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Mosquito larvicidal properties of *Ficus benghalensis* L. (Family: Moraceae) against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae)

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ABSTRACT

Objective: To determine the larvicidal efficacy of different solvent leaf extract of *Ficus benghalensis (F. benghalensis)* against *Culex tritaeniorhynchus (Cx. tritaeniorhynchus)* and *Anopheles subpictus* (*An. subpictus*). **Methods:** Twenty five early third instar larvae of *Cx. tritaeniorhynchus* and *An. subpictus* were exposed to various concentrations and were assayed in the laboratory by using the protocol of WHO 2005. The larval mortality was observed after 24 h of treatment. **Results:** Among three solvent extracts tested the maximum efficacy was observed in the methanol extract. The LC_{50} and LC_{90} values of *F. benghalensis* against early third instar of Cx. *tritaeniorhynchus* and *An. subpictus* were 100.88, 159.76 ppm and 56.66, 85.84 ppm, respectively. No mortality was observed in controls. The *chi*-square values were significant at $P<0.05$ level. **Conclusions:** From the results it can be concluded the crude extract of *F. benghalensis* was an excellent potential for controlling *Cx. tritaeniorhynchus* and *An. subpictus* mosquito larvae.

1. Introduction

Mosquito-borne diseases, like malaria, yellow and dengue fevers, are a major threat to over 2 billion people in the tropics[1]. *Anopheles subpictus (An. subpictus)* is known to transmit malaria and filariasis, in an isolated study of multiple host-feeding in field populations, and its specific role in transmitting malaria in Sri Lanka revealed that multiple blood feeding within the same gonotrophic cycle was attributed to a local "frequent feeding strategy" in this primarily zoophagic and endophilic malaria vector. On the contrary, in Indonesia, *An. subpictus* is a potential vector of bancroftian filariasis and fed on microfilaraemia carriers that harbored *Wuchereria bancrofti* larvae[2]. *An. subpictus* breeds profusely in rainwater accumulations and fallow rice fields[3], waste water disposal systems, and irrigated sites[4], and is also associated with floating and submerged aquatic vegetation in the vicinity of rice plants[5]. Night time human biting collection in Rajasthan, India, showed two feeding peaks for *An. subpictus*, one early in the night and the other just before dawn[6]. *Culex tritaeniorhynchus* (*Cx. tritaeniorhynchus*) Giles is an important vector of JE in India and South East Asian countries. JE is endemic in few states of India and highly endemic in few districts of Tamil Nadu, Southern India[7]. Keiser *et al*[8]. have reported that approximately 1.9 billion people currently live in rural JE prone areas of the world, the majority of them in China (766 million) and India (646 million).

An obvious method for the control of mosquito-borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. However, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. The toxicity problem, together with the growing incidence of insect resistance, has called attention to the need for novel insecticides[9], and for more detailed studies of naturally-occurring insecticides[10,11]. Plants may be a source of alternative agents for control of mosquitoes, because they are rich in bioactive chemicals, are active against a limited number of species including specific target insects, and are bio-degradable. They are potentially suitable for use in integrated pest management programs[12].

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The mosquito larvicidal properties of leaf and seed extract of plant *Agave americana*[13]. Larvicidal efficacy of the crude leaf extract of *Ficus benghalensis* (*F. benghalensis*) with three different solvents like methanol, benzene and acetone were tested against the early second, third, fourth instar larvae of *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi*[14]. The mosquito larvicidal activity in the extract of *Tagetes minuta* (*T. minuta*) flowers against *Ae. aegypti*[15]. The methanolic fraction of leaves of *Mentha piperita* (*M. piperita*), *Phyllanthus niruri* (*P. niruri*), *Leucas aspera* (*L. aspera*) and *Vitex negundo* (*V. negundo*) against larvae of *Cx. quinquefasciatus*[16]. The methanolic extracts of *Solanum suratense* (*S. suratense*), *Azadirachta indica* (*A. indica*) and *Hydrocotyle javanica* (*H. javanica*) exhibited larvicidal activity against *Cx. quinquefasciatus*[17].

The acetone, chloroform, ethyl acetate, hexane and methanol leaf extracts of *A. indica, Achyranthes aspera (A. aspera), L. aspera, Morinda tinctoria (Morinda tinctoria)* and *Ocimum sanctum* (*Ocimum sanctum*) were studied against the early fourth-instar larvae of *Ae. aegypti* L and *Cx. quinquefasciatus* Say[18]. Larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf of five species of cucurbitaceous plants, *Citrullus colocynthis (C. colocynthis), Coccinia indica (C. indica), Cucumis sativus (C. sativus), Momordica charantia (M. charantia),* and *Trichosanthes anguina (T. anguina)*, were tested against the early fourth instar larvae of *Ae. aegypti* L. and *Cx. quinquefasciatus*[19]. The benzene and methanol extracts of *Artemisia vulgaris (A. vulgaris)* has been repellent activity against *Ae. aegypti*[20]. The *Zanthoxylum armatus (Z. armatus), Z. alatum* (Rutaceae), *A. indica* (Mailiaceae) and *Curcuma aromatica (C. aromatica)* (Zingiberaceae) were possess repellent properties against mosquitoes[21]. The repellent activity of active compound Octacosane from *Moschosma polystachyum* against the vector *Cx. quinquefasciatus*[22]. The essential oil of *Zingiber officinalis (Z. officinalis)* as a mosquito larvicidal and repellent agent against the filarial vector *Cx. quinquefasciatus*[23]. Govindarajan[24] reported that the leaf methanol, benzene, and acetone extracts of *Cassia fistula (C. fistula)* were studied for the larvicidal, ovicidal, and repellent activities against *Ae. aegypti*. The leaf extract of *Acalypha indica* with different solvents *viz*., benzene, chloroform, ethyl acetate, and methanol were tested for larvicidal, ovicidal activity, and oviposition attractancy against *An. stephensi*[25].

Ficus benghalens L. (Moraceae, Mulberry family) is commonly known as Banyan tree or Vata or Vada tree in Ayurveda. There are more than 800 species and 2 000 varieties of *Ficus* species, most of which are native to the old World tropics. *F. benghalensis* a remarkable tree of India sends down its branches and great number of shoots, which take root and become new trunk. This tree is considered to be sacred in many places in India. Earlier glucoside, 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitostirolalpha-D-glucose and meso-inositol have been isolated from the bark of *F. benghalensis*[26] Leaves contain crude protein 9.63%, crude fibres-26.84%, CaO-2.53%, and phosphorus-0.4%. It yields latex containing caoytchoue (2.4%), resin, albumin, cerin, sugar and malic acid. It is used in Ayurveda for the treatment of diarrhoea, dysentery and piles[27], Rheumatism, skin disorders like sores[28], to boost immune system[29], as a hypoglycemic[30]. The extracts of *F. benghalensis* were also reported to inhibit insulinase activity from liver and kidney[31]. In the present study, the larvicidal efficacy of different solvent leaf extracts of *F. benghalensis* against *An. subpictus* and *Cx. tritaeniorhynchus*.

2. Materials and methods

2.1. Plant collection

Fully developed leaves of the *F. benghalensis* were collected from different regions of Cuddalore District, Tamilnadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is deposited at the herbarium of plant phytochemistry division, Department of Zoology, Annamalai University.

2.2. Preparation of the extract

The leaves were washed with tap water, shade dried and finely ground. The finely ground plant material (3.0 kg/solvent) was loaded in soxhlet apparatus and was extracted with three different solvents namely benzene, acetone, and methanol individually. The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. The crude residue of this plant varies with the solvents used. The *F. benghalensis* with three different solvents yielded 75.25, 89.50 and 102.50 g of crude residue respectively. Standard stock solutions were prepared at 1% by dissolving the residues in appropriate solvent. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal bioassay.

2.3. Test organisms

Cx. tritaeniorhynchus and *An. subpictus* were reared in the vector control laboratory, Department of zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at (28±2) ℃, 70%-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark.

2.4. Larvicidal bioassay

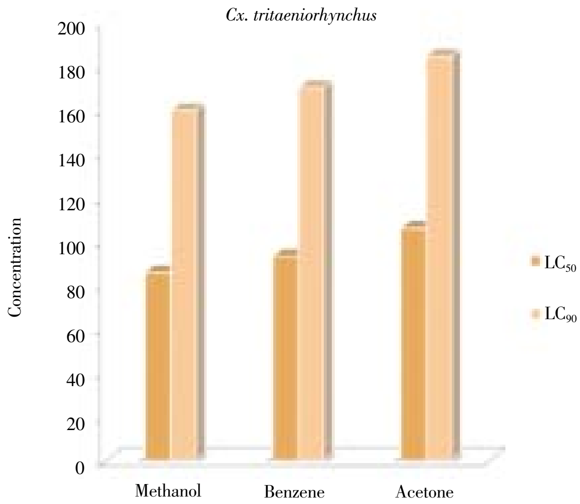
The Larvicidal activity of the plant crude extracts was evaluated as per the method recommended by WHO[32]. Batches of 25 third instar larvae were transferred to a small disposable test cups, each containing 200 mL of water. The appropriate volume of dilution was added to 200 mL water in the cups to obtain the desired target dosage (concentration ranging from 25 to 200 ppm), starting with the lowest concentration. Four replicates were set up for each concentration and an equal number of controls were set up simultaneously using tap water. To this 1 mL of appropriate solvent was added. The LC_{50} value was calculated after 24 h by probit analysis [33].

2.5. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit and *chi*-square values were calculated using the SPSS12.0 (Statistical Package of Social Sciences) software. Results with $P<0.05$ were considered to be statistically significant.

3. Results

The efficacy of *F. benghalensis* was tested against the early third larvae of *Cx. tritaeniorhynchus* and *An. subpictus*. The data were recorded and stastical data regarding the LC_{50}, LC_{90}, *Chi*-square and 95% confidence limits were calculated (Table 1-2 & Figure 1). The methanolic extract of *F. benghalensis* showed highest larvicidal activity against *Cx. tritaeniorhynchus* and *An. subpictus*. The LC_{50} values of *F. benghalensis* against early third larvae of *Cx. tritaeniorhynchus* and *An. subpictus* were 85.84 and 56.66 ppm, respectively. No mortality was observed in control. The *chi*-square value were significant at $P<0.05$ level.



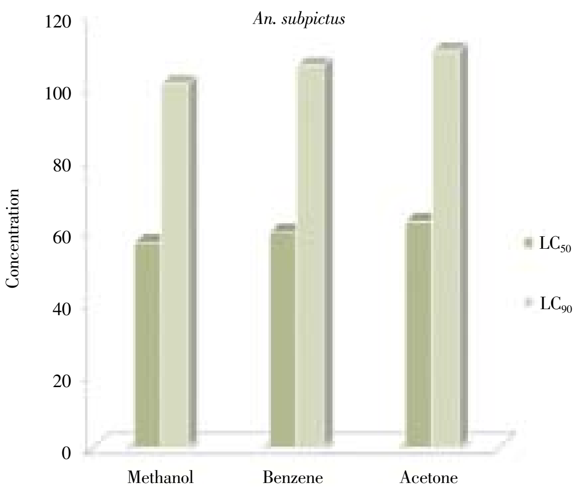


Figure 1. Graph showing the LC_{50}, LC_{90} values of *Cx. tritaeniorhynchus* and *An. subpictus*.

4. Discussion

Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available in many areas of the world. According to Bowers *et al*[34] 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. Different parts of plants contain a complex of chemicals with unique biological activity[35] which is thought to be due to toxins and secondary metabolites, which act as mosquitocidal agent[36]. Our result showed that the crude benzene, acetone and methanol

Table 1

Larvicidal activity of different solvent leaf extracts of *F. benghalensis* against *Cx. tritaeniorhynchus*.

Solvents	Concentration(ppm)	Mortality(%)	LC_{50}(LCL-UCL)	LC_{90}(LCL-UCL)	χ^2
Methanol	Control	0.0			
	40	31.7			
	80	48.8	85.84 (58.28-111.04)	159.76 (130.47-22.08)	21.29
	120	71.8			
	160	83.4			
	200	99.9			
Benzene	Control	0.0			
	40	26.4			
	80	45.2	93.25 (70.08-115.37)	170.10 (142.75-222.36)	16.03
	120	68.1			
	160	79.8			
	200	98.1			
Acetone	Control	0.0			
	40	18.5			
	80	37.8	106.07 (88.01-124.41)	184.60 (160.19-226.11)	10.66
	120	60.5			
	160	74.1			
	200	95.6			

*Significant at $P<0.05$ level. LC_{50}: Lethal concentration; LCL: Lower confidence limit; UCL: Upper confidence limit.

Table 2
Larvicidal activity of different solvent leaf extracts of *F. benghalensis* against *An. subpictus*.

Solvents	Concentration(ppm)	Mortality(%)	LC_{50}(LCL-UCL)	LC_{90}(LCL-UCL)	χ^2
Methanol	Control	0.0	56.66 (43.42-69.27)	100.88 (85.43-129.26)	15.05
	25	23.6			
	50	48.5			
	75	69.3			
	100	83.2			
	125	99.6			
Benzene	Control	0.0	59.70 (47.22-71.77)	105.90 (90.64-132.87)	13.13
	25	20.2			
	50	47.6			
	75	66.8			
	100	80.2			
	125	97.8			
Acetone	Control	0.0	62.68 (50.63-74.13)	109.93 (94.85-135.91)	11.80
	25	17.6			
	50	45.2			
	75	65.3			
	100	77.8			
	125	96.1			

*Significant at $P<0.05$ level. LC_{50}: Lethal concentration; LCL: Lower confidence limit; UCL: Upper confidence limit.

extracts of leaf of *F. benghalensis* have significant larvicidal, properties against two important vector mosquitoes *viz*., *An. subpictus*, and *Cx. tritaeniorhynchus*. This result is also comparable to earlier reports of Singh *et al*[37] who observed the larvicidal activity of *Ocimum canum* oil against vector mosquitoes namely, *Ae. aegypti* and *Cx. quinquefasciatus* (LC_{50} 301 ppm) and *An. stephensi* (234 ppm). Ansari *et al*[38] who observed larvicidal activity of *Pinus longifolia* oil against three vector mosquitoes namely *Ae. aegypti* (LC_{50} – 82.1 ppm), *Cx. quinquefasciatus* (LC50 – 85.7 ppm) and *An. stephensi* (LC_{50} – 112.6 ppm). Cavalcanti *et al*[39-42] reported that the larvicidal activity of essential oils of Brazilian plants against *Ae. aegypti* and observed the LC_{50} to range from 60 to 533 ppm. Prajapati *et al*[43] reported that the larvicidal activity of different plants essential oil showed varied LC_{95} values against *Cx. quinquefasciatus*. They were *Pimpinella anism* (149 μg/mL), *Z. officinalis* (202 μg/mL), *Junipers macropoda* (204 μg/mL), *Cinnamomum zeylanicum* (277 μg/mL), *Curcuma longa* (292 μg/mL), *Cyperus scariosus* (408 μg/mL), *O. basilicum* (315 μg/mL), *Cuminum cyminum* (344 μg/mL) and *Nigella sativa* (365 μg/mL).

Larvicidal activity of crude extract of *Sida acuta* against *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* with LC_{50} values ranging between 38 to 48 mg/L[44]; the lethal concentration (LC_{50}) values of *F. benghalensis* against early second, third and fourth larvae of *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi* were 41.43, 58.21 and 74.32 ppm, 56.54, 70.29 and 80.85 ppm and 60.44, 76.41 and 89.55 ppm respectively[14]. The leaf extract of *C. fistula* with different solvens *viz*, methanol, benzene and acetone were studied for the larvicidal, ovicidal and repellent activity against *Ae. aegypti*. The 24 h LC_{50} concentration of the extract against *Ae. aegypti* were observed at 10.69, 18.27 and 23.95 mg/L respectively[24].

Compared with earlier authors reports, our results revealed that the experimental plant extracts were effective to control *An. subpictus* and *Cx. tritaeniorhynchus*. From these results it was concluded that the plant *F. benghalensis* exhibits larvicidal activity against two important vector mosquitoes. Further analysis to isolate the active compound for larval control is under way in our laboratory. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants.

Conflict of interest statement

We declare that we have no conflict of interest.

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