Green synthesis of silver nanoparticles and its application for mosquito control

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

Methods: The silver nanoparticles were generated using root extract of \textit{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 \textit{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 \textit{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 \textit{Cx. quinquefasciatus}. This is the first report on the mosquito larvicidal activity of the nano particle synthesized by \textit{P. hysterophorus}.

KEYWORDS
Silver nanoparticles, Larvicidal potential, \textit{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\textsuperscript{[1,2]}. Among several species of mosquitoes \textit{Culex quinquefasciatus (Diptera: Culicidae) (Cx. quinquefasciatus)} is main periodic vector of filarial parasite \textit{Wuchereria bancrofti}, accredited for human lymphatic filariasis transmission\textsuperscript{[3]}. \textit{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\textsuperscript{[4]}. Persistent application of the synthetic chemical products mostly available in local markets causes undesirable consequences including production of resistant strains of...
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 larvicideal activity of Ag NPs against Heteroscodra maculata, 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 (Merek, USA) was dissolved in 100 mL double distilled water for stock solution of AgNO3.

2.3. Preparation of nanoparticles

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, Lamda 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 ± 5)% relative humidity in cages (60 cm³) 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 AgNO₃, 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 absolutely 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 synthesized Ag NPs magnified at 5000x (Figure 4).

![Fluorescent micrograph of synthesized nanoparticles.](image1)

**Figure 3.** Fluorescent micrograph of synthesized nanoparticles.

![SEM of synthesized nanoparticles.](image2)

**Figure 4.** SEM of synthesized 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.

![FTIR of synthesized nanoparticles with the silver nitrate and root extract (1:7).](image3)

**Figure 5.** FTIR of synthesized nanoparticles with the silver nitrate and root extract (1:7).

![FTIR of synthesized nanoparticles with the silver nitrate and root extract (1:9).](image4)

**Figure 6.** FTIR of synthesized 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±0.033)%, (8.690±1.602)%; and (17.390±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 larvacidal 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.

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 *Annamonia 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 AgNO₃ 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 AgNO₃ 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 AgNO₃ 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 paradisaical* and aqueous AgNO₃ 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. Sathoshkumar et al.[33] reported that the color of the solution of AgNO₃ and aqueous extract of *Boswellia ovalifoliolata* changed within 10 min, where as *Shorea tumbbaggaia* and *Svensonia hyderobadensis* 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.

**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

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reported that the green synthesized silver nanoparticles have tremendous potentiality to kill the mosquito larvain[36]. Recent studies demonstrated that silver nanoparticles induce embryonic injuries and reduce survival in zebrafish[4,8,37–41]. However, nanoparticles synthesized from other sourceses like double–walled carbon nanotube showed larvicultural activity about 85% of mortality rate with a concentration of 500 mg/L[42]. Baum et al. indicated the toxicity of C. quinquefasciatus to ag 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 larvicultural 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.

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