

Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Document heading

doi: 10.1016/S2222-1808(14)60440-0

© 2014 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

Green synthesis of silver nanoparticles and its application for mosquito control

Naba Kumar Mondal^{1*}, Arnab Chowdhury¹, Uttiya Dey¹, Priyanka Mukhopadhyay², Soumendranath Chatterjee², Kousik Das¹, Jayanta Kumar Datta¹

¹Department of Environmental Science, The University of Burdwan, Burdwan-713104, India

²Parasitology and Microbiology Laboratory, Department of Zoology, The University of Burdwan, Burdwan-713104, India

PEER REVIEW

ABSTRACT

Peer reviewer

Prof. Bikash Mondal, Department of Chemistry, Bejoy Narayan Mahavidyalaya, Hooghly, India.
Tel: +919474778062
E-mail: bmondal.bnm@gmail.com

Comments

This particular research highlighted the insight of bioactive agent in the formation of nanoparticles and their possible role towards the control of mosquitoes. It has a great application in community health improvement. Details on Page S209

Objective: To synthesize and characterize silver nanoparticles from aqueous root extract of *Parthenium hysterophorus* (*P. hysterophorus*) and also to evaluate the potentiality of synthesized silver nanoparticles as larvicidal agent against *Culex quinquefasciatus* (*Cx. quinquefasciatus*).

Methods: The silver nano particles were generated using root extract of *P. hysterophorus*. The characterization of synthesized nanoparticles was done by visual color change, UV-Vis spectrum, scanning electron micrograph, fluorescent microscope and Fourier transform infrared spectroscopy.

Results: It was found that aqueous silver ions can be reduced by aqueous root extract of *P. hysterophorus* to generate extremely stable silver nanoparticles in aqueous medium. Larvae were exposed to varying concentrations of plant extracts, aqueous silver nitrate solution and synthesized silver nanoparticles for 0, 24 and 48 h separately. Aqueous root extract showed moderate larvicidal effects; however, the maximum efficacy (60.18%) was observed with the synthesized silver nanoparticles against the larvae of *Cx. quinquefasciatus*.

Conclusions: These results suggest that the green synthesis of silver nanoparticles have the potential to be used as an ideal eco-friendly approach for the control of the *Cx. quinquefasciatus*. This is the first report on the mosquito larvicidal activity of the nano particle synthesized by *P. hysterophorus*.

KEYWORDS

Silver nanoparticles, Larvicidal potential, *Culex quinquefasciatus*, Biological control

1. Introduction

Mosquito have the ability of carrying and transmitting human and animal diseases across the countries causing hundreds of millions of clinical cases and millions of death annually^[1,2]. Among several species of mosquitoes *Culex quinquefasciatus* (Diptera: Culicidae) (*Cx. quinquefasciatus*) is main periodic vector of filarial parasite *Wuchereria bancrofti*, accredited for human lymphatic filariasis transmission^[3]. *Cx. quinquefasciatus* is a cosmopolitan mosquito with worldwide distribution, especially in the

tropical and subtropical areas and is associated with human dwellings. The adult females lay eggs preferentially in relatively large, permanent aquatic habitats with high concentrations of decomposing organic matter, such as sewage effluents and septic tanks.

Several insecticides namely, DDT, dieldrin, organophosphorous, fenithothion and propoxur were widely used in India to mitigate this dangerous problem^[4]. Persistent application of the synthetic chemical products mostly available in local markets causes undesirable consequences including production of resistant strains of

*Corresponding author: Naba Kumar Mondal, Department of Environmental Science, The University of Burdwan, Burdwan-713104, India.

Tel: +919434545694

Fax: 0342-2634200

E-mail: nkmenvbu@gmail.com

Article history:

Received 14 Nov 2013

Received in revised form 20 Nov, 2nd revised form 24 Nov, 3rd revised form 29 Nov 2013

Accepted 12 Dec 2013

Available online 28 Jan 2014

mosquitoes, ecological imbalance and elimination of non-target organism in the environment[5].

Therefore, a demand stems out for the synthesis of bio-origin mosquito repellent[6,7]. In this juncture the field of nanotechnology is one of the most active areas of research in modern material sciences[8]. Nanoparticles are particles with a size of 100 nm which has a vast application in pharmaceutical, industrial and biotechnological fields[9]. Silver nanoparticles (Ag NPs) are emerging as one of the fastest growing materials due to their unique physical, chemical and biological properties; small size and high specific surface area. Ag NPs are reported to possess anti-viral, anti-bacterial and anti-fungal properties[10–12]. Presently Jayaseelan *et al.*[13] investigated the larvaecidal activity of Ag NPs against *Heteroscodra maculate*, which was synthesized by using aqueous leaf extract of *Musa paradisiaca*. The efficacies of synthesized Ag NPs using aqueous leaf extract of *Mimosa pudica* against the larvae of *Rhipicephalus microplus*[14]. Suman *et al.*[9] synthesized silver nanoparticle from aqueous aerial extract of *Ammannia baccifera* that can effectively inhibit larval activity of the larvae *Anopheles subpictus* (LC₅₀=257.61 ppm) and the larvae *Cx. quinquefasciatus* (LC₅₀=257.61 ppm) and synthesized Ag NPs showed significant toxic effects against the larvae of *A. subpictus* with an LC₅₀=29.54 ppm and against the larvae of *Cx. quinquefasciatus* at LC₅₀=22.32 ppm.

The use of environmentally benign materials such as silver nanoparticles offer numerous benefits of eco-friendliness and compatibility for larvicidal application. Keeping in mind the above fact the present aim of this study was to investigate the bioactive components present in the root extract of *Parthenium hysterophorus* (*P. hysterophorus*) plant which help the biosynthesis of silver nanoparticles and to analyze the larvicidal effects of the extract as well as silver nanoparticles on one mosquito species *Cx. quinquefasciatus*.

2. Materials and methods

2.1. Preparation of plant extract

A total of 0.84 g NaCl (Merck, USA) was dissolved in 100 mL of distilled water to prepare the saline water. The parthenium roots were collected from the road side of G. T. Road, Burdwan (23.2383° N, 87.8608° E) and washed with the saline water. The roots were then soaked in tissue paper for drying. A total of 4 g of dried root were cut into small pieces and smashed with 100 mL sterilized distilled water. Then it was boiled in the water bath for three minutes and the solution

was collected through Whatmann No. 42 filter paper and kept into the refrigerator.

2.2. Preparation of AgNO₃ solution

Initially 0.17 g AgNO₃ (Merck, USA) was dissolved in 100 mL double distilled water for stock solution of AgNO₃.

2.3. Preparation of nanoparticle

The root extract of *Parthenium* and the AgNO₃ solution were mixed in the ratio of 1:3, 1:5, 1:7, and 1:9 and kept at room temperature for 72 h for the development of reddish brown color. The best color was formed from the 1:9 ratio of solution.

2.4. Observation of color change at different time interval

The root extract, having no color, became white after 8 h of mixing with AgNO₃ solution and became deep white after 18 h. A light red color appeared after 24 h of incubation and finally the solution became deep reddish brown after 72 h. After 8 d the solution became colorless because the particles were precipitated.

2.5. Characterization of nanoparticles

The solution in each beaker were dried and sent for scanning electron micrograph (SEM). The SEM characterization was carried out using a scanning electron microscope (HITACHI, S-530). Infrared photograph was recorded by Fourier transform infrared spectroscopy (FTIR) (BRUKER, Tensor 27), absorbance was measured by UV-Vis spectrophotometer (Perkin Elmer, Lambda 35) and fluorescent spectrophotometer (SD 1000).

2.6. Collection of mosquito larvae and maintenance of mosquito culture

Cx. quinquefasciatus larvae were collected from stagnant and slow moving water bodies of the submerged rice field from different areas of Burdwan district, West Bengal following the method of Service and Laird[15,16]. During each survey, a habitat was first examined for the presence of mosquito larvae visually, and then captured by using a standard dipper (11.5 cm diameter and 350 mL capacity), pipettes and white plastic pans[15,17]. Larvae were brought to the laboratory and morphological features were recorded through stereoscopic binocular and light microscopic

study. The larvae were allowed to become adult and again they were characterized and identified following standard identification keys like Service, 1976 and Laird, 1998. *Cx. quinquefasciatus* culture was maintained on honey soaked cotton pads at (28 ± 2) °C and $(80 \pm 5)\%$ relative humidity in cages (60 cm^3) in the Parasitology and Microbiology Research Laboratory of the University of Burdwan, West Bengal, India^[18,19].

2.7. Determination of the mosquitocidal activity of the silver nanoparticle

For each test, 30 late third instars *Cx. quinquefasciatus* larvae were released in 100 mL rice field water with 15% sucrose solution containing a mixture of nanoparticle at different doses (0.5 mL, 1 mL and 2 mL) for 24 h and rest 30 larvae were kept in 100 mL water with only sucrose solution without having any nanoparticle considered as control. Three replications were used for both treated and untreated larvae. Survival of larvae was observed at 12 h intervals and % mortality was determined following Abbott's formula^[20].

2.8. Statistical analysis

The average larval mortality data were calculated with respect to control and other statistics at 95% confidential limits of upper confidence limit and lower confidence limit by following standard statistical book^[21]. A comprehensive statistical software package (SPSS 16.0) was used to calculate ANOVA and DMRT test.

3. Results

3.1. Visual color change of AgNPs

The color intensity of synthesized Ag NPs increased with duration of incubation (Figure 1). The color of the extract changed to light brown after 12 h of incubation for the synthesis of nanoparticles. The bioreduction of silver ions in the solution was monitored periodically by measuring the UV-Vis spectroscopy of the solutions. The reaction was rapid as the yellowish brown color appeared within 48 h and the reaction confirmed the formation of Ag NPs and there was no color change further. The optimum time required for the completion of reaction from our study was 48 h (Figure 1). It was observed that the reduction of silver ions reaches saturation within 48 h of incubation.



Figure 1. Synthesised nanoparticles changes color after mixing of silver nitrate and *Parthenium* root extract (1:9) during 48 h.

3.2. UV-Vis analysis of synthesized Ag NPs

After the addition of root extract of *P. hysterophorus*, the color of AgNO_3 changed from light yellow to reddish brown, which indicated the synthesis of Ag NPs in the aqueous solution. The production of silver nanoparticles synthesized from aqueous root extract of *P. hysterophorus* was evaluated through spectrophotometer in a range of wavelength from 300 to 600 nm (Figure 2). This revealed a peak at 420 nm in root extract of *P. hysterophorus* indicating the production of silver nanoparticles.

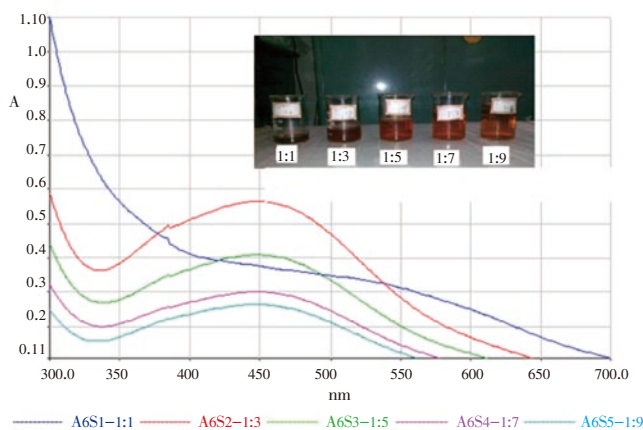


Figure 2. Absorbance change in different proportion of root extract with fixed dose of silver nitrate solution.

UV-Vis spectra (Figure 2) revealed that concentration of aqueous extract of *P. hysterophorus* plays an important role in the formation of Ag NPs. On the other hand SEM study clearly revealed that the shape of the AgNPs is spherical.

3.3. Morphology of synthesized silver nanoparticles

The fluorescent microscope study of the nanoparticles revealed that the nano-Ag predominates absolute spherical in shape (Figure 3). Most of the nanoparticles were roughly spherical in shape with smooth edges. The surface morphology of nanoparticles was investigated using SEM and observation showed synthesized nanoparticles was not in direct contact even within the aggregates, indicating stabilization of the synthesized nanoparticles. The experimental results showed that the reduction of silver ions was held on the metal surface. SEM picture of synthesised Ag NPs magnified at 5000 \times (Figure 4).



Figure 3. Fluorescent micrograph of synthesised nanoparticles.

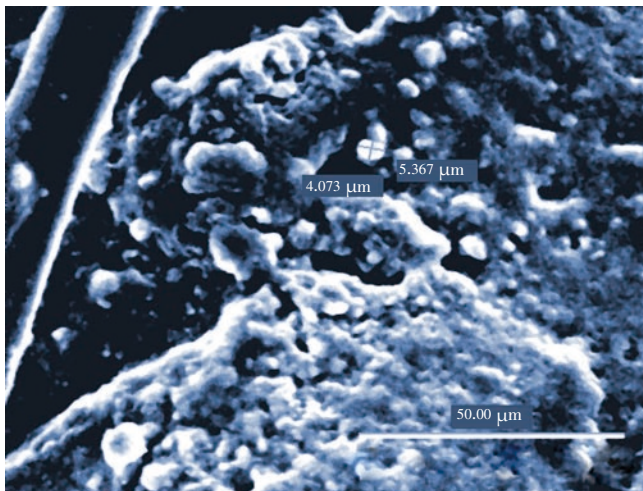


Figure 4. SEM of synthesised nanoparticles.

3.4. FTIR analysis of synthesized Ag NPs

FTIR spectra of silver nanoparticles exhibited prominent peaks at 1635 cm^{-1} and 3280 cm^{-1} (Figures 5 and 6). The spectra showed sharp and strong band at 1635 cm^{-1} assigned to the stretching vibration of (NH) C=O group. The band 3280

cm^{-1} developed for phenolic–OH stretching.

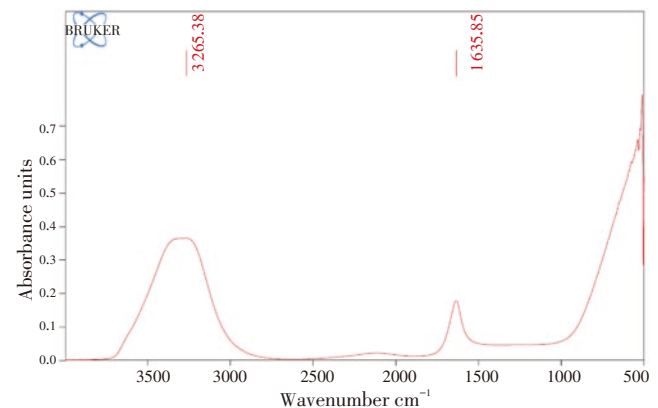


Figure 5. FTIR of synthesised nanoparticles with the silver nitrate and root

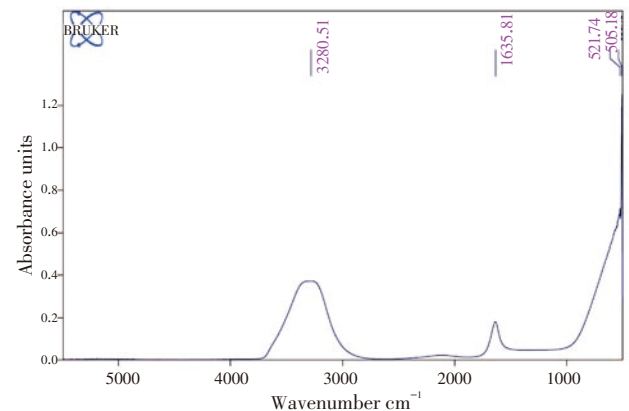


Figure 6. FTIR of synthesised nanoparticles with the silver nitrate and root extract (1:9).

3.5. Mosquito larvicidal activity

The analysis of larvicidal activity of *P. hysterophorus* root extract against *Cx. quinquefasciatus* are shown in Figure 7 and it was found dose and time dependent. The percent mortality increased by increasing the dose and time of exposure of larvae. The aqueous root extract of *P. hysterophorus* of 100 mg/L showed (4.000 \pm 0.033)%, (8.690 \pm 1.602)%; and (17.390 \pm 0.003)% mortality after 24 h, 48 h and 72 h respectively. Therefore, from the experimental data it was clear that aqueous extract of *P. hysterophorus* root dose not show mortality above 18% during 72 h of incubation. Again, pure silver nitrate solution of 10 mg/L concentration showed 12.5%, 13.04% and 21.74% of mortality in 24 h, 48 h and 72 h of incubation respectively (Figure 8). However, little higher percentage of mortality increases with increasing concentration and duration of exposure. The maximum mortality was recorded 60.87% with 100 mg/L of silver nitrate solution during 72 h of incubation. The larvicidal activity of synthesized nanoparticle (1:1) showed significant difference ($P<0.05$) from both pure plant extract and pure metal solution (10 mg/L) during 24 h of incubation. Similar significant mortality percentage was recorded during 48 h and 72 h of

incubation (Figure 7). However, nanoparticle synthesized from different ratios of plant extract and metal solution showed different mortality percentage (Figure 7). Results revealed that during 24 h of incubation about 20.84% and 26.17% higher mortality were obtained using nanoparticle of 1:3 ratio than 1:1 and 1:9 ratio respectively. However, in 48 h, the mortality percentage was recorded in following order as 52.17% (1:3)>30.2% (1:9)>26.09% (1:1) and in 72 h 60.87% (1:3)>55.5% (1:9)>34.78% (1:1).

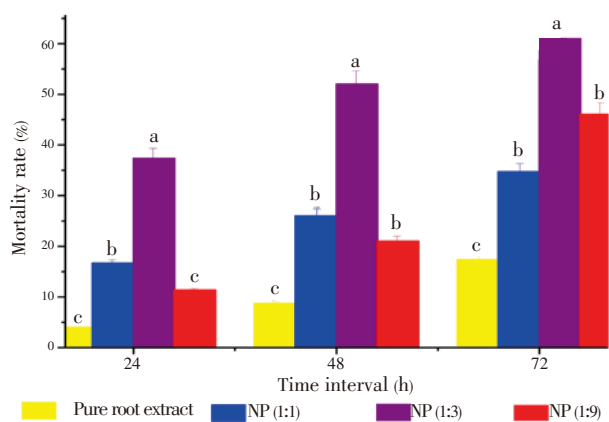


Figure 7. Mosquito larva mortality with pure root extract and synthesized Ag NPs from different ratios of root extract and silver nitrate solution. Different letters indicate significant differences at $P < 0.01$ according to the Tukey–HSD.

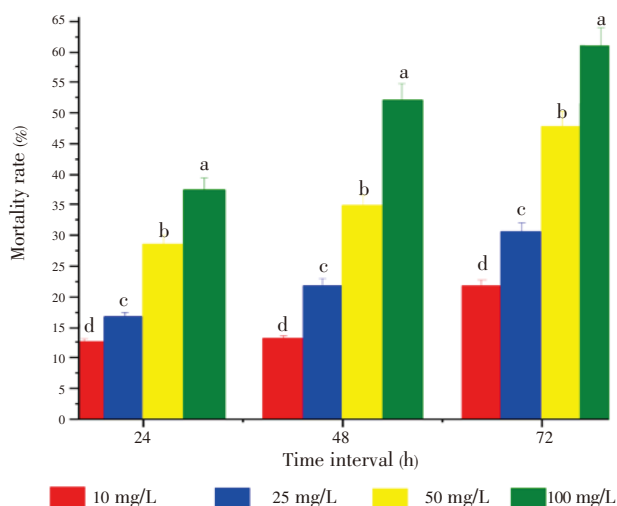


Figure 8. Mosquito larva mortality with pure silver nitrate solution of different concentration. Different letters indicate significant differences at $P < 0.01$ according to the Tukey–HSD.

4. Discussion

From the previous literature it was revealed that soluble carbohydrate present in the aqueous extracts of biomaterial is the key determining factor for reduction of silver ions and formation of corresponding nanoparticles[22]. Mulvaney[23] reported in his paper that when *Parthenium* leaf extract was mixed with aqueous solution of silver ions, it started

to change color from water color to yellowish brown and the color was changed due to extraction of surface plasmon vibrations which indicated formation of AgNPs. Certain carbohydrates present in the aqueous extract of *Ammannia baccifera* are proposed to play the key role for the reduction of silver ions to form silver nanoparticles[24]. However, biochemical response towards the formation of nanoparticles by using plant materials is yet to be elucidated. Phytoextracts are emerging as potential mosquito control agents, with low-cost, easy-to-administer, and risk-free properties[25,26]. This is exactly similar to surface plasmon vibration with characteristic peak of silver nanoparticles produced by chemical reduction[27,28]. However, large volume of plant extract when added to the AgNO_3 solution the intensity of the color changes abruptly which is perhaps due to enhancement in the number of nanoparticles in the reaction medium[29]. Both plant extract and AgNO_3 concentration in the reaction medium influenced the formation of Ag NPs[30]. Formation of Ag NPs started within 1 min of the mixing of the extract with AgNO_3 solution and increased up to 30 min[31], but after that only slight variation can be observed and comparatively less reaction time than earlier report[32]. On the other hand Jayaseelan *et al.*[13] observed the synthesis of Ag NPs from the mixture of aqueous peel extract of *Musa paradisiacal* and aqueous AgNO_3 solution, the color of the extract changes to dark brown during 30 min of incubation period. Therefore it can be speculated that the intensity of the color for the synthesized Ag NPs can vary from plant to plant with time. Santhoshkumar *et al.*[33] reported that the color of the solution of AgNO_3 and aqueous extract of *Boswellia ovalifoliolata* changed within 10 min, whereas *Shorea tumbuggaia* and *Svensonia hyderabadensis* took 15 min to synthesized nanoparticles.

The structures of nanoparticles were identical with that of the silver nanoparticles produced from the sun dried leaf powder of *Cinnamomum camphora*, which was attributed to a similarity in the reductive agents present in the plant species[29]. Similarly many other researchers also said that silver nanoparticles can be easily synthesized from the aqueous leaf extract of *Nelumbo nucifera* Gaertn[31]. Tian *et al.*[32] reported that the numerous flavonoids including quercetin or quercetin 3-O-glycosides were isolated from lotus leaves that were used for silver nanoparticles synthesis.

Similar work was reported by Santhoshkumar *et al.*[33] by using silver nanoparticles in which they used different ratios of plant extract for the reduction of Ag (I) ions.

The main reservoir of bioactive compounds is plant which consists less toxic, minimum chance to cause resistant and fully biodegradable[34]. Phytochemicals have many advantages over synthetic insecticides. Neem is one of such best example with rich phytochemicals mainly steroids, tannins and alkaloids which are responsible for high antimosquitodical activity[35]. Rajakumar and Rahuman

reported that the green synthesized silver nanoparticles have tremendous potentiality to kill the mosquito larva[36]. Recent studies demonstrated that silver nanoparticles induce embryonic injuries and reduce survival in zebrafish[4,8,37–41]. However, nanoparticles synthesized from other sources like double-walled carbon nanotube showed larvicidal activity about 85% of mortality rate with a concentration of 500 mg/L[42]. Baun *et al.* indicated the toxicity of C₆₀, carbon nanotubes, and titanium dioxide to an aquatic invertebrate, *Daphnia magna*[43].

Green synthesis of Ag NPs in present study show that the aqueous root extracts of *P. hysterophorus* can be used an effective reducing agent for the synthesis of Ag NPs. The synthesized Ag NPs are highly stable and have significant mosquito larvicides against *Cx. quinquefasciatus*. The results reported in this study open the possibility for further investigation of the efficacy of larvicidal and repellent properties of natural product extracts. The isolation and purification of crude extract of root aqueous and methanol extract of *P. hysterophorus* are in progress. The advantageous point of silver nanoparticles as larvicides is that the drug resistance due to the overuse of pesticides can be overcome.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

The Field of nanotechnology and its application is immense important now-a-days. The present paper nicely explained with adequate previous literature.

Research frontiers

Present research highlighted on the synthesis of *P. hysterophorus* and utilize the same for mosquito control.

Related reports

All the cited references in this paper are very much relevant with present study.

Innovations & breakthroughs

The innovation part of the present paper is the possibility of effective utilization of noxious weed for the synthesis of nanoparticles.

Applications

Present research highlighted the effective utilization of nanoparticles as larvicidal agent against *Cx. quinquefasciatus*.

Peer review

This particular research highlighted the insight of bioactive agent in the formation of nanoparticles and their possible role towards the control of mosquitoes. It has a great application in community health improvement.

References

- [1] Dhanasekaran D, Sakthi V, Thajuddin N, Panneerselvam A. Preliminary evaluation of *Anopheles* mosquito larvicidal efficacy of mangrove actinobacteria. *Int J Appl Biol Pharm Technol* 2010; **1**(2): 374–381.
- [2] Roth D, Henr B, Mak S, Fraser M, Taylor M, Li M, et al. West Nile virus range expansion into British Columbia. *Emerg Infect Dis* 2010; **16**: 1251–1258.
- [3] Oduola AO, Awe OO. Behavioural biting preference of *Culex quinquefasciatus* in human host in Lagos metropolis Nigeria. *J Vector Borne Dis* 2006; **43**: 16–20.
- [4] Zhang H, Cui H, Yao S, Zhang K, Tao H, Meng H. Ionic liquid-stabilized non-spherical gold nanofluids synthesized using a one-step method. *Nanoscale Res Lett* 2012; **7**(1): 583.
- [5] Mathivanan T, Govindarajan M, Elumalai K, Krishnappa K, Annandan A. Mosquito larvicidal and phytochemical properties of *Ervatamia coronaria* Staf. (Family: Apocynaceae). *J Vector Borne Dis* 2010; **47**: 178–180.
- [6] Niraimathi S, Balaji N, Venkataramanan N, Govindarajan M. Larvicidal activity of alkaloid from *Sida acuta* against *Anopheles subpictus* and *Culex tritaeniorhynchus*. *Int J Curr Res* 2010; **11**: 34–38.
- [7] Samidurai K, Jebanesan A, Saravanakumar A, Govindarajan M, Pushpanathan T. Larvicidal, ovicidal and repellent activities of *Pemphis acidult* Frost. (Lythraceae) against filarial and dengue vector mosquitoes. *Acad J Entomol* 2009; **2**(2): 62–66.
- [8] Shameli K, Ahmad MB, Zamanian A, Sangpour P, Shabanzadeh P, Abdollahi Y, et al. Green biosynthesis of silver nanoparticles using *Curcuma longa* tuber powder. *Int J Nanomedicine* 2012; **7**: 5603–5610.
- [9] Suman TY, Elumali D, Kaleena PK, Rajasree SRR. GC-MS analysis of bioactive components and synthesis of silver nanoparticle using *Ammannia baccifera* aerial extract and its larvicidal activity against malaria and filariasis vectors. *Ind Crop Prod* 2013; **47**: 239–245.
- [10] Rogers JV, Parkinson CV, Choi YW, Speshock JL, Hussain SM. A preliminary assessment of silver nanoparticle inhibition of monkeypox virus plaque formation. *Nanoscale Res Lett* 2008; **3**: 129–133.
- [11] Sathishkumar M, Sneha K, Won SW, Cho CW, Kim S, Yun YS. *Cinnamon zeylanicum* bark extract and powder mediated green synthesis of nanocrystalline silver particles and its bactericidal activity. *Colloids Surf B Biointerfaces* 2009; **73**: 332–338.
- [12] Panáček A, Kolár M, Vecerová R, Pucek R, Soukupová J, Krystof V, et al. Antifungal activity of silver nanoparticles against *Candida* spp. *Biomaterials* 2009; **30**: 6333–6340.

- [13] Jayaseelan C, Rahaman AA, Rajkumar G, Vishnu KA, Santhoskumar T, Marimuthu S, et al. Synthesis of pediculocidal and larvicidal silver nanoparticles by leaf extract from heart leaf moonseed plant, *Tinospora cordifolia* Miers. *Parasitol Res* 2011; **109**: 185–194.
- [14] Marimuthu S, Rahuman AA, Rajkumar G, Santhoshkumar T, Kirthi AV, Jayaseelan C, et al. Evaluation of green synthesized silver nanoparticles against parasites. *Parasitol Res* 2011; **108**: 1541–1549.
- [15] Service MW. *Mosquito ecology: field sampling methods*. USA: Springer; 1976.
- [16] Laird M. *The natural history of larval mosquito habitats*. New York, USA: Academic Press; 1988.
- [17] Sharma VP. Role of fishes in vector control in India. In: Sharma VP, Ghosh A, editors. *Larvivorous fishes of inland ecosystems: proceedings of the MRC–CICFRI workshop, New Delhi, 27–28 September 1989*. Delhi: Malaria Research Centre; 1994, p. 1–19.
- [18] Chatterjee S, Chandra G. Role of *Anopheles subpictus* as a primary vector of malaria in an area in India. *Jpn J Trop Med Hyg* 2000; **28**: 177–181.
- [19] Yeo MK, Kang M. Effects of nanometer sized silver materials on biological toxicity during zebrafish embryogenesis. *Bull Korean Chem Soc* 2008; **29**: 1179–1184.
- [20] Abbott W. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 1925; **18**: 265–267.
- [21] Gomez KA, Gomez AA. *Statistical procedures for agricultural research*. 2nd ed. New York: John Wiley and Sons; 1984.
- [22] Amin M, Anwar F, Ramzan Seed MR, Janjua A, Iqbal MA, Rashid U. Green synthesis of silver nanoparticles through reduction with *Solanum xanthocarpum* L. Berry extract: characterization, antimicrobial and urease inhibitory activities against *Helicobacter pylori*. *Int J Mol Sci* 2012; **13**: 9923–9941.
- [23] Mulvaney P. Surface plasmon spectroscopy of nanosized metal particles. *Langmuir* 1996; **12**: 788–800.
- [24] Tripathy S, Pradhan D, Anjan M. Anti-Inflammatory and Antiarthritic potential of *Ammania baccifera* Linn. *Int J Pharma Bio Sci* 2010; **1**: 1–7.
- [25] Govindarajan M, Sivaakumar R, Rajeswary M, Yogalakshmi K. Adulticidal activity of *Pithecellobium dulce* (Roxb.) Benth against *Culex quinquefasciatus* (Say). *Asian Pac J Trop Dis* 2012; **2**: 124–128.
- [26] Sivakumar R, Jebanesan A, Govindarajan M, Rajasekar P. Larvicidal and repellent activity of tetradecanoic acid against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say) (Diptera:Culicidae). *Asian Pac J Trop Med* 2011; **4**(9): 706–710.
- [27] Kong H, Jang J. One-step fabrication of silver nanoparticle embedded polymer nanofibers by radical-mediated dispersion polymerization. *Chem Commun* 2006; **8**: 3010–3012.
- [28] Kathiresan K, Manivannan S, Nabeel MA, Dhivya B. Studies on silver nanoparticles synthesized by a marine fungus, *Penicillium fellutanum* isolated from coastal mangrove sediment. *Colloids Surf B Biointerfaces* 2009; **71**: 133–137.
- [29] Cruz D, Fale PL, Mourato A, Vaz PD, Serralheiro ML, Lino AR. Preparation and physicochemical characterization of Ag nanoparticles biosynthesized by *Lippia citriodora* (Lemon Verbena). *Colloids Surf B Biointerfaces* 2010; **81**: 67–73.
- [30] Santhoshkumar T, Rahuman AA, Bagavan A, Marimuthu S, Jayaseelan C, Kirthi AV, et al. Evaluation of stem aqueous extract and synthesized silver nanoparticles using *Cissus quadrangularis* against *Hippobosca maculate* and *Rhipicephalus* (*Boophilus*) microplus. *Exp Parasitol* 2012; **132**: 156–165.
- [31] Huang J, Li Q, Lu Y, Su Y, Yang X, Wang H, et al. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotechnol* 2007; doi: 10.1088/0957-4484/18/10/105104.
- [32] Tian N, Liu Z, Huang J, Luo G, Liu S, Liu X. [Isolation and preparation of flavonoids from the leaves of *Nelumbo nucifera* Gaertn by preparative reversed-phase high-performance liquid chromatography]. *Se Pu* 2007; **25**: 88–92. Chinese.
- [33] Santhoshkumar T, Rahuman AA, Rajkumar G, Marimuthu S, Bagavan A, Jayaseelan C, et al. Synthesis of silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. *Parasitol Res* 2011; **108**: 693–702.
- [34] Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles Stephensi* Liston (Insect: Diptera: Culicidae). *Asian Pacific J Trop Biomed* 2011; **1**: 124–129.
- [35] Mgbemena IC. Comparative evaluation of larvicidal potentials of three plant extracts on *Aedes aegypti*. *J Am Sci* 2010; **6**: 435–440.
- [36] Rajakumar G, Abdul Rahuman A. Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrata* leaf extract against filariasis and malaria vectors. *Acta Trop* 2011; **118**: 196–203.
- [37] Griffitt RJ, Luo J, Gao J, Bonzongo JC, Barber DS. Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. *Environ Toxicol Chem* 2008; **27**: 1972–1978.
- [38] Millstone JE, Hurst SJ, Métraux GS, Cutler JL, Mirkin CA. Colloidal gold and silvertriangular nanoprisms. *Small* 2009; **5**: 646–664.
- [39] Murphy CJ, Gole AM, Hunyadi SE, Stone JW, Sisco PN, Alkilany A, et al. Chemical sensing and imaging with metallic nanorods. *Chem Commun (Camb)* 2008; **7**: 544–557.
- [40] Lee KS, El-Sayed MA. Gold and silver nanoparticles in sensing and imaging: sensitivity of plasmon response to size, shape, and metal composition. *J Phys Chem B* 2006; **110**: 19220–19225.
- [41] Zhong L, Jiao T, Liu MA. A facile method to the synthesis of gold nanoprisms using a series of gemini amphiphiles. *J Nanosci Nanotechnol* 2009; **9**: 2726–2730.
- [42] Mouchet F, Landois P, Sarremejean E, Bernard G, Puech P, Pinelli E, et al. Characterisation and *in vivo* ecotoxicity evaluation of double-wall carbon nanotubes in larvae of the amphibian *Xenopus laevis*. *Aquat Toxicol* 2008; **87**: 127–137.
- [43] Baun A, Hartmann NB, Grieger K, Kusk KO. Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. *Ecotoxicology* 2008; **17**: 387–395.