Parasitol Res (2016) 115:751–759
DOI 10.1007/s00436-015-4799-y

ORIGINAL PAPER

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*

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Received: 2 October 2015 / Accepted: 19 October 2015 / Published online: 26 October 2015
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Abstract *Aedes albopictus* is an important arbovirus vector, including dengue. Currently, there is no specific treatment for dengue. Its prevention solely depends on effective vector control measures. In this study, silver nanoparticles (AgNPs) were biosynthesized using a cheap leaf extract of *Berberis tinctoria* as reducing and stabilizing agent and tested against *Ae. albopictus* and two mosquito natural enemies. AgNPs were characterized by using UV–vis spectrophotometry, X-ray diffraction, and scanning electron microscopy. In laboratory conditions, the toxicity of AgNPs was evaluated on larvae and pupae of *Ae. albopictus*. Suitability Index/Predator Safety Factor was assessed on *Toxorhynchites splendens* and *Mesocyclops thermocyclopoides*. The leaf extract of *B. tinctoria* was toxic against larval instars (I–IV) and pupae of *Ae. albopictus*; LC_{50} was 182.72 ppm (I instar), 230.99 ppm (II), 269.65 ppm (III), 321.75 ppm (IV), and 359.71 ppm (pupa). *B. tinctoria*-synthesized AgNPs were highly effective, with LC_{50} of 4.97 ppm (I instar), 5.97 ppm (II), 7.60 ppm (III), 9.65 ppm (IV), and 14.87 ppm (pupa). Both the leaf extract and AgNPs showed reduced toxicity against the mosquito natural enemies *M. thermocyclopoides* and *T. splendens*. Overall, this study firstly shed light on effectiveness of *B. tinctoria*-synthesized AgNPs as an eco-friendly nanopesticide, highlighting the concrete possibility to employ this newer and safer tool in arbovirus vector control programs.

Keywords Arbovirus · Copepods · Culicidae · Dengue · Predaceous mosquito larvae · Predatory safety factor · SEM · Suitability index · XRD

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Introduction

Mosquitoes (Diptera: Culicidae) represent a key threat for millions of people worldwide, since they act as vectors for devastating parasites and pathogens, including malaria, yellow fever, dengue, West Nile, chikungunya, and filariasis (Mehlhorn et al. 2012; Benelli 2015a). *Aedes albopictus* (Skuse), commonly known as the Asian tiger mosquito, is currently retained the most invasive mosquito species in the world, since it is able to rapidly adapt to different anthropogenic environments, thanks to its ecological and physiological

plasticity. Recently, the Asian tiger mosquito has invaded many countries, spreading rapidly to Europe, North and South America, the Caribbean, Africa, and the Middle East (Caminade et al. 2012; Murugan et al. 2016a). *Ae. albopictus* is both a nuisance and a disease vector. Its medical importance is mainly due to the aggressive daytime human-biting behavior and to its ability to transmit many diseases. It works as a vector for many viruses, including dengue, yellow fever, West Nile, Japanese encephalitis, St. Louis, encephalitis virus (*Flaviridae*, genus *Flavivirus*), chikungunya, eastern equine encephalitis, Venezuelan equine encephalitis, western equine encephalitis, Ross River, Sindbis, Mayaro, Getah (*Togaviridae*, genus *Alphavirus*), Potosi, San Angelo, La Crosse, Jamestown Canyon (*Bunyaviridae*, genus *Bunyavirus*), Rift Valley fever (*Bunyaviridae*, genus *Phlebovirus*), and Orungo virus (*Reoviridae*, genus *Orbivirus*). *Ae. albopictus* is also the vector of different filariases, such as *Dirofilaria immitis* Leidy, *D. repens* Railliet and Henry, and *Setaria labiatopapillosa* Perroncito (Paupy et al. 2009; Benelli 2015b).

Mosquito young instars are usually targeted using organophosphates, insect growth regulators, and microbial control agents. Indoors residual spraying and insecticide-treated bed nets are also employed to reduce transmission of malaria in tropical countries (Benelli 2015a). However, these chemicals have strong negative effects on human health and/or the environment and induce resistance in a number of mosquito species (Hemingway and Ranson 2000). On this basis, eco-friendly tools have been recently implemented to enhance control of mosquito vectors. Particularly, important efforts have been carried out investigating the efficacy of botanical products against mosquito vectors. Many plant-borne compounds have been reported as excellent toxics against Culicidae, acting as ovicides, larvicides, pupicides, adulticides, oviposition deterrents, adult repellents, growth and/or reproduction inhibitors (Amer and Mehlhorn 2006a, b, c, d; Pavela 2008, 2009; see Benelli 2015c; Pavela 2015 and Benelli et al. 2015 for recent reviews).

In a biological control perspective, mosquito young instar populations may be controlled by a number of aquatic predators, including odonate nymphs, water bugs, tadpoles, fishes, and copepods (Bowatte et al. 2013; Kalimuthu et al. 2014; Murugan et al. 2015a, b, c, d). Copepods are small aquatic crustaceans, most of them are omnivorous and can prey on immature mosquitoes, especially first instar larvae (Hurlbut 1938; Marten et al. 1989; Rawlins et al. 1997; Manrique-Saide et al. 1998; Williamson 1999). *Mesocyclops thermocyclopoides* is a common copepod species in tropical and subtropical areas, evaluated as a biocontrol agent against *Aedes* mosquitoes (Mahesh Kumar et al. 2012). This species feeds on the first and second instars of mosquito larvae, fatally wounding about seven individuals per day (Schaper and Hernández 1998).

Interestingly, there are also some Culicidae species that contribute to the control of mosquito vectors of public health importance. *Toxorhynchites*, also called "elephant mosquito" or "mosquito eater," is a cosmopolitan genus of mosquitoes. The genus includes the largest known species of mosquito, and it is among the few kinds of mosquito that do not consume blood. The adults subsist on carbohydrate-rich materials, such as honeydew, or saps and juices from damaged plants, refuse, fruit, and nectar. The larvae of *Toxorhynchites* prey on the larvae of other mosquitoes and similar nektonic prey. In this respect, they contrast with blood-sucking species of mosquitoes. *Toxorhynchites* larvae live on a protein- and fat-rich diet of aquatic animals such as mosquito larvae. They have no need to risk their lives sucking blood in adulthood, having already accumulated the necessary materials for oogenesis and vitellogenesis. Most species occur in forests. The larvae of one jungle variety, *Toxorhynchites splendens*, consume larvae of other mosquito species occurring in tree crevices, particularly *Aedes* ones. The adults of these mosquitoes are larger than *Aedes* and are harmless to humans. The larvae of *Toxorhynchites splendens* are cannibalistic only if other preys are unavailable (Steffan 1975; Steffan and Evenhuis 1981; Focks 1985). Overall, studies on *T. splendens* as biocontrol agents for larval mosquito control conducted in several countries reported promising results (Focks 1985; Rawlins et al. 1991; Aditya et al. 2006).

The development of green processes for the biosynthesis of nanoparticles is evolving into an important branch of nanotechnology (Shin et al. 2007). Plants and microbes are currently used for nanoparticle synthesis. The use of plants to fabricate nanoparticles is rapid, low cost, eco-friendly, and a single-step method for biosynthesis process (Huang et al. 2007; Kumar and Yadav 2009). Recently, silver nanoparticles have been biosynthesized using various plant extracts (e.g., *Aloe vera* (Dinesh et al. 2015), *Phyllanthus niruri* (Suresh et al. 2015), *Moringa oleifera* (Sujitha et al. 2015), *Caulerpa scalpelliformis* (Murugan et al. 2015a)) and have been found highly effective against important mosquito vectors, even if tested at low doses (see Benelli 2016 for a review).

Berberis tinctoria Lesch. is an evergreen erect shrub with yellow wood belonging to Berberidaceae. The leaves of *B. tinctoria* have been evaluated for hepatoprotective activity and antioxidant activity (Murugesh et al. 2005). They contain berberine, an important alkaloid, which is effective for various infectious diseases and possess antibacterial property (Sasikumar et al. 2007). The use of berberine has been described in Indian and Chinese medicine for the treatment of diarrhea and intestinal parasitic infections (Saha et al. 2011). In this research, we investigated the mosquitocidal properties of *B. tinctoria* leaf extract and green-fabricated silver nanoparticles. The biosynthesized silver nanoparticles were characterized by UV–vis spectrophotometry, scanning electron microscopy (SEM), and X-ray diffraction (XRD). Mosquitocidal properties were assessed in laboratory against larvae (I–IV instar) and pupae of the arbovirus vector *Ae.*

albopictus. Green-synthesized AgNPs and *B. tinctoria* leaf extract were tested against the two mosquito predators *T. splendens* and *M. thermocyclopoides*, and the Suitability Index/Predator Safety Factor was calculated.

Materials and methods

Plant material and preparation of the extract

B. tinctoria was collected from Kattabettu (11° 24' 44.8" N 76° 48' 47.6" E; Kothagiri, Tamil Nadu, India). Leaves were identified at the Department of Botany, Bharathiar University (Coimbatore, India). Voucher specimens (ID: BERTIN1-3) were stored in our laboratories and are available upon request. The plant leaves were washed with distilled water and shade-dried for 2 days at 28 °C. Ten grams of washed and finely cut leaves were stored in a 300-ml Erlenmeyer flask filled with 100 ml of sterile distilled water. The mixture was boiled for 5 min before finally decanted it. The aqueous extract was stored at 4 °C and used within 5 days.

Biosynthesis and characterization of silver nanoparticles

To reduce Ag^+ ions to Ag^0, 10 ml of plant aqueous extract were added to 190 ml of aqueous $AgNO_3$ (1 mM). The effect of reaction time on synthesis rate and size of AgNPs was studied by carrying out the reaction in a water bath at 95 °C with reflux (elapsed time, from 10 min to 4 h). AgNPs were subjected to repeated centrifugation at 15,000 rpm for 20 min followed by re-dispersion of the pellet in de-ionized water. UV–vis spectra were recorded as a function of reaction time on a UV-3600 Shimadzu spectrophotometer operated at a resolution of 1 nm. After freeze-drying of the purified AgNP, the structure and composition were analyzed by 10 kV Ultra High Resolution SEM (FEI QUANTA-200 SEM). XRD using Cukα radiation (PAN anlyticalX'pert Pro MPD diffractometer) was used to determine the crystalline structure of AgNPs. In addition, X-ray analysis on dried AgNPs was carried out using a Philips Model PW 1050/37 diffractometer, operating at 40 kV and 30 mA, with a step size of 0.02° (2θ) (Suresh et al. 2015).

Tested concentrations

From the aqueous extract, the concentrations were made (50, 150, 250, 350, and 450 ppm), and 2 ml of *B. tinctoria*-synthesized AgNPs was diluted in 100 ml of distilled water for the preparation of 2 % (*v*:*v*) stock solution. Then, the experimental concentrations (i.e., 2.5, 5, 10, 20, and 40 ppm) were prepared by subsequent dilution of stock solution in distilled water. All stocks and dilutions were kept refrigerated at −4 °C and tested within 8 weeks.

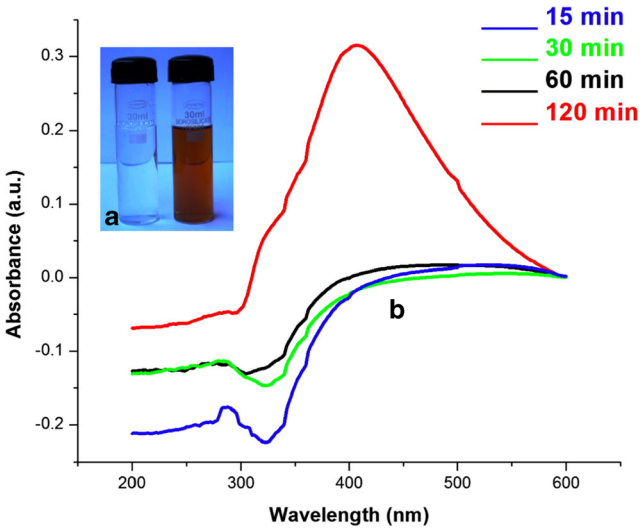

Fig. 1 **a** Chromatic variations of silver nitrate solution (1 mM) before (*left*) and after (*right*) the process of reduction of Ag^+ to Ag^0 nanoparticles using the *B. tinctoria* leaf extract. **b** UV visualization of the absorption spectrum of the biosynthesized silver nanoparticles after different time intervals

Ae. albopictus rearing

Experiments were conducted using laboratory-reared pathogen-free strains of *Ae. albopictus* colonies that were originally established as described by Subramaniam et al. (2015). Batches of 100–110 eggs were transferred to 18 cm l× 13 cm W×4 cm D enamel trays containing 500 ml of water where they were allowed to hatch in laboratory conditions (27± 2 °C and 75–85 % R.H.;14:10 (L:D)) photoperiod. Mosquito larvae were fed daily with 0.5 g of ground dog biscuit (Pedigree, USA) and brewer's yeast (Sigma-Aldrich,

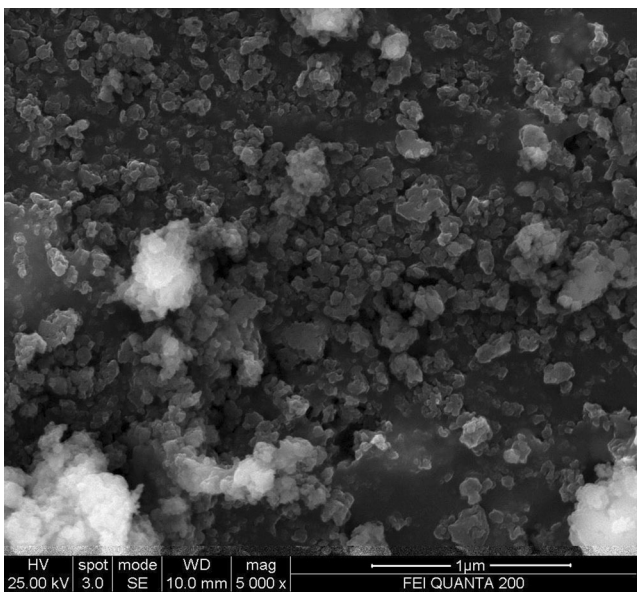

Fig. 2 SEM micrograph showing the morphological characteristics of silver nanoparticles synthesized using the *B. tinctoria* leaf extract

Germany) in a 3:2 (*w*:*w*) ratio. *Ae. albopictus* larvae and pupae were used for acute toxicity experiments. Furthermore, each container was placed inside a cubic chiffon cage (90×90×90 cm) to wait for adult emergence. Adults of both species were fed on 10 % (*w*:*v*) sucrose solution. Five days after emergence, *Ae. albopictus* adults were deprived of sugar feeding for 12 h and then supplied with artificial blood feeding. The blood meal was furnished, by means of a professional heating blood (lamb blood), at fixed temperature of 38 °C and provided with a membrane of cow gut. After 30 min, the blood meal was removed, due to blood drying phenomena, and gut membrane was substituted with a new fresh one for the following utilization (Nicoletti et al. 2012).

Larvicidal and pupicidal activity against *Ae. albopictus*

Following the methods reported by Murugan et al. (2015a, b), 25 *Ae. albopictus* larvae (I, II, III, or IV instar) or pupae were exposed for 24 h in a glass beaker filled with 250 ml of de-chlorinated water plus *B. tinctoria* aqueous extract (i.e., 50, 150, 250, 350, and 450 ppm) and *B. tinctoria*-synthesized AgNPs (i.e., 2.5, 5, 10, 20, and 40 ppm). Larval food was provided for each tested concentration. Each concentration was replicated five times against all instars. In the control, for both species, 25 larvae or pupae were transferred in 250 ml of de-chlorinated water. No mortality was observed in the control. Percentage mortality was calculated as follows:

$$\text{Percentage mortality} = \left(\text{Number of dead individuals} \Big/ \text{Number of treated individuals}\right) * 100.$$

LC_{50} and LC_{90} were calculated by probit analysis, following the method by Finney (1971). SPSS software package 16.0 version was used for all analyses.

Toxicity against mosquito natural enemies

Following the method by Sivagnaname and Kalyanasundaram (2004), here, the effect of *B. tinctoria* leaf extract and AgNPs was tested on two non-target mosquito natural enemies, *T. splendens* (IV instar larvae) and *M. thermocyclopoids* (adults). Both species were collected from rural ponds in Coimbatore (Tamil Nadu, India) and maintained at 27±3 °C and R.H. 85 % in cement tanks (120-cm diameter, 60-cm depth) filled with de-chlorinated water (Murugan et al. 2015c). Leaf extracts of *B. tinctoria* and green-synthesized AgNPs were evaluated at a concentration of five times higher than the LC_{50} dose of mosquito larvae. Five replicates were performed for each concentration, along with negative controls. The tested organisms were observed for mortality and other abnormalities such as sluggishness and reduced swimming activity after 48 h of exposure. The exposed predators were also observed continuously for 10 days to understand the post-treatment effect of this extract on survival and swimming activity. LC_{50} and LC_{90} values were obtained by probit analysis, and the Suitability Index/Predator Safety Factor

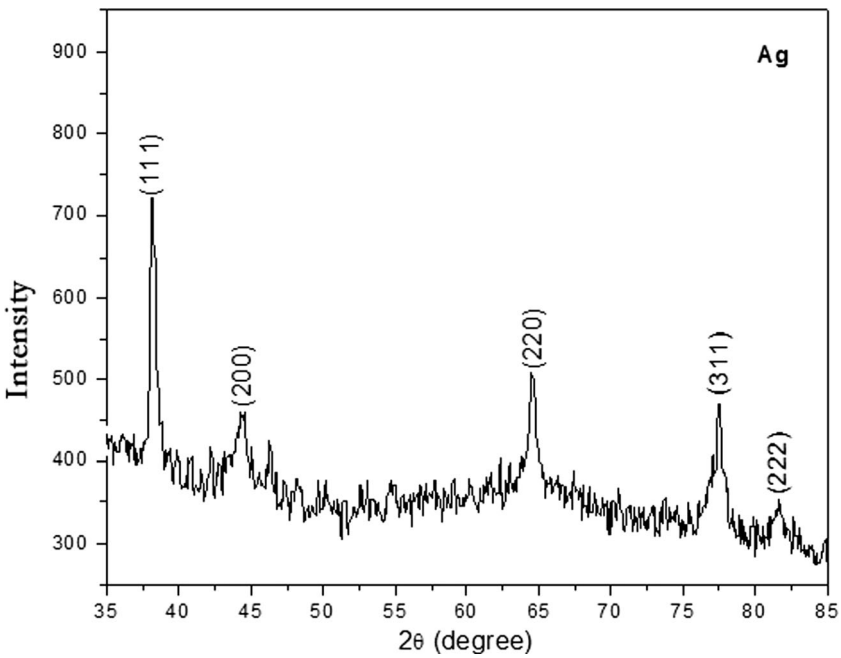

Fig. 3 XRD pattern of biosynthesized silver nanoparticles using the *B. tinctoria* leaf extract

Table 1 Larval and pupal toxicity of *B. tinctoria* leaf extract against the arbovirus vector *Ae. albopictus*

Target	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL–UCL)	Regression equation	χ^2 (*d.f.* = 4)
Larva I	182.72 (108.89–237.54)	448.44 (368.10–622.70)	$y=0.881+0.005x$	5.42 NS
Larva II	230.99 (201.09–259.59)	543.36 (485.26–629.23)	$y=0.948+0.004x$	1.38 NS
Larva III	269.65 (238.51–302.55)	613.09 (540.85–724.46)	$y=1.006+0.004x$	0.27 NS
Larva IV	321.75 (287.53–363.62)	694.61 (604.06–840.86)	$y=1.106+0.003x$	0.91 NS
Pupa	359.71 (322.95–408.679)	733.20 (635.21–893.40)	$y=1.234+0.003x$	0.93 NS

No mortality was observed in the control

LC_{50}, lethal concentration that kills 50 % of the exposed organisms

LC_{90}, lethal concentration that kills 90 % of the exposed organisms

χ^2 chi-square value, *d.f.* degrees of freedom, *NS* not significant ($\alpha=0.05$)

was calculated for each tested species using the following formula (Deo et al. 1998).

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

Results and discussion

Biosynthesis and characterization of silver nanoparticles

When the *B. tinctoria* leaf extract was added to the $AgNO_3$ aqueous solution, the color changed from pale yellow to reddish-brown (Fig. 1a), indicating the reduction from Ag^+ to Ag^0, thus the formation of AgNPs. The darker color could be due to the excitation of surface plasmon vibrations, which is typical of AgNPs (Ahmad et al. 2003; Krishnaraj et al. 2010). UV–vis spectrophotometry can be used to examine size and shape-controlled nanoparticles in aqueous suspensions (see Shrivastava and Dash 2010; Govindarajan et al. 2016). The absorption spectrum of *B. tinctoria*-synthesized AgNPs showed a maximum absorption peak at 420 nm after 120 min (Fig. 1b), broadening of the peak indicated that the particles are poly-dispersed (Prasad and Elumalai 2011). In agreement with our results, Sujitha et al. (2015) reported that the synthesis of AgNPs using *M. oleifera* leads to a maximum absorbance peak at 450 nm. The *B. tinctoria* leaf extract without $AgNO_3$ did not show any change in color over time for at least 4 weeks.

SEM analysis showed that *B. tinctoria*-synthesized AgNPs were predominantly spherical in shape (Fig. 2), with a mean size of 65–70 nm. In XRD trials, Bragg reflections corresponding to the 111, 200, 220, 311, and 222 sets of lattice planes were observed (Fig. 3). The XRD pattern showed that the AgNPs formed by the reduction of $AgNO_3$ by *B. tinctoria* leaf extract were crystalline in nature. Notably, these sharp Bragg peaks might be due to the capping agents stabilizing AgNPs. XRD results also suggest that crystallization of the bioorganic phase occurs on the surface of the AgNPs. Similarly, Sathyavathi et al. (2010) reported diffraction peaks at 44.50°, 52.20°, and 76.7° 2θ, which correspond to the 111, 200, and 220 facets of the face-centered cubic crystal structure.

Larvicidal and pupicidal activity against *Ae. albopictus*

In our laboratory assays, the leaf extract of *B. tinctoria* was toxic against larval instars (I–IV) and pupae of the arbovirus vector *Ae. albopictus*. LC_{50} values were 182.72 ppm (I instar larvae), 230.99 ppm (II), 269.65 ppm (III), 321.75 ppm (IV), and 359.71 ppm (pupae), respectively (Table 1). AgNPs synthesized from the leaf extract of *B. tinctoria* were highly

Table 2 Larval and pupal toxicity of green-synthesized silver nanoparticles against the arbovirus vector *Ae. albopictus*

Target	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL–UCL)	Regression equation	χ^2 (*d.f.* = 4)
Larva I	4.97 (2.60–6.85)	23.00 (19.85–27.84)	$y=0.354+0.071x$	2.00 NS
Larva II	5.97 (3.77–7.80)	24.07 (20.85–28.95)	$y=0.423+0.071x$	2.18 NS
Larva III	7.60 (4.78–10.01)	32.89 (28.50–39.45)	$y=0.386+0.051x$	1.83 NS
Larva IV	9.65 (6.53–12.39)	39.61 (34.06–48.14)	$y=0.413+0.043x$	3.12 NS
Pupa	14.87 (11.15–18.60)	55.27 (45.99–71.13)	$y=0.472+0.032x$	4.70 NS

No mortality was observed in the control

LC_{50}, lethal concentration that kills 50 % of the exposed organisms

LC_{90}, lethal concentration that kills 90 % of the exposed organisms

χ^2 chi-square value, *d.f.* degrees of freedom, *NS* not significant ($\alpha=0.05$)

Table 3 Biotoxicity of the *B. tinctoria* leaf extract and silver nanoparticles against the predatory mosquito *T. splendens*

Treatment	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL–UCL)	Regression equation	χ^2 (*d.f.* = 4)
B. tinctoria	552.28 (470.67–706.88)	999.32 (810.70–1378.36)	$y=1.302+0.003x$	0.92 NS
Ag nanoparticles	234.48 (185.51–340.42)	535.04 (404.25–838.08)	$y=0.975+0.005x$	1.51 NS

No mortality was observed in the control

LC_{50}, lethal concentration that kills 50 % of the exposed organisms

LC_{90}, lethal concentration that kills 90 % of the exposed organisms

χ^2 chi-square value, *d.f.* degrees of freedom, *NS* not significant ($\alpha=0.05$)

effective against *Ae. albopictus* young instars, with LC_{50} of 4.97 ppm (I), 5.97 ppm (II), 7.60 ppm (III), 9.65 ppm (IV), and 14.87 ppm (pupae) (Table 2). A dose-dependent effect was found, in agreement with a number of previously reported plant-borne pesticides (e.g., Amer and Mehlhorn 2006c, d; Bagavan et al. 2009; Benelli 2015c). Recently, a growing number of green-synthesized AgNPs showed comparable larvicidal and pupicidal toxicity against different mosquito vectors (Santhoshkumar et al. 2011; Subramaniam et al. 2015; Benelli 2016; Murugan et al. 2016b). For example, Suresh et al. (2015) highlighted that AgNPs synthesized using the aqueous extract of *P. niruri* are highly effective against larvae and pupae of *Ae. aegypti*, with LC_{50} values ranging from 3.90 ppm (I) to 13.04 ppm (pupae). Low doses of *C. scalpelliformis*-synthesized AgNPs are highly toxic also against the filariasis vector *Culex quinquefasciatus*, with LC_{50} values ranging from 3.08 ppm (I) to 7.33 ppm (pupae) (Murugan et al. 2015a). We hypothesize that the toxicity of AgNPs against arbovirus vectors may be enabled by the small size of these nanoparticles, which allows passage through the insect cuticle and into individual cells where they interfere with molting and other physiological processes (Murugan et al. 2015e, f). Further research on the impact of sub-lethal doses of green-fabricated AgNPs on mosquito fecundity and longevity is urgently needed (Roni et al. 2015).

Toxicity against mosquito natural enemies

B. tinctoria was tested against the non-target mosquito predators *T. splendens* and *M. thermocyclopoids*, with LC_{50} values of 552.28 and 480.92 ppm, respectively (Tables 3 and 4, respectively). Experiments conducted testing AgNPs on *T. splendens* and *M. thermocyclopoids* lead to LC_{50} values of 234.48 and 218.16 ppm, respectively. The Safety Index/Predator Safety Factor calculated for the leaf extract of *B. tinctoria* was 3.02 and 2.63 for *T. splendens* and *M. thermocyclopoids*, respectively, while for AgNPs, it was 47.1 and 43.8, respectively. Currently, moderate knowledge is available about the acute toxicity towards aquatic non-target species (Benelli 2016). *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 *Ae. aegypti* and *Anopheles stephensi* (Patil et al. 2012a, b). 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 *An. stephensi* and *C. quinquefasciatus*. Similarly, Haldar et al. (2013) did not detected toxicity of AgNPs produced using dried green fruits of *Drypetes roxburghii* against *P. reticulata*, after 48 h of exposure to LC_{50} of IV instar larvae of *An. 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 *An. stephensi* and *C. quinquefasciatus* larvae. AgNPs biosynthesized 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).

Table 4 Biotoxicity of the *B. tinctoria* leaf extract and silver nanoparticles against the predatory copepod *M. thermocyclopoides*

Treatment	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL–UCL)	Regression equation	χ^2 (*d.f.* = 4)
B. tinctoria	480.92 (413.02–604.77)	990.23 (802.08–1370.02)	$y=1.210+0.003x$	0.26 NS
Ag nanoparticles	218.16 (173.60–311.80)	516.20 (391.98–799.43)	$y=0.938+0.004x$	4.98 NS

No mortality was observed in the control

LC_{50}, lethal concentration that kills 50 % of the exposed organisms

LC_{90}, lethal concentration that kills 90 % of the exposed organisms

χ^2 chi-square value, *d.f.* degrees of freedom, *NS* not significant ($\alpha=0.05$)

Conclusions

Overall, we biosynthesized AgNPs using a cheap aqueous extract of *B. tinctoria* leaves as a reducing and stabilizing agent. Bio-fabricated AgNPs were mostly spherical in shape, crystalline in nature, with face-centered cubic geometry, and their mean size was 65–70 nm. This study highlighted that *B. tinctoria*-synthesized AgNPs are easy to produce, stable over time, and may be employed at low dosages to strongly reduce young instar populations of arbovirus mosquito vectors, with little impact on vector natural enemies such as copepods and *Toxorhynchites* predaceous larvae. Further research is ongoing to shed light on the toxicity mechanism(s) of AgNPs against mosquito predators and their potential long-term toxicity effects on arthropod longevity and fecundity.

Acknowledgments The authors would like to thank the financial support rendered by King Saud University, through Vice Deanship of Research Chairs. The authors are grateful to the UGC-MRP, New Delhi, India (No. F. No.36-250/2008 (SR) 24/03/2009) and the Department of Physics and Astronomy, King Saud University (project no. RGP-1435-057) for their financial support.

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.

Conflict of interest The authors declare no conflicts of interest. G. Benelli is an Editorial Board Member of *Parasitology Research*. This does not alter the author's adherence to all the *Parasitology Research* policies on sharing data and materials.

Informed consent Informed consent was obtained from all individual participants included in the study.

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