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STUDIES ON MOSQUITOCIDAL ACTIVITY OF *FICUS RACEMOSA* L. EXTRACTS

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ABSTRACT

Mosquitocidal activity of family Moraceae plant, *Ficus racemosa* was tested against *Ae. aegypti* mosquito. The LC_{50} and LC_{90} values of *F. racemosa* methanol extract against *A. aegypti* were 64.76 and 130.48 ppm, severally. The ovicidal activity of *F. racemosa* exerted 100 percent mortality at 120, 160 and 200 ppm against *A. aegypti* and for repellency activity was definite against *A. aegypti* species at three concentration viz., 1.0, 2.0 and 3.0 mg/cm^2 underneath the laboratory conditions. The wood spirit extract of *F. racemosa* establish to additional repellent than the extra extracts. A better concentration of 3.0 mg/cm^2 provided 100 percent protection up to 160 and two hundred minutes, severally. The result clearly shows that larvicidal ovicidal and repellent activity was dose dependent. From the results it is often all over the crude oil ether extract of *F. racemosa* was an impressive potential for dominant the vector mosquito *A. aegypti*.

KEYWORDS: Larvicidal, ovicidal, repellent activity, *Ficus racemosa*, and *Aedes aegypti*.

INTRODUCTION

Mosquitoes are the most importance single cluster of insects in terms of public health importance, that transmit variety of diseases, like malaria, filariasis, dengue, Japanese

encephalitis, *etc.*, inflicting millions of deaths once a year. A recent estimate shows that over 50 million people square measure in danger of break bone fever virus exposure worldwide. Annually, there square measure two million infections, 5, 00, 000 cases of viral haemorrhagic fever, and 12, 000 deaths.[1] Yellow-fever mosquito (*Ae. aegypti*), a vector of dengue is cosmopolitan within the tropical and subtropical zones. Dengue fever occurrence has increased fourfold since 1970 and nearly half the world's population is currently in danger. *Ae. aegypti* is additionally the vector of dengue viral haemorrhagic fever, that is endemic to South East Asia, the Pacific islands area, Africa and therefore the America.[2] Indeed, the present irruption of those diseases is due to the upper range of breeding place in today's throwaway the general public and conjointly increasing resistance of mosquitoes to current industrial pesticides. Though yellow fever has been fairly brought under control with its vaccine, no vaccine is offered for dengue. The sole method of decreasing the incidence of this disease is so the demolition of *Ae. aegypti*.[3] Presently, regarding 400th of the world's population is in danger and there are 50-100 million cases once a year. A calculable 500 000 individuals with severe dengue need hospitalization every year and regarding 2.5% of these affected die. Recently the amount of reportable cases has continued to extend. In 2015, 2.35 million cases of dengue fever were reportable within the Americas alone, of that 10 200 cases were diagnosed as severe dengue fever inflicting 1181 deaths. The year 2015 was characterized by massive dandy fever outbreaks worldwide, with the Philippines coverage over 169 000 cases and Malaya extraordinary 111 000 suspected cases of dengue fever, representing a 59.5% and 16 pf increase just in case numbers to the previous year, severally. Brazil alone reportable over one.5 million cases in 2015, just about three times above in 2014. Conjointly in 2015, Delhi, India, recorded its worst irruption since 2006 with over 15 000 cases.[4] Within the last few years, dengue has re-emerged within the United States of America and has created inroads into Europe.[5] In India, dengue is widespread and endemic in most major cities.[6]

Currently, most pesticides are non-selective and may be harmful to different organisms and to the atmosphere. there's an imperative have to be compelled to develop new materials for controlling mosquitoes in an environmentally safer way, exploitation perishable and target-specific insecticides against them.[7,8,9] Expertise has shown that, aerial toxicants for the obliteration of this mosquito don't seem to be effective, since it's extremely domesticated and lots of adults rest inside in hidden places like closets. The sole successful means of reducing two-winged Insects densities to tier wherever dengue fever epidemics don't occur is by

offensive the larval breeding places. Bioactive organic compounds made by plants will act as repellent, oviposition or food deterrents, growth inhibitors and toxins.[10,11] The Moraceae plants family's square measure cosmopolitan throughout the tropical and subtropical elements of Asia. The family contains a spread of compounds, which showed toxicity and antimicrobial.[12,13] *Ficus racemosa* could be a massive deciduous tree distributed throughout India, notably in evergreen forests and wet localities.[14] In keeping with Ayurvedic system of medicine, bark and fruits square measure accepted to be helpful in polygenic disease. Paste of bark is applied doubly on a daily basis for 2-3 days to cure swellings of foot and hands. Bark boiling is gargled to cure mouth ulceration. Stem-bark is hypoglycaemic and medication. Bark is tonic and employed in rinder pest diseases of cattle. The bark is antiseptic, antipyretic and vermicidal and a boiling of the bark is employed in treating varied skin diseases and ulceration. It's additionally effective within the treatment of piles, dysentery, asthma, gonorrhea, haemoptysis and urinary diseases.[15] Therefore, this study provides fist report on the dipterous insect larvicidal activity impact of *F. racemosa* leaf extract against *A. aegypti* as target species.

MATERIAL AND METHODS

Collection of plant material

Plant sampling was distributed throughout the season (February - March) of 2014 from totally different places of Karaikal union of the Pondicherry. Bulk samples were dried within the shade and once drying every sample was ground to a fine powder. At the time of assortment, two pressed voucher herbarium specimens were prepared per species and identified with the assistance of Plant taxonomist, Department of botany, Annamalai University, whenever doable, flowering or fruiting specimens were collected to facilitate taxonomic identification.

Extraction methodology

The dried leaves (100g) were pulverised automatically using industrial electrical stainless-steel mixer and extracted consecutive with methanol, hexane, diethylether and acetone (500 ml, Ranchem), in an exceedingly Soxhlet equipment on an individual basis till exhaustion. The extract was focused beneath reduced pressure of 22-26 mm Hg at 45oC by 'Rotavapour' and therefore the residue obtained was hold on at 4oC by "Rotavapour'.

Mosquito rearing

Eggs of *A. aegypti* were collected from ICMR center Virudachalam. The egg rafts were then delivered to the laboratory. The eggs were placed in enamel trays (30 cm×24× cm×5 cm) every containing 2 L of water and unbroken at temperature (28 ± 2)) ^{o}C with a photoperiod of 16:8 h (L:D) for larval hatching. The larvae of every mosquito species were maintained in separate trays beneath a similar laboratory conditions and fed with a pulverized feed containing a combination of dog biscuit and baker's yeast (3:1 ratio). The trays with pupae of every mosquito species were maintained in separate mosquito cages at (26 ± 2) ^{o}C and ratio of (85 ± 3) % beneath a photoperiod of 16:8 h (L:D) for adult emergence. Cotton soaked in 100% liquid saccharine resolution in an exceedingly Petri dish to feed adult mosquitoes was additionally placed in every mosquito cage. An immobilized young chick was placed for three h within the cage order to supply feed particularly for female mosquitoes. A plastic tray (11 cm×10 cm ×4 cm) stuffed with water with a lining of partly immersed filter paper was then placed within every cage to enable the female mosquitoes to get their eggs. The eggs obtained from the laboratory-reared mosquitoes were right away used for toxicity assays or allowed to hatch out beneath the controlled laboratory conditions delineated higher than. Solely the recently hatched larvae/pupae of *A. aegypti* were employed in all bioassays.

Larvicidal activity

The larvicidal activity of crude extract was assessed as per the protocol antecedently described by WHO.[16] From the stock solution, six totally different take a look at concentrations (40, 80, 120, 160, and 200 mg/L) were ready and tested against the freshly moulted (0–6 h) III arthropod larvae of *A. aegypti*. The take a look at medium (500 ml plastic cups) was prepared by adding one ml of applicable dilution of take a look at concentrations and mixed with 249 ml of dechlorinated water to create up 250 ml of take a look at solution. The larvae were fed with dry yeast powder on the water surface (50 mg/l). The management (without plant extracts) experiments were conjointly run parallel with every replicate. For every experiment, six replicates were maintained at a time. A minimum of twenty five larvae per concentration was used for all the tests. The larval mortality was determined and recorded once 24 h post-treatment. Percent mortality was corrected for management mortality exploitation probit analysis.

Ovicidal activity

The method of Su and mulla was slightly changed and used to take a look at the ovicidal activity.[17] The varied concentrations as expressed within the previous experiments were ready from the stock solution. Before treatment, the eggs/eggs raft of *A. aegypti* is counted severally with the help of hand lens. Freshly hatched eggs (100) were exposed to DMSO in water served as management. When treatment, the eggs from every concentration were severally transferred to distilled water cups for hatching assessment when reckoning the eggs beneath a magnifier. Every take a look at was replicated 5 times. The hatchability was assessed 48 h post treatment.

$$\% \text{ Mortality} = \frac{\text{Mortality at treatment-Mortality at control}}{\text{100-Mortality at control}} \times 100$$

Repellent activity

The repellent study was following the ways of WHO.[18] 3-4 days old previous blood-starved female *A. aegypti* mosquito (100) was unbroken during a net cage (45×45×40cm). The volunteer had no contact with lotion, perfumes or perfumed soaps on the day of the bioassay. The arms of the take a look at person were cleansed with isopropyl alcohol. Since air drying the arm solely 25 cm^2 of the dorsal aspect of the skin on every arm was exposed, the remaining space being coated by rubber gloves. The chosen healthful plant leaf extract at 1.0 to 3.0 mg/cm^2 concentration was applied. The management and treated arms were introduced at the same time into the cage. The numbers of bited were counted over 5 min each 30 min and also the experiment were conducted 5 times. It had been discovered that there was no skin annoyance from the plant extract. The percentage of repellency was calculated by the subsequent formula.

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

Where T_a is the number of mosquitoes in the control group and T_b is the number of mosquitoes in the treated group.

Statistical analysis

The average larval mortality knowledge were subjected to probit analysis for calculating LC_{50}, LC_{90} and alternative statistics at 95^{th} confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL), regression, chi-square, mean and variance values were

calculated victimization the SPSS (Statistical Package of Social Sciences) 12.0 software. The LC_{50} and LC_{90} value were calculated by victimization probit analysis.[19] Results with $p<0.05$ were thought of to be statistically vital.

RESULTS AND DISCUSSIONS

In the preparatory shows of botanical extracts, result revealed that the methanol extract of *F. racemosa* was effective equated with the other solvent hexane, diethylether and acetone extracts. Therefore our present study was aimed to evaluate the efficacy of *F. racemosa* leaf extract against the selected vector mosquito *A. aegypti*. The effect of leaf methanol, hexane, diethylethr and acetone extracts *F. racemosa* are tested at 40-200 ppm and showed larvicidal activity against the larvae of A. aegypti are presented in Table 1. The plant extracts exhibited moderate larvicidal effects after 24 h. The experiments conducted for evaluating larvicidal efficacy of leaf of *F. racemosa*. Among the extracts tested, the highest larvicidal activity was observed in methanol, hexane, diethyl ether and acetone extracts of *F. racemosa* against *A. aegypti* with the LC_{50} values being 64.76, 184.70, 80.31 and 128.55; LC_{90} values of 130.48, 334.51, 150.25 and 231.74 ppm, respectively. The data is statistically significant at $p < 0.05$. The mean percent of egg hatchability of *A. aegypti* are tested with four different solvents at different concentrations of *F. racemosa* leaves extracts, and the results are listed in Table 2. Among the extracts tested for ovicidal activity against *A. aegypti*, the methanol extract of *F. racemosa* exerted 100% mortality (i.e., no hatchability was recorded; Table 2) at 120, 160 and 200, respectively. Control eggs exhibited the 100% hatchability. The repellent activity of the leaf extracts *F. racemosa* showed repellent against *A. aegypti*, in Table 3. A higher concentration of 3.0 mg/cm^2 provided 100% up to 120, 160 and 200 min against *A. aegypti*, respectively. The results of present study are comparable with earlier reports the larvicidal activity of the flower extract of *Calotropis procera* on the larvae of *Anopheles sp*. and *Culex sp*. with transmit malaria and filariasis was investigated.[20] The evident larvicidal activity of ethyl acetate extract followed by hexane, chloroform and acetone extracts of *Commiphora caudata* showed LC_{50} values of *A. aegypti* are 97.19, 112.85, 99.17 and 109.67 mg/L; *An. stephensi* are 96.04, 104.16, 97.13 and 106.53 mg/L; *C. quinquefasciatus* are 94.76, 102.95, 95.98 and 105.09 mg/L, respectively.[21] The LC_{50} and LC_{90} values of methanol *Annona reticulata* leaf extract against *A. aegypti*, *An. stephensi and C. quinquefasciatus* were 62.82, 74.36 and 80.44 ppm, respectively.[22] Larvicidal and ovicidal efficacy of different solvent leaf extract of *Ariitolochia indica* against *An. stephensi*. The LC_{50} and LC_{90} values of acetone, benzene, chloroform, hexane and methanol extracts of *Ariitolochia indica* against *An.*

stephensi larvae in 24 h were 76.29, 58.82, 53.59, 65.84, 51.78 and 205.85, 193.23, 185.16, 196.72 and 181.00 ppm, respectively.[23]

To evaluated the larvicidal activity of crude and chloroform: methanol extracts of *Allium sativum, Cuminum cyminum, Zingiber offinale, Curcuma longa* and *Solanum tuberosum* against *An. stephensi* and *Cx. quinquefasciatus*. The mortality rate of both larval of *An. stephensi* and *Cx. quinquefasciatus* were recorded in the *Cuminum cyminum, Allium sativum, Zingiber offinale, Curcuma longa* and *Solanum tuberosum* for crude extracts. Followed by, *Curcuma longa, Zingiber offinale, Solanum tuberosum, Cuminum cyminum* and *Allium sativum* for chloroform and methanol extracts.[24] The larvicidal efficacy of *Annona squamosa*, *Cynodon dactylon* and *Melia azedarach* and root of *Hemidesmus indica* acetone, ethyl acetate, chloroform and butanol extracts against *C. quinquefasciatus* and *A. aegypti*. The LC_{50} values of *Melia azadarach* were 264.87, 65.27, 88.39 and 514.65 ppm, respectively against *C. quinquefasciatus*.[25]

Table 1: Larvicidal activity of the *F. racemosa* extract against A. aegypti.

Extracts	Concentration	%mortality ±SD	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	Chi-square
Methanol	40 80 120 160 200	33.6±4.15 60.8±2.94 82.4±3.28 98.2±1.78 100.0±0.0	64.76 (55.54-72.57)	130.48 (120.65-143.41)	2.303
Hexane	40 80 120 160 200	13.2±2.58 15.4±3.57 28.4±2.50 41.6±3.18 56.4±2.50	184.70 (167.66-209.51)	334.51 (290.76-407.46)	1.325
Diethyl ether	40 80 120 160 200	24.2±2.68 51.8±2.96 71.4±2.60 92.8±3.42 100.0±0.0	80.31 (71.86-87.87)	150.25 (139.84-163.74)	3.217
Acetone	40 80 120 160 200	16.4±2.70 27.6±2.30 40.8±2.94 61.2±2.77 86.2±2.28	128.55 (118.88-138.68)	231.74 (212.56-258.51)	3.989

Significant at $p<0.05$ level

Table 2: **Ovicidal activity of the *F. racemosa* extract against A. aegypti.**

Plant extract	Percentage of egg hatch ability					
	Concentration used (ppm)					
	Control	40	80	120	160	200
Methanol	100±0.0	55.8±3.4	29.4±2.6	NH	NH	NH
Hexane	100±0.0	88.6±2.5	67.6±1.9	53.6±2.1	36.8±1.6	16.4±1.8
Diethyl ether	100±0.0	59.8±2.2	41.6±2.6	19.6±2.3	NH	NH
Acetone	100±0.0	78.4±2.3	59.4±1.8	41.2±2.7	19.8±2.2	NH

Values represents mean of five replications.

Table 3: Repellent activity of the *F. racemosa* extract against A. aegypti.

Plant extract	Concentration (mg/cm2)	% of repellency					
		30	40	80	120	160	200
Methanol	1.0	100±0.0	100±0.0	95.4±3.5	86.8±1.6	71.6±2.3	61.4±1.1
	2.0	100±0.0	100±0.0	100±0.0	90.4±1.1	81.6±1.5	73.2±1.7
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	93.4±2.6	81.8±2.2
Hexane	1.0	90.6±1.9	82.4±2.6	65.4±1.6	46.8±1.4	32.6±1.8	20.2±1.3
	2.0	100±0.0	90.8±2.9	70.2±2.7	56.8±2.3	41.2±2.8	20.8±2.5
	3.0	100±0.0	98.4±0.8	87.8±2.2	67.8±2.1	50.4±1.8	38.6±1.9
Diethyl ether	1.0	100±0.0	95.6±1.8	82.6±1.9	70.4±1.5	63.8±2.1	51.6±1.5
	2.0	100±0.0	100±0.0	93.2±1.4	83.4±2.1	72.8±1.6	64.2±2.5
	3.0	100±0.0	100±0.0	100±0.0	97.6±1.9	84.4±3.1	71.2±2.6
Acetone	1.0	100±0.0	86.8±1.3	66.2±2.2	50.8±1.6	39.2±1.4	23.4±1.8
	2.0	100±0.0	97.2±1.0	83.2±1.7	71.4±1.5	59.8±2.6	49.4±2.5
	3.0	100±0.0	98.4±0.8	87.8±2.2	67.8±2.1	50.4±1.8	38.6±1.9

Mean ± SD value of the replications.

CONCLUSION

In general, it could be concluded that methanol and diethylether extracts of *F. racemosa* used on the present study act as larvicidal, ovicidal and repellent inhibitng against the mosquito vector, *Aedes aegypti*. Further studies on the screening, isolated and purification of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are needed as the present study shows that there is scope to use *F. racemosa* leaf extracts to control the immature stages of vector mosquitoes.

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REFERENCE

1. Guha-sapir D, Schimme B. Dengue fever: new paradigms for a changing epidemiology, Emerg. Themes Epidemiol, 2005; 12-13.
2. Senthilkumar A, Kannathasan K, Venkatesalu V. Chemical constituents and larvicidal property of the essential oil of *Blumea mollis* (D. Don) Merr. against *Culex quinquefasciatus*, Parasitol Res, 2008; 103: 959-962.
3. Ravikumar S, Syed Ali M, Margaret Beula J. Mosquito larvicidal efficacy of seaweed extracts against *Aedes aegypti*. Asian pac J trop med, 2011; 143-146.
4. World Health Organization. Dengue and severe dengue. Fact sheet no. 117, March 2014, Geneva: WHO.
5. Alves MJ, Fernandes PL, Amaro F, Osório H, Luz T, Parreira P, Andrade G, et al. Clinical presentation and laboratory findings for the first autochthonous cases of dengue fever in Madeira island, Portugal, October 2012, Euro Surveill, 2013; 18: 20398.
6. National Vector Borne Disease Control Programme. Dengue/ dengue haemorrhagic fever, 2013.
7. Isman MB. Botanical insecticides, deterrent, and repellent in modern agriculture and increasingly regulated world. Annu Rev Entomol, 2006; 51: 45–66.
8. Pavela R. Larvicidal effects of various Euro-Asiatic plants against *Cx. quinquefasciatus* Say larvae (Diptera: Culicidae). Parasitol Res, 2007; 36: 821–823.
9. Jawale C, Kirdak R, Dama L. Larvicidal activity of *Cestrum nocturnum* on *Aedes aegypti*. Bangladesh J Pharmacol, 2010; 5: 39–40.
10. Ezeonu FC, Chidume GI, Udedi SC. Insecticidal properties of volatile extracts of orange peels. Bioresour Technol, 2001; 76: 273–274.
11. Carlini CR, Grossi-de-Sa MF. Plant toxic proteins with insecticidal properties. A review on their potential as bioinsecticides. Toxicon, 2002; 40: 1515–1539.
12. Rocha GDG, Simões M, Lúcio KA, Oliveira RR, Kaplan MAC, Gattass CR. Natural triterpenoids from *Cecropia lyratiloba* are cytotoxic to both sensitive and multidrug resistant leukemia cell lines. Bioorg Med Chem, 2007; 15: 7355–7360.
13. Kuete V, Metuno R, Ngameni B, Tsafack AM, Ngandeu F, Fotso GW, Bezabih M, Etoa FX, Ngadjui BT, Abegaz BM, Beng VP. Antimicrobial activity of the methanolic extracts and compounds from *Treculia obovoidea* (Moraceae). J Ethnopharmacol, 2007; 112: 531–536.

14. Kirtikar KR, Basu BD. Indian medicinal plants, vol. 10, 2nd edn. Oriental Enterprises, Uttaranchal, 2001; 3216–3219.
15. Husain A, Virmani OP, Popli SP, Misra LN, Gupta MM, Srivastava GN, Abraham Z, Singh AK. Dictionary of Indian medicinal plants. CIMAP, Lucknow, 1992; 546.
16. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides, Communicable disease control, prevention and eradication, WHO pesticide evaluation scheme, WHO, Geneva, *WHO/CDS/WHOPES/GCDPP* 2005; 1.3.
17. Su T, Mulla MS. Ovicidal activity of neem products (Azadirachtin) against *Culex tarsalis* and *Culex quinquefasciatus* (Diptera: Culicidae). J Am Mosq Control Assoc, 1998; 14: 204-209.
18. World Health Organization. Guidelines for efficacy testing of mosquito repellents for human skins, *WHO/HTM/NTD/WHOPES.*, 2009; 4: 4-18.
19. Finney DJ. A statistical treatment of the sigmoid response curve. In: Probit analysis, Cambridge University Press, London., 1971; 633.
20. Azmathullah NMD, Asrar Sheriff M, Sultan Mohideen AK. Phytochemical screening and larvicidal efficacy of *Calotropis procera* flower extract against *Culex sp*. and *Anopheles sp*. mosquito larvae. *J Environ Scie Comp Scie Enginee & Tech*, 2013; 2: 938-943.
21. Baranitharan M, Dhanasekaran S. Mosquito larvicidal properties of *Commiphora caudata* (Wight &Arn.) (Bursaceae) against *Aedes aegypti* (Linn.), *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say). Int J Curr Microbiol App Sci, 2014; 3: 262-268.
22. Balu Selvakumar, Gokulakrishnan J, Elanchezhiyan K, Deepa J. Mosquitocidal activities of Indian medicinal plant *Pavonia odorata* willd (Malvaceae) against selected vector mosquitoes (Diptera: Culicidae). Int J Curr Advan Res, 2015; 4: 221-227.
23. Gokulakrishnan J, Balu Selvakumar, Elumalai K, Krishnappa K. Mosquito larvicidal and ovicidal efficacy of *Ariitolochia indica* Linn (Aristolochiaceae) leaf extracts against malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). Int J Curr Lif Sci, 2012; 2: 48-52.
24. Singha S, Chandra G. Mosquito larvicidal activity of some common spices and vegetable waste on *Culex quinquefasciatus* and *Anopheles stephensi*. Asian Pac J Trop Med, 2011; 288-293.
25. Ramanibai R, Velayutham K. Larvicidal efficacy of medicinal plant extracts for the control of mosquitoe vectors. Int J Pharm Bio Sci, 2014; 5: 707-715.