Larvicidal efficacy of silver nanoparticles synthesized biologically using *Swietenia mahagoni* (L.) Jacq. leaf extract against *Anopheles stephensi*, *Culex quinquefasciatus* and *Cx. vishnui* group

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Mosquito borne diseases are a global crisis, particularly in developing countries. Non-availability of apposite vaccines against these diseases has lead to sole dependence on the vector managerial steps for dropping the incidences. In the present study, we tried to evaluate the larvicidal potential of biologically synthesized silver nanoparticles (Ag NP) using aqueous leaf extracts of *Swietenia mahagoni* (L.) Jacq. against third instar larvae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Cx. vishnui* group. Aqueous extract of leaves reduced the aqueous silver ions to produce stable Ag NP. The characterization of synthesized nanoparticles was done through UV-Vis spectrum, Transmission electron microscope (TEM), X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscope. Third instar larvae of three mosquito species namely *An. stephensi*, *Cx. quinquefasciatus* and *Cx. vishnui* group were exposed to different concentrations of synthesized nanoparticles for 24, 48 and 72 h. TEM measured the range of nanoparticle size as 8-9 nm whereas XRD measured as 6 nm. Cent percent mortality of larvae of *An. stephensi* was recorded at 80 ppm at 48 h. About 96 and 80% mortality of *Cx. vishnui* group and *Cx. quinquefasciatus* larvae respectively were noted at 80 ppm after 72 h of exposure. The result of regression analysis proved that the mortality rate (Y) was positively correlated with the period of exposure (X) and regression coefficients were close to one. Toxicity study on non-target species showed no injurious activity during experimental period. Results indicate, possibly a first report on mosquito larvicidal effect of Ag NP synthesized using *S. mahagoni* leaf extract which may be used to effectively control the larval forms of three important vector mosquitoes.

Keywords: Encephalitis, Elephantiasis, Lymphatic filariasis, Malaria, Mosquitoes, West Indian mahogany, Vector control

Mosquitoes are potential vectors of several severe human diseases like malaria, lymphatic filariasis, dengue, DHF, chikungunya, yellow fever, encephalitis, etc.1. Worldwide, about 860 million people are reported to be suffering from lymphatic filariasis2. In malaria, about 216 million people are reported suffering from 91 countries, and from India, it is estimated to be 13 million cases3,4. The ‘malaria incidence’ in India according to WHO 2016 report is 18.6 per 1000 population5. Japanese encephalitis is also one of the most common diseases in India6. While chemical insecticides are effective in minimizing mosquito population, they lead to development of resistance and resurgence of the vector insects7. They are non-biodegradable and causes harm to non-target organisms8.

In recent years, scientists have focused on new herbal insecticides which are biodegradable and nontoxic to non-targets8-14. In current context, green nanotechnology opens a new horizon in the field of biocontrol of mosquitoes15,16. Nanoparticles have been applied in biological, chemical, physical, industrial, pharmaceutical and environmental sciences. Applications of nanotechnology have been extended in the field of mosquito control by the synthesis of silver nanoparticles (Ag NP) from plant extracts17. In the present study, we evaluated the larvicidal role of Ag NP, synthesized using *Swietenia mahagoni* leaf extract and AgNO3, against three species of vector mosquitoes, *Anopheles stephensi*, *Culex vishnui* group and *Culex quinquefasciatus* including determination of possible structure of this biologically synthesized nanoparticle.

**Materials and Methods**

Fresh, mature leaves of *Swietenia mahagoni* (L.) Jacq. were collected from the campus of the
University of Burdwan. After proper identification of the plant, the voucher specimen was deposited in the Department of Zoology (BUZGU-125), The University of Burdwan, West Bengal, India. The silver nitrate (AgNO₃ AR 99.9%) of Merck, India was used without further purification. De-ionized and double distilled water was utilized for preparation of extracts and solutions. The eggs of *An. stephensi*, *Cx. quinquefasciatus* and *Cx. vishnui* group were collected from fresh water of underground and overhead tanks of Kolkata metropolis, cemented drains, and rice fields surrounding the University campus, respectively. They were reared in the laboratory. The larvae were fed with artificial food (dried yeast powder and powder of dog biscuits in the ratio of 1:3).

### Preparation of leaf extract

Collected leaves of *S. mahagoni* were thoroughly rinsed with distilled water and dried in paper towel. Then the leaves were cut into small pieces by sharp knife. The aqueous leaf extract was prepared by mixing 10 g of the leaves and 200 mL double distilled water taken in a glass conical flask, followed by vigorous shaking with a vortex stirrer (Spinix, India) for an hour.

### Synthesis of silver nanoparticles

A stock solution of AgNO₃ was prepared. Twenty milliliters of the silver nitrate stock solution was mixed with 25 mL of the leaf extracts of *S. mahagoni*, and the volume was adjusted with deionized water to 250 mL so that final concentration of Ag⁺ion become 2×10⁻³ M and leaf extract concentration remained 10% (v/v) of the stock. The mixture was stirred with a vortex stirrer for 5 min and then warmed at 50°C in a thermo stated water bath for 8 h. The resultant dark brown coloured solution was allowed to cool for overnight in air tight container. The final nano colloidal solution was subjected to repeated centrifugation (twice) to get rid of any un-interacted biological molecules at 10000 rpm for 15 min in Remi Research centrifuge instrument. The final pellet was collected, dried in vacuum desiccators and stored for future use.

### Characterization of nanoparticles

The characterizations of the nano particles, synthesized from *S. mahagoni*, were done through the application of modern scientific instruments such as UV-vis spectroscope, TEM, XRD and FTIR spectroscope.

Ag NP shows a characteristic surface plasmon resonance (SPR) absorption in the UV-visible region. The nanoparticle formation kinetics and the SPR absorption spectra of the water dispersed ultracentrifuged pellet of Ag NP were studied on a UV-Vis spectrophotometer (UV 1800 series, Japan) functioned at a resolution of 1 nm within 190 to 1100 nm wavelength range.

Transmission electron microscope (TEM) studies of the particles were carried out at an accelerated voltage of 200 kV using a Philips CM200 TEM equipped with a LaB6 source.

The pellet of purified ultracentrifuged nanoparticles was dusted and subjected to X-ray diffraction analysis (Bruker D8 advance) with a parallel beam optics attachment. The X-ray diffraction profiles of the samples were recorded using Ni-filtered CuKα-radiation (λ=1.5418 Å) from a highly stabilized and automated Philips X-ray generator (PW 1830) operated at 30 mA current and 35 kV voltage. The X-ray diffraction pattern of the sample should reveal the formation of phase pure silver. From the X-ray diffraction pattern, the particle size ($D$) can be found using the Scherrer’s formula given below:

$$D = \frac{k\lambda}{\beta \cos \theta}$$

where $k$ is a constant which is approximately 0.9 and $\beta$ in radians represents the broadening in X-ray diffraction. The full angular width at a point where the intensity has fallen to half its maximum value i.e. full width at half maximum intensity (FWHM) is a measure of broadening of X-ray peaks.

The Fourier transform infrared (FTIR) analysis was done with water dispersed pellet of the ultracentrifuged Ag NP sample produced from *S. mahagoni* leaf extract to get an idea about the chemical framework of nano particles by a FTIR spectrometer (Perkin Elmer Lx 108873). The scanning range was 450-4000 cm⁻¹ at a resolution of 4 cm⁻¹.

### Larvicidal bioassay

The bioassay experiments were carried out according to standard WHO procedure with moderate modifications. Synthesized nanoparticles were treated on 3rd instars larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Cx. Vishnui* group separately for bioassay experiment. Twenty five larvae of each species were kept separately in glass Petridishes (150 mL capacity/9 cm diameter)
containing 100 mL of disinfected tap water. Each of four concentrations of nanoparticles (20, 40, 60 and 80 ppm) was applied separately into Petridishes. The experiments were repeated thrice for accuracy against each species of larva. They were maintained in 29±2°C and 82±1% RH in a photoperiod of 14:10 h light and dark cycle. Tap water was used in the control experiment without any nanoparticle against each concentration. Larval mortalities were noted after 24, 48 and 72 h of exposure.

Effect on non-target organisms

Non-target organisms, such as fish (Gambusia affinis), dragonfly nymph (Diplonychus annulatum) and tadpole of Bufo sp. were collected from field and acclimatized for 3 days in the laboratory. Batches of 25 fishes, dragonfly nymphs and tadpoles were placed in three separate pots, respectively, each containing 500 mL of dechlorinated tap water. In each pot, Ag NP at LC50 concentration of third instars An. stephensi larvae was dissolved. At the same time, the controls with similar set without Ag NP were studied. Experiments on non-targets were done thrice on three separate days. The mortalities or any abnormal activities were studied up to 72 h.

Statistical analysis

The computer software “STAT PLUS 2007” (Trial version) and MS EXCEL 2003 were used to calculate the LC50, LC 90, regression equations (Y=mortality, X=concentration), regression coefficient values, mean mortality and standard error, etc. The percentage of corrected mortality was analyzed by Abbott’s formula22.

Result and Discussion

UV-Vis absorption spectra of the Ag NP showed absorption peak at 450 nm in Fig. 1. TEM analysis proved the presence of nanoparticles in the analyzed sample (Fig. 2). The size of the synthesized nanoparticles was within 8-9 nm range through TEM measurement. XRD is a non-destructive analytical method to uniquely identify the crystalline phases present and to study the structural properties. It is shown in Fig. 3. The major peaks at 37.92° and 44.94° are in good agreement with the joint committee on powder diffraction standard (JCPDS) data respective to silver structure23. The other relatively low intensity peaks at 54.4°, 57.1° and 64.21° also agrees well with JCPDS data. Using the observed value of $\theta = 18.96^\circ$ and utilizing the known value of $\lambda = 1.5418$ Å and measuring the values of $\beta$, the particle size value comes out to be ~58 Å. Similarly, for $\theta = 22.47^\circ$, the particle size value comes out to be ~62 Å. Thus, the average value of particle size in the sample is ~ 60 Å or 6 nm. The X-ray value is on the lower side comparable to TEM value since instrumental and strain broadening was not taken into account. Scherrer equation only considers peak broadening. If the instrumental and strain broadening was taken into consideration, the FWHM value would have been lowered and the particle size would have been higher. FTIR analysis of synthesized nano particles and its respective functional group is shown in Fig. 4 and Table 1, respectively that proves that specific functional group is related to the active principle.
The larvicidal activity of silver nanoparticles against larvae of *An. stephensi*, *Cx. vishnui* group and *Cx. quinquefasciatus* are presented in Table 2. Log probit analysis and regression analysis of larvicidal activity of Ag NP of *S. mahagoni* leaf extract were done. LC$_{50}$ and LC$_{90}$ values, regression equations and R$^2$ values for the 3rd instars larvae of these three species are presented in Table 3. The results of the present study indicate that the mortality rate of 3rd instars of *An. stephensi*, *Cx. vishnui* group and *Cx. quinquefasciatus* at 80 ppm concentration were significantly higher than the mortality rates at 20 ppm, 40 ppm and 60 ppm concentrations of Ag NP of *S. mahagoni* leaf extract at 24, 48 and 72 h of exposure. Higher mortality rate was also recorded in all cases at 72 h bioassay than those at 24 and 48 h. The results of regression analyses revealed that the mortality rate (Y) was positively correlated with the period of exposure (X) having a regression coefficient close to one in each case. The results of log probit analyses (95% confidence level) revealed that LC$_{50}$ values gradually decreased with the exposure period. Cent percent mortality was observed in Ag NP at 80 ppm at 72 h of exposure on 3rd instar larvae of *An. stephensi*. In control experiments, no larval mortality was observed. The results of toxicity test on non target organisms such as fish (*Gambusia affinis*), dragonfly nymph (*Diplonychus annulatum*), and tadpole of *Bufo* indicated no mortality after 24 h.

The viewpoint of using herbal products for synthesizing silver nanoparticles (Ag NP) was to

![Fig. 4 — FTIR analysis of synthesized nanoparticle.](image-url)
control three mosquito species. Different phytochemical constituents have been fractionated from diverse plant sources and used against mosquitoes as repellent, oviposition deterrent, larvicidal, pupicidal and adulticidal agents. However, green synthesized nanoparticles are modern tool facilitating the development of a most effective and environmentally safe mosquito control programmes. Nanotechnology has an ample relevance in vector and pest management in the form of nanocapsules. The application boundary of nanotechnology has been extended in the field of mosquito control by the synthesis of silver nanoparticles from plant extracts. Rajakumar et al. first used silver nanoparticles synthesized from leaf extract of *Eclipta prostrata* against 4th instar larvae of *Cx. quinquefasciatus* and *An. subpictus*. Silver nanoparticles were synthesized by *Nelumbo nucifera* leaf extract and its larvicidal activity was reported against *Cx. quinquefasciatus* and *An. subpictus* 

The larvicidal activity of Ag NP synthesized from aqueous extract of *Drypetes roxburghii* against the larvae of *An. stephensi* and *Cx. quinquefasciatus* was also reported. Elebina et al. studied the larvicidal efficacy of synthesized Ag NP from nanoparticles using aqueous leaf extract of *Eupatorium odoratum* leaf against *Cx. quinquefasciatus* and *An. stephensi*. The larvicidal efficacy of Ag NP had been reported. Adhikari et al. reported the larvicidal potentiality of *S. mahagoni* leaves crude extract against 3rd instar larvae of *Cx. vishnui* group where percent larval death was noticed at 0.4% concentration after 72 h of exposure period with LC50 and LC90 values of 0.05 and 0.28%, respectively. The present study is important as the synthesized nanoparticles prepared by the treatment of AgNO3 with aqueous extract of leaves of *S. mahagoni* have larvicidal potentiality against *An. stephensi*, *Cx. vishnui* group and *Cx. quinquefasciatus*. Cent percent mortality of *An. stephensi* larvae was achieved using aqueous Ag NP after 48 h of exposure. This finding is important in vector control. The toxicity test of the synthesized nanoparticles on non-targets (Gambusia affinis, Diplonychus annulatum and tad pole of Bufo) did not show any abnormal activity or sluggishness and no mortality was observed in control experiments.

The use of green nanoparticles to control mosquito larvae is rapid and ecofriendly approach. It is the first attempt to synthesize Ag NP with the help of *S. mahagoni* and to study their effect on *An. stephensi*, *Cx. vishnui* group and *Cx. quinquefasciatus* larvae.

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#### References


