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Larvicidal Potential of Biologically Synthesised Silver Nanoparticles against Aedes Albopictus

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Abstract

Understanding of biological processes at the nanoscale level is a strong driving force behind development of nanotechnology. Silver nanoaparticles have important applications in the field of biology. Stable silver nanoparticles were synthesized by biological reduction method. The objective of the present study was to evaluate the larvicidal activity of silver nanoparticles synthesized from aqueous leaf extract of Hibiscus rosasinensis against the larvae of Aedes albopictus mosquito reared in rubber plantation. The parasite larvae were exposed to varying concentrations of aqueous extract of Hibiscus rosasinensis and synthesized silver nanoparticles for 24 h as per WHO protocols. Distilled water served as control. Percentage mortality was recorded. The synthesized nanoparticles exhibited significant larvicidal activity. This method is considered as an innovative alternative approach using green nanochemistry technique to control vector parasites and is the first report on mosquito larvicidal activity of Hibiscus rosasinensis leaf mediated synthesized silver nanoparticles.

Keywords: Silver nanoparticles, larvicidal, Aedes albopictus.

Introduction

India is endemic to mosquito-borne diseases due to favorable ecological conditions. Aedes albopictus, a vector for the transmission of many viral pathogens poses serious threat to human health and has proven to be very difficult to control due to their remarkable ability to adapt to various environments, their close contact with humans, and their reproductive biology. The rich organic content, stagnant water, low illumination and small orifice of the coconut shells in rubber plantations favours intense breeding[1]. Insecticide applications, although highly efficacious against the target species vector control, is facing a threat due to the development of resistance to chemical insecticides resulting in rebounding vectorial capacity[2].

Microbial control agents as alternatives to chemical insecticides have been successfully demonstrated[3]. However, significant problems exist with all of these.

Silver nanoparticles 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. Biological synthesis of nanoparticles has received increased attention due to a growing need to develop environmentally benign technologies in material synthesis. Several plant species have been utilized in this regard.

A green approach for the fabrication of stable, bioactive silver nanoparticles using Hibiscus rosasinensis leaf-extract has been reported[4].

Aedes albopictus: Aedes albopictus (Asian tiger mosquito) is an invasive species about 2 to 10 mm length characterized by its black and white striped legs, and small black and white body. It is widespread throughout tropical to temperate regions of the globe. Aedes albopictus is an opportunistic container breeder that is capable of utilizing natural as well as artificial container habitats. Any open container containing water will suffice for larvae development, even with less than an ounce of water in. It can also breed in running water. Their ability to breed in artificial containers facilitated their passive spread in the last decades through main transportation routes[5].

These mosquito species coexist in man-made containers in urban, suburban and rural settlements in tropical and subtropical regions[6, 7, 8]. In addition, Aedes albopictus larvae inhabit natural containers as bromeliads, bamboo stumps and tree-holes[9]. These conditions have contributed largely to the proliferation of this species, which is the chief vector of the alpha viruses causing Chikungunya and Dengue fever. The higher resistance of their eggs to desiccation, their capability to remain alive for many months and the intra ovarian transmission of the virus has made the control of the virus and mosquito extremely difficult. Moreover, the periodically emerging alpha virus variants can thrive well in A.albopictus mosquitoes[10]. Aedes albopictus has been proven to be a severe nuisance species. Many chemical methods tried to eradicate this mosquito have only caused more harm to the environment.

The use of environmentally benign materials such as silver nanoparticles offer numerous benefits of eco-friendliness and compatibility for larvicidal application. In these circumstances, an improvised method using the biologically synthesised silver nanoparticles were evaluated for the destruction of the mosquito larvae.

Material and Methods

Chemicals used: Silver nitrate ($AgNO_3$) cryst. extra pure was procured from Merck, Germany. Normal saline, double distilled water and de-mineralized water were used throughout the experiments.

Synthesis of silver nanoparticles: Fresh leaves of Hibiscus rosasinensis were thoroughly washed, finely cut and stirred with 200 ml of de-mineralized water and filtered to obtain the extract which was used as reducing agent and stabilizer. Silver nitrate ($AgNO_3$) solution was prepared fresh. Double distilled water was used throughout the experiments.

The aqueous extract was challenged with silver nitrate solution (1mM) under controlled parameters and ambient conditions. The bioreduction was monitored by periodic sampling of aliquots of the aqueous component and measuring the UV– Vis spectra of the solution. The synthesized nanoparticles were characterized by UV-Vis Spectroscopic analysis, High Resolution Scanning Electron Microscope (HR-SEM), X-ray diffraction (XRD) and Fourier Transform Infrared (FTIR) spectroscopy.

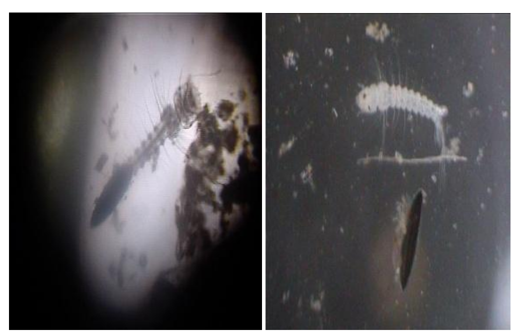
Experimental Model: Aedes albopictus used in this experiment were generated from the latex collecting cups of approximately 1000 larvae and/or eggs. They were reared in the laboratory. These eggs were washed with 0.01% formaldehyde solution for 30–40 minutes[11] so as to prevent microsporidian infections which might interfere with the normal development of the immature stages of mosquitoes[12] and soaked in water to facilitate hatching.

After hatching, first instar larvae were distributed in rubber latex collecting cups. Care was taken to prevent overcrowding until development to early 4th instar larvae required for the study. The liquid after the coagulation of latex is used as natural medium for larval growth. Water in rearing container was refreshed every day by removing a little quantity of water from the rearing buckets and replacing with fresh water. Different stages of the larval rearing are shown in figure 1.

a)Aedes albopictus mosquito b)Aedes albopictus larvae in latex cups

c) Larva d) Pupa

e) Aedes albopictus – Eggs



f)Emerging larva g)Emerged larva

Figure-1
Different stages of larval emergence-Aedes albopictus

Mosquito larvicidal bioassay: Standard methods for testing biologically synthesized nanoparticle toxicity and the susceptibility of mosquito larvae to insecticides was performed as stipulated by WHO[13]. The larvicidal bioassay were performed at a room temperature of 27 ± 1^0C. Randomly, twenty (20) 4th instar larvae were placed into 200 ml of sterilized double-distilled water and set in an environmental chamber at 27°C with a photoperiod 16:8-h light/dark cycle. The effectiveness of silver nanoparticles as mosquito larvicides was determined from all the twenty 4th instar larvae with exposure to time periods. The larvae were separated into 4 small specimen bottles containing 25ml distilled water and the larvae were then exposed to each of the concentrations of the extracts in a final volume of 245ml distilled water taken in 500ml bowls. The nanoparticle solutions were diluted using double distilled water as a solvent according to the desired concentrations (5.0, 4.0, 2.0, 1.0, and 0.5 mg/L). At each tested concentration, four trials were made and each trial consists of four replicates and a control (aqueous plant extracts) were tested for anti–larval effects. The larval mortalities were assessed to determine the acute toxicities on 4th instar larvae of Aedes albopictus at intervals of 1, 3,6, 12,16,and 24 hours of exposure. The number of dead larvae were counted from the 1st hour of exposure, and the percentage of mortality was reported from the average of four replicates. The larval mortality data were corrected for control mortality by the formula of Abbott[14].

Results and Discussion

The use of natural product chemistry coupled with nanotechnology that reduces mosquito populations at the larval stage can provide many associated benefits to vector control. Since silver nanoparticles are considered to be potential agents for various biological applications including antimicrobial, its application as a mosquito larvicidal agent was investigated. The surface plasmon resonance peak in the UV-Vis absorption spectra of the silver nanoparticles synthesized by biological reduction showed an absorption peak at 417 nm. figure 2 High resolution scanning electron microscopic analysis provided information on the morphology and size of the nanoparticles which was found to be on an average of 35nm. figure 3 The larvicidal activity of silver nanoparticles against larvae of Aedes albopictus is presented in figures 4,5 and 6. figures 4,5 and 6. The data obtained from the present study clearly indicate that silver nanoparticles could provide excellent larval control of Aedes albopictus. Greater mortality is seen in larvae treated with silver nanoparticles compared to aqueous extract. It was observed that aqueous extract and distilled water had little or no effect on larval mortality. Higher concentrations of aqueous plant extract caused no death of the larvae until 12 hours of exposure. The nanoparticle at 1.0 mg/l slightly decreased the survival of larvae to 50% after 12 hours of exposure, while 100% mortality of the larval population was observed in a concentration of 5.0mg/l nanoparticles within 3 hours. The nanoparticle of 1.0mg/l killed the larvae slowly and nearly 90% mortality was found after 16 hours of exposure. The maximum efficacy was observed in 5.0 mg/l of silver nanoparticles. The mechanism which causes the death of the larvae could be the ability of the nanoparticles to penetrate through the larval membrane. The silver nanoparticles in the intracellular space can bind to sulphur-containing proteins or to phosphorus containing compounds like DNA, leading to the denaturation of some organelles and enzymes[15]. Subsequently, the decrease in membrane permeability and disturbance in proton motive force causes loss of cellular function and finally cell death.

Conclusion

The prospect of utilizing natural products for synthesizing silver nanoparticles and testing its efficacy in controlling mosquitoes as larvicides is a recent phenomenon facilitating the development of a more potent and environmentally safe pesticide. Identification of the bioactive principles involved and their mode of action and field trials are necessary to recommend an effective formulation as an anti-mosquito product in control programs.

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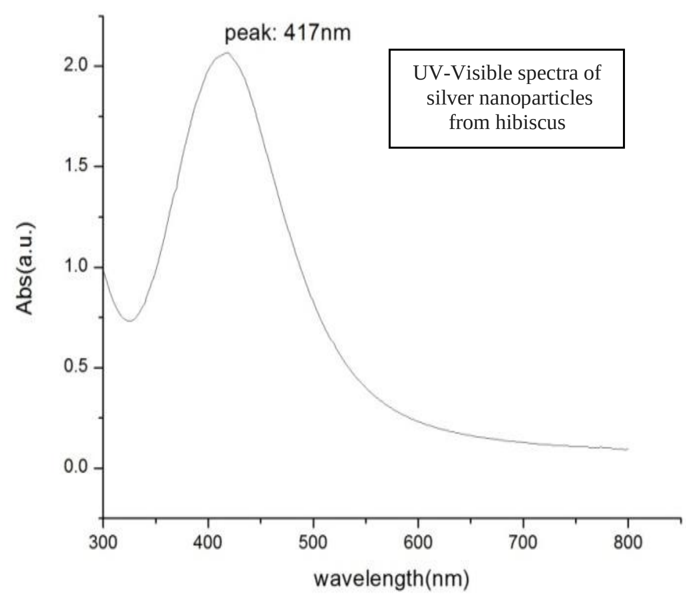

Figure-2
Uv-Vis spectra of silver nanoparticles

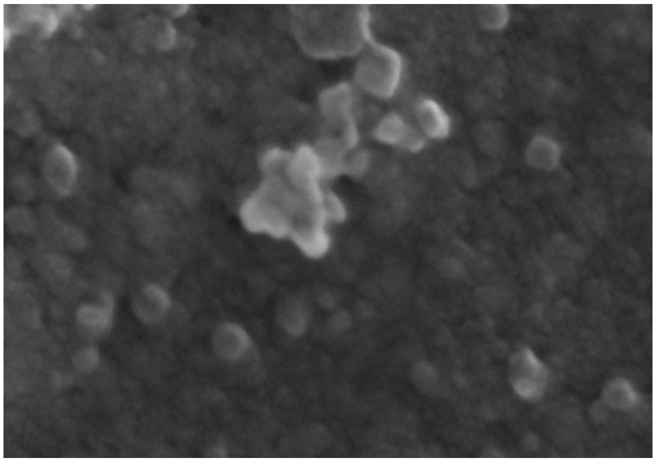

Figure-3
Scanning Electron Microscope image of the synthesized silver nanoparticle

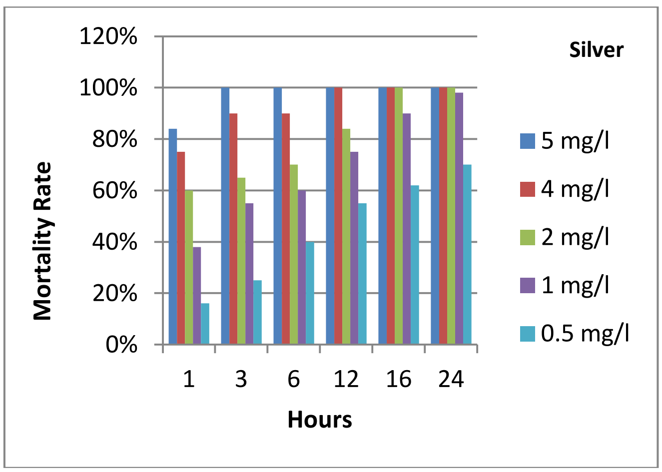

Figure-4
Survival percentage of mosquito larvae after exposure to different concentrations of silver nanoparticles

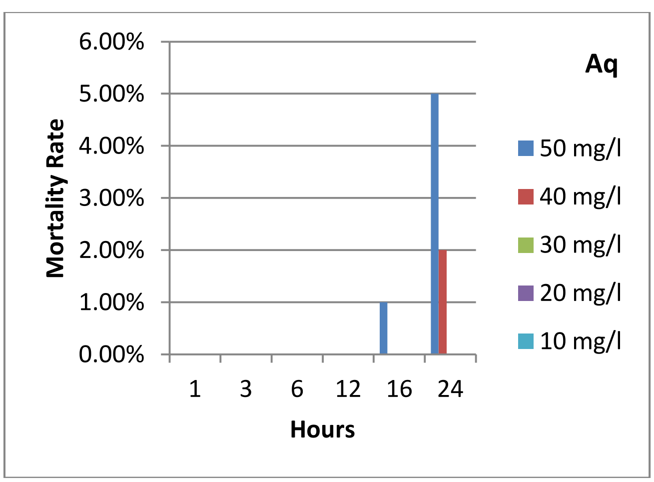

Figure-5
Survival percentage of mosquito larvae after exposure to different concentrations of aqueous extract

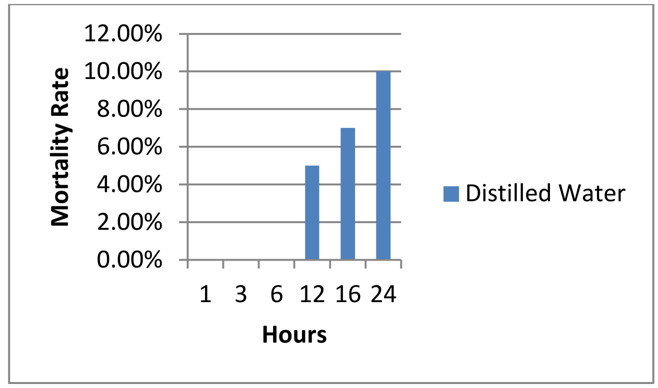

Figure- 6
Survival percentage of mosquito larvaeafter exposure to distilled water

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