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Screening of larvicidal and pupicidal activity of biological synthesised silver nanoparticles [AgNPs] of *Artemisia nilagirica* against the dengue & chikungunya vector, *Aedes aegypti*

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Abstract
In the present investigation the larvicidal and pupicidal activity of silver nanoparticle synthesized by the leaf extracts of *Artemisia nilagirica* against *Aedes aegypti* was attempted in laboratory condition. The synthesis of silver nanoparticles was initially confirmed by formation of brown colour in the reaction mixture within 1 day incubation. The synthesis of silver nanoparticles in the reaction mixture was further confirmed by UV-Vis spectroscopy showed an absorption band at 415.00nm. SEM analysis provided the shape of sliver nanoparticle is spherical and particle size was 8nm. The XRD analysis showed three distinct differentiation peaks can be indexed 2Ɵ values crystalline planes of cubic silver. FTIR analysis showed the functional group of synthesized silver nanoparticles and the absorption bands seen at 3180.4, 1744.49, 1685.67, 1544.88, 1396.37, 1371.29, 988.45, 705.9 and 518.82cm^{-1}.

Keywords: Silver nanoparticles, *Artemisia nilagirica*, larvicidal activity, pupuidal activity, profit analysis

1. Introduction
Nanotechnology is an inter-disciplinary science that involves the production, manipulation and use of materials in the nano-scale range. In recent times, "Nanobiotechnology" has emerged as an important branch of nanotechnology [1]. Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other scientific fields, such as chemistry, biology, physics, materials science, and engineering conducted at the nanoscale, which is about 1 to 100 nanometers. Biological materials have potential to reduce the metal ions into metal NPs. Biosynthesis of AgNps gained considerable attention in the past decade. Moreover, it has been proposed as a less toxic, cost-effective, environmentally friendly alternative to chemical and physical methods. Accordingly, the biological synthesis of nanoparticles involves algae, actinomycetes, bacteria, fungi and plants. The medical properties of silver have been known for over 2,000 years. Silver nanoparticles have important applications in the field of biology. Stable silver nanoparticles were synthesized by biological reduction method. Silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects [2]. Mosquitoes comprise a monophyletic taxon [3] belonging to family *culicidae,* order *Diptera*. Mosquitoes are the principal vector of many vector-borne diseases affecting human beings and animals, in addition to nuisance. Vector-borne diseases in India, e.g., malaria, dengue, chikungunya, filariasis, Japanese encephalitis and leishmaniasis, cause thousands of deaths per diseases, with 2.5 billion people living in areas of risk and many tens of millions of cases occurring each year [4, 5]. *Aedes aegypti* occurs in Asia, Africa and Central and South America. It transmits virus of Flavivirus genus, etiologic agents of human diseases like dengue and yellow fever [6]. Over 2.5 billion people over 40% of the world's population are now at risk from dengue. WHO currently estimates there may be 50-100 million dengue infections worldwide every year (World Health Organization). These diseases can be controlled by targeting the causative parasites and pathogens. It is easier to control vectors than parasites. The chemical control was one of the most widely used conventional methods for mosquito control since chemical pesticides are relatively in expensive usually produces immediate control. Generally, the chemical control is carried out by the indoor residual spraying of insecticides such as dichloro diphenyl tri-chloro ethane, hexa chlorocyclo hexane, benzene hexa chloride, melathion and synthetic pyrothroid. Application of plants for synthesis of nanoparticles can be advantageous over other biological process because it get rid of the complex process of maintaining cell cultures and is also suitable for large-scale nanoparticle synthesis. Green nanoparticle synthesis has been accomplished using biocompatible plant extract, reducing and capping agents. The larvidcidal activity of biosynthesized silver nanoparticles using the plant extracts has been tested against the dengue vector *Ae. Albopictus* and *Ae. Aegypti.*

2. Materials and methods
The fourth instar larvae of the *Aedes aegypti* mosquito was treated with the aqueous leaf extract and the green nanoparticles synthesized from the leaves of *Artemisia nilagirica.*

2.1 Silver nitrate preparation
Silver nitrate was used as precursor for the synthesis of solver nanoparticles. Analytical grade, silver nitrate (AgNo3) was prepared for 16.96 mg of silver nitrate was carefully weighed and dissolved in 90 ml of Milli-Q-water. This aqueous silver nitrate solution was always prepared fresh.

2.2 Preparation of plant extract

The freshly harvested plant leaves were washed thoroughly in tap water, pat dried with paper towel, and shade dried at room temperature (35±1ºC). These dried leaves were powered mechanically using electrical mixer. Aqueous extract was prepared by mixing 10g of dried leaf powder in100 ml of double distilled water. This suspension was mixed well and left for 5 hour without disturbance, then filtered through Whatmann filter paper (no.1), and the filtrate was used to find out the larvicidal and pupicidal activity against the target vector.

2.3 Collection and maintenance of target vector

Different larval instars and pupa of *Ae. aegypti* were collected from the Indian council for Medical Research, Madurai and were brought to the laboratory safely without disturbance. These larvae and pupae were maintained in enamel trays containing deionized water and allowed to feed on brewer's yeast, dog biscuits, and sucrose in a 3:1:1 ratio in the laboratory at room temperature for 24 hours, before start of the experiment.

2.4 Synthesis of silver nanoparticles from aqueous leaf extract

Aqueous leaf extract *Artemisia nilagirica* was prepared by placing 10g chopped fresh leaves in a 250ml Erlenmeyar flask and boiled with 100ml if sterile double distilled water up to 60 min at 60ºC in a water bath. The crude extract was passed through Whatsmann filter paper (no.1), and the filtrates (aqueous aqueous leaf extract) were stored at 4ºC and used within 3 days. Ten millilitre of aqueous leaf extract was treated with 90 ml of prepared 1 mm aqueous AgNO3 solution in an Erlenmeyer flask and incubated in dark at room temperature. The aqueous solution of 1mm of Ag NO3 was greatly reduced from Ag^{I} to Ag^{0} by aqueous leaf extract leading to change of pale yellow to dark brown resulting in synthesis of Ag NPs.

3. Characterization

The formation of Silver nanoparticles was verified by using UV-Vis spectrophotometer was monitored for reaction time on Shimadzu UV-2450, Japan, at a resolution of 1nm between 300 and 800 nm.

To determine the nature and size of the synthesized AgNPs, X-ray diffraction (XRD) was performed. For this, the reaction mixture was centrifuged (20,000rpm, 15 min, 4ºC). The scanning range during the characterization was selected between 10o to 90o.

For FTIR studies, dried powder of the AgNPs was subjected to analyze the presence of possible functional groups for the reduction of Ag^{I} ions resulting in formation of AgNPs using Fourier transform infrared (FTIR) spectroscopy (Shimadzu, Japan). FTIR spectra were recorded at 1 cm^{-1} resolution.

For SEM studies, a drop of aqueous solution containing purified silver nanomaterials obtained after repetitive centrifugation was placed on the carbon coated copper grids and dried under infrared lamp for characterization of their morphology using FEI Quanta 200 Scanning Electron Microscope at accelerating voltage of 20 kV.

The larvicidal and pupicidal activity was evaluated using WHO method (1996) with slight leaf modifications. Different test concentrations of aqueous leaf extract and AgNPs in 200 ml deionized water were prepared in 250 ml capacity autoclaved glass bottles. Mortality rate was recorded after 24 hours of exposure period. The dead larvae in ten replicates were combined expressed as a percentage of larval and pupal mortality for each concentration.

3.1 Statistical analysis

The data on the efficacy of Ag-NP were subjected to Probit analysis [11]. By using the computer software (SPSS, 2007) and MS EXCEL 2007 the LC_{50} value.

4. Results and discussion

4.1 UV-Vis spectroscopy

Artemisia nilagirica aqueous leaf extract was subjected to synthesis of silver nanoparticles and the visible colour change indicates the formation of nanoparticles which is confirmed by UV-visible absorption spectroscopy. The progress of the reaction between metal ions and the aqueous leaf extracts were monitored by UV-visible spectro of silver nanoparticles in aqueous solution with different reaction times that are shown in (Fig. 1) The UV-visible spectro showed an absorption band at 415.00 nm which corresponds to the absorbance of silver nanoparticles. This absorption is unique property of metal nanoparticles called SPR (Surface Palsmon Resonance) that arise due to conduction of electrons on surface of silver nanoparticles. SPR for different metal nanoparticles were reported by [13] 422 to 447 nm silver nanoparticles for *Cardiospermum halicacabum.*

4.2 SEM analysis

SEM results provided the information about morphology and size of the silver nanoparticles. The SEM micrograph shows silver nanoparticles in the size range 8nm. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by the capping agent (Fig. 2) Further SEM image showed the high density of silver nanoparticles by the calculation of XRD analysis. In the present study SEM image showed that synthesized silver nanoparticles are spherical in shape and 8 nm in size. Similar shape of nanoparticles was synthesized from *Musbalbisiana* [12].

4.3 XRD analysis

The crystalline nature of silver nanoparticles was confirmed by the X-ray diffraction analysis. (Fig. 3) shows the XRD pattern with the diffraction peaks of the face centred cubic (FCC) crystal structure. The broadening of the Bragg peaks indicates the formation of nanoparticles. In addition to the Bragg peaks representative of FCC silver nano cryatals, additional and yet unassigned, peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles. The average size of silver nanoparticles formed in the bioreduction process is determined using Scherr's formula and estimated to be 20 nm.

4.4 FTIR analysis

FTIR spectroscopy analysis was carried out to identify the potential bio molecules in the aqueous leaf extract responsible for the reduction and also the capping reagent responsible for the stability of the bio reduced silver nanoparticles. A typical FTIR spectrum of the obtained silver nanoparticles is showed (Fig. 4) the functional group of synthesized silver nanoparticles and the absorption bands

seen at 3180.4, 1744.49, 1685.67, 1544.88, 1396.37, 1371.29, 988.45, 705.9 and 518.82cm^{-1}. Similar results was reported by [14] the spectrum exhibits the band at 1635.64 cm-1 corresponding to primary amide groups, bands at 1381.64 cm-1 represents the nitro compounds including primary (CN) and secondary amines (NH) stretch vibration of proteins. Strong bands of phenyl ring compounds indicate the occurrence of proteins with silver nanoparticles synthrsized by *Pongamia notatum.*

4.5 Larvicidal and Pupicidal activity

The silver nanoparticles showed potent maximum larvicidal and pupicidal activity against the larvae of *Aedes aegypti.* LC_{50} values of aqueous leaf extract of *Artemisia nilagirica* against *Aedes aegypti* and silver nanoparticles synthesized from aqueous leaf extract of *Artemisia nilagirica* were 78.665 and 5.582 respectively (Table 1). Larval mortality and total mortality were increased with increasing concentration of AgNPs synthesized from aqueous leaf extract of *Artemisia nilagirica* except adult emergence (Table 2 & Table 3).

The higher LC_{50} value of the aqueous leaf extract of *Artemisia nilagirica* against the *Ae. Aegypti* was 78.65 ppm, LC_{50} value of the synthesized silver nanoparticles against Ae. *Aegypti* was 5.582ppm. This indicates the toxicity found in aqueous leaf extract of *Artemisia nilagirica.* The larvicidal property of silver nanoparticles may be accouted for its effect on digestive tract enzymes, structural deformation in DNA, generation of atractive oxygen species [8, 9]. Increased larvicidal spectrum may also be due to synergistic combination of silver nanoparticles and proteins and other secondary metabolites adhering on silver nanoparticles surface during reduction and stabilization and silver nanoparticles.

Similar results were observed in larvicidal activity of synthesized silver nanoparticles utilizing an aqueous extract from Eclipta prostrate was observed in crude aqueous and synthesized silver nanoparticles against *Culex quinquefasciatus* (LC_{50}=27.49 and 4.56 ppm; LC_{90}=70.38 and 13.14 ppm) and against Anopheles subpicus (LC_{50}=27.85 and 5.14 ppm; LC_{90}=71.45 and 25.68 ppm) respectively [10].

Table 1: LC_{50} values of the test solutions for aqueous leaf extract and silver nanoparticles synthesized aqueous leaf extract against IV instar larvae of the mosquito *Aedes aegypti.*

Types of leaf extract	Test solution	LC50 values in ppm
Leaf extract	Aqueous leaf extract	78.67
Silver nanoparticles synthesized leaf extract	silver nanoparticles synthesized from extract of leaf	5.58

Table 2: Effect of different concentrations of aqueous leaf extract on the larval and pupal period, pupal and adult mortality, percentage of total mortality and adult emergence on the IV instar larvae of *Aedes aegypti.*

S. No	Parametres	Control	Concentration of aqueous leaf extract in ppm									
			1	2	3	4	5	6	7	8	9	10
1	Larval period in days	4	4	4	4	4	4	4	4	4	4	4
2	Pupal period in days	2	2	2	2	2	2	2	2	2	2	2
3	Larval mortality	0	1	4	4	5	6	8	9	10	11	14
4	Pupal mortality	0	1	0	1	2	1	1	1	2	1	1
5	Adult mortality	0	0	0	0	0	0	0	0	0	0	0
6	Total mortality	0	10	20	25	35	35	45	45	60	60	75
7	Adult emergence %	100	90	80	75	65	65	55	55	40	40	25

Table 3: Effect of different concentrations of silver nanoparticles synthesized from aqueous leaf extract on the larval and pupal period, pupal and adult mortality, percentage of total mortality and adult emergence on the IV instar larvae of *Aedes aegypti.*

S. No	Parametres	Control	Concentration of silver nanoparticles synthesized from aqueous leaf extract in ppm									
			1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
1	Larval period in days	4	4	4	4	4	4	4	4	4	4	4
2	Pupal period in days	2	2	2	2	2	2	2	2	2	2	2
3	Larval mortality	0	1	1	3	5	9	10	13	16	18	20
4	Pupal mortality	0	0	1	1	2	0	2	1	1	1	0
5	Adult mortality	0	0	0	0	0	0	0	0	0	0	0
6	Total mortality	0	5	10	20	35	45	60	80	85	95	100
7	Adult emergence %	100	95	90	80	65	65	40	20	15	5	0

5. Conclusion

From the present investigation, it was augmented that the silver nanoparticles synthesized from the aqueous leaf extract of *Artemisia nilagirica* had a telling effect on the mortality of larvae and pupae of filarial mosquito *Aedes aegypti*. This research focused on eco-friendly nano-synthetic routes to mosquitocidal.

6. References

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