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Synthesis by silver nanoparticle activity of Larvicidal Useing leaf extracts of *Andrographis alata* against *Culex quinquefasciatus*

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Abstract
In the present study, the Larvicidal activity of biosynthesized silver nanoparticle using leaf extracts of *Andrographis alata* was used against *Culex quinquefasciatus* in order to carry out larvicidal activity. Methanolic leaf extract of *Andrographis alata* along with AgNPs was significantly inhibits the adult emergence. The percentage of adult emergence inhibition were 08.0±1.3, 14.0±1.3, 30.0±1.5, 45.5±1.5, 58.0±3.2, 76.0±2.4 and 96.0±2.3 % at different concentration viz., 0.1, 0.2, 0.3, 0.4 and 0.5 ppm against the larvae of *Cx.quinquefasciatus.* The LC_{50} values of the methanolic leaf extract of *Andrographis alata* along with silver nanoparticle was 460.05 ppm against late third larva *Cx. quinquefasciatus*. The chi-square value 0.25 was significant at $p<0.05$ level. In AgNPs, the LI_{50} value was 623.12 ppm and the EI_{90} value was 1254.35. The chi –square value 0.03.65 was significant at $p<0.05$ level. From the result it is concluded that the plant extract and silver nanoparticles were effectively inhibit the emergence of adult from larvae of *C. quinquefasciatus.*

Keywords: Larvicidal activity, Nanoparticles, *Andrographis alata, Culex quinquefasciatus*

Introduction
The major challenges of public health in a vast region of our planet are considerably affecting the lives of hundreds of millions of people every year and moreover even today [1]. The Larvicides are among the main tools to control mosquito populations. The most widely used larvicides are to stop the growth and act as inhibitors [2, 3]. To bring an alternative to the conventional method, the silver nanoparticles from plants is possible. The production of AgNPs synthesized using plant extracts as reducing, stabilizing, and capping agents, the Larvicidal activity of the selected plant via NPs, and a high efficiency due to the favorable surface area to volume ratio due to the small size of the particles (1–100 nm) [4, 5]. The use of chemical vector for control brings hazards towards humans, animals, non-target species, and the development of resistance. The restrictions of insecticidal agents and rapid development of vector resistance which is associated with synthetic insecticides have prompted the necessity of searching for new insecticidal substances and alternative methods, as novel biological tools [6, 7].Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered environmental and human health concern, which initiated a search for alternative control measures. The Mosquito's canister spreads more diseases than any other group of arthropods and affects Millions of people throughout the world. Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting several people of the Indian population every year [8].

The *C. quinquefasciatus* is one such mosquito species that spread disease in an estimated population of 120 million, among these 44 million people have chronic manifestation [9,10]. Hence there is a need for developing eco-friendly agents from the natural sources such as plants. Plants constitute a number of substances with medicinal as well as insecticidal activities. An attempt was made to find larvicidal properties silver nanoparticles of against filarial vector *C. quinquefasciatus*. Can able to transmit diseases like, malaria, dengue fever, yellow fever, filariasis, chickungunya, etc., in worldwide (Matasyoh *et al*. 2011).
Mosquitoes are the most prevalent vector species, which can able to transmit diseases like, malaria, dengue fever, yellow fever, filariasis, chickungunya, etc., in worldwide (Matasyoh *et al*. 2011).

Materials and methods
In the present study the activity of larvicidal of the using leaf extracts of *Andrographis alata* against *Culex quinquefaciatus* was carried out in the laboratory.

Plant description of *Andrographis alata*

Kingdom	**:**	Plantae
Unranked	**:**	giosperms
Unranked	**:**	Eudicots
Order	**:**	Lamiates
Family	**:**	Acanthacea**e**
Genus	**:**	*Andrographis*
Species	**:**	*A.alata*

Fig 1: *Andrographis alata*

Sterilization of glassware and chemicals

All types of glassware such as conical flask, petri plates, test tubes, pipettes, centrifuge tubes, tip boxes, saline bottles etc., were sterilized at 121ºC for 1 min in an autoclave.

Collection of mosquito larvae

The mosquito larvae were collected from stagnant water samples from roadside ditches, irrigation canals, drainage canals, temporary water pools and ponds around Puthanampatti village (N11º 03. 845'; E078º 41. 007'), Tiruchirapalli district, Tamil Nadu, India, using sterile wide mouth container.

Fig 2: Mosquito larvae

Collection of plant materials for Namakkal Distract Kolli Hills Area Map

The plant *Andrographis alata* leaves (Plate I) were collected from Kolli Hills a part of Eastern Ghats. The Kolli Hills is located in Namakkal district of Tamil Nadu, India. The plant was taxonomically identified at the Department of Botany.

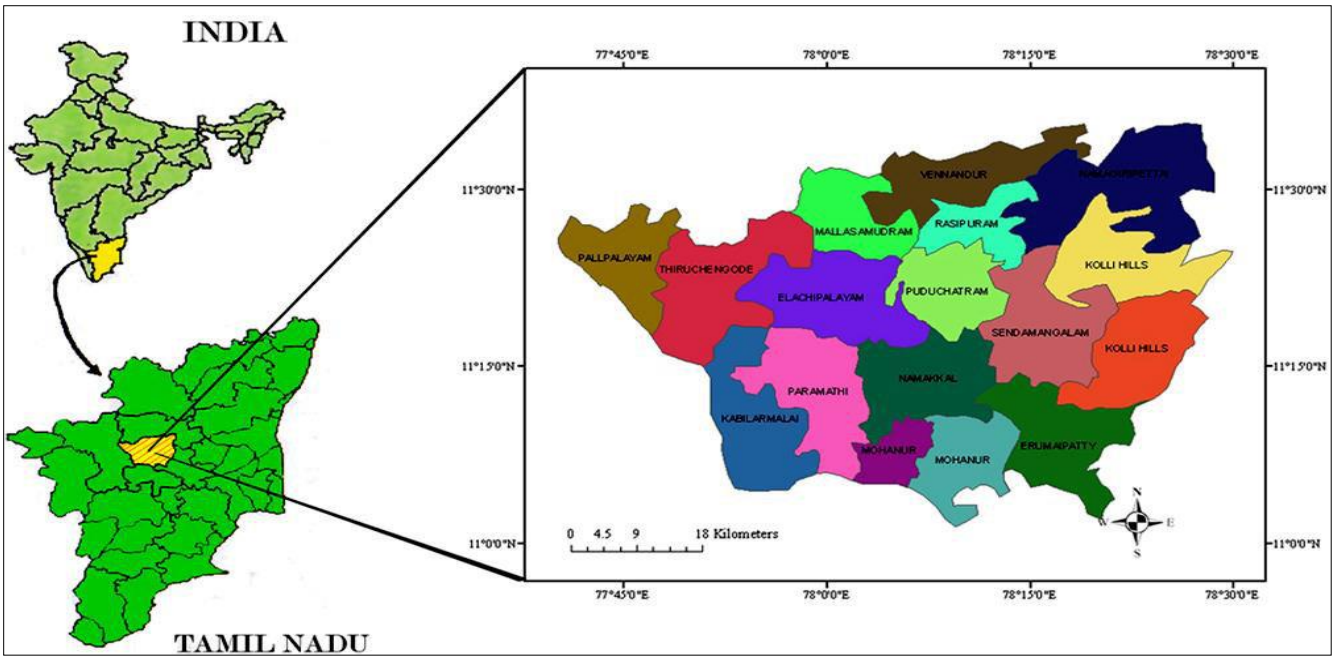

Fig 3: Collection of plant Kolli Hills Area Map

Preparation of plant extract

The leaves of *Andrographis alata* was carefully examined and old leaf, insect damaged, and fungus infected leaves were carefully removed. The fresh and healthy leaves were washed with tap water and shade dried at room temperature (27-31ºC) for 5 to 10 days or until they broke easily by hand.

Laboratory colonization of mosquitoes

The egg rafts of *Culex quinquefaciatus* were collected in local drainage of Puthanampatti village (Plate III).The mosquito colony maintained at 70-85% RH,28±ºC temperature and 14:10 light and dark photo period cycle. The larvae were fed on powdered mixture of dog biscuits and yeast tablets in 3:1 ratio. The blood meal was given to the female adult mosquitoes (Plate IV) and 5.0%glucose solution and honey were given to the Male adult mosquitoes.

Test for larvicidal activity

Testing of the plant extracts along with silver nanoparticles for larvicidal activity was carried out at different concentration by

preparing the required stock solution by following the standard procedure. Six beakers were taken and 50ul (0.1%, 20ppm) of silver nanoparticles was taken out and added to Plant extracts in to the plates. 5 replica were have been noticed down by giving 20 larvae in to the plate and the results were noticed down for every 24 hours and calculation was carried down by using the formula.

Results and Discussion

In the present study the Larvicidal activity of Silver nanoparticle synthezed by the leaf extracts of *Andrographis alata* against *Culex quinquefasciatus.* Larvicidal activity of leaf extract of *Andrographis alata* along with silver nanoparticle against *Culex quinquefasciatus* was experimented successfully. The result of the larvicidal activity of methanolic extract of leaf of *Andrographis alata* along with silver nanoparticle against larval mosquitoes of *Culex quinquefasciatus* is presented in Table 1, 2 and 3 ; Fig 1.

Table 1: Larvicidal activity leaf extract of *Andrographis alata* along with silver nanoparticle against *Culex quinquefasciatus* in 24 hrs

% solution	Mortality of larva in 24 hrs (Replica N=5)					Average	% Mortality
	1	2	3	4	5		
0.1 % (3,000µl)	0	0	2	5	8	3.40	13 %
0.2 % (4,000µl)	0	2	3	8	9	5.00	22 %
0.3 % (5,000µl)	1	1	5	11	15	5.60	32 %
0.4 % (6,000µl)	2	4	7	14	16	8.40	40 %
0.5 % (7,000µl)	2	3	8	15	19	10.20	46 %
Control	0	0	0	0	0	0	0%

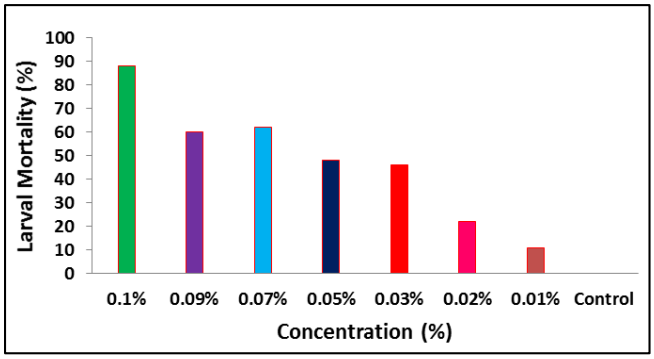

Fig 4: Larvicidal activity leaf extract of *Andrographis alata* along with silver nanoparticle against *Culex quinquefasciatus* in 24 hrs

The LC_{50} values of the methanolic leaf extract of *Andrographis alata* along with silver nanoparticle was 0.460.05 ppm against late third larva *Cx. quinquefasciatus* (Table 2). The LC_{90} and regression equation were 850.30 ppm, Y=05.70+0.18X. The 95% lower and upper confidence limit of LC50, LC90 (LCL-UCL) were (425.11 – 610.02) and (775.01 – 940.20) ppm respectively. The chi –square value 0.25 was significant at $p<0.05$ level. In the present study one leaf extract was chosen with ethanol extract along with silver nanoparticle (0.017 mg in 100 ml distilled water). Some other technique like reflux extraction method may be applied to get 100% mortality at various ppm in different hours like 24, 42, and 72 hrs. However LC50 value of crude obtained by soxhlet extraction showed higher larval mortality.

Table 2: Larvicidal activity leaf extract of *Andrographis alata* along with silver nanoparticle against *Culex quinquefasciatus* in 24 hrs

Concentration (%)	Larval mortality (%)	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	Chi square
0.1 %	85 %	460.05 (425.11-610.02)	850.30 (775.01-940.20)	Y=05.70+0.18X	0.25*
02 %	68 %				
0.3 %	60 %				
0.4 %	45 %				
0.5 %	40 %				
Control	0%				

*significant at $P<0.05$

Values in parenthesis represent 95% confidence interval

Table 3: Probit analysis of Larvicidal activity leaf extract of *Andrographis alata* along with silver nanoparticle against *Culex quinquefasciatus* in 24 hrs

Observed and Expected Frequencies					
Number of		Observed		Expected	
CON	Subjects	Responses	Responses	Residual	Prob
.10	20.0	17.6	16.021	1.579	.80105
.20	20.0	12.0	14.875	-2.875	.74373
.30	20.0	12.4	12.158	.242	.60792
.04	20.0	9.6	9.147	.453	.45736
.05	20.0	9.2	6.255	2.945	.31275

Culex quinquefasciatus

The methanolic leaf extract of *Andrographis alata* along with silver nanoparticle was significantly inhibits the adult emergence. The percentage of adult emergence inhibition were 08.0±1.3, 14.0±1.3, 30.0±1.5, 45.5±1.5, 58.0±3.2, 76.0±2.4 and 96.0±2.3 % at different concentration viz., 0.1, 0.2, 0.3, 0.4 and 0.5 ppm against the larvae of *Cx. quinquefasciatus* (Table 4, Fig 2). The LI_{50} value was 623.12 ppm and the EI_{90} value was 1254.35. The regression equation was Y=12.15+0.615X. The 95% lower and upper confidence limit of LC_{50}, LC_{90} (LCL-UCL) were (510.23-750.40) and (1115.15-1452.05) ppm respectively. The chi – square value 0.03.65 was significant at $p<0.05$ level (Table 4).

Table 4: Insect growth regulator activity leaf extract of *Andrographis alata* along with silver nanoparticle against *Culex quinquefasciatus*

Concentration (%)	Larval mortality (%)±SD	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	Chi square
0.1	96.0±2.3	623.12 (510.23-750.40)	1254.35 (1115.15-1452.05)	Y=12.15+0.615X	0.03.65*
0.2	76.0±2.4				
0.3	58.0±3.2				
0.4	45.5±1.5				
0.5	30.0±1.5				
Control	00.0± 0.00				

*Significant at $P<0.05$
Values in parenthesis represent 95% confidence interval

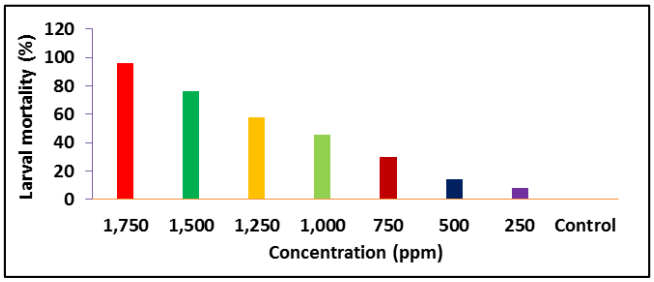

Fig 5: Insect growth regulator activity leaf extract of *Andrographis alata* along with silver nanoparticle against *Culex quinquefasciatus*

Discussion

The activity of crude plant extracts is often attributed to the complex mixture of active compounds. In the preliminary screening, potential larvicidal activity of plant extract was noted. The ethanol extract of leaf extract was tested against the mosquito larvae and 100 per cent larval mortality was observed in the ethanol extract. Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of outrageous diseases like Malaria, Filariasis, Dengue, Japanese encephalitis, etc. causing millions of deaths every year. Eliminating the source of infection is an essential step in the control of mosquito-borne diseases. Synthesized within plants in varying proportions.

These compounds, either independently or jointly contribute to larvicidal activity of mosquitoes. The current revise has opened up prospects for large scale extraction of active ingredients of plant origin for effective mosquito control. Also the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health. In concise, the present study elucidated the larvicidal properties of plant extract along with silver nanoparticles in controlling vector mosquito. As stated earlier, the results reported in the present study open up the possibility of further investigations on evaluation, identification and isolation of the bioactive components of these plant extracts and its systematic effects on target mosquitoes, which would eventually facilitate the application of the extract as larvicidal, adult emergence inhibition and ovicidal agent in small-volume aquatic habitats or breeding sites of limited size in and around human dwellings.

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