IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Effect of Medicinal Plants on the Mosquito Vectors from the Different Agroclimatic Regions of Tamil Nadu, India.

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ABSTRACT

Vector control plays a key role in prevention and control of major vector-borne infectious diseases in tropical and subtropical regions. The three main climatic factors that affect malaria transmission and distribution are temperature, precipitation and relative humidity. *Aedes aegypti*, commonly known as the Yellow Fever Mosquito, is a mosquito that can host the dengue fever, Chikungunya and yellow fever viruses. Medically most important species, *Culex quinquefasciatus*, breeds in waters polluted with organic debris such as rooting vegetation, household refuse and excreta. Chemical control use of pesticides is still the most important element in the integrated approach to vector control. But they are non-selective and harmful to other beneficial organisms. Hence, botanical have grown very important in controlling the mosquito vectors. Laboratory and field investigations have been made to evaluate the combined effect *Clerodendron inerme, Acanthus ilicifolius* on three species of mosquito vectors, *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus.* Different concentrations of *Clerodendron inerme* and *Acanthus ilicifolius* have been tested on the various stages of species of mosquito vectors. Lethal concentrations (LC_{50} and LC_{90}) were also worked for the different larval stages of mosquitoes. Significant increased mortality was evident after the plant extracts. The lethal effect on mosquito larvae may be due to the active plant compounds on the gut lining of the mosquito larvae. Thelarval density was decreased after the treatment of plant extracts at the breeding sites (drinking water anddditches water), and hence, these plant extracts of the suitable alternatives of synthetic insecticides for the mosquito vector management.

Key words: *Clerodendron inerme, Acanthus ilicifolius, Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus,* mosquitocidal activity.

Introduction

Mosquitoes are one of the most medically significant vectors, and they transmit parasites and pathogens, which continue to have devastating impact on human beings. The vector borne diseases caused by mosquitoes are one of the major health problems in many countries. Malaria, Dengue, yellow fever, filariasis and chikungunya are some of the deadly diseases spread by mosquitoes. *Anopheles stephensi* is recognized as a major vector for urban malaria in India. This species prefers to breed in small synthetic water collections and is responsible for frequent outbreaks of malaria, particularly at construction sites in urban areas [14]. *Culex quinquefasciatus* is important vector of *Brancraftian filariasis* in tropical and subtropical regions. According to WHO[31]about 90 million people worldwide are infected with

Wuchereria bancrafti the lymphatic dwelling parstite and ten times more people ate at the risk of being infected. In India alone 25 million people harbor microfilaria (mf) and 19 million people suffer from filarial disease manifestations [19,13]. *Aedes aegypti*, a vector of dengue, is widely distributed in the tropical and subtropical zones. About two-thirds of the world's population lives in areas infested with dengue vectors. Dengue haemorragic fever occurs in Asia, the Americas and some Pacific islands. Dengue is endemic in all continents except Europe, and epidemic Dengue viruses, causative agents of dengue fever and more severe dengue haemorragic fever/dengue shock syndrome, infect over 100 million people every year [8].

Over 2000 extracts of higher plants prepared from 325 different plant species were screened for insecticidal activity against the larvae of *A. aegypti*. There are a good number of reports on the successful use of neem and its byproducts against mosquitoes. *Clerodendron inerme* Gaertn. (Verbinaceae), commonly known as Kashmir bouquet is a biennial, hardy plant and widely grown as a hedge plant along home gardens. The leaf extract of the plant has been shown to contain insecticidal properties against mosquitoes [9]. Review of the literature revealed that various solvent extracts of plant materials have been tested against mosquitoes. Therefore, it was thought rewarding to investigate the dry powder of leaf material as source of insecticidal properties against the mosquito larvae. *Acanthus ilicifolius* Linn. (Acanthaceae) is relatively lesser-known, yet important medicinal plant of Herbal Material Medica. The plant is used in traditional systems of medicine, including Traditional Indian Medicine (TIM) or Ayurveda and Traditional Chinese Medicine (TCM) [5]. *A. ilicifolius* (sea holly) occurs in tropical Asia and Africa, through Malaya to Polynesia. It is a viny shrub or tall herb, upto 1.5 m high, scarcely woody, bushy, with very dense growth. Shallow tap roots, but occasionally stilt roots are conspicuous. Leaf simple opposite, decussate, cauline, exstipulate, petiole short, flattened, glabrous, pulvinous to sheathing base Flower bisexual, typically zygomorphic, complete, erect, sessile, hypogynous. Fruit 1 cm green and 2.5 - 2.0 cm long, kidney shaped 4 seed drupe, Seed 0.5 - 1.0 cm long [32].

This species is found from intermediate to upstream estuarine zones in the mid to high intertidal regions. It is shade tolerant with a maximum pore water salinity of 65ppt and a salinity of optimal growth of 8ppt [21]. It is found on all soil types, especially muddy areas along inertial banks. This species leaves tend to be less spiney in deeper shade and also on the older leaves and is often sympatric with *Acanthus bracteates*. This species is a low sprawling shrub let ranging from 50-120cm tall. It naturally reproduces vegetatively and also by seeds. Due to this, generation length is difficult to determine for this species.

Thangam and Kathiresan, [25] Insecticides of plant origin have been receiving attention in recent years to overcome the environmental hazards in using synthetic insecticides. Large numbers of plant samples have been screened for their insecticidal and/or repellent activities and a few of them have been found promising and their products are commercially available. They have investigated for the first time seaweeds, seagrasses and mangrove plants for their larvicidal, skin and smoke repellent activities against mosquito species. Some of them were effective in killing the larvae or repelling adult female mosquitoes. Leaves of *Escoecaria agalloclla* and *Acantllus iliciJolius* were found to show smoke repellent activity. Isolation and identification of active compounds from the effective samples would be useful in synthesising mosquito larvicides or repellents on a large scale.

In the present investigation, we have examined the effect of sundried leaf powder of *Clerodendron inerme* and *Acantllus iliciJolius* against mosquitocidal activity of *Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus.*

Materials and Methods

Colonization of mosquito vectors

Collection of eggs

The eggs of *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* were collected from local (in and around Tenkasii, India) different breeding habitats with the help of a 'O' type brush. The eggs were then brought to the laboratory and transferred to 18 x 13 x 4 cm size enamel trays containing 500 ml water and kept for larval hatching.

Maintenance of Larvae

The freshly hatched larvae were fed with dog biscuits and yeast at 60:40 ratios. The feeding was continued till the larvae transformed into the pupal stage.

Maintenance of pupae and adult

The pupae were collected from culture trays and were transferred to glass beakers containing 500 ml of water with help of a sucker. The glass beaker containing pupae were then kept in 90 x 90 x 90 cm size mosquito cage for adult emergence.

The cage was made up of wooden frames and covered with polythene sheets on four sides (two laterals, one back and other one upper) and the front part was covered with a muslin cloth. The bottom of

the cage was fitted with strong cardboard. The freshly emerged adults were maintained at 27 ± 2°C, 75-85% RH, under 14L: 10D photoperiod cycles. The adults were fed with 10% sugar solution for a period of three days before they were provided an animal for blood feeding.

Blood feeding of adult Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus and egg laying

The adult female mosquitoes were allowed to feed on the blood of Rabbit (shaved on the dorsal side) for two days, to ensure adequate blood feeding for five days. After blood feeding, ovitraps were placed inside the cage for the adults to lay eggs.

Collection of plant materials

The fresh plants *Clerodendron inerme, Acanthus ilicifolius* was collected from in and around Tenkasi, India. The plants were authentified at BSI (Botanical Survey of India) and the specimens were deposited at Zoology Department lab,Vyasa Arts and Science Womens college,Tenkasi.

Preparation of plant extracts

Clerodendron inerme, Acanthus ilicifolius fresh leaves were washed with tap water and shade dried at room temperature. An electrical blender powdered the dried plant materials (leaves and seeds). From the powder 200g of the plant materials were extracted with 2.5 liters of organic solvents of methanol for 8h, in a Soxhlet apparatus [27]. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator.

Preparation of required plant extract concentration

One gram of the plant residue was dissolved in 100 ml of methanol (stock solution) considered as 1% stock solution. From this stock solution different concentrations were prepared ranging from 2 to 10%, respectively.

Larval toxicity test of plant extract

A laboratory colony of *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* larvae were used for the larvicidal activity. Twenty-five numbers of first, second, third and fourth instar larvae were kept in 500 ml glass beaker containing 249 ml of dechlorinated water and 1ml of desired concentration of plant extracts were added. Larval food was given for the test larvae. At each tested concentration 2 to 5 trials were made and each trial consists of three replicates. The control was setup by mixing 1ml of acetone with 249 ml of dechlorinated water. The larvae exposed to dechlorinated water without acetone served as control. The control mortalities were corrected by using Abbott's formula [1].

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

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

LC_{50}, LC_{90} were calculated from toxicity data by using probit analysis [7].

Pupal toxicity test of plant extract

A laboratory colony of *Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus* pupae were used for pupicidal activity. Twenty numbers of freshly emerged pupae were kept in 500 ml glass beaker containing 249 ml of dechlorinated water and 1ml of desired plant extract concentrations was added. Five replicates were setup for each concentration and control was setup by mixing 1ml of acetone with 249ml of dechlorinated water. The control mortality was corrected by Abbott's formula [1].

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

$$\text{Percentage mortality} = \frac{\text{Number of dead pupae}}{\text{Number of pupae introduced}} X100$$

LC_{50}, LC_{90} were calculated from toxicity data by using probit analysis [7].

Field trail

For the field trial the quantity of plant residues required (Based on laboratory LC_{90} values) for each treatment was determined by calculating the total surface area of the water in each habitat. The required quantities of *Clerodendron inerme, Acanthus ilicifolius* were mixed thoroughly with water in a bucket with constant agitation. Teepol was used as emulsifying agent (0.05%). Field applications of the *Clerodendron inerme, Acanthus ilicifolius* were done with the help of a knapsack sprayer and uniformly on the surface of the water in each habitat. Dipper sampling and counting of larvae monitored the larval density before 24 hrs, 48 hrs and 72 hrs after the treatment. A separate sample was taken to determine the species composition of each larval habitat. Twelve trails were conducted for *Clerodendron inerme, Acanthus ilicifolius* alone the treatment. The percentage of reduction was calculated by the following formula:

$$= \frac{C-T}{C} \times 100$$

Where,
C – is the total number of Mosquitoes in control
T – is the total number of Mosquitoes in treatment

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90} values were calculated by using Finney's Method [7] SPSS 13.00 versions were used.

Results

Larval and pupal mortality of *Anopheles stephensi* after the treatment of methanolic extract of *Clerodendron inerme* leaf extract is shown in Table 1. 22% mortality was noted at I instar larvae by the treatment at 20 ppm whereas it has been increased to 81% at 100 ppm of *C. inerme* leaf extract. The LC_{50} and LC_{90} values were represented as follows: LC_{50} value of I instar was 55.042%, II instar was 63.338%, III instar was 73.050% and IV instar was 80.167%. LC_{90} value of I instar was 125.502%, II instar was 137.168%, III instar was 153.544% and IV instar was 156.931%, respectively. The LC_{50} value of pupae was 74.355% and LC_{90} value of pupae was 199.206% respectively.

Table 2 illustrates the larval and pupal mortality of *Aedes aegypti* (I to IV instars and pupae) after the treatment of methanolic extract of *Clerodendron inerme* at different concentrations (20 to 100 ppm). 30% mortality was noted at I instar larvae by the treatment of *C. inerme* at 20 ppm whereas it has been increased to 88% at 100 ppm of *C. inerme* leaf extract treatment. The LC_{50} and LC_{90} values were represented as follows: LC_{50} value of I instar was 45.749%, II instar was 51.042%, III instar was 57.170% and IV instar was 68.166%, respectively. LC_{90} value of I instar was 110.392%, II instar was 116.758%, III instar was 128.697% and IV instar was 145.587%. The LC_{50} value of pupae was 56.444% and LC_{90} value of pupae was 184.556% respectively.

Table 3 provides the considerable larval and pupal mortality of *Culex quinquefasciatus* (I to IV instars) after the treatment of methanolic extract of *Clerodendron inerme* leaf extract at different concentrations (20 to 100 ppm). 40% mortality was noted at I instar larvae by the treatment of *C. inerme* at 20 ppm whereas it has been increased to 91% at 100 ppm. The LC_{50} and LC_{90} values were represented as follows: LC_{50} value of I instar was 34.767%, II instar was 39.299%, III instar was 47.311% and IV instar was 54.395%, respectively. LC_{90} value of I instar was 99.894%, II instar was 108.553%, III instar was 131.860% and IV instar was 150.486%, respectively. The LC_{50} value of pupae was 55.100% and LC_{90} value of pupae was 190.363% respectively.

Table 4 illustrates the larval and pupal mortality of *Anopheles stephensi* (I to IV instars) after the treatment of methanolic extract of *Acanthus ilicifolius* at different concentrations (20 to 100 ppm). 23% mortality was noted at I instar larvae by the treatment of *A. ilicifolius* at 20 ppm whereas it has been increased to 89% at 100 ppm of *A. ilicifolius* leaf extract treatment. LC_{50} value of I instar was 52.765%, II instar was 57.764%, III instar was 63.368% and IV instar was 70.185%, respectively. LC_{90} value of I instar was 108.301%, II instar was 115.835%, III instar was 125.248% and IV instar was 131.288%, respectively. The LC_{50} value of pupae was 62.784% and LC_{90} value of pupae was 141.035% respectively.

Table 5 provides the considerable larval and pupal mortality of *Aedes aegypti* (I to IV instars) after the treatment of methanolic extract of *Acanthus ilicifolius* leaf extract at different concentrations (20 to 100 ppm). 19% mortality was noted at I instar larvae by the treatment of *A. ilicifolius* at 20 ppm whereas it has been increased to 78% at 100 ppm of *A. ilicifolius* leaf extract treatment. The LC_{50} and LC_{90} values were represented as follows: LC_{50} value of I instar was 69.579%, II instar was 76.635%, III instar was 82.692% and IV instar was 88.230%, respectively. LC_{90} value of I instar was 131.813%, II instar was 143.171%, III instar was 150.588% and IV instar was 155.707%, respectively. The LC_{50} value of pupae was 87.287% and LC_{90} value of pupae was 199.466% respectively.

Table 6 illustrates the larval and pupal mortality of *Culex quinquefasciatus* (I to IV instars) after the

Table 1: Larval and pupal toxicity effect of *Clerodendrone inerme* against on malarial vector, *Anopheles stephensi*

Larval and pupal stages	% of mortality Concentration (ppm) 20	40	60	80	100	LC_{50} and LC_{90} (%)	Regression equation	95% Confidence limit LCL LC_{50} LC_{90} (%)	UCL LC_{50} LC_{90} (%)	Chi- square value
I	22	46	55	62	81	55.0425 125.502	Y= -1.00114 X= 0.01819	48.2069 111.7097	61.401 146.9242	4.472
II	18	40	50	59	73	63.3387 137.1685	Y= -1.09945 X= 0.01736	56.6562 121.1242	70.3442 162.7326	3.148
III	14	36	47	51	65	73.0505 153.5547	Y= -1.16290 X= 0.01592	56.6799 117.3448	100.9596 286.2014	5.654
IV	10	30	43	48	60	80.1671 156.9319	Y= -1.33835 X= 0.01669	64.2592 120.1046	113.5892 291.7177	5.925
Pupa	26	38	47	53	58	74.3557 199.2067	Y= -.76323 X=0.01026	63.4073 159.2947	90.119 288.8586	1.119

LCL: 95% of Lower Confidence Limit; UCL: 95% of Upper Confidence Limit;

Table 2: Larval and pupal toxicity effect of *Clerodendrone inerme* against on dengue vector, *Aedes aegypti*

Larval and pupal stages	% of mortality Concentration (ppm) 20	40	60	80	100	LC_{50} and LC_{90} (%)	Regression equation	95% Confidence limit LCL LC_{50} LC_{90} (%)	UCL LC_{50} LC_{90} (%)	Chi- square value
I	30	48	60	72	88	45.7491 110.3928	Y= -.90697 X= 0.01982	38.5843 99.3206	51.7809 126.9239	1.219
II	26	45	56	68	85	51.0428 116.7588	Y= -.99541 X= 0.01950	44.3432 104.8303	57.0188 134.7089	1.467
III	24	40	52	65	78	57.1706 128.6971	Y= -1.02434 X= 0.01792	50.4013 114.3078	63.6637 151.1792	0.305
IV	18	36	46	57	69	68.1664 145.5872	Y= -1.12836 X= 0.01655	61.2459 127.504	76.0115 175.1563	1.509
Pupa	30	48	56	60	63	56.4447 184.5568	Y= -.56464 X= 0.01000	43.5133 147.8371	68.042 268.2409	3.814

LCL: 95% of Lower Confidence Limit; UCL: 95% of Upper Confidence Limit;

Table 3: Larval and pupal toxicity effect of *Clerodendrone inereme* against on filarial vector, *Culex quinquefasciatus*

Larval and pupal stages	% of mortality Concentration (ppm) 20	40	60	80	100	LC_{50} and LC_{90} (%)	Regression equation	95% Confidence limit LCL LC_{50} LC_{90} (%)	UCL LC_{50} LC_{90} (%)	Chi- square value
I	40	52	70	80	91	34.7672 99.8343	Y= -.68477 X= 0.01970	25.7896 89.7323	41.5636 114.9292	100
II	35	50	68	77	86	39.2991 108.5534	Y= -.72723 X= 0.01850	30.528 97.0309	46.0993 126.1946	0.563
III	32	48	59	67	79	47.3119 131.8605	Y= -.71714 X= 0.01516	37.921 114.7187	54.9079 160.8749	0.668
IV	30	45	54	63	72	54.3957 150.4863	Y= -.72547 X= 0.01334	44.8255 127.9704	62.9172 191.6393	0.61
Pupa	34	48	52	60	65	55.1003 190.3638	Y= -.52205 X= 0.00947	40.927 150.5368	67.2586 285.942	1.048

LCL: 95% of Lower Confidence Limit; UCL: 95% of Upper Confidence Limit;

Table 4: Larval and pupal toxicity effect of *Acanthus ilicifolius* against on malarial vector, *Anopheles stephensi*

Larval and pupal stages	% of mortality Concentration (ppm) 20	40	60	80	100	LC_{50} and LC_{90} (%)	Regression equation	95% Confidence limit LCL LC_{50} LC_{90} (%)	UCL LC_{50} LC_{90} (%)	Chi- square value
I	23	39	57	69	89	52.7651 108.3014	Y= -1.21760 X= 0.02308	47.2242 98.8274	57.9124 121.7204	1.736
II	20	37	51	65	85	57.7646 115.8351	Y= -1.27480 X= 0.02207	52.2512 105.24	63.1504 131.1084	1.397
III	18	34	46	60	80	63.3688 125.2488	Y= -1.31239 X= 0.02071	57.6952 112.9446	69.287 143.469	1.235
IV	13	31	39	56	75	70.1859 131.2886	Y= -1.47206 X= 0.02097	64.5587 118.3212	76.5245 150.5541	1.921
Pupa	22	37	50	62	71	62.7845 141.0358	Y= -1.02825 X= 0.01638	55.7053 123.5903	70.171 169.553	0.71

LCL: 95% of Lower Confidence Limit; UCL: 95% of Upper Confidence Limit;

Table 5: Larval and pupal toxicity effect of *Acanthus ilicifolius* against on dengue vector, *Aedes aegypti*

Larval and pupal stages	% of mortality Concentration (ppm) 20	40	60	80	100	LC_{50} and LC_{90} (%)	Regression equation	95% Confidence limit LCL LC_{50} LC_{90} (%)	UCL LC_{50} LC_{90} (%)	Chi- square value
I	19	25	39	55	78	69.579 131.8131	Y= -1.43280 X= 0.02059	63.8659 118.5759	76.0079 151.5501	3.225
II	15	23	37	51	69	76.6356 143.1713	Y= -1.47609 X= 0.01926	70.3272 127.4693	84.3722 167.4262	0.415
III	12	21	33	48	63	82.6928 150.5585	Y= -1.56154 X= 0.01888	75.8609 133.3175	91.6203 177.6935	0.012
IV	10	17	30	45	58	88.2307 155.7074	Y= -1.67572 X= 0.01899	80.9143 137.5281	98.2329 184.5996	0.17
Pupa	18	31	42	51	50	87.287 199.4663	Y= -0.99718 X= 0.01142	76.1473 162.2213	105.8331 277.3153	3.958

LCL: 95% of Lower Confidence Limit; UCL: 95% of Upper Confidence Limit;

Table 6: Larval and pupal toxicity effect of *Acanthus ilicifolius* against on filarial vector, *Culex quinquefasciatus*

Larval and pupal stages	% of mortality Concentration (ppm) 20	40	60	80	100	LC_{50} and LC_{90} (%)	Regression equation	95% Confidence limit LCL LC_{50} LC_{90} (%)	UCL LC_{50} LC_{90} (%)	Chi- square value
I	33	45	63	72	93	44.1244 103.7729	Y= - 0.9480 X= 0.02149	37.3806 94.0383	49.8068 117.8928	4.515
II	28	41	59	70	85	50.2586 114.8283	Y=-0.9975 X= 0.01985	43.6081 103.3163	56.1545 132.0051	0.481
III	25	38	55	64	79	56.5538 127.4368	Y= -1.0224 X= 0.01808	49.8032 113.3329	62.9771 149.3748	0.55
IV	22	33	51	58	70	65.5576 145.0219	Y= -1.0572 X=0.01613	58.4508 73.3233	126.6749 175.3045	1.073
Pupa	38	45	52	60	66	53.7219 194.8983	Y= -.48767 X= 0.00908	38.3099 152.5323	66.3112 301.0476	0.02

LCL: 95% of Lower Confidence Limit; UCL: 95% of Upper Confidence Limit;

treatment of methanolic extract of *Acanthus ilicifolius* at different concentrations (20 to 100 ppm). 33% mortality was noted at I instar larvae by the treatment of *A. ilicifolius* at 20 ppm whereas it has been increased to 93% at 100 ppm of *A. ilicifolius* leaf extract treatment. The LC_{50} and LC_{90} values were represented as follows: LC_{50} value of I instar was 44.124%, II instar was 50.258%, III instar was 56.553% and IV instar was 65.557%, respectively. LC_{90} value of I instar was 103.772%, II instar was 114.828%, III instar was 127.436% and IV instar was 145.021%, respectively. The LC_{50} value of pupae was 53.761% and LC_{90} value of pupae was 194.898% respectively.

Table 7, 8, 9, 10, 11, 12 provides larval mortality at field after applying methanloic extracts of *Clerodendron inerme* and *Acanthus ilicifolius* extract on three species of mosquito vectors namely malarial vector, *Anopheles stephensi*, dengue vector *Aedes aegypti* and filarial vector, *Culex quinquefasciatus*. The selected breeding habitats were Vadavalli, Mettupalayam, Navavoor privu, Pommanam palayam, Ooty, Mettupalayam (Kallaru), at our province Tamil Nadu, India. *Clerodendron inerme* and *Acanthus ilicifolius* extract were prepared at required concentrations and sprayed by using knapsack sprayer. Bio-efficacy of plant extracts have been noted. The percentage of larval reduction was noticed during 24 hrs, 48 hrs and 72 hrs at the breeding sites. There was complete reduction of larval density was noted after the treatment of plant extracts. Similarly, larval reduction was also noted after the treatment of plant extract at the different breeding habitats of mosquito vectors.

Discussion

Malaria is the largest single component of disease burden; epidemic malaria, in particular, remains a major public health concern in tropical countries. In many developing countries, and especially in Africa, malaria exacts an enormous toll in lives, in medical costs, and in days of labor lost [12]. *Aedes aegypti*, a vector of Dengue and Dengue hemorrhagic fever, which is a widely distributed tropical and subtropical disease, is now endemic in more than 100 countries and threatens the health of approximately 2.5 billion people. Worldwide, around 80 million people are infected annually at an attack rate of 4% [18]. In recent years, *A. aegypti* (Diptera: Culicidae) spread the virus of chikungunya which affected the southwest Indian Ocean islands in 2005, spread out to India, and resulted in an ongoing outbreak that has involved >1.5 million patients [24]. *Culex quinquefasciatus*, the potential vector of bancroftian filariasis is the most widely distributed mosquito in India. It is responsible for major public health problem in India with around 31 million microfilaraemics, 23 million cases of symptomatic filariasis, and about 473 million individuals potentially at risk of infection[3].

Table 7: Field trail by using methanolic extract of *Clerodendrone inerme* on malarial vector, *Anopheles stephensi*

Place	:	Vadavalli panchayat, Coimbatore
Habitat	:	Used pot
Size	:	2 X 1.5 m
Depth	:	1 cm
Species	:	*Anopheles stephensi*
Stage	:	Larvae
Calculation	:	0.5x0.5=0.25x4410=11.1
Required Concentration	:	55.2

S. No.	Larval Density			
	Before Treatment	After Treatment		
		24 hrs	48 hrs	72 hrs
1	35	2	2	--
2	40	8	5	--
3	25	6	3	--
4	18	3	2	--
5	15	4	3	--
6	8	2	1	--
Total	141	25	16	--
Average	23.5	4.2	2.6	--
% Reduction	-	82.3%	88.7%	100%

Table 8: Field trail by using methanolic extract of *Clerodendrone inerme* on dengue vector, *Aedes aegypti*.

Place	:	Mettupalayam
Habitat	:	Waste Tyers
Size	:	20cm (width)
Depth	:	10cm
Species	:	*Aedes aegypti*
Stage	:	larval stage

S. No.	Larval Density			
	Before Treatment	After Treatment		
		24 hrs	48 hrs	72 hrs
1	98	5	2	-
2	85	6	1	-
3	79	5	1	-
4	91	5	2	-
5	72	5	1	-
6	81	7	2	-
Total	506	33	9	-
Average	84.9	5.5	1.5	-
% Reduction	-	93.47%	98.20%	100%

Table 9: Field trail by using methanolic extract of *Clerodendrone inerme* on filarial vector, *Culex quinquefasciatus*

Place	:	Coimbatore (Navavoor privu)
Habitat	:	Waste soil tank
Size	:	1m (width)
Depth	:	10cm
Species	:	*Culex quinquefasciatus*
Stage	:	larval stage

S. No.	Larval Density			
	Before Treatment	After Treatment		
		24 hrs	48 hrs	72 hrs
1	44	10	-	-
2	40	16	-	-
3	54	20	16	-
4	56	10	-	-
5	60	14	10	-
6	40	10	1	-
Total	-	294	80	27
Average	-	49	13	4.5
% Reduction	-	72.78%	90.81%	100%

Table 10: Field trail by using methanolic extract of *Acanthus ilicifolius* on malarial vector, Anopheles stephensi

Place	:	Pommanam palayam Vadavalli panchayat, Coimbatore
Habitat	:	Water Tank
Size	:	2 X 1.5 m
Depth	:	1 cm
Species	:	*Aedes aegypti*
Stage	:	Larvae
Calculation	:	2 X 1.5 = 3 X 1.84 X 10 = 55.2
Required Concentration	:	55.2

S. No.	Larval Density			
	Before Treatment	After Treatment		
		24 hrs	48 hrs	72 hrs
1	128	61	12	--
2	157	49	7	--
3	168	19	13	--
4	189	34	4	--
5	196	26	4	--
6	147	29	6	--
Total	985	218	48	--
Average	164.1	36.30%	8	--
Reduction	-	77.80%	95.10%	100%

Table 11: Field trail by using methanolic extract of *Acanthus ilicifolius* on dengue vector, *Aedes aegypti*.

Place	:	Ooty
Habitat	:	Tyers
Size	:	10cm (width)
Depth	:	10cm
Species	:	*Aedes aegypti*
Stage	:	larval stage

S. No.	Larval Density			
	Before Treatment	After Treatment		
		24 hrs	48 hrs	72 hrs
1	32	1	-	-
2	41	-	-	-
3	35	-	-	-
4	27	-	-	-
5	18	-	-	-
6	40	1	-	-
Total	193	2	-	
Average	32.2	0.3	-	-
% Reduction	-	99%	100%	100%

Table 12: Field trail by using methanolic extract of *Acanthus ilicifolius* on filarial vector, *Culex quinquefasciatus*

Place	:	Mettupalayam(Kallaru)
Habitat	:	Drainage
Size	:	20cm (width)
Depth	:	10cm
Species	:	*Culex quinquefasciatus*
Stage	:	larval stage

S. No.	Larval Density			
	Before Treatment	After Treatment		
		24 hrs	48 hrs	72 hrs
1	95	9	3	-
2	90	6	-	-
3	80	4	-	-
4	95	4	2	-
5	75	6	1	-
6	85	14	2	-
Total	-	520	43	8
Average	-	86.6	7.16	1.3
% Reduction	---	91.73%	98.46%	100%

Murugan *et al.,* [17] established the neem seed kernel extract possess antipupational property for mosquito species. Babu and Murugan [6] investigated that the larvicidal effect of resinous exudate from the tender leaves of *Azadirachta indica*. Vahitha *et al*. studied the larvicidal efficacy of *Pavonia zeylamica* L. and *Acacia ferruginea* D.C. against *Culex quinquefasciatus* Say.

They results clearly suggested that the *C. inerme* interfered with developmental processes of the fourth instar larvae and pupae of *A. aegypti*. In this context, the observations that exposure of fourth instar mosquito *Culex quinquefasciatus* to ether extract of *C. inerme* leaves resulted in death at larval–pupal molt and pupal–adult eclosion and suggesting inhibition of the moulting process [20] lend further support to our observations. EI_{50} and EPQ_{50} were found to be 40.8 mg and 144.8 mg respectively. In the present study, *Clerodendron inerme* plant extracts showed mosquitocidal activity on the three species of mosquito vectors namely malarial vector, *Anopheles stephensi*, dengue vector *Aedes aegypti* and filarial vector, *Culex quinquefasciatus.*

There have been numerous reports on the mosquito larvicidal activity of terrestrial plants. Ours was the first study on mosquito larvicidal and repellent activity of marine plants [11]. Subsequently the mosquito larvicidal activity of seaweeds, *Plocamium telfairiae* and *Laurencia nipponica* was reported by [28,29]. Mosquito larvicidal compounds were also isolated by them. Effective repellent compounds, like dimethyl pthalate, available in the market are very costly and moreover they can give protection only for a short period of one or two hours [10]. In view of these facts, the purified active compounds from the most effective samples found in our studies could -be effective in killing mosquito larvae or repelling adult female mosquitoes in an economic and safe manner. This finding would be useful in the field of mosquito control without polluting the environment. [26] while *Acanthus ilicifolius* was most effective against *Ae. Aegypti* by giving 74% of protection [25]. In the present study, *Acanthus ilicifolius* treatment of good larval and pupicidal activity of against three species of mosquito vectors namely malarial vector, *Anopheles stephensi*, dengue vector *Aedes aegypti* and filarial vector, *Culex quinquefasciatus.*

Rao *et al*. [22] reported that the field-tested relatively stable lipid-rich fractions of neem products, which were as effective as good quality crude neem products in the control of culicine vectors of Japanese encephalitis and produced a slight but significant reduction in population of anopheline pupae. In the present study, the field trials were conducted by using *Clerodendron inerme* and *Acanthus ilicifolius* treatment in different habitats of three species of mosquito vectors namely malarial vector, *Anopheles stephensi*, dengue vector *Aedes aegypti* and filarial vector, *Culex quinquefasciatus* (Vadavalli, Mettupalayam, Navavoor privu, Pommanam palayam, Ooty, Mettupalayam (Kallaru) in Tamil Nadu, India. The percentage reduction of larval mortality also showed the variations among the different breeding habitats of mosquito vectors. This may due to the impact of geographical distribution of *A. stephensi*, *Aedes aegypti*, *Culex quinquefasciatus* at the breeding sites.

The finding of the present of investigation revealed that *Clerodendron inerme* and *Acanthus ilicifolius* good larvicidal and pupicidal activity against three species of mosquito vectors namely malarial vector, *Anopheles stephensi*, dengue vector *Aedes aegypti* and filarial vector, *Culex quinquefasciatus* at different Agro-climatic regions of Tamil Nadu, India. Their mode of action and effect on non target organism are presently under the investigations. These plant extracts showed that has good effective mosquito control properties and also can act as an eco-friendly, bio-pesticide for further vector control programs.

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