



## LABORATORY EFFICACY OF FICUS RELIGIOSA EXTRACTS AGAINST MOSQUITO-BORNE DISEASE

Subash B\*

Department of Zoology, Annamalai University, Annamalainagar-608 002, Cuddalore, Tamilnadu, India. \*Corresponding Author

Vijayan P

Department of Zoology, Annamalai University, Annamalainagar-608 002, Cuddalore, Tamilnadu, India.

Baranitharan M

Department of Zoology, Annamalai University, Annamalainagar-608 002, Cuddalore, Tamilnadu, India.

**ABSTRACT** To determine the mosquitocidal activity of *Ficus religiosa* leaf extracts was tested against three mosquitoes. The ovicidal activity of *F. religiosa* leaf extracts at 240, 300 and 360 ppm and for repellency activity was definite against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* species at three concentration viz., 1.0, 2.0 and 4.0 mg/cm<sup>2</sup> under the laboratory conditions. Mean percent hatchability of the ovicidal activity was observed and ethanol extract exerted 240, 300 and 360 ppm against *Cx. quinquefasciatus*. The petroleum ether extract of *F. religiosa* establish to more repellent than the additional extracts. A higher concentration of 4.0 mg/cm<sup>2</sup> provided 100% protection up to 120, 160 and 200 minutes, respectively. The outcome clearly shows that ovicidal and repellent activity was dose reliant. From the results it can be concluded the petroleum ether extract of *F. religiosa* was an outstanding potential for controlling the vector mosquito *Cx. quinquefasciatus*.

**KEYWORDS :** *Anopheles stephensi*, *Culex quinquefasciatus*, *Aedes aegypti*, ovicidal and repellent activity, *Ficus religiosa*

### 1. INTRODUCTION

Mosquito-borne diseases menace the living and livelihoods of millions of people worldwide. In terms of dengue, 2.5 billion people live at danger of infection with one or more of the four serotypes of the virus, which cause an estimated 390 million infections a year, and the affected area has increased rapidly in the past 30 years. In 2015, 2.35 million cases of dengue were reported in the America alone of which 10 200 cases were identify problem as severe dengue caused 1181 deaths<sup>1,2</sup>. Malaria slaughtered an expected 306,000 under-fives comprehensively, incorporating 292,000 kids in the African Region<sup>3</sup>. Malaria incidence rate has declined by 41% since 2000, and 2010. Mortality has fallen by 61% since 2000 and 29% since 2015. Seventeen countries eliminated malaria between 2000 and 2015, with a further 13 countries "approaching elimination."<sup>4,5</sup>. *Culex quinquefasciatus* is an important vector of filariasis and worldwide, 25 million men clumsy people with sex organ sickness and over 15 million folks are afflicted with lymphodema<sup>6,7</sup>.

These diseases challenge the developed and developing countries of the world for irradiation. Pesticides have numerous beneficial effects. These include crop protection, preservation of food and material and prevention of vector-borne diseases. The ripe fruits of *Ficus religiosa* are edible and rich source of proteins and minerals. Value added products prepared from *F. religiosa* are also availability, nutritional composition, phytochemicals and related health benefits can help food researchers to design new functional foods and nutraceuticals<sup>8</sup>. Visible of the recently inflated interest in developing plant origin pesticides as another to chemical pesticide, this study was undertaken to assess the insecticidal activities with special regard to ovicidal and repellent potential of compounds from the medicinal plant oil *F. religiosa* against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*.

### 2. MATERIAL AND METHODS

#### 2.1 PLANT COLLECTION AND EXTRACTION

*Ficus religiosa* was collected from the natural population in and North Poigainallur, Nagapattinam district of Tamil Nadu, India, and identified in Department of Zoology, Annamalai University, Annamalai Nagar, Tamil Nadu, and India. The leaves were washed many times with water to remove all the unwanted impurities. Then, the leaves were shade-dried under room temperature and kept in a hot air oven for 50 °C for half an hour. After that, the material was ground by using electric blender. Later, 500g of powdered plant material was packed inside a Soxhlet apparatus, and successive extraction was carried out by using solvents such as methanol and ethyl acetate for 72 hrs. The solvent was evaporated under rotary evaporator (Heidolph, Germany), and the dried extracts were stored at 4°C until further bioassay.

#### 2.2 OVICIDAL ACTIVITY

The method of Su & Mulla, 1998<sup>9</sup> was followed to check the ovicidal

activity. The leaf extracts was diluted within the several solvent to attain completely different concentrations (60, 120, 180, 240, 300 and 360 ppm). The freshly ordered egg raft containing 100 eggs of *A. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were exposed to every dose of leaf extract till they hatched or died. Every concentration was replicated six times. Eggs exposed to several solvents in water served 48 h post treatment by the subsequent formula.

$$\text{Egg hatchability (\%)} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} \times 100$$

#### 2.3 REPELLENT ACTIVITY

The minutes of protection in admiration to measurement method was utilized<sup>10</sup>. Three-day-old blood-starved female three important vector mosquitoes (100) were unbroken during a net cage (45cm × 30cm × 45cm). The volunteer had no contact with lotion, perfumes or perfumed soaps on the day of the assay. The arms of volunteer, solely 25 cm<sup>2</sup> dorsal facet of the skin on every arms was exposed and therefore the remaining space lined by rubber gloves. The crude extracts were applied at five.0 mg/cm<sup>2</sup> singly within the exposed area of the fore arm. The time of the check enthusiastic about whether or not are the target mosquitoes day or night biters. *An. stephensi*, *Cx. quinquefasciatus* are testing throughout the getting dark from 20:00 to 4:00, while *A. aegypti* was tested throughout the day time 8:00 to 16:00. The management and treated arm were introduced at the same time in to the experimental cages, the mosquitoes were activated. Every check concentration was continual six times. The volunteer conducted their check of every concentration by inserting the treated and management arm in to a similar cage for one full minute for each 5 minutes. The mosquitoes that landed on the hand were recorded so agitated off before imbibing any blood; creating out a five minutes protection. The proportion of repellency was calculated by the subsequent formula.

$$\% \text{ Repellency} = [(T_a - T_b) / T_a] \times 100$$

Where  $T_a$  is the number is that the variety of mosquitoes within the management group and  $T_b$  is that the number of mosquitoes within the treated group.

#### 2.4 DATA ANALYSIS

Mortality data were subjected to probit analysis. ANOVA analysis, followed by DMRT test ( $P < 0.05$ ) were employed to investigate ovicidal and repellence data.

### 3. RESULTS AND DISCUSSION

#### 3.1 OVICIDAL ACTIVITY

Medicinal plants are produce on vector control. These plants can be used to develop environmentally safe vector and pest managing

agents. Ovicidal activity of *F. religiosa* ethanol extract highest eggs hatchability was found to be at 300 and 360 ppm with *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* and lowest hatchability were acetone, benzene, hexane extracts at 300 ppm (Table 1). The present studies are on par with earlier findings such as, the highest (100%) no eggs hatchability were exerted by *Coleus aromaticus* 11-octadecenoic acid, methyl ester compound tested at 40 ppm on *Cx. quinquefasciatus* <sup>6</sup>. Mortality (zero hatchability) of 100% with methanol extract of *Acalypha alnifolia* was exerted at 125 and 300 ppm against *An. stephensi* <sup>11</sup>. Ovicidal activity of *Sesamum indicum* methanol extract was found to be most effective at 250, 300 ppm on *Cx. quinquefasciatus* <sup>12</sup>. Further, maximum ovicidal activity of *Coleus aromaticus* diethyl ether extract at 120, 160 and 200 ppm <sup>13</sup>.

### 3.2 REPELLENT ACTIVITY

Repellent activity of *F. religiosa* ethanol, acetone, benzene and hexane extracts was tested against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*. In *F. religiosa* ethanol extract showed highest repellent activity was found to be 4.0 mg/cm<sup>2</sup> with protection time upto 120, 160 and 200 minutes against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* (Figure 1). The present investigation comparable with the *Ficus racemosa* methanol extract, highest repellent activity was provided 100% protection up to 160 and 200 minutes at 3.0 mg/cm<sup>2</sup>

against *Ae. aegypti* <sup>14</sup>. The repellency of citronellal compound was contained to be mainly effective and the maximum action was observed at 0.75 and 1.50 mg/cm<sup>2</sup> concentrations provided 100% protection up to 210 minutes against *An. stephensi* <sup>15</sup>. Further, high repellent activity of *Coleus aromaticus* methanol fraction 4 tested at 2.5 mg/cm<sup>2</sup> was observed tested for at least 320 minutes against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti* <sup>6</sup>.

### 4. CONCLUSION

In conclusion, the present study results that medicinal plant *F. religiosa* have the potential for the development of new and safe control products and exhibits ovicidal and repellent activity against important mosquitoes. Furthermore, the results of the present study may donate to a diminution in the relevance of synthetic insecticides, which in turn increases the opening for nature control of various medically significant pests by botanical pesticides. Also, our results open the opportunity for further investigations of the isolation and identification of bioactive compounds from natural product extracts.

### 5. ACKNOWLEDGEMENTS

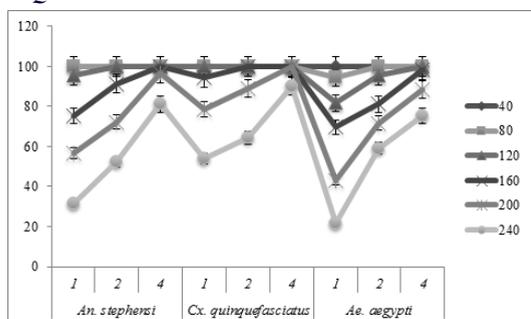
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TABLE 1 OVICIDAL ACTIVITY OF THE M. OFFICINALIS EXTRACTS AGAINST MOSQUITO SPECIES

Species	Extract	Percentage of egg hatch ability					
		Concentration used (ppm)					
		60 ppm	120 ppm	180 ppm	240 ppm	300 ppm	360 ppm
<i>An. stephensi</i>	Ethanol	71.3±2.5 <sup>c</sup>	53.8±2.5 <sup>c</sup>	32.5±3.1 <sup>b</sup>	11.6±2.3 <sup>a</sup>	NH	NH
	Acetone	92.1±2.2 <sup>d</sup>	71.5±2.3 <sup>c</sup>	59.1±2.1 <sup>c</sup>	38.6±2.5 <sup>b</sup>	20.8±2.4 <sup>a</sup>	8.6±2.3
	Benzene	88.5±3.8 <sup>cd</sup>	67.8±3.0 <sup>c</sup>	43.3±2.9 <sup>b</sup>	21.6±2.6 <sup>a</sup>	9.8±2.4	NH
	Hexane	97.5±1.7 <sup>d</sup>	78.1±2.5 <sup>cd</sup>	59.8±2.1 <sup>c</sup>	43.5±1.7 <sup>b</sup>	28.8±2.1 <sup>ab</sup>	16.3±1.8 <sup>a</sup>
	Ethanol	66.1±2.7 <sup>c</sup>	39.5±2.5 <sup>ab</sup>	10.5±2.1	NH	NH	NH
<i>Cx. quinquefasciatus</i>	Acetone	89.3±1.6 <sup>c</sup>	67.3±2.3 <sup>c</sup>	54.1±2.9 <sup>b</sup>	31.6±1.0 <sup>ab</sup>	12.1±1.9	NH
	Benzene	80.1±2.0 <sup>cd</sup>	59.8±1.7 <sup>b</sup>	31.3±2.0 <sup>ab</sup>	10.3±1.8	NH	NH
	Hexane	94.5±1.7 <sup>d</sup>	79.8±1.3 <sup>cd</sup>	61.5±2.0 <sup>c</sup>	50.6±1.8 <sup>b</sup>	34.3±2.3 <sup>ab</sup>	11.5±1.7
	Ethanol	85.3±1.8 <sup>cd</sup>	60.8±2.4 <sup>c</sup>	43.8±2.1 <sup>b</sup>	25.8±1.6 <sup>a</sup>	8.1±1.3	NH
<i>Ae. aegypti</i>	Acetone	95.3±1.7 <sup>d</sup>	77.8±2.5 <sup>cd</sup>	64.6±2.7 <sup>c</sup>	42.8±2.3 <sup>b</sup>	27.3±1.8 <sup>ab</sup>	12.6±1.7
	Benzene	92.5±1.7 <sup>d</sup>	76.1±2.1 <sup>cd</sup>	61.6±1.8 <sup>c</sup>	43.6±1.9 <sup>b</sup>	22.3±2.7 <sup>a</sup>	5.6±1.5
	Hexane	98.8±0.9 <sup>d</sup>	82.3±1.6 <sup>cd</sup>	67.1±2.3 <sup>c</sup>	34.5±2.3 <sup>ab</sup>	20.8±1.9 <sup>a</sup>	10.5±2.1

NH- No hatchability; values are mean of six replicates ±SD. of five replications. Different alphabets in the column are statistically significant at P≤0.05 level DMRT Test.

FIGURE 1 REPELLENCY OF THE M. OFFICINALIS ETHANOL EXTRACT AGAINST AN. STEPHENSI CX. QUINQUEFASCIATUS AND AE. AEGYPTI



### 6. REFERENCES

- WHO. Dengue and severe dengue. Fact sheet no. 117, March 2014; Geneva.
- Baranitharan M, Sawicka B, Gokulakrishnan J. Phytochemical profiling and larval control of *Erythrina variegata* methanol fraction against malarial and filarial vector. *Advances in Preventive Medicine* 2019, ID 2641959, 1-9.
- WHO. Malaria. Fact sheet December 2015.
- WHO. World Malaria Report 2016. Geneva.
- Baranitharan M, Tamizhazhagan V, Kovendan K, "Medicinal Plants as Potent Power for Malaria Control: Review", *Entomology and Applied Science Letters* 2019, 6, 28-44.
- Baranitharan M, Dhanasekaran S, Murugan K, Kovendan K, Gokulakrishnan J, Benelli G. *Coleus aromaticus* leaf extract fractions: A source of novel ovicides, larvicides and repellents against *Anopheles*, *Aedes* and *Culex* mosquito vectors?. *Process Safety and Environmental Protection* 2017. 106, 23-33.
- WHO. Lymphatic filariasis. Fact sheet N° 2014. 102.
- Sandeep, Ashwani Kumar, Dimple, Vidisha Tomer, Yogesh Gat and Vikas Kumar. *Ficus religiosa*: A wholesome medicinal tree. *Journal of Pharmacognosy and Phytochemistry* 2018, 7, 32-37.
- Su, T., Mulla MS. Ovicidal activity of neem products (Azadirachtin) against *Cx. tarsalis* and *Cx. quinquefasciatus* (Diptera: Culicidae). *Journal of the American Mosquito Control Association* 1998, 14, 204-209.
- World Health Organization. "Guidelines for efficacy testing of mosquito repellents for

- human skins," WHO/HTM/NTD/WHOPEP. 2009, 4, 4-18.
- Kovendan, K., Murugan K, Mahesh Kumar P, Thiagaraja P, William SJ. Ovicidal, repellent, adulticidal and field evaluations of plant extract against dengue, malaria and filarial vectors. *Parasitology Research* 2013, 112, 1205-1219.
- Baranitharan M, Dhanasekaran S, Murugan K, Kovendan K, Gokulakrishnan J, Jeyasankar A. Experimental investigations of Nagapattinam indigenous medicinal plant extracts against dengue, malaria and filarial disease. *International Journal of Zoology and Applied Bioscience* 2015, 2, 155-161.
- Baranitharan, M., Dhanasekaran S. Mosquitocidal efficacies of medicinal plant of *Coleus aromaticus* Benth (Lamiaceae) leaf extracts Chikungunya vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae). *International Journal of Current Research in Chemistry and Pharmaceutical Sciences* 2014, 1, 61-67.
- Baranitharan M, Dhanasekaran S, Jeyasankar A, Arivoli S, Gokulakrishnan J. Studies on mosquitocidal activity of *Ficus racemosa* L. extracts. *World Journal of Pharmaceutical and Life Sciences* 2016, 2, 199-208.
- Baranitharan M, Dhanasekaran S, Murugan K, Kovendan K, Gokulakrishnan J. Chemical composition and laboratory investigation of *Melissa officinalis* essential oil against human malarial vector mosquito, *Anopheles stephensi* L. (Diptera: Culicidae). *Journal of Coastal Life Medicine* 2016, 4, 969-973.