nanocrystals against *Plasmodium berghei* and *Plasmodium falciparum*, and their potential against malaria mosquitoes. Res Vet Sci 106:14–22

Murugan K, Panneerselvam C, Subramaniam J, Madhiyazhagan P, Hwang JS, Wang L, Dinesh D, Suresh U, Roni M, Higuchi A, Nicoletti M, Benelli G (2016b) Eco-friendly drugs from the marine environment: spongeweed-synthesized silver nanoparticles are highly effective on *Plasmodium falciparum* and its vector *Anopheles stephensi*, with little non-target effects on predatory copepods. Environ Sci Pollut Res. doi:10.1007/s11356-016-6832-9

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

Naik BR, Gowreeswari GS, Singh Y, Satyavathi R, Daravath RR, Reddy PR (2014) Bio-synthesis of silver nanoparticles from leaf extract of *Pongamia pinnata* as an effective larvicide on dengue vector *Aedes albopictus* (Skuse) (Diptera: Culicidae). Adv Entomol 2:93–101

Naqqash MN, Gökçe A, Bakhsh A, Salim M (2016) Insecticide resistance and its molecular basis in urban insect pests. Parasitol Res 115: 1363–1373

Panneerselvam C, Murugan K, Roni M, Aziz AT, Suresh U, Rajaganesh R, Madhiyazhagan P, Subramaniam J, Dinesh D, Nicoletti M, Higuchi A, Alarfaj AA, Munusamy MA, Kumar S, Desneux N, Benelli G (2016) Fern-synthesized nanoparticles in the fight against malaria: LC/MS analysis of *Pteridium aquilinum* leaf extract and biosynthesis of silver nanoparticles with high mosquitocidal and antiplasmodial activity. Parasitol Res 115:997–1013

Parashar V, Parashar R, Sharma B, Pandey AC (2009) *Parthenium* leaf extract mediated synthesis of silver nanoparticles: a novel approach towards weed utilization. Dig J Nanomater Biostruct 4:45–50

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

Patil CD, Borase HP, Patil SV, Salunkhe RB, Salunke BK (2012b) Larvicidal activity of silver nanoparticles synthesized using *Pergularia daemia* plant latex against *Aedes aegypti* and *Anopheles stephensi* and non-target fish *Poecillia reticulata*. Parasitol Res 111:555–562

Paulsamy S, Senthilkumar P, Anandakumar AM, Sathishkumar P (2010) Utilization of forest flora as agricultural tools and other domestic goods by the villagers adjoining the foot hills of Anamalai, the western Ghats, Coimbatore district. J Non-Timb Forest Prod 17: 339–334

Pavela R, Benelli G (2016a) Ethnobotanical knowledge on botanical repellents employed in the African region against mosquito vectors—a review. Exp Parasitol 167:103–108

Pavela R, Benelli G (2016b) Essential oils as eco-friendly biopesticides? Challenges and constraints. Tr Plant Sci 21(12):1000–1007

Pavela R, Govindarajan M (2016) The essential oil from *Zanthoxylum monophyllum* a potential mosquito larvicide with low toxicity to the non-target fish *Gambusia affinis*. J Pest Sci. doi:10.1007/s10340-016-0763-6

Pullaiah T (2006) Encyclopaedia of world medicinal plants. Daya Books, New Delhi

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

Raman N, Sudharsan S, Veerakumar V, Pravin N, Vithiya K (2012) *Pithecellobium dulce* mediated extra-cellular green synthesis of larvicidal silver nanoparticles. Spectrochim Acta A 96:1031–1037

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

Rashmi S, Preeti V (2009) Biomimetic synthesis and characterization of protein capped silver nanoparticles. Bioresour Technol 100:501–504

Rastogi L, Arunachalam J (2011) Sunlight based irradiation strategy for rapid green synthesis of highly stable silver nanoparticles using aqueous garlic (*Allium sativum*) extract and their antibacterial potential. Mater Chem Phy 129:558–563

Raut RW, Niranjan SK, Jaya RL, Vijay DM, Sahebrao BK (2010) Extracellular synthesis of silver nanoparticles using dried leaves of *Pongamia pinnata* (L.) Pierre. Nano-Micro Lett 2:106–113

Rawani A, Ghosh A, Chandra G (2013) Mosquito larvicidal and antimicrobial activity of synthesized nano-crystalline silver particles using leaves and green berry extract of *Solanum nigrum* L. (Solanaceae: Solanales). Acta Trop 128:613–622

Reda M, Sheshtwy EI, Abdullah M, Nayera A (2011) In situ production of silver nanoparticles on cotton fabric and its antimicrobial evaluation. Cellulose 18:75–82

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

Roopan SM, Rohit MG, Rahuman AA, Kamaraj C, Bharathi A, Surendra TV (2013) Low-cost and eco-friendly phyto-synthesis of silver nanoparticles using *Cocos nucifera* coir extract and its larvicidal activity. Ind Crop Prod 43:631–635

Sanghi R, Verma P (2009) Biomimetic synthesis and characterization of protein capped silver nanoparticles. Bioresour Technol 100:501–504

Sathishkumar M, Sneha K, Won SW, Cho CWS, Kim Yun YS (2009a) *Cinnamon zeylanicum* bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. Colloid Surface B 73:332–338

Sathishkumar P, Paulsamy S, Anandakumar AM, Senthilkumar P (2009b) Effect of habitat variation on the content of certain secondary metabolites of medicinal importance in the leaves of the plant: *Acacia caesia* Willd. Adv Plant Sci 22:451–453

Semmler M, Abdel-Ghaffar F, Al-Rasheid KAS, Mehlhorn H (2009) Nature helps: from research to products against blood sucking arthropods. Parasitol Res 105:1483–1487

Shivshankar S, Ahmad A, Sastry M (2003) Geranium leaf assisted biosynthesis of silver nanoparticles. Biotechnol Prog 19:1627–1631

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

Soni N, Prakash S (2014) Silver nanoparticles: a possibility for malarial and filarial vector control technology. Parasitol Res 113:4015–4022

Su T, Mulla MS (1998) Ovicidal activity of neem products (azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). J Am Mosq Control Assoc 14:204–209

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

Subramaniam J, Murugan K, Panneerselvam C, Kovendan K, Madhiyazhagan P, Dinesh D, Mahesh Kumar P, Chandramohan B (2016) Multipurpose effectiveness of *Couroupita guianensis*-synthesized gold nanoparticles: high antiplasmodial potential, field efficacy against malaria vectors and synergy with *Aplocheilus lineatus* predators. Environ Sci Poll Res. doi:10.1007/s11356-015-6007-0

Suganya G, Karthi S, Shivakumar MS (2014) Larvicidal potential of silver nanoparticles synthesized from Lucas aspera leaf extracts against dengue vector *Aedes aegypti*. Parasitol Res 113:1673–1679

Sujitha V, Murugan K, Paulpandi M, Panneerselvam C, Suresh U, Roni M, Nicoletti M, Higuchi A, Madhiyazhagan P, Subramaniam J, Dinesh D, Vadivalagan C, Chandramohan B, Alarfaj AA, Munusamy MA, Barnard DR, Benelli G (2015) Green synthesized silver nanoparticles as a novel control tool against dengue virus (DEN-2) and its primary vector *Aedes aegypti*. Parasitol Res 114:3315–3325

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

Thambiraj J, Paulsamy S (2010) Antimicrobial activity of the folklore medicinal plant, *Acacia caesia* (L.) Wild. Plant Archives 10:675–678

Thirunavokkarasu M, Balaji U, Behera S, Panda PK, Mishra BK (2013) Biosynthesis of silver nanoparticles from extract of *Desmodium gangeticum* (L.) DC. and its biomedical potential. Spectrochim Acta Part A 116:424–427

Thovhogi N, Diallo A, Gurib-Fakim A, Maaza M (2015) Nanoparticles green synthesis by *Hibiscus sabdariffa* flower extract: main physical properties. J Alloys Compd 647:392–396

Tripathi A, Chandrasekaran N, Raichur AM, Mukherjee A (2009) Antibacterial applications of silver nanoparticles synthesized by aqueous extract of *Azadirachta indica* (Neem) leaves. J Biomed Nanotechnol 5(1):93–98

Veerakumar K, Govindarajan M, Rajeswary M, Muthukumaran U (2014a) 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

Veerakumar K, Govindarajan M, Rajeswary M, Muthukumaram U (2014b) Mosquito larvicidal properties of silver nanoparticles synthesized using *Heliotropium indicum* (Boraginaceae) against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquifasciatus* (Diptera: Culicidae). Parasitol Res 113:2663–2673

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

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

Vijayakumar M, Priya K, Nancy FT, Noorlidah A, Ahmed ABA (2013) Biosynthesis, characterisation and anti-bacterial effect of plant-mediated silver nanoparticles using *Artemisia nilagirica*. Ind Crop Prod 41:235–240

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

Vivekanandhan S, Misra M, Mohanty AK (2009) Biological synthesis of silver nanoparticles using *Glycine max* (soybean) leaf extract: an investigation on different soybean varieties. J Nanosci Nanotechnol 9(12):6828–6833

Ward M, Benelli G (2017) Avian and simian malaria: do they have a cancer connection? Parasitol Res. doi:10.1007/s00436-016-5352-3

World Health Organization (1981) Instruction for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides. WHO/VBC/81.806

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 2005; WHO/CDS/WHOPES/GCDPP/1.3

Yugandhar P, Savithramma N (2016) Biosynthesis, characterization and antimicrobial studies of green synthesized silver nanoparticles from fruit extract of *Syzygium alternifolium* (Wt.) Walp. an endemic, endangered medicinal tree taxon. Appl Nanosci 6:223–233

Zargar M, Hamid AA, Bakar FA, Shamsudin MN, Shameli K, Jahanshiri F, Farahani F (2011) Green synthesis and antibacterial effect of silver nanoparticles using *Vitex negundo* L. Molecules 16:6667–6676

Zhang W, Chen Z, Liu H, Zhang L, Gao P, Li D (2011) Biosynthesis and structural characteristics of selenium nanoparticles by *Pseudomonas alcaliphila*. Colloids Surf B 88:196–201

Zhu L, Tian Y (2011) Chemical composition and larvicidal activity of *Blumea densiflora* essential oils against *Anopheles anthropophagus*: a malarial vector mosquito. Parasitol Res 109:1417–1422

Zhu L, Tian Y (2013) Chemical composition and larvicidal activity of essential oil of *Artemisia gilvescens* against *Anopheles anthropophagus*. Parasitol Res 112:1137–1142