WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 1383-1393. Research Article ISSN 2277– 7105

LARVICIDAL EFFICACY OF *AZIMA TETRACANTHA* LEAVES AND ITS SILVER NANOPARTICLES AGAINST *ANOPHELES STEPHENSI*

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Article Received on
30 April 2015,

Revised on 20 May 2015,
Accepted on 11 June 2015

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ABSTRACT

Laboratory experiments were conducted to evaluate the larvicidal activity of *Azima tetracantha* leaves extract and its silver nanoparticle (AgNPs) under 4 concentrations as 2, 4, 6 and 8% against the 4th instar larvae of *Anopheles stephensi*. The different larval mortality percentages were recorded after 96 hours. The larvicidal effect of *Azima tetracantha* leaves extract and its silver nanoparticle were compared. The *Azima tetracantha* leaves extract and its silver nanoparticle in their different concentrations have shown larvicidal effects on *Anopheles stephensi* larvae. Statistical analysis showed no significant differences between the higher concentration of *Anopheles stephensi* that all concentrations recorded 100 % mortality of the tested larvae. The results indicated that, silver nanoparticle had the greatest larvicidal effect against *Anopheles stephensi* larvae with the lowest LC50 (0.18%) followed by plant. This study suggests that silver nanoparticle should be considered as promising larvicidal activity against *Anopheles stephensi* larva.

KEYWORDS: *Anopheles stephensi*, *Azima tetracantha* leaves, Silver nanoparticle.

INTRODUCTION

Mosquitoes are among the most important insect pests affecting health of people and animals. Biting female mosquitoes not only irritate people and animals, but they can transmit several diseases than any other group of arthropods and affect millions of people throughout the

world. Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700 million people every year globally and 40 million Indian populations.[1, 2]

Approximately 3500 mosquito species are reported from the tropical and subtropical regions of the world yet only a small fraction of the mosquito species transmit diseases to mankind,[3] Among this *Anopheles* (malaria, filariasis), *Aedes* (yellow fever, dengue, chikungunya) and *Culex* (West Nile, Japanese encephalitis, filariasis) mosquitoes are distributed globally. Malaria, filariasis and dengue hemorrhagic fever (DHF) are still major public health problems in Southeast Asian countries including India because of tropical or subtropical climate prevailing in this region, poor drainage system, presence of many fish ponds, irrigation ditches and rice fields.[4] Mosquito transmitted diseases are very important for human health as many fall vulnerable to these diseases every year and a large number of them die annually. Malaria infects 500 million people every year and more than one million die. Dengue fever infects 50 million people annually with 25,000 death cases. Yellow fever infects 200,000 every year and 30.000 die.

Due to disadvantages associated with synthetic pesticides, there is a renewed effort to develop substances of plant origin which are considered to be more environmentally friendly due to their innate biodegradability and lower toxicity to most organisms. Many of the reported tropical plants came under scrutiny, leading to extraction and characterization of their active constituents and its nanoparticles for various uses. The most important of these constituents are alkaloids, terpenoids, steroids, phenols, saponins and tannins. A large number of plant products have been reported to have repellent activity.[5-6] Keeping these in mind the present study was undertaken to evaluate the larvicidal activity of *Azima tetracantha* leaves extract and its silver nanoparticle against *Anopheles stephensi* mosquitoe.

MATERIALS AND METHODS

LARVICIDAL ACTIVITY

Sampling and culture of mosquito larvae

Anopheles stephensi mosquito larvae were collected from a stagnant water source located adjacent to a residential area at Thanjavur, Thanjavur District, Tamil Nadu. The collected larva was identified by Zoologist Dr. S.S. Rajendran, Department of Zoology, Serfoji colloge, Thanjavur. After washing the larvae with tap water, they were transferred to a sterilized plastic container and transported to Harman Research laboratory, Thanjavur. Among the larvae, 4th instar stages were segregated and kept in plastic containers and fed with artificial

food i.e. mixture of dog biscuits and dried yeast powder at the ratio of 3:1. All other larval stages and pupae found in the water samples were discarded properly. Colonies were kept free from exposure to pathogen, insecticides or repellents.

Collection of plant materials

The mature *Azima tetracantha* leaves were collected in May 2013 from Kodaikanal, Dindugal district, Tamil Nadu, India. The leafs were identified and authenticated by Botanist, Prof. Dr. S. John Britto, Director, The Rapinat Herbarium, St. Josephs College, Tiruchirappalli, Tamil Nadu, India.

Preparation of leaf extract

The dried leafs were pulverized well with mortar and pestle to make a powder. Twenty grams of powder sample was mixed into 100 ml of deionized water and the mixture was boiled for 10 min. After cooling the leaf extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4°C for further use.

Synthesis of Ag nanoparticles using leaf extracts

For the Ag nanoparticles synthesis, 5 ml of *Azima tetracantha* is leaf extract was added to 45 ml of 1 mM aqueous $AgNO_3$ solution in a 250 ml Erlenmeyer flask. The flask was then incubated in the dark at 5hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without leaf extract. The Ag nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the Ag nanoparticles were freeze dried using larvicidal activity. Our earlier reports confirm the synthesis and characterization of silver nanoparticles.[7]

Larvicidal test

Method for testing larvicidal action of the *Azima tetracantha* extract and Silver nanoparticles was slightly modified from those of World Health Organization (WHO, 1996). A stock solution was prepared dissolving a known amount of crude extract in water and stored in a refrigerator at 15°C. Twenty healthy late 4^{th} instar larvae were introduced into each testing cup (sterilized plastic cup of 150 ml capacity) containing 100 ml of dechlorinated tap water with stock solution. A measured volume of stock solution was added to obtain the desired concentrations. Experiments were carried out with a series of four concentrations viz. 2, 4, 6 and 8 % in triplicates. Each batch of replicates contained one control of 100 ml of water alone

and another of 100 ml of water containing a volume of solvent corresponding to the maximum volume of extract tested. As very few larvae succumbed within 12 hours of exposure to the test solutions, mortality was recorded after 6 hours of exposure, during which no feed was given to the larvae. The mortalities of mosquito larvae were recorded if moribund larvae were incapable of rising to the surface or moving when they were disturbed.

RESULTS

Extensive use of chemical agents for the control of vectors has created adverse environmental problems and developed physiological resistance in mosquitoes. Searching for new control agents from natural products such as plant secondary metabolites has gained popularity among researchers in countries with a strong herbal tradition and large numbers of plants have been reported to possess insecticidal activity. India possesses good plant diversity which provides a range of natural products. Botanicals considered as safer alternatives have provided a variety of phytochemicals, which have a wide range of benefits ranging from pharmaceutical products to insecticides.

Keeping this in view the present study was aimed to discover cost-effective, eco-friendly alternative for mosquito control by evaluating the larvicidal activity of different extracts of *Azima tetracantha* and Silver nanoparticles against larva of *Anopheles stephensi* mosquitoes.

Larvicidal activity

Larvicidal activity of *Azima tetracantha* against *A. stephensi* was presented in table 1 and Fig 1. The mortality rate of larvae at 8 ml was significantly higher than the mortality rates at 2, 4 and 6 % concentrations of crude plant extract at 12, 24, 48, 72 and 96 hrs of exposure. Higher mortality rate was also recorded at 96 hrs bioassay than those at 24 hrs. No mortality was observed in 12 h exposure period for plant extract at 2 and 4% concentrations.

Table 1 % of larvicidal activity of *Azima tetracantha* against larva of *A. Stephensi*

Plant extract (%)	12 hrs	24 hrs	48 hrs	72 hrs	96hrs
2	0 %	10%	20%	40%	60%
4	0%	20%	30%	50%	70%
6	10%	20%	30%	60%	80%
8	20%	40%	60%	80%	100%
Control	-	-	-	10%	10%

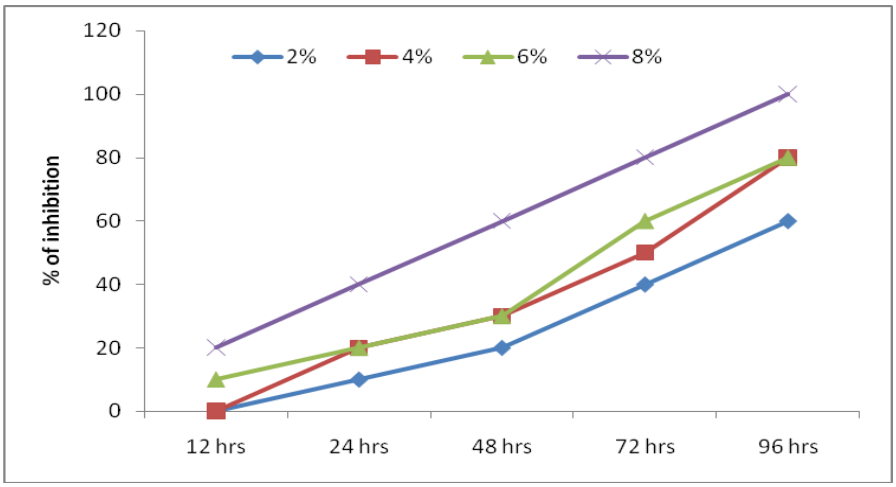
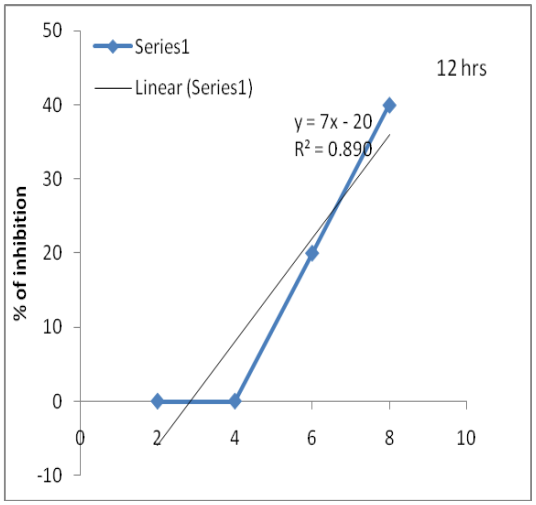
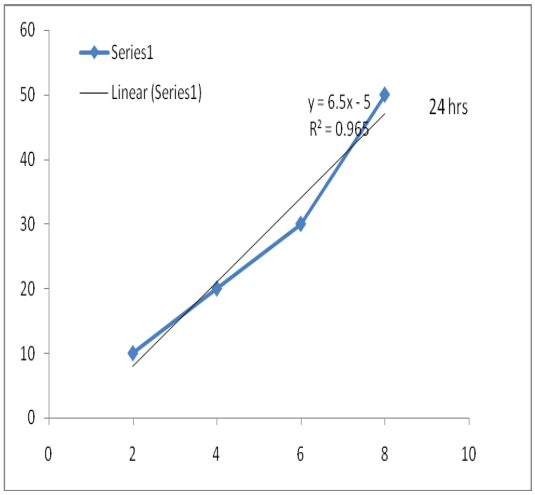
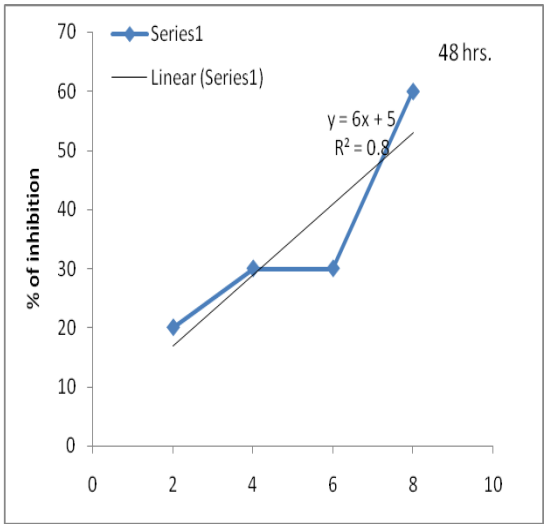
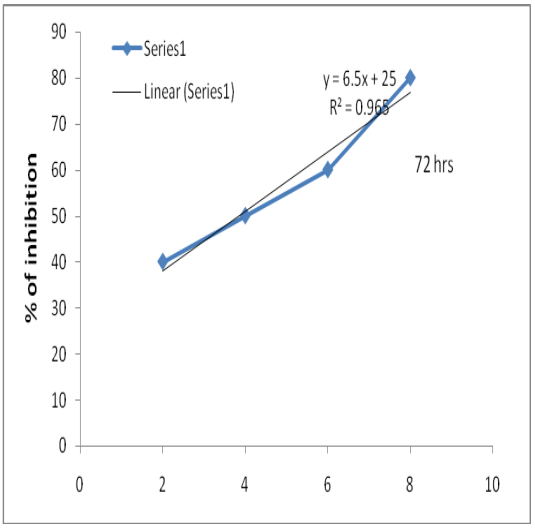


Figure: 1% of Larvacidal activity of *Azima tetracantha* against larva of *A. Stephensi*









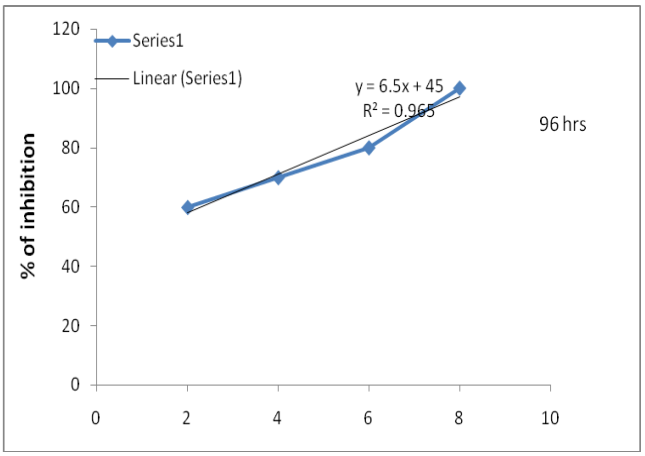


Fig 2 shows the regression analysis of *Azima tetracantha* against larva of *A. Stephensi*

Table 2 % of larvacidal activity of Silver nanoparticles against larva of *A. Stephensi*

AgNPs (%)	12 hrs	24 hrs	48 hrs	72 hrs	96 hrs
2	0%	0%	10%	50%	70%
4	10%	10%	30%	60%	80%
6	20%	20%	40%	70%	100%
8	20%	50%	60%	90%	100%
Control	-	-	-	10%	10%

Larvicidal activities of Silver nanoparticles against *A. stephensi* were presented in table 2 and fig 3. The mortality rate of larvae at 6 and 8 ml was significantly higher (P< 0.05) than the mortality rates at 2 and 4 % concentrations of crude plant extract at 12, 24, 48, 72 and 96 hrs of exposure. Higher mortality rate was also recorded at 96 hrs.

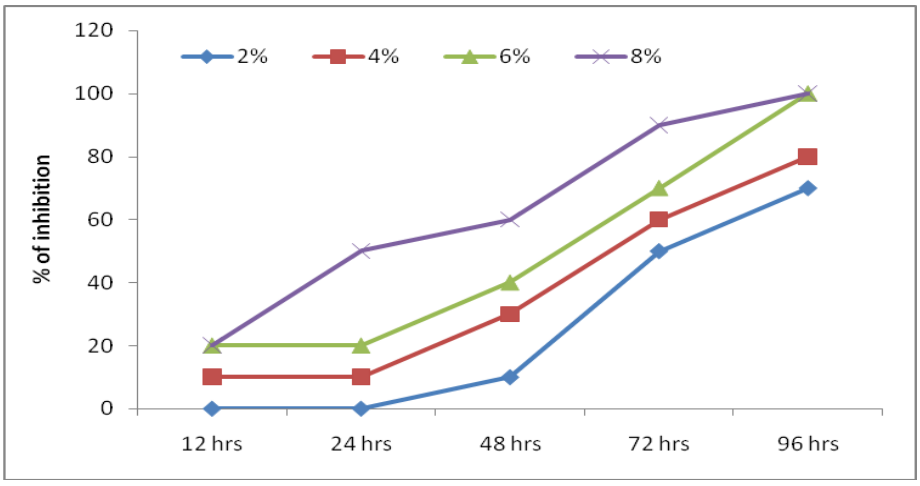
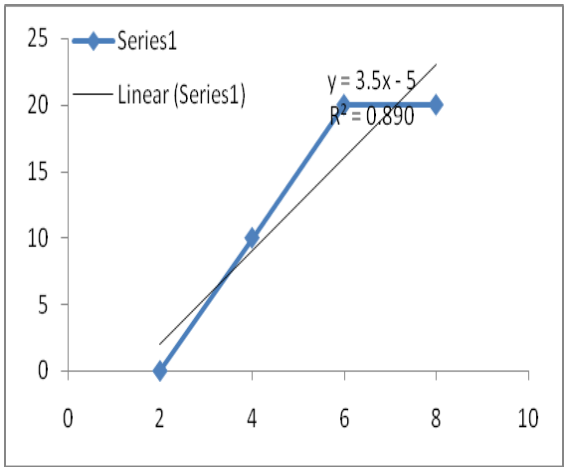
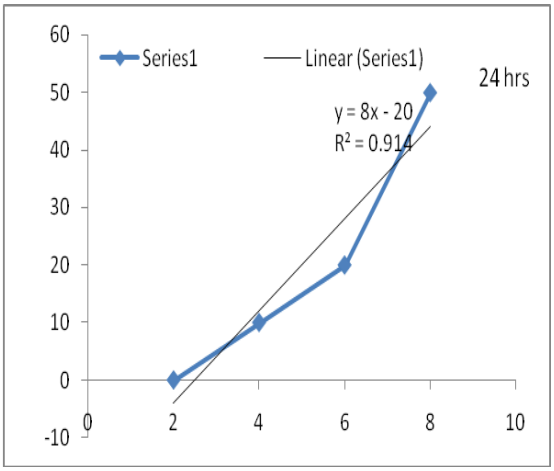
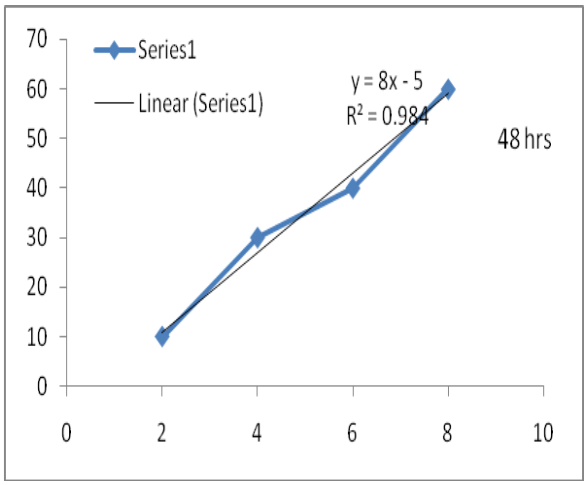
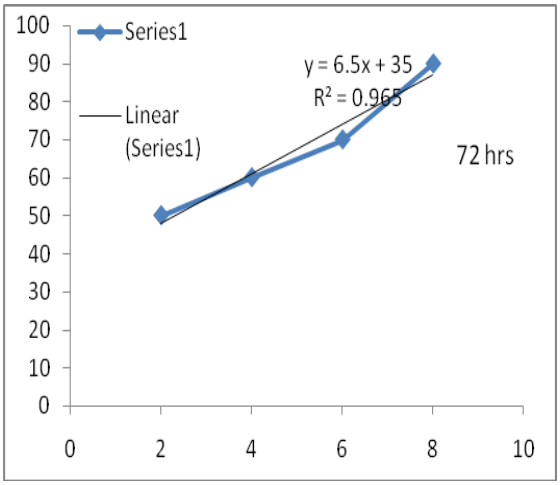


Figure: 3% of larvacidal activity of Silver nanoparticles against larva of *A. Stephensi*









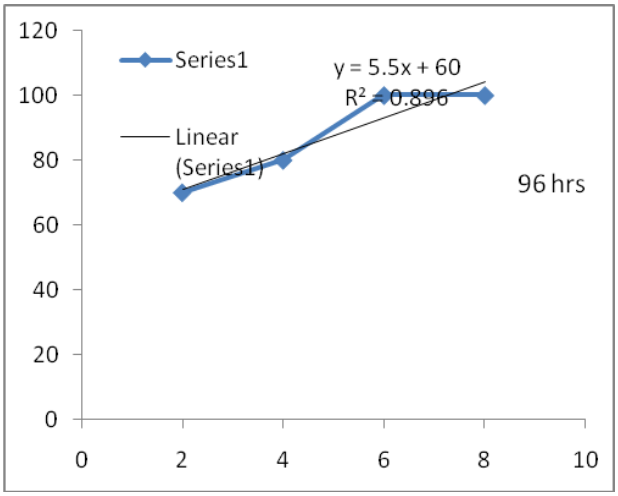


Fig 4 shows the regression analysis of silver nanoparticle against larva of *A. Stephensi*

The result given in table 3 revealed that LC_{50} values were gradually increased with exposure period and there is a positive correlation between mortality (Y) and concentration (X) having a regression coefficient value close to one in each case. LC_{50} value of ethanolic extract of *Azima tetracantha* were 10 for12h, 8.46 for(24 h, 7.50 for 48 h and 3.84% for 72 h

respectively, after 72 hrs of exposure but the LC_{50} value of ethanolic extract of *Azima tetracantha* were 0.833% , after 96 h of exposure.

The result given in table 3 revealed that LC_{50} values were gradually increased with exposure period and there is a positive correlation between mortality (Y) and concentration (X) having a regression coefficient value close to one in each case. LC_{50} value of silver nanoparticle was 15.71 for 12h, 8.75 for 24 h, 6.87 for 48 h and 2.30% for 72 h respectively, after 72 hrs of exposure but the LC_{50} value of silver nanoparticle was 0.18%, after 96 h of exposure.

Table 3. LC_{50} of 96 hrs value and regression analysis of *Azima tetracantha* and AgNPs against larva of *A. stephensi.*

Exposure hours	LC_{50}	Regression equation	R^2 Value
Azima tetracantha			
12	10	Y = 7x – 20	0.890
24	8.46	Y = 6.5x – 05	0.965
48	7.50	Y = 6x + 05	0.800
72	3.84	Y = 6.5x + 25	0.965
96	0.833	Y = 6.5x +45	0.965
AgNPs			
12	15.71	Y = 3.5x – 5	0.890
24	8.75	Y = 8x – 20	0.914
48	6.87	Y = 8x - 05	0.984
72	2.30	Y = 6.5x + 35	0.965
96	0.18	Y = 5.5x +60	0.896

DISCUSSION

The present investigation revealed that the *Azima tetracantha* and Silver nanoparticles possess larvicidal activity against *A. stephensi* larvae. Silver nanoparticles showed maximum larvicidal activity followed by *Azima tetracantha* extract at lower exposure periods of 12, 24 and 48 hours (Table 1-2). Silver nanoparticles exhibited high larvicidal activity at higher exposure period of 72 and 96 hours (Table 2). The larvicidal activity of extract of *Azima tetracantha* and Silver nanoparticles attained 100% at 96 hours exposure period. These irregular patterns of toxicity might be due to the interactions of compounds and solute with the water medium where the larvae were reared. The LC50 values of *Azima tetracantha* and Silver nanoparticles represented in table 3.

Chandrashekhar *et al.*[8] study the activity of silver nanoparticles (AgNPs) synthesized using *Plumeria rubra* plant latex against second and fourth larval instar of *Aedes aegypti* and

Anopheles stephensi was determined. Range of concentrations of synthesized AgNps (10, 5, 2.5, 1.25, 0.625, 0.3125 ppm) and aqueous crude latex (1,000, 500, 250, 125, 62.50, 31.25 ppm) were tested against larvae of *A. aegypti* and *A. Stephensi*. The synthesized AgNps from P. rubra latex were highly toxic than crude latex extract in both mosquito species. The LC values for second and fourth larval instars after 24 h of crude latex exposure were 1.49, 1.82 ppm against A. aegypti and 1.10, 1.74 ppm against A. stephensi respectively. These figures were 181.67, 287.49 ppm against *A. aegypti* and 143.69, 170.58 ppm against A. stephensi respectively for crude latex extract. The mortality rates were positively correlated with the concentration of AgNPs. The characterization studies of synthesized AgNPs by UV-Vis spectrophotometry, transmission electron microscopy (TEM), Particle size analysis (PSA) and zeta potential confirmed the spherical shape and size (32-200 nm) of silver nanoparticles alongwith stability. Toxicity studies carried out against non-target fish species Poecilia reticulata, the most common organism in the habitats of A. aegypti and A. stephensi showed no toxicity at LC and LC doses of the AgNPs.

Rajakumar and Abdul Rahuman,[9] examined the Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrata* leaf extract against *filariasis* and malaria vectors. Insecticides of synthesized natural products for vector control have been a priority in this area. In this study, larvicidal activity of synthesized silver nanoparticles (AgNPs) utilizing aqueous extract from *Eclipta prostrata*, a member of the Asteraceae was investigated against fourth instar larvae of filariasis vector, *Culex quinquefasciatus* say and malaria vector, *Anopheles subpictus* Grassi (Diptera: Culicidae). The synthesized AgNPs characterized by UV–vis spectrum, scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared (FTIR) and X-ray diffraction (XRD). SEM analyses of the synthesized AgNPs were clearly distinguishable measured 35–60 nm in size. Larvae were exposed to varying concentrations of aqueous extract of synthesized AgNPs for 24 h. The maximum efficacy was observed in crude aqueous, and synthesized AgNPs against *C. quinquefasciatus* (LC_{50} = 27.49 and 4.56 mg/L; LC_{90} = 70.38 and 13.14 mg/L), and against *A. subpictus* (LC_{50} = 27.85 and 5.14 mg/L; LC_{90} = 71.45 and 25.68 mg/L) respectively. The chi-square value were significant at $p < 0.05$ level. These results suggest that the synthesized AgNPs have the potential to be used as an ideal eco-friendly approach for the control of the *Culex tritaeniorhynchus* and *A. subpictus*.

Kalimuthu *et al.*[10] have carried out to establish the properties *Cadaba indica lam* leaf extracts with different solvents viz. ethanol, hexane, chloroform and petroleum ether against dengue vector *Aedes aegypti*. Among the extracts, highest larval mortality is reported for ethanolic extract with the LC_{50}value of 144.5 ppm and LC_{90} value of 260. 85 ppm.

Govindarajan *et al*.[11] have reported the mosquito ovicidal and repellent activity of *Eratamia coronaria* and *Caesalpinia pulcherrima* leaf extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi.* The eggs have lost 100% hatchability due to *E. coronaria* leaf extract at concentrations between 150 and 250 ppm while for *C.pulcherrima* it is between 225 and 375 ppm. *E. coronaria* has show greater repellency than *C.pulcherrima*, provided 100% protection for 150, 180 and 210 min at 5 mg/cm^2.

CONCLUSION

Larvicidal efficacy of *Azima tetracantha* and Silver nanoparticles was tested against the *A. stephensi* larvae. The mortality rate of *A. Stephensi* larvae were tested against 2, 4, 6 and 8% concentrations of each Silver nanoparticle and plant extract at 12, 24, 48, 72 and 96 hours interval. The larvicidal activity was observed at 96 hours. Silver nanoparticles possess has significant larvicidal activity than *Azima tetracantha* extract. The potential larvicidal activity of Silver nanoparticles might be due to the synergistic effects of phytochemicals and silver present in it. Identifying bio-insecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. Overall, Silver nanoparticle is a source of natural larvicidal activity that can be important in insect prevention.

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