JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Larvicidal and Pupicidal Activities of *Plumeria alba* Against Fourth Instar Larvae of *Ades aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*.

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ABSTRACT

Larvicidal and pupicidal activities of latex water crude extracts of *Plumeria alba* were studied against *Ades aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* following the treatment on larvae and pupae. The results of the total larval and pupal mortality rate progressively increased in the different concentrations of 1%, 0.5%, 0.250%, and 0.125%. Showed significant larval mortality effect was recorded in *Culex quinquefasciatus* of 73.33, 55.32, 39.26, 21.32 % at 24hrs and 88.23, 71.65, 46.56, 37.25% at 48hrs respectively. Pupicidal activity is higher in *Culex quinquefasciatus* than maximum pupal mortality was observed in 81.12, 67.45, 41.34, 27.65% and then concentration of 1%, 0.5%, 0.250%, and 0.125% at 24 hrs respectively. Larval and pupal mortality more than 80% observed from crude extract of *Plumeria alba* at 1% concentrationand control of *Ades aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* mosquito vector.

Keywords: *Plumeria alba, Ades aegypti, Anopheles stephensi, Culex quinquefasciatus,* Larvicidal activity, Pupicidal activity.

Introduction

The three blood feeding genera and very dangerous mosquitoes are *Anopheles stephensi, Aedes aegypti* and *culex quinquefaciatus* and they are widely distributed in the tropical and sub tropical zones and they are transmitted disease like malaria, dengue, filariasis, japanesse encephalitis, yellow fever and chikungunya[1]. *Ades aegypti* mosquito is a major vector of viral disease for Zika, chikungunya, dengue, yellow fever, these diseas are mainly transmitted by holometabolous insectof *Ades aegypti* mosquitoes. Dengue virus is tranasmitted in to infected female mosquito[2]. Kingdom: Animalia; Phylum: Arthropoda; Class: Insect; Order: Dipteral; Family: Culicidae; Genus: *Aedes; Species: aegypti.*

The 90 million people worldwide were once infected with *Wuchereria bancrofti*. In India alone is 25 million people microfilaria and 19 million people suffer from filarial diseases[3]. Respiratory trumpets of Culex mosquito are long and narrow. Adult *Cx. quinquefasciatus* vary from 3.96 to 4.25 mm in length[4]. Kingdom: Animalia; Phylum: Arthropoda; Class: Insect; Order: Dipteral; Family: Culicidae; Genus: *culex ; Species: quinquefasciatus.*

An. stephensi is one of the dominant malaria vectors in Middle East, the Indian subcontinent, Iran, Iraq, Bangladesh, south China, Myanmar, Thailand and Ethiopia[5]. worldwide malaria cases rising from 217 million in 2016 to 219 million in 2017 and around 229 million in 2019 WHO.

Chemical pesticide is proved to be efficient in mosquito control program. In the environment to use more controlling artificial insecticides to get direct results in the control of mosquitoes[6]. Several residential, developing countries are searching environmentally safe products for vector control program. This has led to intensify look for tools and display eco-friendliness and target specificity and this has been establish with plant extracts otherwise recognized as botanicals. The utilize of plant products on alternative for mosquito control and various plant products have been tried in past days before the discovery of chemical pesticides[7]. Nevertheless, high cost of synthetic environment and food safety concerns, unacceptability and toxicity of many organophosphates and organochlorines and have argue for stimulated research towards the advance of possible insecticides of botanic origin[8].

Therefore, the blood feeding contact or comeback is prohibited. Consequently, with the use of the phytochemical extract on the skin, the mosquito could not bite because the vigorous ingredients does not allow it to smell the attractantand could not thus identify the human as its source of meal[9]. Latex of *P. pudica* show inflammatory ulcerative colitis Latex of Thevetia peruviana show activity against antifungal activity[10]. Different part of *P. alba* are used in Indian traditional medical system for the treatment of various diseases[11]. Although *P. alba* is worldwide used in traditional medicine, toxicological data on the plant are scarce. In view of the above facts an attempt has been made to evaluate the controlling of mosquito vectors, *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* by using the plant, *Plumeria alba.*

MATERIALS AND METHOD

Collection of *Plumeria alba* Latex

The *Plumeria alba* was collected and identified as botanist, Department botany, Arignar anna government arts college, Namakkal District,Tamil Nadu,India. Fresh latex of *Plumeria alba* was collected from in around our college, Arignar anna government arts college, Namakkal District,Tamil Nadu, India.The latex was collected from the plant by making small incisions near the youngest bud and the latex was left flow in to the glass tube with wide mouths. The latex was gently handled and maintains homogeneity. The latex was kept in the laboratory at 40ºC and weighted the latex.

Preparation of Plant Extract

The fresh latex was mixed well with the dechlorinated water, each 100 ml of the latex was extracted twice with 300ml of dechlorinated water at room temperature then the latex was filtered with the help of N.o1 Whitman filter paper and crude extract was air dry under room temperature (28±2ºC). After then the dried crude extract was scrape with help of spatula. The scraped cured extract were weighted and calculate how many grams of cured available from the latex1 gram of crude mixed with 100ml of dechlorinated water. Then crude were prepared in different concentration. Then the residues were used for bioassay against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciantus* vector mosquito mosquito's.

Insect Rearing

The mosquito's larvae's and eggs were collected from muthurajapalaiyam, musiri, trichy, tamilnadu, india. The *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciantus* larvae collected and reared in department of zoology, Arignar anna government arts college, Namakkal District,Tamil Nadu, India. After third generation mosquito fourth instars larvae of *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciantus* against *Plumeria alba* plant latex.

The collected eggs were maintained in plastic trays containing tap water. Once the larvae are hatched, those were fed with Dog biscuit and Baker's yeast in the ratio of 3:1 every day. The pupae formed were transferred in a cup containing tap water and placed in the oviposition cage (44 × 44 × 43). Emerged adults were continuously fed with 10% sucrose solution. Adults were given blood meal from a Broiler chicken from fifth day on wards. Small plastic bowls of tap water lined with filter paper were placed inside the cage for oviposition. The whole setup was maintained at 28 ± 2oC and 70-80% relative humidity under the 14:10 light and dark cycles

Bioassay of Larvicidal activity of *Plumeria alba* Latex crude extract against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciantus* mosquito vector.

The larvicidal activity of *Plumeria alba* Latex plant crude extract assessed by using the standard method as prescribed by WHO (2005). From the stock solution, four different concentrations viz., 0.125%, 0.250%, 0.500% and 1% for crude extracts was prepared and tested against the freshly moulted (0-6 hours) 4th instar larvae of *A. aegypti, A. stephensi and C. quinquefasciatus.* Polysorbate 20 used as emulsifier and distilled water treated as control. Twenty five larvae of each mosquito species were introduced in 250 ml plastic cups containing 100 ml of aqueous and the required amount of plant extracts were added. The larval mortality were observed and recorded after 12, 24 and 48 hours of post treatment. For each experiment, five replicates were maintained at a time. The percentage of larval mortality was calculated by using Abbott's formula

8.

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \text{ X } 100$$

$$\text{Percentage mortality} = \frac{\text{Numbere of dead larvae}}{\text{No of larvea introduced}} \text{X}100$$

Bioassay of pupicidal activity of *Plumeria alba* Latex crude extract against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciantus* mosquito vector.

The pupicidal activity of *Plumeria alba* Latex plant crude extract of using thestandard method as prescribed by WHO (2005). Similartest concentrations as stated in the previous experiments wereprepared and tested against the pupae of *A. aegypti, A. stephensiand C. quinquefasciatus* Tween 20 in water weretreated as control. The pupae of these mosquito species (10pupae) were introduced in 250 ml plastic cups containing 100ml of aqueous medium and the required amount of plant extract wereadded. The pupal mortality were observed and recorded after24 hours of post treatment. For each experiment, five replicateswere maintained at a time.
The percentage of mortality wascalculated by using Abbott's formula[8].

$$\text{Percentage mortality Pupae} = \frac{\text{Numbere of dead pupae}}{\text{No of pupae introduced}} \text{X}100$$

Statistical analysis

Data analysis was carried out using Microsoft Excel 2007. For all the experimental data from that LC50, LC90 was carried out using SPSS 16.00.

RESULTS AND DISSCUSSION

Larvicidal and pupicidal activities of *Plumeria alba* against the freshly moulted (0-6hrs old) fourth instar larvae of *A. aegypti, A. stephensi* and *C. quinquefasciatus*

In the present study *Plumeria alba* was tested against the fourth instar larvae of *A. aegypti, A. stephensi* and *C. quinquefasciatus* and the data pertaining to the experiments are shown in table 1 and 2, figar 1 and 2. Perusal of the data clearly indicated that maximum larval mortality were recorded in *C. quinquefasciatus mosquito at* 12 hrs values was recorded in different concentrations of 0.125%, 0.25%, 0.5%, 1% the values in 63.32, 45.30, 31.23,19.12% and then LC_{50} (LCL-UCL), LC $_{90}$(LCL-UCL) X^2 values for 0.673(0.561-0.826), 1.748(1.439-2.304)0.898 and the following larval mortality values were recorded in *A. stephensi* in different concentrations of 0.125%, 0.25%, 0.5%, 1% the values in 54.10, 38.64, 21.33, 16.40 % and the values in LC_{50}

(LCL-UCL), LC_{90}(LCL-UCL) X^2values for 0.871(0.730-1.100), 2.015(1.631-2.744) 1.607 and the following larval mortality values were recorded in *A. aegypti* in different concentrations of 0.125%, 0.25%, 0.5%, 1% the values in 43.23, 30.21, 23.20,17.30% and the values in LC_{50} (LCL-UCL), LC_{90}(LCLUCL) X^2 values for 1.208(0.935-1.879), 2.879(2.106-4.964) 0.054 respectively.

Larvicidal activity in Perusal of the data clearly indicated that maximum larval mortality were recorded in *C. quinquefasciatus mosquito at* 24 hrs values was recorded in different concentrations of 0.125%, 0.25%, 0.5%, 1% the values in 73.33, 55.32,39.26, 21.32% and the values in LC_{50} (LCL-UCL), LC_{90}(LCL-UCL) X^2 values for 0.488(0.394-0.589), 1.416(1.1921.780) 2.191in 24hrs. The following larval mortality values were recorded in *A. stephensi* in different concentrations of 0.125%, 0.25%, 0.5%, 1% the values in 68.65, 48.44, 22.32,18.20 % and the values in LC_{50} (LCL-UCL), LC_{90}(LCL-UCL) X^2 values for 0.639(0.548-0.752),1.502(1.283-1.853) 3.722 and the following larval mortality values were recorded in *A. aegypti* in different concentrations of 0.125%, 0.25%, 0.5%, 1% the values in 53.43, 37.65, 26.50, 19.21% and the values in LC_{50} (LCL-UCL), LC_{90}(LCL-UCL) X^2values for 0.886(0.723-1.177), 2.222(1.740-3.244) 0.263 respectively.

Larvicidal activity in Perusal of the data clearly indicated that maximum larval mortality were recorded in *C. quinquefasciatus mosquito at* 48 hrs values was recorded in different concentrations of 0.125%, 0.25%, 0.5%, 1% the values in 88.23, 71.65, 59.56, 37.25 % and the values in LC_{50} (LCL-UCL), LC_{90}(LCL-UCL) X^2values for 0.886(0.723-1.177), 2.222(1.7403.244) 0.263. The following larval mortality values were recorded in *A. stephensi* in different concentrations of 0.125%, 0.25%, 0.5%, 1% the values in 71.35, 59.24, 31.82, 23.40 % and the values in LC_{50} (LCL-UCL), LC_{90}(LCL-UCL) X^2 values for 0.639(0.548-0.752),1.502(1.2831.853) 3.722 and the following larval mortality values were recorded in *A. aegypti* in different concentrations of 0.125%, 0.25%, 0.5%, 1% the values in 66.13, 48.15, 31.40, 20.41% and the values in LC_{50} (LCL-UCL), LC_{90}(LCL-UCL)X^2 values for 0.488(0.394-0.589), 1.416(1.192-1.780) 2.191in 48 hrs respectively.

Pupicidal activity in Perusal of the data clearly indicated that maximum pupal mortality were recorded in *C. quinquefasciatus mosquito at* 24 hrs values was recorded in different concentrations of 0.125%, 0.25%, 0.5%, 1% the values in 81.12, 67.45, 41.34, 27.65 % and the values in LC_{50} (LCL-UCL), LC_{90}(LCL-UCL) X^2 values for 0.379(0.287-0.487), 1.235(1.0541.525) 0.621 in 24hrs. The following larval mortality values were recorded in *A. stephensi* in different concentrations of 0.125%, 0.25%, 0.5%, 1% the values in 71.23, 43.14, 28.76, 19.21 % and the values in LC_{50} (LCL-UCL), LC_{90}(LCL-UCL) X^2 values for 0.618(0.526-0.732), 1.504(1.279-1.868) 0.075 and the following larval mortality values were recorded in *A. aegypti* in different concentrations of 0.125%, 0.25%, 0.5%, 1% the values in 67.12, 42.45, 31.20, 20.22% and the values in LC_{50} (LCL-UCL), LC_{90}(LCL-UCL) X^2 values for 0.648(0.544-0.786), 1.659(1.381-2.143) 0.020 respectively. The observed data was subjected to probit analysis to use in SPSS 16.0.

The use of biologically active plant based products with insecticidal properties has attracted considerable interest of scientists in all over the world. Extensive survey of the flora was undertaken to search for the potential plant extracts or compounds which could be used in the management of important human vector mosquitoes[15]. Moreover, investigations on the insecticidal properties of the plant latex crude extracts have gained great impetus because of imposition of restrictions on the use of chemical pesticides for vector control programmed. Similar type of activity was already reported by various authors[17,18]. The obtained results are corroborates with earlier reports. The investigation of the larvicidal efficacy of the crude leaf ethyl acetate extract of *T. procumbens* was tested against *Cx. tritaeniorhynchus* showed promising larvicidal activity[12].

Larvicidal activity of acetone, ethyl acetate, chloroform and butanol and butanol dried leaf extract of *Melia azedarach* tested against 3rd instar *Culex quinquefasciatus* and *Aedes aegypti*[14]. The result suggested that the ethyl acetate of *M. azedarach* leaf extract was an excellent larvicidal potential in controlling mosquito vectors.[3]. Have reported that the ethyl acetate extract of *Phyllanthus Emblica* Linn. Exhibited more than 90% larval mortality at 250ppm on *C. Quinquefaciatus*[7].

Plants belonging to the family Lamiaceae have been screened and studied for their larvicidal activity against mosquitoes. Plants that showed promising larvicidal activity were ethanolic aerial extracts of *Teucrium divaricatum* (LC_{50} 18.6ppm), *Mentha longifolia* (LC_{50} 26.8ppm), *Melissa officinalis* (LC_{50} 39.1ppm), *Salvia sclarea* (LC_{50} 62.7ppm) and *Mentha pulegium* (LC_{50} 81.0ppm)[11]. The ethyl acetate leaf extract of the experimental plant has also showed ovicidal activity of mosquito species[16]. The ovicidal activity by ethyl acetate, aqueous solution, ethanol leaf extract of *Nerium oleander* against *A. stephensi* at 100, 150, 200, 250, and 300ppm were considered[13]. With each extract at a concentration of 100ppm, the take of hatchability was very high and nil hatchability was recorded as the concentration of extract was better to 300ppm in the case of aqueous and ethanol extract[19].

Table 1. Larvicidal activity of *Plumeria alba* gainst mosquito vector of *A. aegypti, A. stephensi* and *C. quinquefasciatus* at *12* hrs

Mosquitoes	Concentratio n (%)	Larvicidal activity	95% Confidence Limits (ppm)		
			LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	χ^2
A. aegypti	0.125%	17.30±2.45	1.208(0.935-1.879)	2.879(2.106-4.964)	0.054
	0.25%	23.20±5.21			
	0.5%	30.21±4.24			
	1%	43.23±2.32			
A. stephensi	0.125%	16.40±1.40	0.871(0.730-1.100)	2.015(1.631-2.744)	1.607
	0.25%	21.33±2.53			
	0.5%	38.64±1.36			
	1%	54.10±5.63			
C. quinquefasciatus	0.125%	19.12±3.42	**0.673(0.561-0.826)**	1.748(1.439-2.304)	0.898
	0.25%	31.23±2.21			
	0.5%	45.30±3.31			
	1%	63.32±1.50			

The value represents mean ± SD of five replications. $LC_{50, 90}$ =Lethal Concentration brings out 50, 90% Mortality, LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table 2. Larvicidal activity of *Plumeria alba* against mosquito vector of *A. aegypti, A. stephensi* and *C. quinquefasciatus* at 24 hrs

Mosquitoes	Concentratio n (%)	Larvicidal activity	95% Confidence Limits (ppm)		
			LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	χ^2
A. aegypti	0.125%	19.21±2.35	0.886(0.723-1.177)	2.222(1.740-3.244	0.263
	0.25%	26.50±3.53			
	0.5%	37.65±3.74			
	1%	53.43±2.45			
A. stephensi	0.125%	18.20±6.54	0.639(0.548-0.752)	1.502(1.283-1.853)	3.722
	0.25%	22.32±1.33			
	0.5%	48.44±4.32			
	1%	68.65±4.73			
C. quinquefasciatus	0.125%	21.32±1.44	0.488(0.394-0.589)	1.416(1.192-1.780)	2.191
	0.25%	39.26±3.23			
	0.5%	55.32±3.46			
	1%	73.33±2.37			

The value represents mean ± SD of five replications. $LC_{50, 90}$ =Lethal Concentration brings out 50, 90% Mortality, LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table 3. Larvicidal activity of *Plumeria alba* against mosquito vector of *A. aegypti, A. stephensi* and *C. quinquefasciatus* at 48 hrs

Mosquitoes	Concentratio n (%)	Larvicidal activity	95% Confidence Limits (ppm)		
			LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	χ^2
A. aegypti	0.125%	20.41±1.15	0.639(0.541-0.765)	1.59(1.33-2.05)	2.536
	0.25%	31.40±0.43			
	0.5%	48.15±3.14			
	1%	66.13±2.31			
A. stephensi	0.125%	23.40±3.12	0.588(0.498-0.695)	1.470(1.246-1.861)	0.403
	0.25%	31.82±4.42			
	0.5%	59.24±1.51			
	1%	71.35±2.43			
C. quinquefasciatus	0.125%	37.25±3.24	0.276(0.184-0.350)	0.993(0.857-1.208)	2.581
	0.25%	59.56±3.23			
	0.5%	71.65±2.41			
	1%	88.23±4.61			

The value represents mean ± SD of five replications. $LC_{50, 90}$ =Lethal Concentration brings out 50, 90% Mortality, LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table 4. Pupicidal activity of *Plumeria alba* against mosquito vector of *A. aegypti, A. stephensi* and *C. quinquefasciatus* at 24 hrs

Mosquitoes	Concentratio n (%)	Pupicidal activity	95% Confidence Limits (ppm)		
			LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)	χ^2
	0.125%	20.22±2.15	0.648(0.544-0.786)	1.659(1.381-2.143)	0.020

A. aegypti	0.25%	31.20±3.54			
	0.5%	42.45±2.17			
	1%	67.12±1.34			
A. stephensi	0.125%	19.21±2.31	0.618(0.526-0.732)	1.504(1.279-1.868)	0.075
	0.25%	28.76±4.63			
	0.5%	43.14±3.31			
	1%	71.23±4.23			
C. quinquefasciatus	0.125%	27.65±3.24	0.379(0.287-0.487)	1.235(1.054-1.525)	0.621
	0.25%	41.34±5.33			
	0.5%	67.45±2.17			
	1%	81.12±3.32			

The value represents mean ± SD of five replications. $LC_{50, 90}$ =Lethal Concentration brings out 50, 90% Mortality, LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

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