Mosquito larvicidal and silver nanoparticles synthesis potential of plant latex

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Abstract

Silver nanoparticles (AgNPs) were synthesized from the latex of the medicinally important plants *Euphorbia milii, Euphorbia hirta, Ficus racemosa* and *Jatropha curcas.* Synthesized AgNPs were characterized by UV-Vis spectrophotometry, scanning electron microscopy, energy dispersive X-ray analysis, X-ray diffraction, Fourier transformed infrared spectroscopy, particle size, and zeta potential analysis. Potency of latex and latex-synthesized AgNPs was evaluated against the 2nd and 4th instar larvae of *Aedes aegypti* and *Anopheles stephensi*. The lowest lethal concentration 50 (LC_{50}) value among the different types of plant latex studied was observed for latex of *E. milii* (281.28±23.30 and 178.97±37.82 ppm, respectively) against 2nd instar larvae of *Ae. aegypti* and *An. stephensi*. *E. milii* latex-synthesised AgNPs showed a high reduction in LC_{50} compared with its latex; *i.e.*, 8.76±0.46 and 8.67±0.47 ppm, respectively, for 2nd instars of *Ae. aegypti* and *An. stephensi*. LC_{50} values of AgNPs synthesized using the latex of *E. hirta, F. racemosa* and *J. curcas* were lower than those of the latex of the respective plants; *i.e.*, 10.77±0.53, 9.81±0.52, 12.06±0.60 and 8.79±0.51, 9.83±0.52, 9.60±0.51 ppm, respectively, for 2nd instars of *An. stephensi* and *Ae. aegypti*. Similarly, as compared with the plant latex, lower LC_{50} values were reported for latex-synthesized AgNPs against 4th instars of *Ae. aegypt* and *An. stephensi*. Results showed that all the types of plant latex investigated have the potential to convert silver nitrate into AgNPs showing a spectrum of potent mosquito larvicidal effects, indicating the possibility of further exploration of the bioefficacy of latex and latex-synthesized AgNPs against vectors of public health concerns.

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Introduction

About 3.3 and 2.5 billion people, respectively, are at risk of malaria and dengue worldwide, with a higher frequency in the population of sub-Saharan Africa (SSA) (WHO, 2009, 2011). In India, 1.49 million cases of malaria, 28,292 cases of dengue, 767 and 108 deaths were reported from malaria and dengue in 2010 (NVBDCP, 2011). The above figures indicate the global impact of mosquito-transmitted diseases with respect to loss of national productivity due to mortality and morbidity. Mosquito species such as *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* are widely distributed in the tropical and subtropical zones, acting as vectors of diseases like malaria, dengue, filariasis, Japanese encephalitis, yellow fever, and chikungunya (WHO, 2009). To control the outbreak of mosquito-borne diseases, attention should be given to targeting the larval stage of mosquitoes, which are unable to fly and are present in the breeding habitat. Devising a control methodology should therefore be relatively easy for the larval stage. During the past several decades, organophosphates such as temephos and fenthion, and insect growth regulators such as diflubenzuron and methoprene, have been used to control mosquito larvae (Yang *et al*., 2002). Insecticides of microbial origin, such as *Bacillus thuringiensis,* have also been employed for larval control (Raghvendra *et al*., 2011). However, continued and indiscriminate use of these insecticides creates problems such as insecticide resistance, environmental pollution and toxicity to human and non-target organisms (Raghvendra *et al*., 2011). To combat these shortcomings of chemical insecticides, research has shifted toward products of biological origin (Patil *et al*., 2012a; Karunamoorthi, 2013).

Use of products of plant origin to control mosquito larvae has been shown to be an exciting alternative to traditional methods of larval management, as they are not associated with the problems noted above (Shaalan *et al*., 2005, Borase *et al*., 2013). For example, root and leaf extracts of *Plumbego zeylanica* and *Cestrum nocturnum* (Patil *et al*., 2011b), leaf extracts of *Ocimum sanctum, Phyllanthus emblica* (Murugan *et al*., 2012), and hydrodistillate extracts of *Mentha piperita, Ocimum basilicum, Zingiber officinale,* and *curcuma longa* (Kalaivani *et al*., 2012) have been used against mosquito larvae of *An. stephensi, Ae. aegypti* and *Cu. quinquefasciatus.*

The use of phytosynthesized silver nanoparticles as a larvicidal

agent instead of chemical insecticides is gaining importance because of their safety to users as well as nontarget species, and the novelty of their mechanism of action (Marimuthu *et al*., 2011; Patil *et al*., 2012b). Several plants have been screened successfully for silver nanoparticle synthesis, such as *Plumeria rubra.* (Patil *et al*., 2011a), *Pergularia daemia* (Patil *et al*., 2012a), *Acacia arabica* (Thakur *et al*., 2013), *Cadaba indica* lam leaf extract (Kalimuthu *et al*., 2013), *Euphorbia tirucalli*, and *Alstonia macrophylla* (Borase *et al*., 2013), as described in a review by Gan & Li (2012). Chemical and physical methods of nanosynthesis have shortcomings such as the use of toxic chemicals and high temperatures. To address these, the use of living organisms such as plants and microorganisms (bacteria and fungi) for nanoparticle synthesis is gaining momentum.

Latex is a milky to transparent sap produced in some plants and studied mostly with respect to rubber production, interactions with insects as a plant defense mechanism, and in explorations of different pharmacological activities (Kekwick, 2007). The latex-producing plants *E. milii*, *E. hirta*, *F. racemosa* and *J. curcas* used in the present study are available in large quantities locally in India and have been reported in the literature for their medicinal applications as well as for their active biochemical constituents (Table 1). For these reasons and because of the potent mosquito larvicidal activity showed by plant *Plumeria rubra* and *Pergularia daemia* and synthesized AgNPs in our earlier study (Patil *et al*., 2011a; 2012a), we wanted to investigate the potential of other types of plant latex as eco-friendly mosquito larvicidal agents, and as precursors for environmentally benign silver nanoparticle synthesis.

Materials and methods

Plant material

E. milii, *E. hirta*, *F. racemosa* and *J. curcas* growing in the vicinity of Jalgaon, India, were used as sources of fresh latex. Latex was collected in the early morning during March, 2013, by making a small incision near the youngest leaves and at the ends of branches. Extruded latex was collected in sterile tubes (10 mL). Tubes were kept at 4°C to stop coagulation until the time of the experiments.

Phytochemical characterisation of latex

Latex samples were subjected to qualitative tests for the presence of different metabolites as reported by Kokate (1999) and Patil *et al.* (2012b).

Synthesis of silver nanoparticles

One mL of fresh latex was added to 100 mL of an aqueous solution of silver nitrate (100 ppm). The flask was incubated on a rotary shaker (28°C at 120 rpm). Simultaneously, controls containing latex with Milli-Q deionized water and silver nitrate solution alone were maintained under the same conditions. Solutions were observed periodically for any colour change.

Test organisms

For the laboratory trials, locally collected early 2nd and 4th instar larvae of *Ae. aegypti* and *An. stephensi* were used as experimental specimens. The larvae were kept in plastic enamel trays containing dechlorinated tap water, and were maintained as reported by Kalimuthu *et al*. (2013).

Mosquito larvicidal bioassay

Different concentrations of latex and AgNPs were prepared in dechlorinated tap water. Larvicidal activity was assessed using the procedure of WHO (1996) with some modifications and as per the methods of Patil *et al.* (2011b, 2012b). Twenty five 2nd and 4th instar larvae were taken in four batches in 249 mL of water, and 1.0 mL of the desired concentration of latex plus AgNPs were added. The control was set up with dechlorinated tap water. The numbers of dead larvae were counted after 24 h of exposure, and the percent mortality was recorded for the average of four replicates. The experimental media, in which 100% mortality of larvae occurred, was selected for the dose-response bioassay (data not shown).

Dose response bioassay

Based on the preliminary screening results, crude latex extract of the experimental plants plus synthesized AgNPs were subjected to a dose-response bioassay for larvicidal activity against the larvae of *Ae. aegypti* and *An. stephensi*. Different concentrations ranging from 62.25

Table 1. Medicinal properties and chemical constituents of latex producing plants used for analysing larvicidal and silver nanoparticle synthesis potential.

Botanical name	Common name (vernacular name)	Family	Medicinal property	Chemical constituents	References
Euphorbia milii	Milli Crown of throrn (Christ Plant)	Euphorbiaceae	Molluscicidal	Miliin, serine proteas, flavons, triterpenoids, steroids, steroidal glycoside, alkaloids	Yadav *et al.* (2006);
Euphorbia hirta	Tawa-tawa (Dudhi)	Euphorbiaceae	Antihelminthic, repellent, antifeedant and controlling *Plutella xylostella* and nematicidal and against roundworm like guinea worm	Sterols, alkaloids, tannins, glycosides, triterpenoids, alkenes, phenolic acids, choline and shikimic acid	Iwu (1993); Wei *et al.* (2005); Kumar *et al.* (2002); Parekh & Chanda (2007); Rajeh *et al.* (2012)
Ficus racemosa	Cluster Fig Tree (Udumbara)	Moraceae	Anti-inflammatory, antidiarrheal, clears horsevoice and chemomodulatory, larvicides	Racemosic acid, triterpenes	Khan & Sultana (2005); Li *et al.* (2004); Rahuman *et al.* (2008)
Jatropha curcus	Bagbherenda (Jungli erand)	Euphorbiaceae	Nematicidal, fungicidal, mosquito (*Ochlerotatus triseriatus*) larvicidal, insecticidal activities	Triglycerols, sterols, oils, phorbal esters, glucanase protein	Sharma & Trivedi (2002); Gübitz *et al.* (1999)

to 2000 ppm (for the latex) and 0.625 to 20 ppm (for the synthesized AgNPs) were prepared, and numbers of dead larvae were counted after 24 h of exposure; percent mortality was reported from the average of four replicates.

Statistical analysis

Mortality was calculated using Abbott's formula (Abbott, 1925). The dose-response data were subjected to probit regression analysis (Finney, 1971). The lethal concentrations in parts per million (LC_{50}, LC_{90}) and the 95% confidence intervals of LC_{50} (upper confidence limit) and (lower confidence limit) were calculated.

Characterisation of silver nanoparticles

AgNPs solutions were centrifuged at 10,000 rpm for 10 min (REMI, Cooling centrifuge, C-24 BL, India); the pellet obtained was resuspended in water and used to analyse surface plasmon resonance of the silver nanoparticles using a UV-Vis spectrophotometer (Shimadzu 1601, Tokyo, Japan) at the resolution of 1 nm from 200 to 800 nm. Other techniques used for AgNPs characterization included Fourier-transformed infrared spectroscopy (FT-IR) (Shimadzu, Prestige 21, Tokyo, Japan), scanning electron microscopy (SEM), energy dispersive X-ray (EDX) (HITACHI- S4800, Tokyo, Japan), X-ray diffraction (XRD) (Brucker D8 Advance, Karlsruhe, Germany), particle size, and zeta potential analysis (Zetasizer, Malvern Instrument Ltd, Westborough, MA, USA).

Results

Synthesis and characterisation of AgNPs

Transformations of $AgNO_3$ to AgNPs were clearly indicated by a colour change of $AgNO_3$ from colourless to yellowish brown, depleting all the plant latex within 5 to 20 min of latex addition, without agglomeration and indicating synthesis of stable AgNPs (Figure 1A). Latex of *E. milii* showed the fastest colour change among all types of latex tested (within 5 min). Synthesized nanoparticles were characterized by UV-Visible spectroscopy showing a surface plasmon resonance band at 410 to 450 nm, which arises due to the conduction of free electrons on the surface of AgNPs (Figure 1B) (Smitha *et al*., 2008). Absorption maxima at 440, 433,419, and 444 nm were observed for AgNPs synthesized from *E. milii*, *E. hirta*, *F. racemosa* and *J. curcas,* respectively. Similar results have been shown by Borase *et al*. (2013) and Thakur *et al*. (2013). Absorbance of AgNPs synthesized from the latex of *E. milii* was found to be higher than the other types of plant latex under study. *E. milii* latex-fabricated AgNPs show a smaller size of 208 nm with a zeta potential of –9.19 mV (Figure

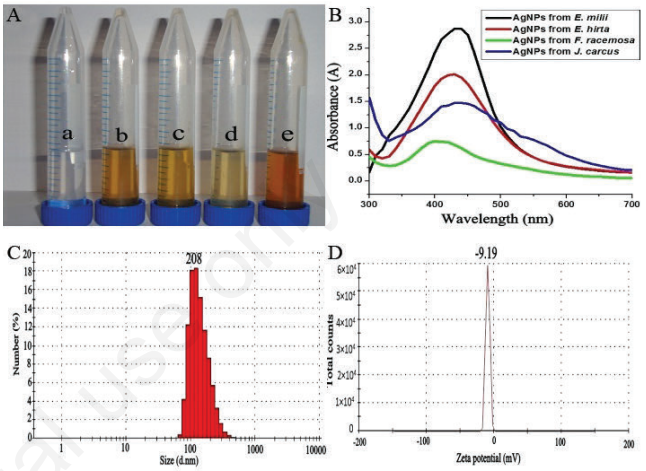

Figure 1. A) Tubes showing colour change of AgNPs: a. silver nitrate solution, b. *E. milii*, c. *E. hirta*, d. *F. Racemosa*, and e. *J. curcas*. B) UV spectra of AgNPs. C and D) Particle size and zeta potential analysis of AgNPs produced from *E. milii* latex.

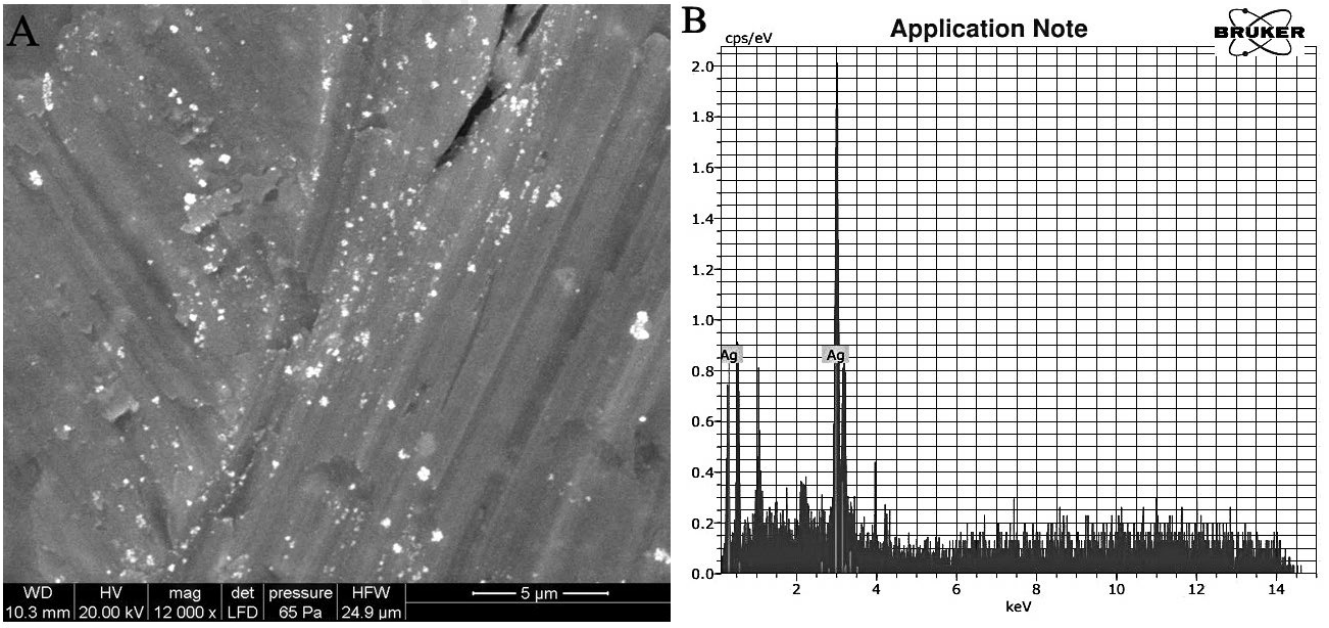

Figure 2. A and B) Scanning electron microscopy and energy dispersive X-ray image of AgNPs synthesized from *E. milii*.

1C and D). AgNPs from *E. hirta*, *F. racemosa* and *J. curcas* showed larger size particles having low stability, as compared with *E. milii*-synthesized AgNPs (data not shown). SEM imaging showed high density of spherical size, monodispersed AgNPs (Figure 2A). EDX spectra confirmed the presence of elemental silver in the samples, as there was a strong signal for the silver atom (Figure 2B).

Fourier transformed infrared spectroscopy analysis showed the presence of different functional groups corresponding to proteins, alkaloids, tannins, saponins and other plant metabolites (Figure 3A). A peak at 670.03 cm^{-1} was assigned to N-H wag of amines of proteins, 701.91 cm^{-1} as a C-H deformation in carbohydrates, 3439.96 cm^{-1} for Ar-OH, O-H and N-H for phenols, alcohols and amides, and 2997.48 cm^{-1} for the C=O bond found in terpenoids and flavonoids. The remaining peaks also indicate the presence of proteins, flavonoids, saponins and other plant metabolites, as evidenced by qualitative phytochemical analysis (Table 2). XRD analysis revealed the crystalline nature of AgNPs. Other peaks in the XRD may arise due to biomolecules capped on the AgNPs surface (Figure 3B).

Mosquito larvicidal bioassay

The plant latex under study and AgNPs fabricated from the latex were used to analyse their potency against the 2nd and 4th instar larvae of *Ae. aegypti* and *An. stephensi.* The results of larvicidal bioassays of the plant latex are presented in Tables 3 and 4, and that of the plant latex-synthesized AgNPs are presented in Tables 5 and 6. All tested plant latex and synthesized AgNPs showed larvicidal efficacy within 24 h of exposure. Mortality rate (Y) was positively related to the dose (X), indicating that mortality is dose-dependent. Latex materials from all the plants tested were less toxic than the synthesized AgNPs to both mosquito species.

Among the AgNPs tested, the AgNPs synthesized from the latex of *E. milii* were highly effective against *An. stephensi* (LC_{50}=8.76 ppm, LC_{90}= 17.11 ppm), and the AgNPs from *J. curcas* was highly effective against *Ae. Aegypti* (LC_{50}=9.43ppm, LC_{90}=18.20 ppm). All the plants used in the present study showed LC_{50} values less than 13 ppm, which could be an important factor in determining a practical larvicidal dose.

Discussion

Latex producing plants secrete milky fluid from a network of laticifer cells, in which subcellular organelles intensively synthesize proteins and secondary metabolites (Lopes *et al*., 2009). The biological importance of latex fluids is still unclear and knowledge of their physiological role is still limited (Ramos *et al*., 2007). Ramos *et al*. (2009) presented first evidence for the use of *Calotropis procerra* (Ait.) R.Br.-secreted proteolytic enzymes as chemical agents against *Ae. aegypti* larvae. Plant latex has been reported to have a negative effect on several insect functions such as egg hatch, larval growth and survival (Giridhar *et al*., 1984; Morsy *et al*., 2001; Ramos *et al*., 2006). The chemico-physical method of nanoparticle synthesis involves the use of toxic substances (sodium borohydrate, polyvinylpyrrolidone) that are harmful to the environment. Our method of AgNPs synthesis using latex, which has an abundance of proteins, enzymes and secondary metabolites, is novel, eco-friendly, and does not require toxic chemicals. Previous studies have demonstrated the involvement of proteins, polyphenols and carbohydrates in the synthesis of

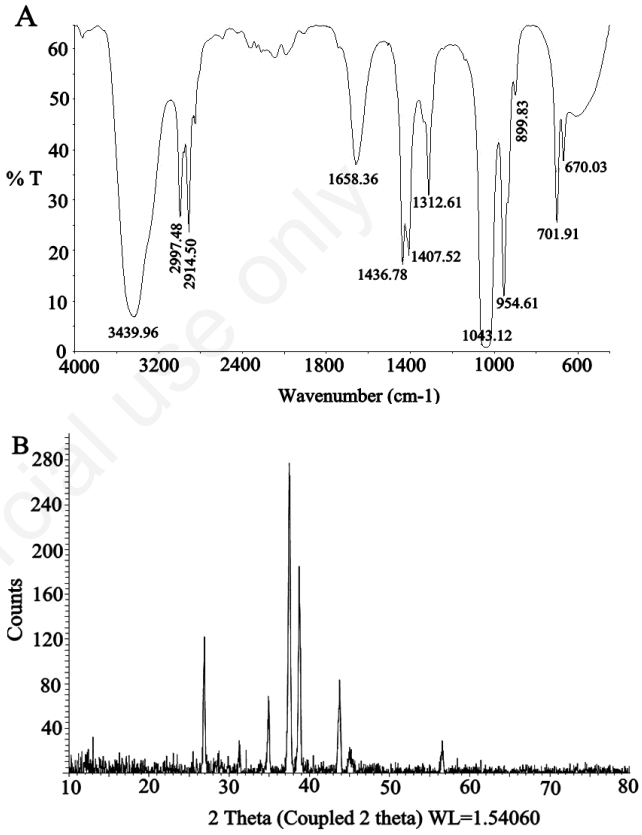

Figure 3. A and B) Fourier transformed infrared spectroscopy and X-ray diffraction spectrum of AgNPs synthesized from *E. milii*.

Table 2. Phytochemical analysis of plant latex.

Sr. No.	Metabolites	*E. milii*	*E. hirta*	*F. racemosa*	*J. curcas*
1	Protein	+	+	+	+
2	Carbohydrates	+	–	+	–
3	Terpenoids	+	+	+	+
4	Alkaloids	–	+	+	+
5	Phenolics	+	+	+	+
6	Flavonoids	+	+	+	+
7	Tannin	+	+	–	–
8	Saponins	+	+	+	+
9	Glycosides	+	–	+	–

+=present; –= absent. Sr., serial number.

metal nanoparticles (Gan & Li, 2012). Nanoparticles produced using chemical methods are of a defined size and shape due to the use of a single reducing and capping agent. In biological synthesis, diverse particle size and shape is observed because of multiple reducing and capping agents. Consequently, isolation, purification and scale-up of compounds responsible for nanoconversion of silver represent potentially valuable alternatives to chemical synthesis.

Duran *et al*. (2011) discussed involvement of the enzyme NADPH-dependent nitrate reductase in production of AgNPs, while Vigneshwaran *et al*. (2006) showed the role of reducing sugars in AgNPs production, AgNPs synthesis were also reported from combination of reducing agents and terpenoids (Shankar *et al*., 2004), polyols, eugenol, quinines and Phyllanthin (Jha *et al*., 2009; Kasthuri *et al*., 2009; Singh *et al*., 2010). The plant latex used in the present study also showed the presence of proteins and secondary metabolites (terpenoids, tannins, alkaloids and others), so we may preliminarily conclude there is an interaction of enzymatic and non-enzymatic compounds in AgNPs formation.

Corbel *et al*. (2007) showed that increased insecticide resistance in mosquitoes is due to increased activity of enzymes involved in insecticide metabolism (*e.g*., esterases, oxidases, glutathione-S-transferase) and mutation in the target sites of insecticide action. This can be corroborated with how AgNPs exhibit their larvicidal action. AgNPs have a high surface area-to-volume ratio, which imparts to them many types of biocidal and catalytic activities. Also, in latex-mediated nanosynthesis, capping of latex metabolites on the surface of the AgNPs, in addition to imparting stability, also increases their larvicidal action. The higher mortality at lower doses is consistent with earlier reports of AgNPs produced from leaf extracts of *Nelumbo nucifera* (LC_{50}=0.69 ppm, LC_{90}=2.15 ppm) against *An. subpictus* and *Cu. quinquefasciatus* (LC_{50}=1.10 ppm, LC_{90}=3.59 ppm) Thirunavukkarasu *et al*. (2010). Marimuthu *et al*. (2011) reported bioactivity of *Mimosa pudica*-synthesized AgNPs against the larvae of *An. subpictus*, *Cu. quinquefasciatus*, and *R. microplus* (LC_{50}=13.90, 11.73 and 8.98 ppm), respectively. AgNPs synthesized using *Tinospora cordifolia* extract were tested against the larvae of *An. subpictus* (LC_{50}=6.43 mg/L) and *Cu. quinquefasciatus* (LC_{50}=6.96 mg/L) (Jayaseelan *et al*., 2011).

Shaalan *et al*. (2005) reported that varying results obtained in lethal concentration values can be due to differences in the levels of toxicity among the insecticidal components of different plants, and the effect of plant extracts can vary significantly depending on plant species, plant part, age of the plant part, extraction solvent, seasonal variation, and mosquito species

In prokaryotic systems, AgNPs have multiple targets for biocidal effects by causing structural damage (Kim *et al*., 2007), generation of reactive oxygen species, interfering with DNA replication, and reacting with the thiol enzyme group (Liau *et al*., 1997; Feng *et al*., 2000). Patil *et al*. (2012b) also pointed out the antagonistic effect of AgNPs on enzymes and proteins regardless of the Gram characteristics in bacteria. The mechanism of larvicidal action of silver nanoparticles requires more detailed study.

Conclusions

Studies were conducted to evaluate the potential mosquito larvicidal activity of plant latex and latex-synthesized AgNPs. Our results suggest the possibility of addressing the problem of emerging mosquito resistance to chemical insecticides by using latex, latex-synthesized AgNPs, or combinations of chemical insecticides with latex and AgNPs, which could be considered an alternative larval eradication tactic that could help reduce the burden of toxic chemical insecticides on the environment and non-target organisms.

Table 3. Larvicidal activity of latex against 2nd instars larvae of *Aedes aegypti* and *Anopheles stephensi*.

Mosquito species	Plant latex	LC_{50}±SE (mg L^{-1})	95% fiducial limits (LCL-UCL)	LC_{90}±SE (mg L^{-1})	95% fiducial limits (LCL-UCL)	Regression equation
Aedes aegypti	*E. milii*	281.28±23.30	234.87-327.91	752.27±51.56	665.59-874.59	Y=9.58+0.00941X
	E. hirta	675.26±39.73	601.94-760.27	1422.69±88.19	1272.29-1626.91	Y=33.63+0.0112X
	F. racemosa	726.69±42.33	647.66-815.91	1555.16±90.48	1399.16-1761.71	Y=3.49+0.0111X
	J. carcus	746.98±48.52	655.56-848.55	1768.99±109.92	1580.74-2022.16	Y=4.49+0.00993X
Anopheles stephensi	*E. milii*	178.97±37.82	95.93-248.31	909.88±73.06	788.93-1087.58	Y=11.9+0.00772X
	F. racemosa	549.52±54.24	441.85-658.39	1809.71±134.66	1584.20-2130.08	Y=7.62+0.00820X
	E. hirta	568.74±46.84	477.61-664.21	1621.64±111.01	1433.66-1881.56	Y=6.70+0.00916X
	J. carcus	755.70±49.04	294.70-391.19	1772.58±112.93	1579.869-2033.902	Y=4.37+0.00985X

LC_{50}, 50% lethal concentration; SE, standard error; LCL, lower confidence limit; UCL, upper confidence limit; LC_{90}, 90% lethal concentration.

Table 4. Larvicidal activity of latex against 4th instars larvae of *Aedes aegypti* and *Anopheles stephensi*.

Mosquito species	Plant latex	LC_{50}±SE (mg L^{-1})	95% fiducial limits (LCL-UCL)	LC_{90}±SE (mg L^{-1})	95% fiducial limits (LCL-UCL)	Regression equation
Aedes aegypti	*E. milii*	638.11±36.53	571.00-716.69	1299.02±80.07	1162.58-1484.72	Y=3.45+0.0116X
	E. hirta	683.69±39.32	611.31-768.07	1408.23±86.27	1260.96-1607.78	Y=3.33+0.0114X
	F. racemosa	777.43±43.49	697.64-870.85	1563.74±93.01	1404.19-1777.41	Y=2.55+0.0113X
	J. carcus	798.89±46.00	713.78-896.79	1678.54±99.45	1507.56-1906.32	Y=3.00+0.0108X
Anopheles stephensi	*E. milii*	761.11±43.43	680.80-853.64	1580.75±93.51	1420.08-1795.11	Y=3.02+0.0111X
	E. hirta	783.42±42.89	704.43-875.06	1560.04±89.73	1405.42-1764.95	Y=2.38+0.0115X
	F. racemosa	884.69±45.65	800.79-982.26	1681.22±92.00	1521.86-1889.82	Y=1.43+0.0115X
	J. carcus	919.31±52.52	822.32-1031.26	1930.60±113.96	1734.54-2191.33	Y=2.68+0.010X

LC_{50}, 50% lethal concentration; SE, standard error; LCL, lower confidence limit; UCL, upper confidence limit; LC_{90}, 90% lethal concentration.

Table 5. Larvicidal activity of latex synthesized AgNPs against 2nd instars of *Aedes aegypti* and *Anopheles stephensi*.

Mosquito species	Plant AgNPs	LC_{50}±SE (mg L^{-1})	95% fiducial limits (LCL-UCL)	LC_{90}±SE (mg L^{-1})	95% fiducial limits (LCL-UCL)	Regression equation
Anopheles stephensi	*E. milii*	8.76±0.46	7.91-9.74	17.11±0.94	15.48-19.24	Y=1.82+1.13X
	E. hirta	10.77±0.53	9.78-11.91	20.11±1.06	18.27-22.5	Y=0.84+1.07X
	F. racemosa	9.81±0.52	8.85-10.93	19.34±1.07	17.47-21.78	Y=1.69+1.06X
	J. carcus	12.06±0.60	10.97-13.36	22.00±1.19	19.94-24.71	Y=0.36+1.01X
Aedes aegypti	*E. milii*	8.67±0.47	7.81-9.68	17.62±1.01	15.89-19.92	Y=2.04+1.11X
	E. hirta	8.79±0.51	7.82-9.87	19.51±1.17	17.50-22.18	Y=3.21+1.01X
	F. racemosa	9.83±0.52	8.88-10.93	19.14±1.06	17.31-21.54	Y=1.53+1.07X
	J. carcus	9.60±0.51	8.67-10.69	18.96±1.05	17.14-21.35	Y=1.81+1.07X

LC_{50}, 50% lethal concentration; SE, standard error; LCL, lower confidence limit; UCL, upper confidence limit; LC_{90}, 90% lethal concentration.

Table 6. Larvicidal activity of latex synthesized AgNPs against larvae of 4th instars of *Aedes aegypti* and *Anopheles stephensi*.

Mosquito species	Plant AgNPs	LC_{50}±SE (mg L^{-1})	95% fiducial limits (LCL-UCL)	LC_{90}±SE (mg L^{-1})	95% fiducial limits (LCL-UCL)	Regression equation
Aedes aegypti	*J. carcus*	9.43±0.48	8.53-10.46	18.20±0.97	16.50-20.41	Y=9.08+0.009X
	E. milii	9.49±0.48	8.61-10.53	17.60±0.96	15.93-19.79	Y=0.884+1.14X
	E. hirta	10.67±0.54	9.57-11.85	20.00±1.10	18.08-22.51	Y=0.808+1.06X
	F. racemosa	11.44±0.65	10.26-12.86	23.07±1.43	20.63-26.38	Y=1.89+0.925X
Anopheles stephensi	*E. milii*	9.95±0.49	9.05-11.02	18.13±0.97	16.45-20.33	Y=0.515+1.14X
	J. carcus	10.01±0.51	9.07-1.10	19.01±1.02	17.24-21.33	Y=1.13+1.10X
	F. racemosa	11.76±0.60	10.66-13.7	21.98±1.22	19.86-24.77	Y=0.723+1.00X
	E. hirta	12.63±0.66	11.44-14.07	23.39±1.35	21.07-26.49	Y=0.525+0.95X

LC_{50}, 50% lethal concentration; SE, standard error; LCL, lower confidence limit; UCL, upper confidence limit; LC_{90}, 90% lethal concentration.

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