

Swift Fabrication of Silver Nanoparticles Using *Bougainvillea glabra*: Potential Against the Japanese Encephalitis Vector, *Culex tritaeniorhynchus* Giles (Diptera: Culicidae)

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Abstract Mosquitoes are the most critical group of insects in the context of public health, since they transmit key parasites and pathogens, causing millions of deaths annually. *Culex tritaeniorhynchus* is an important vector of Japanese encephalitis (JE) across urban and semi-urban areas of Asia. In this study, we bio-fabricated silver nanoparticles (Ag NP) using the leaf extract of *Bougainvillea glabra* as reducing and stabilizing agent. The synthesis of Ag NP was confirmed analyzing the excitation of surface Plasmon resonance using ultraviolet–visible (UV–vis) spectrophotometry. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) showed the clustered and irregular shapes of Ag NP. The presence of silver was determined by energy dispersive X-ray (EDX) spectroscopy.

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Fourier transform infrared (FTIR) spectroscopy analysis investigated the identity of secondary metabolites, which may act as Ag NP capping agents. The acute toxicity of *B. glabra* extract, synthesized Ag NP and a combined treatment testing blends of both mosquitocidal was evaluated against larvae and pupae of *Cx. tritaeniorhynchus*. *B. glabra* showed LC₅₀ of 198.93 (larva I), 234.50 (II), 309.18 (III), 371.69 (IV) and 466.09 (pupa) µg/ml, Ag NP LC₅₀ ranged from 7.77 (I) to 19.44 µg/ml (pupa). Combined treatments with *B. glabra* leaf extract plus 5.12.5 µg/ml of Ag NP lowered the botanical LC₅₀ to 66.09 (I), 76.48 (II), 99.02 (III), 133.43 (IV) and 179.74 µg/ml (IV), respectively. The effectiveness of green-fabricated Ag NP against the JE vector was confirmed in adulticidal tests, as well as evaluating the impact of Ag NP on fecundity and longevity of adult mosquitoes. Lastly, the larvicidal effectiveness of Ag NP was confirmed in the field, treating sewage water bodies. Overall, this study suggests that the green-synthesized Ag NP fabricated using *B. glabra* can be considered a potential mosquito control device against the JE vector, *C. tritaeniorhynchus* in Asian regions.

Keywords Arbovirus · Green synthesis · Larvicides · UV–vis spectrophotometry · FTIR · EDX, XRD, SEM

Introduction

Mosquitoes are the dangerous vectors of deadly pathogens and parasites, which may hit as epidemics or pandemics in the increasing world population of humans and animals [1, 2]. *Culex quinquefasciatus*, commonly known as the southern house mosquito, is a major vector of lymphatic filariasis, a neglected tropical disease commonly known as elephantiasis. Lymphatic filariasis is caused by Filarioidea nematodes, namely *Wuchereria bancrofti*, which is responsible for 90 % of cases, *Brugia malayi*, and *Brugia timori*. Recently, more than 1.4 billion people in 73 countries are living in areas where lymphatic filariasis is transmitted and are at risk of being infected. Globally, an estimated 25 million men suffer with genital disease and over 15 million people are affected with lymphoedema. Eliminating lymphatic filariasis can prevent unnecessary suffering and contribute to the reduction of poverty [3]. *Culex tritaeniorhynchus* is a primary vector of Japanese encephalitis (JE) virus, with a distribution throughout Southeast Asia and South Asia. Keiser et al. [4] have reported that global annual incidence and mortality estimates for JE are 30,000 to 50,000 and 10,000 respectively. *Cx. tritaeniorhynchus* bites throughout the night with minor peaks in activity at 9 pm and 2.30 am [5]. Females have a preference for cattle and pigs, but also occasionally feed on birds and humans [6]. In India, *Cx. tritaeniorhynchus* predominantly feed on cattle, and to lesser extent on ducks, fowl, goats and humans [7]. In southern India this species is predominantly collected resting outdoors [8] however it has been found occurring in unusually high numbers resting indoors during the daytime [9].

The genus *Bougainvillea* comprises 18 species, some of them are used in traditional medicine to treat disorders such as diarrhea, stomach acidity, cough, sore throat, leucorrhoea, hepatitis, and as an anti-inflammatory, antiviral and

antibacterial agent [10, 11]. Leaves and inflorescences of *B. glabra* are also used in Mexican traditional medicine as a remedy for such respiratory illnesses as cough, cold, bronchitis and asthma [12, 13]. Its antimicrobial effect can be associated with the presence of betalains [14], as well as steroidal compounds with anti-inflammatory activity [15].

Bougainvillea glabra (Nyctaginaceae) is a common plant in Asia, used as a source of effective natural dyeing agents and botanicals used against the pests attacking stored rice. As an additional advantage, these plants can be grown easily in the farmer's residential or cropping area. Pesticides of natural origin are economically viable and environmentally safe are easily available for the user [16, 17]. Kalirajan et al. [18] showed the flower extract of *B. glabra* has a promising potential as natural colouring agent and as a biopesticide. Recently, eco-friendly control tools have been implemented to enhance mosquito control. Significant efforts have been carried out to investigating the efficacy of botanical products, and many plant-borne compounds have been reported as excellent toxins against mosquitoes, acting as adulticidal, larvicidal, ovicidal, oviposition deterrent, growth and/or reproduction inhibitors and/or adult repellents [19–22]. In particular, green-synthesized silver nanoparticles are emerging as multi-purpose materials, since their biosynthesis is easy and cheap; they are stable over time, and highly effective against mosquito vectors [23–28].

In this study, we focused on the swift fabrication of silver nanoparticles using *B. glabra* and on its potential against the JE vector *Cx. tritaeniorhynchus*. From a biophysical point of view, the synthesized Ag NP were characterized by UV–vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD). Furthermore, the toxicity of *B. glabra* extract, synthesized Ag NP and a combined treatment testing blends of both mosquitocidals was evaluated against larvae and pupae of *Cx. tritaeniorhynchus*. The effectiveness of green-fabricated Ag NP against the JE vector was confirmed in adulticidal tests, as well as evaluating the impact of Ag NP on fecundity and longevity of adult mosquitoes. Lastly, larvicidal effectiveness of Ag NP was validated in the field treating sewage water bodies.

Materials and Methods

Collection of Plant Materials

The fresh leaves of *B. glabra* were collected in Kerala (Thenjippalam, Malappuram, India). The plants were identified at Department of Botany, Bharathiar University, Coimbatore. Voucher specimens were deposited in our laboratory and are available upon request.

Synthesis and Characterization of Silver Nanoparticles

Leaves of *B. glabra* were thoroughly washed with distilled water and dried for 2 days at room temperature. A plant leaf extract was prepared placing 10 g of leaves

in a 300-mL Erlenmeyer flask with 100 mL of sterile distilled water. The mixture was boiled at 5 min, decanted for 2 h, stored at -4°C , and used within 5 days. The crude extract was treated with an aqueous solution of AgNO_3 (1 mM) and incubated at room temperature with dark condition. The solution turned to yellow-brownish, indicating the formation of Ag NP. The effects of reaction time on synthesis rate and particle size of the Ag NP were studied carrying out the reaction in a water bath (from 10 min to 4 h at 95°C with reflux). AgNO_3 solution was purified by repeated centrifugation at 15,000 rpm for 20 min, followed by re-dispersion of the pellet in deionizer water. The presence of synthesized Ag NP was confirmed by sampling the reaction mixture at regular intervals and absorption maxima was scanned by ultraviolet–visible (UV–vis) spectrum at the wavelength of 200–800 nm in a UV-3600 Shimadzu spectrophotometer operating at a resolution of 1 nm. Furthermore, the reaction mixture was subjected to centrifugation at 15,000 rpm for 20 min. The resulting pellet was dissolved in deionized water and filtered through a Millipore filter (0.45 μm). After freeze-drying of the purified Ag NP, the structure and composition were analyzed by 30-kV ultra-high resolution SEM (FEI QUANTA-200 SEM), TEM and EDX spectroscopy. The surface groups of the Ag NP were qualitatively confirmed using FTIR spectroscopy. FTIR spectra were recorded on a Perkin-Elmer 2000 FTIR spectrophotometer. To investigate the crystalline structure of silver nanoparticles, XRD analyses were conducted using a $\text{CuK}\alpha$ radiation (PAN analytical X'pert Pro MPD diffractometer [27]).

Mosquito Rearing

Following the method reported by Murugan [25, 26] the eggs of *Cx. tritaeniorhynchus* were collected from Kallar at Mettupalayam, Tamil Nadu, India. Batches of 100–110 eggs were transferred into 18 cm L \times 13 cm W \times 4 cm enamel trays containing 500 mL of water and they were allowed to hatch at laboratory conditions [$27 \pm 2^{\circ}\text{C}$ and 75–85 % R.H.; 14:10 (L:D) photoperiod]. Larvae were daily fed with 5 g of dog biscuits (Pedigree, USA) and brewer's yeast (Sigma–Aldrich, Germany) in 3:1 ratio. I to IV instar larvae and recently pupated individuals (i.e., pupation occurred within 24 h) were collected and used in the experiments.

Larvicidal and Pupicidal Potential

Larvicidal activity of the aqueous extract and Ag NP from *B. glabra* was evaluated according to WHO protocol [29] with slight modification [25, 26]. 25 *Cx. tritaeniorhynchus* larvae (I, II, III, IV instar) and pupae were placed for 24 h in a 500-mL glass beaker filled with 249 mL of dechlorinated water plus 1 mL of the desired concentration of *B. glabra* aqueous extract or *B. glabra*- synthesized Ag NP. Larval food (0.5 mg) was also provided. Each concentration was replicated five times against all larval instars and pupae. In control treatments, 25 larvae or pupae were transferred in 250 mL of dechlorinated water. Percentage mortality was calculated as follows:

$$\text{Mortality (\%)} = (\text{Number of dead individuals} / \text{Number of treated individuals}) \times 100.$$

Impact on Longevity and Fecundity

Following the method reported by Naresh Kumar et al. [30] the longevity and fecundity experiments were conducted by taking an equal number of male and female larvae which had emerged from the control and treated sites. They were settled in wooden cages (30 × 30 cm) individually for each concentration. Three days after the blood meal, eggs were collected daily from the small plastic bowls containing water kept in the cages. The fecundity was calculated by the number of eggs laid in ovitrap divided by number of females let to mate (20 n, the death of adults in the experiments was also considered). Longevity was calculated by the number of days lived by the adult. The emergence day and mortal days of the adults were recorded and the mean longevity in days was calculated.

Field Experiments

The aqueous leaf extract of *B. glabra* and green-synthesized Ag NP were applied into sewage water bodies at Kalveerampalaym, Coimbatore, using a knapsack sprayer (Private Limited 2008, Ignition Products, India). Post-treatment observations were conducted after 24, 48, and 72 h using a larval dipper. Toxicity was assessed against larvae. All larvae were counted and identified to specific level Almirón and Brewer [31]. More than 91 % of all surveyed larvae belong to *Cx. tritaeniorhynchus*. Six trials were conducted for each site over similar weather conditions (28 ± 2 °C; 80 % RH). The required concentration of mosquitocidal was calculated on the basis of the total surface area and volume (0.25 m³ and 250 L) using 10 × LC₅₀ values [32]. After 24 h, the percentage reduction of the larval density was calculated using the formula:

Percentage reduction = $(C - T)/C \times 100$, where C is the total number of mosquitoes in the control and T is the total number of mosquitoes in the treatment [27].

Data Analysis

All mosquito toxicity data were subjected to ANOVA followed by Tukey's HSD test [33]. Mosquito mortality data were also subjected to probit analysis. LC₅₀ and LC₉₀, were calculated using the method by Finney [34]. Data were analyzed using the SPSS Statistical Software Package version 17.0. A probability level of P < 0.05 was used for the significance of differences between values.

Results and Discussion

Bio-Physical Characterization of Nanoparticles

Biofabricated silver nanoparticles were characterized by UV–vis spectrophotometry, which could be used to examine size and shape-controlled nanoparticles in aqueous suspensions [39]. When the AgNO₃ solution and leaf extract were mixed, the colour changed from light pale yellow to brownish indicating the reduction from

Ag^+ to Ag (Fig. 1a-b). The color changes were noted by visual observation in the leaf extracts when incubated with AgNO_3 solution. The absorption spectrum of Ag NP showed a maximum absorption peak at 430 nm (Fig. 1c). Manusadianas et al. [35] previously reported that the lethality response of aquatic organisms (*Nitellopsis obtusa* algae, *Thamnocephalus platyurus* shrimps, and *Brachionus calyciflorus* rotifers) induced by sonicated and non-sonicated nano ZnO suspensions with various particle sizes (10 and 20–30 nm) and nano ZnO particles showed LC_{50} values of 438, 0.21, and 0.6 mg/L for 20–30 nm, respectively. Here the main peak is probably due to the excitation of longitudinal plasmon vibrations of Ag NP in the suspension [36, 37]. The synthesis of Ag NP was confirmed through visual assessment. Similarly, the *S. muticum* aqueous extract (5 % w/v) changed from yellowish light brown to dark brown after the addition of 1 mM AgNO_3 . [38].

Representative SEM micrograph (Fig. 2) of synthesized nanoparticles showed spherical and cubic structures with a size range of 30–70 nm. The latter may originate from the capping biomolecules bound to the surface of Ag NP [40, 41]. In agreement with our research, Ankanna et al. [42] reported SEM micrographs of Ag NP indicating that they were well-dispersed and ranged in size 30–40 nm. Also Ag NP produced using *Emblica officinalis* were predominantly spherical with an

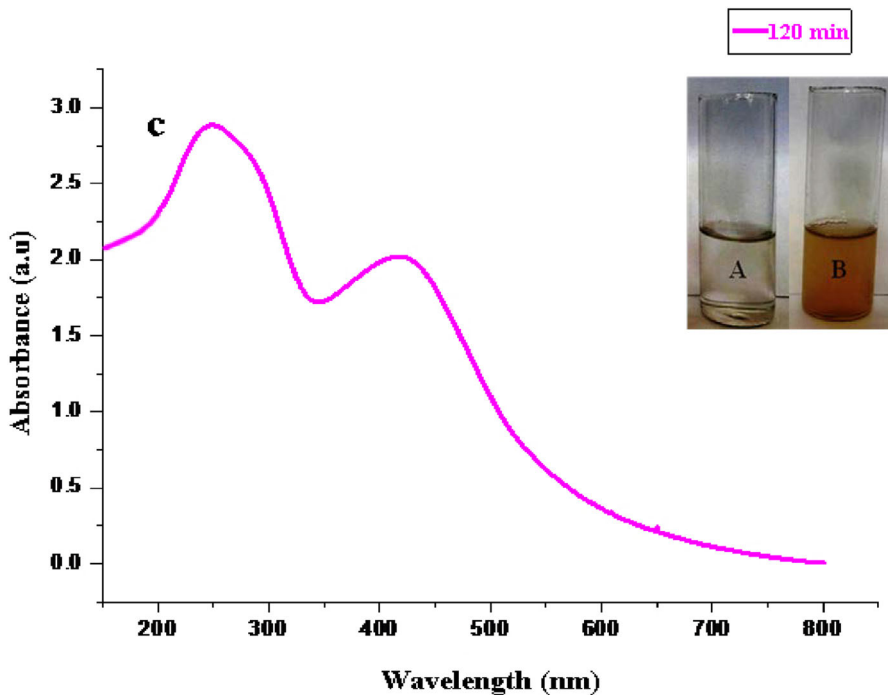


Fig. 1 The pictures show the color change (a) before and after (b) the process of reduction of Ag^+ to Ag nanoparticles. c UV-visualization of the absorption spectrum of silver nanoparticles synthesized using the *B. glabra* leaf extract after 120 min

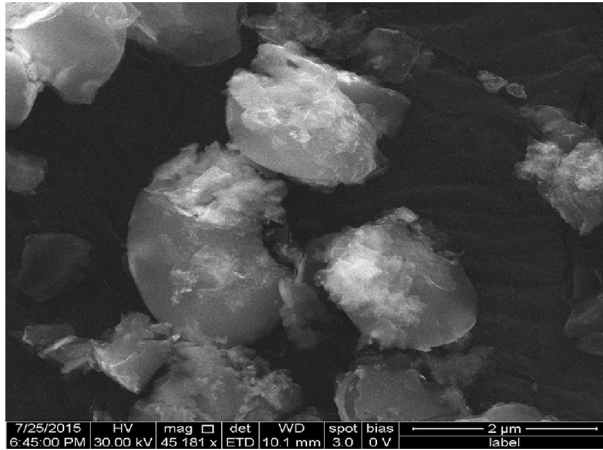


Fig. 2 SEM showing the morphological characteristics of silver nanoparticles synthesized using the *B. glabra* extract

average size of 16.8 nm [43]. Murugan et al. [44] reported that *Aristolochia indica* leaf extract can be used for effective synthesis of Ag NP with 20–35 nm in size. The *Sargassum muticum*-mediated synthesis led to the production of well dispersed Ag NP with a size range of 43–79 nm [38].

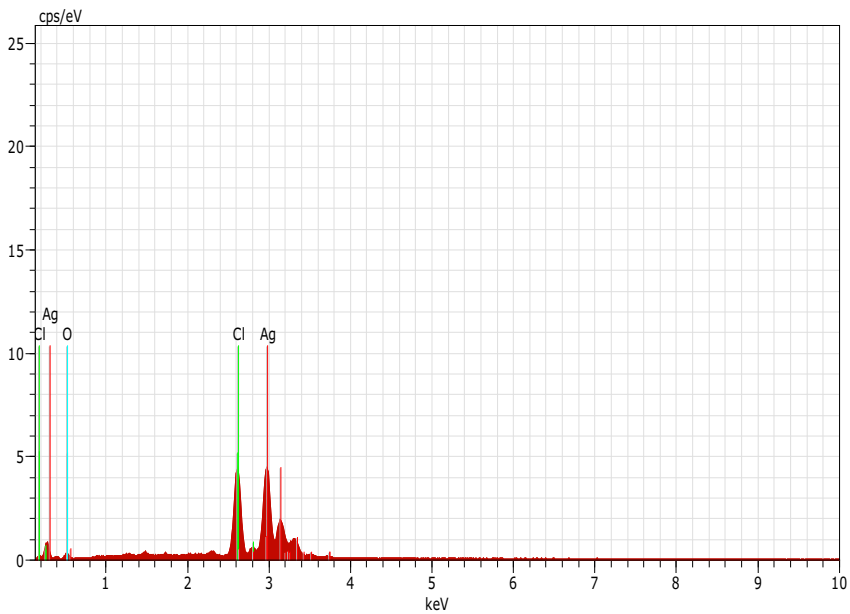


Fig. 3 EDX profile of *Bougainvillea glabra*-synthesized silver nanoparticles

Notably, the Ag NP formed was irregularly cubical or spherical in shape. It is known that the shape of metal nanoparticles considerably changes their optical and electronic properties [45]. The EDX spectrum recorded from *B. glabra* synthesized Ag NP revealed a distinct signal and high atomic percent values for silver. EDX spectroscopy confirmed the presence of Ag in the analyzed samples, showing a sharp optical absorption band peak at 5 keV (Fig. 3), typical for the absorption of metallic silver nanocrystallites [46–49]. Figure 4 shows TEM of Ag NP synthesized using *B. glabra* leaf extract. Among shapes, spheres dominated, an average size lower than 20 nm. Most of the Ag NP was roughly circular in shape with smooth edges. In previous findings, Ag NP from *Annona squamosa* leaf extract were spherical in shape with an average size ranging from 20 to 100 nm [50] while Thirunavokkarasu et al. [51] have reported spherical nanoparticles with size ranging from 8 to 90 nm following one-pot fabrication with *Desmodium gangeticum*.

Nanoparticles in XRD patterns exhibited different size dependent features leading to anomalous peak position, height and width (Fig. 5). The sharp Bragg's peaks may be due to the presence of capping agent stabilizing Ag NP [52]. Our findings are in agreement with previous research conducted on Ag NP synthesized using leaf extract of *Acalypha indica* [53]. The XRD pattern of pure silver ions was known to display peaks at $2\theta = 7.9^\circ, 11.4^\circ, 17.8^\circ, 30.38^\circ,$ and 44° [54]. Dubey et al. [55] showed that the size of silver nanocrystals, as estimated from the full width at half maximum of (111) peak of silver using the Scherrer's formula, was 20–60 nm. In agreement with our results, a peak with maximum absorption at

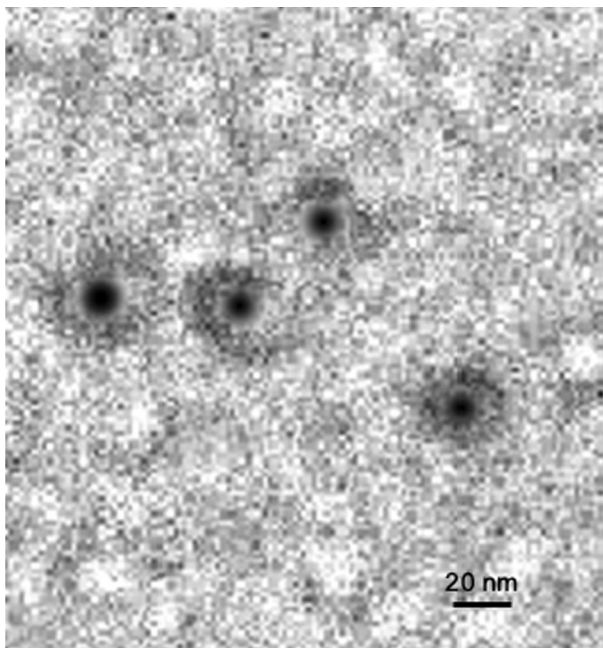


Fig. 4 TEM micrograph of silver nanoparticles biosynthesized using the *Bougainvillea glabra* aqueous extract

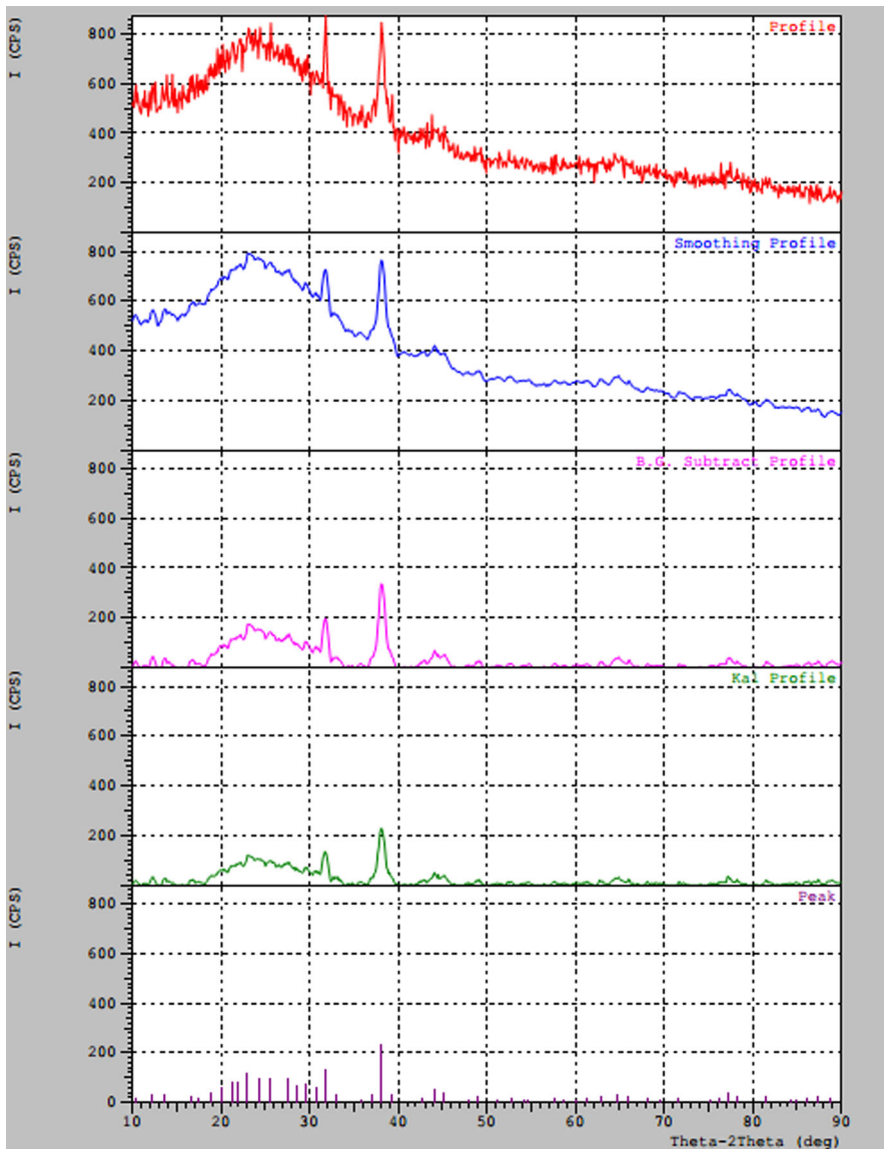


Fig. 5 XRD pattern of silver nanoparticles synthesized using the *Bougainvillea glabra* leaf extract

410 nm characterized the synthesis of *Aloe vera*-fabricated Ag NP [37]. The XRD patterns indicated that the structure of Ag NP is face-centered cubic [56]. Overall, from the XRD pattern, it can be noted that Ag NP synthesized using *B. glabra* leaf extract were essentially crystalline. Recently, XRD analyses aimed at the characterization of Ag NP fabricated using the extract of *Mimusops elengi* leaves and *S. muticum* also pointed out the production of silver nanocrystallites [38, 57].

Our XRD results also highlights that the crystallization of the bioorganic phase occurs on the surface of the green-synthesized Ag NP [58].

FTIR spectroscopy was carried out to identify the possible bio-molecules from the *B. glabra* leaf extract, which may be responsible for the green synthesis and stabilization of Ag NPs. Figure 6 reports that the FTIR spectrum of aqueous Ag NP prepared from the *B. glabra* leaf extract have major transmittance peaks at 3369.87, 2975.46, 2937.18, 2132.62, 1921.04, 1738.50, 1654.34, 1438.79, 1368.48, 1218.23, 1086.28, 1045.30, 879.68, 655.22, 598.92, and 544.49 cm^{-1} . They indicate that these different functional groups from bio molecules have probably capped Ag NP to prevent agglomeration, thus stabilizing the medium [25, 26, 59]. In detail, the peaks at 1,620–1,636 cm^{-1} may represent carbonyl groups from polyphenols such as catechin gallate, epicatechin gallate, epigallocatechin, epigallocatechin gallate, gallic acid and theaflavin; the results suggest that molecules interacting with Ag NP have free and bound amide groups. These amide groups may also be in the aromatic rings [60]. The peak at 1,381 cm^{-1} is probably due to the C–N stretching of the aromatic amine group [61]. The peaks at 1,027–1,092 cm^{-1} could correspond to the C–N stretching vibration of aliphatic amines or to alcohols/phenols, representing the presence of polyphenols [62]. The broad intense band close to 3400 cm^{-1} may be assigned to the N–H stretching arising from peptide linkages present in the proteins of the extract [63], while the peak located close to 1640 cm^{-1} could be assigned to the C=O stretching in carboxyl groups or C=N bending in amide groups [64, 65].

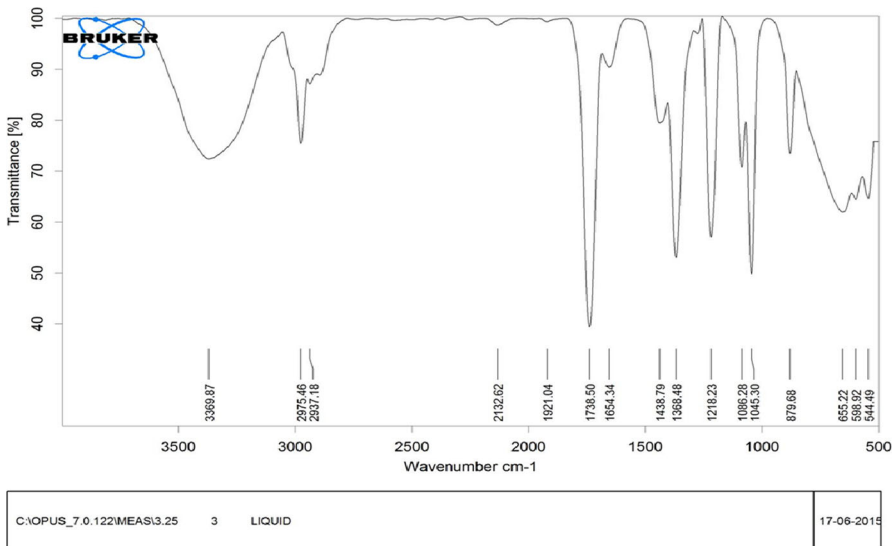


Fig. 6 Fourier transform infrared (FTIR) spectrum of vacuum-dried powder of silver nanoparticles synthesized using the extract of *Bougainvillea glabra* leaves

Ovicidal, Larvicidal and Pupicidal Potential

Egg hatchability of *Cx. tritaeniorhynchus* post-exposure to different doses of *B. glabra* extract and Ag NP was showed in Table 1. The percent hatchability was inversely proportional to the concentration of extract. Among the extracts tested for ovicidal activity on *C. tritaeniorhynchus*, the ovicidal activities of crude benzene and ethyl acetate extracts of *Ervatamia coronaria* (leaf) and *Caesalpinia pulcherrima* were highly effective [82]. Also, 100 % mortality was found post-treatment with methanol extract of *A. alnifolia* (125 ppm) [21].

The leaf extract of *B. glabra* was effective also against larvae (I-IV) and pupae of *Cx. tritaeniorhynchus*. LC₅₀ values were 198.93, 234.50, 309.18, 371.69 and 466.09 µg/ml, respectively. LC₉₀ values were 477.28, 563.56, 711.75, 772.26 and 936.36 µg/ml, respectively (Table 2). The potential role of botanical as nove mosquito larvicides and pupicides has been reported for a wide number of species [2, 19, 37, 66, 67]. Kovendan et al. [68] showed that methanolic leaf extract of *Jatropha curcas* exhibited dose-dependent larval toxicity against *Cx. quinquefasciatus*. Mahesh Kumar et al. [69] reported that *S. xanthocarpum* was toxic for larvae and pupae of *Cx. quinquefasciatus* with LC₅₀ of 155.29, 198.32, 271.12, 377.44 and 448.41 ppm. Results of the present study are consistent with earlier findings [70] where the LC₅₀ values of ethyl acetate extract of *Leucas aspera* were 75.40, 93.09, 132.20, and 138.60 ppm against the first, second, third, and fourth larval instars of *Cx. quinquefasciatus*. Also, the leaf extract of *A. alnifolia* with different solvents (hexane, chloroform, ethyl acetate, acetone and methanol) tested for larvicidal activity *C. quinquefasciatus* led to LC₅₀ of 198.79, 172.48, 151.06, 140.69 and 127.98 ppm and LC₉₀ = 458.73, 430.66, 418.78, 408.83 and 386.26 ppm, respectively [71]. Unfortunately, most of these researches failed to show insights on toxicity mechanisms exterted by the tested botanicals.

Green-synthesized Ag NP were highly effective in laboratory experiments conducted on *Cx. tritaeniorhynchus* larvae, with LC₅₀ of 7.77, 9.40, 13.19, 15.67 and 19.44 µg/ml; LC₉₀ were 19.28, 25.12, 30.80, 33.50 and 41.63 µg/ml concentration of 5 to 25 µg/ml, respectively (Table 3). Recently, the larvicidal activity of Ag NP synthesized using an aqueous extract from neem cake were highly effective against *A. aegypti* LC₅₀ of 3.96, 4.52, 5.42, 6.71, and 8.30 ppm, respectively [28].].

Table 1 Ovicidal activity of the *B. glabra* leaf extract and biofabricated silver nanoparticles against the JE vector *Culex tritaeniorhynchus*

Mosquitocidal treatment	Egg hatchability (%)					
<i>B. glabra</i> leaf extract	Control	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL
	100 ± 0.00 ^a	58.6 ± 1.16 ^b	45.3 ± 1.17 ^c	34 ± 1.33 ^d	28.2 ± 1.28 ^e	NH
Silver nanoparticles	Control	5 µg/mL	10 µg/mL	15 µg/mL	20 µg/mL	25 µg/mL
	100 ± 0.00 ^a	43.2 ± 1.28 ^c	22.5 ± 1.19 ^f	NH	NH	NH

Values were mean ± SD of five replicates Different letters indicated significant differences (ANOVA followed by Tukey's HSD, $P < 0.05$)

NH no hatchability

Table 2 Larvicidal and pupicidal toxicity of the *B. glabra* leaf extract against the JE vector *Culex tritaeniorhynchus*

Target	Mortality (%)					Regression equation	LC ₅₀ (LC ₉₀)	95 % confidence limit		χ^2
	100 µg/mL	200 µg/mL	300 µg/mL	400 µg/mL	500 µg/mL			LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)	
Larva I	35.1 ± 1.10 ^a	51.8 ± 1.24 ^a	62.3 ± 1.18 ^a	78.5 ± 1.29 ^a	96.2 ± 1.01 ^a	$y = 0.916 + 0.005x$	198.93 (477.28)	(105.26–257.32)	(396.16–662.18)	5.495 n.s.
Larva II	31.4 ± 1.07 ^b	45.6 ± 1.36 ^b	58.2 ± 1.06 ^b	69.5 ± 1.04 ^b	88.7 ± 1.31 ^b	$y = -0.913 + 0.004x$	234.50 (563.56)	(198.78–264.89)	(505.91–650.34)	2.447 n.s.
Larva III	28.6 ± 1.20 ^c	34.2 ± 1.15 ^c	46.5 ± 1.23 ^c	58.7 ± 1.35 ^c	76.4 ± 1.07 ^c	$y = -0.984 + 0.003x$	309.18 (711.75)	(272.68–346.97)	(621.04–862.05)	1.963 n.s.
Larva IV	22.2 ± 1.28 ^d	27.9 ± 1.34 ^d	38.5 ± 1.20 ^d	49.3 ± 1.23 ^d	70.6 ± 1.07 ^d	$y = -1.189 + 0.003x$	371.69 (772.26)	(335.13–417.40)	(671.17–941.06)	2.602 n.s.
Pupa	16.8 ± 1.28 ^e	24.5 ± 1.36 ^e	29.1 ± 1.34 ^e	39.6 ± 1.07 ^e	57.4 ± 1.02 ^e	$y = -1.287 + 0.003x$	466.09 (930.36)	(414.32–548.75)	(781.77–1206.20)	1.640 n.s.

No mortality was observed in the control, Mortality is expressed as mean ± SD of five replicates, Within a column means followed by the same letter(s) are not significantly different at 5 % level by Tukey's HSD test

LCL lower confidence limit

UCL upper confidence limit

χ^2 Chi square

n.s. not significant ($\alpha = 0.05$)

Table 3 Larvicidal and pupicidal toxicity of green-synthesized silver nanoparticles against the JE vector *Culex tritaeniorhynchus*

Target	Mortality (%)					Regression equation	LC ₅₀ (LC ₉₀)	95 % confidence limit		χ ²
	5 µg/mL	10 µg/mL	15 µg/mL	20 µg/mL	25 µg/mL			LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)	
Larva I	41.3 ± 1.38 ^a	58.7 ± 1.16 ^a	75.1 ± 1.29 ^a	89.5 ± 1.62 ^a	100 ± 0.00 ^a	y = -0.866 + 0.111x	7.77 (19.28)	(6.104-9.09)	(17.672-21.47)	4.697 n.s.
Larva II	38.8 ± 1.06 ^b	49.4 ± 1.32 ^b	67.3 ± 1.36 ^b	77.6 ± 1.77 ^b	92.5 ± 1.23 ^b	y = -0.767 + 0.082x	9.40 (25.12)	(7.376-10.99)	(22.653-28.77)	1.974 n.s.
Larva III	31.4 ± 1.32 ^c	38.8 ± 1.28 ^c	52.6 ± 1.43 ^c	64.2 ± 1.42 ^c	85.5 ± 0.88 ^c	y = -0.961 + 0.073x	13.19 (30.80)	(11.451-14.78)	(27.417-36.05)	3.858 n.s.
Larva IV	26.2 ± 1.06 ^d	31.5 ± 1.38 ^d	45.7 ± 1.24 ^d	57.6 ± 1.07 ^d	79.9 ± 1.41 ^d	y = -1.127 + 0.072x	15.67 (33.50)	(14.060-17.36)	(29.717-39.44)	3.743 n.s.
Pupa	22.6 ± 1.20 ^e	28.4 ± 0.92 ^d	36.8 ± 1.28 ^e	49.7 ± 1.36 ^e	65.5 ± 0.81 ^e	y = -1.123 + 0.058x	19.44 (41.63)	(17.390-22.20)	(35.555-52.38)	1.248 n.s.

No mortality was observed in the control, Mortality is expressed as mean ± SD of five replicates, Within a column means followed by the same letter(s) are not significantly different at 5 % level by Tukey's HSD test

LCL lower confidence limit

UCL upper confidence limit

χ² Chi square

n.s. not significant (α = 0.05)

L. aspera-synthesized Ag NP showed LC₅₀ ranging from 13.06 (I) to 25.54 ppm (pupae) towards *A. aegypti* [77]. Besides the effectiveness of green-synthesized Ag NP, they are often eco-friendly. Nowadays, moderate knowledge is available about the acute toxicity of mosquitocidal nanoparticles towards non-target aquatic species [56]. Notably, Subarani et al. [72] did not reported toxicity effects of *Vinca rosea*-synthesized Ag NP against *P. reticulata*, after 72 h of exposure to dosages toxic against *A. stephensi* and *C. quinquefasciatus*. Similarly, Haldar et al. [73] did not detected toxicity of Ag NP produced using dried green fruits, *D. roxburghii* against *P. reticulata*, after 48 h exposure to LC₅₀ of IV instars larvae of *A. stephensi* and *C. quinquefasciatus*. Similarly, the larvicidal effect of aqueous crude leaf extracts, silver nitrate solution, and Ag NP of *Mimosa pudica* showed that the highest mortality was achieved by synthesized Ag NP both against the larvae of *A. subpictus* (LC₅₀ = 8.89, 11.82, and 0.69 ppm) and *C. quinquefasciatus* (LC₅₀ = 9.51, 13.65, and 1.10 ppm) [74]. High mortality was reported for treatments on *A. subpictus* using *Nelumbo nucifera* plant extract methanol extract, aqueous extract, and green-synthesized Ag NP (LC₅₀ = 8.89, 11.82, and 0.69 ppm; LC₉₀ = 28.65, 36.06, and 2.15 ppm) and also against the larvae of *C. quinquefasciatus* (LC₅₀ = 9.51, 13.65, and 1.10 ppm; LC₉₀ = 28.13, 35.83, and 3.59 ppm) [75]. As regards to other arthropods, ZnO nanoparticles showed the LC₅₀ and r^2 values against *Rhipicephalus microplus* (13.41 mg/L; 0.982), *Pediculus humanus capitis* (11.80; 0.966 mg/L), and larvae of *An. subpictus* (3.19; 0.945 mg/L) and *C. quinquefasciatus* (4.87; 0.970 mg/L) [76].

Combined treatments with *B. glabra* leaf extract plus 5.12.5 µg/ml of Ag NP lowered the botanical LC₅₀ to 66.09 (I), 76.48 (II), 99.02 (III), 133.43 (IV) and 179.74 µg/ml (IV), respectively (Table 4). Similarly, *Morinda citrifolia* tested in blend with the fungus *Metarhizium anisopliae* achieved values of LC₅₀ 3.71, 16.73, 29.71, 40.60, and 138.10 mg/L; LC₉₀ were 122.29, 150.15, 156.90, 211.99 and 806.67 mg/L at 48 h, respectively [78]. Moreover, the combined treatment of *Catharanthus roseus* with *Bacillus thuringiensis* against the malaria vector *An. stephensi* obtained LC₅₀ of 2.18, 2.41, 2.76 and 3.22 mg/L, respectively [79]. The larval mortality after a combined treatment of *B. thuringiensis* subsp. *israelensis* plus *Jatropha curcas* leaf extract (JCLE) was found toxic for all mosquito larval instars. At a dose of 14 µg/ml + 1.0 % combined *B. thuringiensis* subsp. *israelensis*, the treatment led to IV instar larval mortality of 83 %. The LC₅₀ values were 1.20, 1.29 1.35 and 1.44 %, respectively [80]. The larvicidal activity of aqueous crude bark extracts and synthesized Ag NP from *F. racemosa* was tested against fourth instars larvae of the filarial vector *C. quinquefasciatus* and the JE vector *C. gelidus* showing LC₅₀ of 12.00 and 11.21 mg/l, respectively [81].

Impact on Longevity and Fecundity

Adult longevity and fecundity of *Cx. tritaeniorhynchus*, after the treatment of *B. glabra* extract and green synthesized Ag NP, is shown in Table 5. Significant reduction in adult longevity and fecundity was recorded in experiment when compared with the control. Longevity was reduced to 8.1 days post-treatment at 500 µg/ml concentration of aqueous extract of *B. glabra*, whereas control longevity

Table 4 Combined treatment of *B. glabra* leaf extract plus green-synthesized silver nanoparticles against the JE vector *Culex tritaeniorhynchus*

Target	Mortality (%)					Regression equation	LC ₅₀ (LC ₉₀)	95 % confidence limit		χ^2
	50 + 2.5 µg/mL	100 + 5 µg/mL	150 + 7.5 µg/mL	200 + 10 µg/mL	250 + 12.5 µg/mL			LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)	
Larva I	45.2 ± 2.77 ^a	67.4 ± 2.88 ^a	83.3 ± 1.81 ^a	93.7 ± 2.24 ^a	100.0 ± 0.0 ^a	$y = -0.750 + 0.011x$	66.09 (178.98)	(46.95–80.63)	(163.5–199.7)	1.886 n.s.
Larva II	41.3 ± 2.12 ^b	59.5 ± 2.06 ^b	75.0 ± 2.73 ^b	84.6 ± 2.07 ^b	91.8 ± 3.03 ^b	$y = -0.590 + 0.008x$	76.48 (242.56)	(50.54–95.41)	(218.4–278.1)	0.295 n.s.
Larva III	37.5 ± 2.14 ^c	51.2 ± 2.38 ^c	68.7 ± 3.14 ^c	71.4 ± 2.07 ^c	83.1 ± 2.80 ^c	$y = -0.585 + 0.006x$	99.02 (315.99)	(69.69–120.2)	(276.3–382.1)	1.754 n.s.
Larva IV	29.8 ± 2.38 ^d	45.4 ± 2.40 ^d	56.3 ± 1.97 ^d	67.7 ± 2.88 ^d	76.6 ± 2.70 ^d	$y = -0.785 + 0.006x$	133.43 (351.27)	(110.7–152.8)	(306.2–426.7)	0.419 n.s.
Pupa	24.6 ± 2.40 ^e	38.5 ± 2.35 ^e	49.4 ± 2.07 ^e	53.8 ± 1.30 ^e	64.2 ± 1.92 ^e	$y = -0.845 + 0.005x$	179.74 (373.16)	(155.7–208.5)	(320.7–461.6)	1.324 n.s.

No mortality was observed in the control, Mortality is expressed as mean ± SD of five replicates, Within a column means followed by the same letter(s) are not significantly different at 5 % level by Tukey's HSD test

LCL lower confidence limit

UCL upper confidence limit

χ^2 Chi square

n.s. not significant ($\alpha = 0.05$)

Table 5 Effect of *B. glabra* leaf extract and green-synthesized silver nanoparticles on fecundity and longevity of the JE vector *Culex tritaeniorhynchus*

Treatment ($\mu\text{g/mL}$)	Adult longevity (days)		Fecundity (n)
	Male	Female	
Control	23.2 ± 1.06^a	39.5 ± 0.99^a	165.3 ± 0.96^a
<i>B. glabra</i>			
100 $\mu\text{g/mL}$	19.8 ± 1.28^b	35.2 ± 1.49^b	159.6 ± 1.07^b
200 $\mu\text{g/mL}$	15.7 ± 1.00^c	32.5 ± 1.22^c	148.8 ± 1.57^c
300 $\mu\text{g/mL}$	13.4 ± 1.07^d	29.8 ± 1.06^d	139.2 ± 1.35^d
400 $\mu\text{g/mL}$	10.7 ± 1.17^e	25.7 ± 1.27^e	126.4 ± 1.07^e
500 $\mu\text{g/mL}$	8.1 ± 1.15^f	21.1 ± 1.44^f	117.7 ± 1.49^f
Ag nanoparticles			
5 $\mu\text{g/mL}$	16.4 ± 0.92^c	31.6 ± 1.07^c	151.8 ± 1.28^c
10 $\mu\text{g/mL}$	12.6 ± 1.20^d	28.4 ± 1.20^d	143.4 ± 1.36^{cd}
15 $\mu\text{g/mL}$	9.3 ± 1.28^e	23.7 ± 1.06^{ef}	135.2 ± 1.39^d
20 $\mu\text{g/mL}$	7.2 ± 1.15^f	19.3 ± 1.21^f	128.6 ± 1.20^e
25 $\mu\text{g/mL}$	4.7 ± 1.00^g	14.5 ± 1.47^g	112.5 ± 1.28^g

Within each column, values followed by the same letter(s) are not significantly different (ANOVA, Tukey's HSD, $\alpha = 0.05$)

was 23.2 days. The fecundity was also reduced post-treatment with *B. glabra*-synthesized Ag NP. One hundred and fifty eggs were recorded in control and the numbers of eggs recorded in treatment were 151.8, 143.4, 135.2, 128.6 and 112.5 eggs when doses of 5, 10, 15, 20, and 25 $\mu\text{g/ml}$ were tested, respectively. Similarly, Naresh Kumar et al. [83] reported a reduction in adult longevity (4.2 in male and 11.7 in female at 10 ppm) in *An. stephensi* after the treatment with Ag NP fabricated using *A. squamosa*. Furthermore, Poopathi and Tyagi [84] showed a reduction of adult longevity (17 in male and 27 in female) in *C. quinquefasciatus* after the treatment with *B. sphaericus* (GR strain). Also, Makowski et al. [85, 86] noted a reduction in adult longevity (17 days for males and 27 for females) in *C. quinquefasciatus* after the treatment with *B. sphaericus* (GR strain).

Adulticidal Potential on *Cx. tritaeniorhynchus*

In laboratory assays, the adulticidal potential of *B. glabra* leaf extract and Ag NP against *Cx. tritaeniorhynchus* showed LC_{50} and LC_{90} of 302.54 and 647.70 $\mu\text{g/ml}$; and 10.87 and 26.83 $\mu\text{g/ml}$, respectively (Table 6). Concerning other botanicals, the adulticidal activity of the essential oil isolated from *Mentha longifolia* was screened by fumigant toxicity assay against the house mosquito, *C. pipiens* by Oz et al. [87]. Also, the bio-surfactant products released by *Bacillus subtilis* subsp. *subtilis* (VCRC B471), are a potential bio adulticide for ULV spray against *A. stephensi* [88]. The adult mortality exerted by ethanol extract of *C. sinensis* showed LC_{50} and LC_{90} of 272.19 and 457.14 ppm, *A. stephensi* of 289.62 and 494.88 ppm, and *A. aegypti* of 320.38 and 524.57 ppm, respectively [89]. The methanol extract of *Andrographis*

Table 6 Adulticidal activity of *B. glabra* leaf extract and green-synthesized silver nanoparticles against the JE vector *Culex tritaeniorhynchus*

Treatment	Dose ($\mu\text{g}/\text{mL}$)	Mortality (%)	Regression equation	LC ₅₀ (ppm) (LCL-UCL)	LC ₉₀ (ppm) (LCL-UCL)	χ^2
<i>B. glabra</i> extract	Control	0.0 \pm 0.0 ^a	$y = 1.123 + 0.004x$	302.54 (270.75–334.58)	647.70 (577.31–756.21)	0.226 n.s.
	100	22.3 \pm 2.20 ^b				
	200	36.7 \pm 2.78 ^c				
	300	48.2 \pm 2.58 ^d				
	400	63.6 \pm 2.70 ^f				
	500	77.5 \pm 3.01 ^g				
Ag nanoparticles	Control	0.0 \pm 0.0 ^a	$y = 0.874 + 0.080x$	10.87 (9.04–12.39)	26.83 (24.16–30.79)	1.493 n.s.
	5	34.6 \pm 3.04 ^c				
	10	46.2 \pm 1.92 ^d				
	15	59.1 \pm 2.68 ^e				
	20	76.3 \pm 3.00 ^g				
	25	89.4 \pm 2.40 ^h				

No mortality was observed in the control, Mortality is expressed as mean \pm SD of five replicates, Within a column means followed by the same letter(s) are not significantly different at 5 % level by Tukey's HSD test

LCL lower confidence limit

UCL upper confidence limit

χ^2 Chi square

n.s. not significant ($\alpha = 0.05$)

paniculata on the adults of *C. quinquefasciatus* and *A. aegypti* showed LC₅₀ and LC₉₀ of 149.81, 172.37 ppm and 288.12, 321.01 ppm, respectively [90]. The LC₅₀ and LC₉₀ values of *A. alnifolia* leaf extracts (hexane, benzene, ethyl acetate, acetone and methanol) on *C. quinquefasciatus* adults were 383.59, 354.13, 327.74, 314.33 and 291.71 ppm, respectively [21]. The LC₅₀ and LC₉₀ values of *Cassia tora* leaf extracts (hexane, chloroform benzene, acetone, and methanol) against *C. quinquefasciatus* adults led to LC₅₀ values of 338.81, 315.73, 296.13, 279.23, and 261.03 ppm and LC₉₀ values were 575.77, 539.31, 513.99, 497.06, and 476.03 ppm, respectively [91].

Effectiveness in the Field

In the field, the Ag NP (10 \times LC₅₀) efficacy led to *Cx. tritaeniorhynchus* larval reduction of 17.8, 63.3, and 91.9 %, while the leaf extract of *B. glabra* achieved values of 7.84, 56.9, and 100 % of larval reduction after 24, 48, and 72 h, respectively (Table 7). These results are in agreement with previous research [92], analyzing the field efficacy of biopesticides in tsunami-affected areas of India, which reported strong reduction, or even eradication, of larval populations of several mosquito vectors. A total of 1,400 *C. quinquefasciatus* larvae found were

Table 7 Field efficacy of *B. glabra* leaf extract and green-synthesized silver nanoparticles against *Culex tritaeniorhynchus* larvae in sewage water bodies (study site: Kalveerampalayam; water body size: 1.5×1.0 m, depth: 1.5 m; required dose: $1.5 \times 1.0 \times 1.5 = 2.25$ L; required concentration: $0.402 \times 10 = 4.02$ $\mu\text{g/mL}$)

Site n.	Larval density (n)							
	Before treatment		Post-treatment		Before Treatment		Post-treatment	
	<i>B. glabra</i> leaf extract		<i>B. glabra</i> leaf extract		Ag nanoparticles		Ag nanoparticles	
	24 h	48 h	72 h	24 h	48 h	24 h	48 h	72 h
1	215	184	86	18	223	208	98	0
2	204	163	92	22	209	189	93	0
3	192	152	72	15	198	182	88	0
4	178	136	67	11	184	171	75	0
5	189	159	79	17	210	192	83	0
6	201	188	36	12	213	198	96	0
Total	1179	969	432	95	1237	1140	533	0
Mean \pm SD	$196.5 \pm 0.8a$	$161.5 \pm 1.2b$	$72 \pm 0.8c$	$15.8 \pm 1.4d$	$206.1 \pm 0.6e$	$190 \pm 0.8f$	88.8 ± 1.2	0
Reduction	–	17.8 %	63.3 %	91.9 %	–	7.84 %	56.9 %	100 %

Within the mean row, different letters indicate significant differences (Tukey's HSD test, $P < 0.05$)

observed in the sewage water systems. After being treated with *L. aspera* extracts, the *C. quinquefasciatus* larval density was reduced by 60.4, 81.9, and 99.7 % at 24, 48, and 72 h, respectively; similarly, the reduction of *C. quinquefasciatus* larval densities after treatment with *A. indicum*, *H. suaveolens*, and *J. curcas* were 51.7, 77.6 and 92 %; and 50, 73.5 and 90.4 %; 46.7, 71.7, and 89.9 % at 24, 48, and 72 h, respectively [93]. More recently, the mosquitocidal efficacy of the leaf extract of *E. hirta* was investigated in a field condition against *A. stephensi*, and larval density was reduced by 13.17, 37.64 and 84.00 % after 24, 48, and 72 h, respectively [94]. The larval density decreased rapidly post-treatment with plant extracts and Ag NP at the mosquito breeding sites (sewage water), thus these novel products could be considered as promising alternatives to synthetic insecticides for the mosquito vector management.

Conclusions

To our mind, the plant-mediated reduction of metal ions to nanocomposites could represent a valid tool for the development of clean, nontoxic, and environmentally acceptable control agents. The green-synthesized Ag NP fabricated in this research are hydrophilic in nature, disperse uniformly in water, and highly stable. They also exhibit significant mosquito potential activity against the JE vector *Cx. tritaeniorhynchus*. Indeed, the effectiveness of green-fabricated Ag NP against the JE vector was confirmed in adulticidal tests, as well as evaluating the impact of Ag NP on fecundity and longevity of adult mosquitoes. Larvicidal effectiveness of Ag NP was confirmed in treated sewage water bodies. Overall, this study suggests that the green-synthesized Ag NP fabricated using *B. glabra* can be considered a potential mosquito control device against the JE vector, *Cx. tritaeniorhynchus* in Asian regions.

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Compliance with Ethical Standards

Conflicts of interest We declare that we have no conflict of interest.

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