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Evaluation of larvicidal and pupicidal activity of *Morinda citrifolia* L. (Noni) (Family: Rubiaceae) against three mosquito vectors

K. Kovendan[1*], K. Murugan[1], S. P. Shanthakumar[2] and S. Vincent[3]

[1]*Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore – 641 046, Tamil Nadu, India.*
[2]*Laboratory of Integrated Pest Management (IPM), M.S. Swaminathan Research Foundation, III Cross Road, Institutional Area, Taramani, Chennai – 600 113, Tamil Nadu, India.*
[3]*P.G. Research & Department of Advanced Zoology and Biotechnology, Loyola College, Nungambakkam, Chennai – 600 034, Tamil Nadu, India*

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ABSTRACT

Objective: To evaluate the mosquito larvicidal and pupicidal activity against three important medically mosquito vector such as malarial vector, *Anopheles stephensi (An. stephensi)*, dengue vector, *Aedes aegypti (Ae. aegypti*) and filarial vector *Culex quinquefasciatus (Cx. quinquefasciatus)*. **Methods:** *Morinda citrifolia (M. citrifolia)* leaf was collected in and around Alleppy districts, Kerala, India. *M. citrifolia* leaf was washed with tap water and shade dried at room temperature. An electrical blender powdered the dried plant materials (leaves). From the leaf, 1 kg powdered was macerated with 3.0 L of methanol sequentially for a period 72 h and filtered. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. The larvicidal and pupicidal activity was assayed at various concentrations ranging from (100-500 ppm) under the laboratory as well as field conditions. The LC_{50} and LC_{90} value of the *M. citrifolia* leaf extract was determined by Probit analysis. **Results:** The plant extract showed larvicidal and pupicidal effects after 24 and 48 hrs of exposure; All larval instars and pupae have considerably moderate mortality; however, the highest larval and pupal mortality was methanolic extract of *M. citrifolia* observed in three mosquito vectors at 48 h. The LC_{50} and LC_{90} of *M. citrifolia* against the first to fourth instar larvae and pupae against mosquito vectors. *An. stephensi* had values of LC_{50}=146.08, 159.07, 172.16, 185.08 and 202.68 ppm and LC_{90}=322.12, 363.48, 388.56, 436.51 and 513.56 ppm, respectively. The *Ae. aegypti* had values of LC_{50}=181.27, 210.40, 229.80, 256.73 and 292.01 ppm and LC_{90}=407.99, 485.65, 534.14, 624.16 and 756.79 ppm, respectively. The *Cx. quinquefasciatus* had values of LC_{50}=226.70, 256.97, 290.05, 316.33 and 358.11 ppm and LC_{90}=560.35, 652.07, 733.03, 797.09 and 875.25 ppm, respectively at 24 h. **Conclusions:** The results of the leaf extract of *M. citrifolia* are promising as good larvicidal and pupicidal activity against the mosquito vector, *An. stephensi*, *Ae. aegypti*, *Cx. quinquefasciatus*. This is a new eco-friendly approach for the control of vector control programs. Therefore, this study provides first report on the larvicidal and pupicidal activities against three species of mosquito vectors of this plant extract from India.

1. Introduction

Mosquitoes are the principal vector of many vectorborne diseases affecting human beings and animals, in addition to nuisance. Vector-borne diseases in India, e.g., malaria, dengue, chikungunya, filariasis, Japanese encephalitis and leishmaniasis, cause thousands of deaths per year. India reports 1.48 million malarial cases and about 1,173 deaths; 1.4 million suspected and 1,985 confirmed chikungunya cases; 5,000 Japanese encephalitis cases and approximately 1,000 deaths; 383 dengue cases and six deaths during 2006 and 2007 [1, 2, 3].

The container breeding mosquito, *Aedes aegypti* L. thrives in urban and peridomestic environments where it transmits the dengue virus to humans [4]. More than 50 million people are at risk of dengue virus exposure worldwide. Annually, there are 2 million infections, 500,000 cases of dengue hemorrhagic fever, and 12,000 deaths [5]. *Culex quinquefasciatus* is a

*Corresponding author: Dr. K. Kovendan Division of Entomology Department of Zoology School of Life Sciences Bharathiar University Coimbatore – 641 046, India.
Phone: +91-9962447932
E-mail: gokulloyo@yahoo.co.in

vector of lymphatic filariasis affecting 120 million people worldwide, and approximately 400 million people are at risk of contracting filariasis worldwide, resulting into the annual economic loss of 1.5 billion dollars [6]. Lymphatic filariasis is a serious public health problem in India, comprising of one third of infected population of the world [7]. *Anopheles stephensi* is responsible for transmission of malaria in urban regions of India [8] Traditionally, plants and their derivatives were used to kill mosquitoes and other household and agricultural pests. In all probability, these plants used to control insects contained insecticidal phytochemicals that were predominantly secondary compounds produced by plants to protect themselves against herbivorous insects [9]. In view of the growing concern regarding pollution by chemical insecticides and acquired tolerance among target species, the merits of phytochemicals present in plants as secondary metabolites are increasingly recognized. Recent studies have in sighted the insecticidal properties of chemicals derived from plant material and concluded that they are environmentally safe, degradable, and target specific [10].

Morinda citrifolia L. (Noni) is also known as Indian mulberry, belongs to family; Rubiaceae. *M. citrifolia* fruit has a long history of use as a food in tropical regions throughout the world. It mainly contains saponins, tannins, triterpenes, alkaloids, flavonoids. It is mainly used for the bowel disorders, including arthritis, atherosclerosis, bladder infections, boils, burns, cancer, chronic fatigue syndrome, circulatory weakness, cold, congestion, constipation, diabetes, eye inflammations, fever, fractures, gastric ulcers, gingivitis, headaches, heart diseases, hypertension, immune weakness, indigestion, intestinal parasites, kidney disease, malaria, menstrual cramps, mouth sores, respiratory disorders, ringworms, sinusitis, sprains, stroke, skin inflammation and wounds [11].

A number of major components have been identified in the Noni plant such as scopoletin, octoanoic acid, potassium, vitamin C, terpenoids, alkaloids, anthraquinones (such as nordamnacanthal, morindone, rubiadin, and rubiadin-1-methyl ether, anthraquinone glycoside), b-sitosterol, carotene, vitamin A, flavone glycosides, linoleic acid, Alizarin, amino acids, acubin, L-asperuloside, caproic acid, caprylic acid, ursolic acid, rutin, and a putative proxeronine [12, 13, 14, 15].The structures of the new compounds were determined by spectroscopic data interpretation. Compound 4, borreriagenin, cytidine, deacetylasperuloside, dehydromethoxygaertneroside, epi-dihydrocornin, methyl alpha-d-fructofuranoside, and methyl beta-d-fructofuranoside were isolated for the first time from *M. citrifolia* [16].

The present study would be useful in promoting research aiming at the development of new agent for mosquito control based on plant source of natural products. In view of the recent increased interest in developing plant-based insecticides as an alternative to chemical insecticides, this study was undertaken to assess the mosquitocidal properties of *M. citrifolia* leaf extracts of against the medically important mosquito vectors, *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* as target species.

2. Materials and methods

2.1. Collection of eggs and maintenance of larvae

The eggs of *An. stephensi Ae. aegypti* and *Cx. quinquefasciatus* were collected from National Centre for Disease Control field station of Mettupalayam, Tamil Nadu, India, using an "O"-type brush. These eggs were brought to the laboratory and transferred to 18 × 13 × 4-cm enamel trays containing 500-mL of water for hatching. The mosquito larvae were pedigree dog biscuits and yeast at 3:1 ratio. The feeding was continued until the larvae transformed into the pupal stage.

2.2. Maintenance of pupae and adults

The pupae were collected from the culture trays and transferred to plastic containers (12 × 12 cm) containing 500-mL of water with the help of a dipper. The plastic jars were kept in a 90 × 90 × 90-cm mosquito cage for adult emergence. Mosquito larvae were maintained at 27+2 ℃, 75-85% relative humidity, under a photoperiod of 14:10 (light/dark). A 10% sugar solution was provided for a period of 3 days before blood feeding.

2.3. Blood feeding of adult mosquito vectors

The adult female mosquitoes were allowed to feed on the blood of a rabbit (a rabbit per day, exposed on the dorsal side) for 2 days, to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

2.4. Collection of plant and preparation of extract

The *M. citrifolia* plants were collected from in and around Alleppy (sea sources) districts in Kerala, India. The plants were identified Taxanomist, Department of Botany, University of Madras, Chennai, Tamil Nadu. The voucher specimen has been deposited Department of Zoology, Bharathiar Univeristy, Coimbatore. *M. citrifolia* leaves were washed with tap water and shade dried at room temperature (28±2 ℃) for 10 to 20 days. The air-dried plant materials (leaves) were powdered by an electrical blender. From the leaf, 1 kg powdered was macerated with 3.0 L of methanol sequentially for a period 72 h and filtered. The yield of the *M. citrifolia* crude extract by methanol (21.7 g), respectively. The extracts were concentrated at reduced temperature on a rotary vacumm evaoporator and stored at a temperature of 4 ℃. One gram of the plant residue was dissolved in 100-mL of acetone (stock solution) considered as 1% stock solution. From this stock solution concentrations

were prepared ranging from 100, 200, 300, 400 and 500 ppm, respectively.

2.5. Larval/pupal toxicity test

Laboratory colonies of mosquito larvae/pupae were used for the larvicidal/pupicidal activity. Twenty-five numbers of first to fourth instars larvae and pupae were introduce into 500-mL glass beaker containing 249-mL of de-chlorinated water and 1-mL of desired concentrations of plant leaf extract were added. Larval food was given for the test larvae. At each tested concentration two to five trials were made and each trial consisted of five replicates. The control was setup by mixing 1-mL of acetone with 249-mL of dechlorinated water. The larvae and pupae were exposed to dechlorinated water without acetone served as control. The control mortalities were corrected by using Abbott's formula [17].

The LC_{50} and LC_{90} were calculated from toxicity data by using probit analysis [18].

3. Results

The result shows that mortality effects of metahanol leaf extract of *M. citrifolia* against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* at 24 and 48 h, respectivley (Table 1, 3, 5). The *M. citrifolia* were studied used as eco friendly insecticides instead. A result with first to fourth instars larvae and pupae for the control of mosquito vectors. The *An. stephensi* had values of LC50 =146.08, 159.07, 172.16, 185.08 and 202.68 ppm

Table 1.
Larval and pupal toxicity effect of methanol leaf extract of *M. citrifolia* against *Ae. aegypti* at 24 & 48 hrs

Conc.(ppm)	Hours	First instars	Second Instars	Third Instars	Fourth instars	Pupae
100	24	37.7±1.70 de	35.0±1.20e	32.4±1.21e	31.6±1.88e	29.9±1.18e
	48	48.5±1.17cd	46.3±1.64 d	40.1±1.70d	39.4±1.16d	38.3±1.65ce
200	24	51.3±1.82 d	46.7±1.21 de	43.9±1.32cd	40.8±1.29de	39.6±1.11de
	48	65.6±1.70bc	63.2±1.16cd	57.7±1.42 c	54.7±1.95cd	51.2±1.41cd
300	24	69.5±1.77 b	60.7±1.62c	58.3±1.98cd	53.2±1.10	50.5±1.21d
	48	77.2±1.21ab	70.5±1.58bc	68.8±1.13bc	64.3±1.98c	61.3±1.30bc
400	24	87.5±1.28ab	77.6±1.21b	73.4±1.08ab	68.4±1.10bc	63.3±1.44c
	48	91.7±0.50a	86.4±0.57ab	82.8±1.29 b	79.5±1.82b	76.4±1.60ab
500	24	100.0±0.00a	96.0±0.00a	90.9±0.00a	82.6±1.29ab	70.8±1.51b
	48	100.0±0.00a	100.0±0.00a	98.5±0.00a	89.9±0.00a	81.6±1.36a

Control-Nil mortality; Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT Each (Mean± SD) value of five replicates.

Table 2
Lethal concentration values of methanol leaf extract of *M. citrifolia* against *Ae. aegypti* at 24 & 48 hrs

Mosquito larval instars and pupae	Exposure hours	Regression equation	LC_{50} values (LFL-UFL) (ppm)	LC_{90} values (UFL-UFL) (ppm)	x^2 (df = 4)
First instars	24	Y= -1.025 +0.006X	181.27 (84.37-238.99)	407.99 (337.22-569.33)	7.23*
	48	Y= -0.638 +0.005X	122.49 (80.05-153.86)	368.41 (335.14- 414.35)	4.78*
Second Instars	24	Y= -0.980 +0.005X	210.40 (115.67-271.11)	485.65 (400.35-689.58)	6.18*
	48	Y= -0.654 +0.005X	138.22 (76.27-217.46)	409.22 (321.13- 689.03)	9.06*
Third Instars	24	Y= -0.968 +0.004X	229.80 (196.45-258.33)	534.14 (483.21-608.52)	2.67*
	48	Y= -0.779 +0.005X	165.23 (43.79-228.85)	436.97 (358.30-626.14)	6.18*
Fourth instars	24	Y= -0.903 +0.004X	256.73 (219.71-289.50)	621.16 (550.89-731.64)	1.05*
	48	Y= -0.663 +0.004X	177.38 (130.39-212.63)	520.48 (465.35-604.81)	0.78*
Pupae	24	Y= -0.805 +0.003X	292.01 (248.40-334.24)	756.79 (646.43-954.48)	0.15*
	48	Y= -0.622 +0.003X	197.47 (144.84-236.50)	604.08 (529.37-727.72)	0.60*

LC_{50} - Lethal concentration that kills 50% of the exposed larvae and pupae, LC_{90} - Lethal concentration that kills 90% of the exposed larvae and pupae, LFL = Lower fiducidal limit, UFL = Upper fiducidal limit, x^2 - Chi-square value, df – degrees of freedom, *Significant at P<0.05 level

Table 3
Larval and pupal toxicity effect of methanol leaf extract of *M. citrifolia* against *Cx. quinquefasciatus* at 24 & 48 hrs

Conc. (ppm)	Hours	First instars	Second Instars	Third Instars	Fourth instars	Pupae
100	24	33.0±1.70e	31.7±1.75e	28.5±1.21e	27.7± 1.50e	26.0± 1.82e
	48	47.0±1.10d	45.5±1.27d	42.0± 1.38d	40.5±1.12d	38.0±1.18d
200	24	47.0±1.29de	43.5±1.08de	41.0±1.16de	39.0±1.08de	36.0±1.50de
	48	59.0±1.08cd	54.7±1.21d	51.5±1.50cd	48.4±1.82cd	45.7±1.21cd
300	24	58.0±1.19c	53.0±1.16c	50.7±1.75c	47.7±2.64c	43.2±1.38c
	48	76.0±1.21bc	70.7±1.18bc	66.9±1.64bc	61.3±1.70bc	59.2±1.08bc
400	24	71.0±1.16bc	65.7±1.82b	62.1±1.70bc	58.5±1.29bc	69.0±1.81c
	48	89.0±1.10ab	84.5±1.64ab	80.0±1.17b	74.8±1.20b	82.9±1.58b
500	24	89.0±0.00a	81.0±0.00a	73.0±1.74ab	69.0±2.21ab	64.8±1.16ab
	48	100.0±0.00a	93.0±0.00a	89.0±0.00a	82.0±1.82a	79.7±1.30a

Control–Nil mortality; Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT Each (Mean± SD) value of five replicates.

Table 4
Lethal concentration values of methanol leaf extract of *M. citrifolia* against *Cx. quinquefasciatus* at 24 & 48 hrs

Mosquito larval instars and pupae	Exposure hours	Regression equation	LC_{50} values (LFL–UFL) (ppm)	LC_{90} values (UFL–UFL) (ppm)	x^2 (df = 4)
First instars	24	Y= –0.871 +0.004X	226.70 (189.45–257.76)	560.35 (502.30–648.21)	2.39*
	48	Y= –0.716 +0.005X	139.30 (14.59–201.41)	388.58 (319.40–545.06)	6.03*
Second Instars	24	Y= –0.834 +0.003X	256.97 (216.60–292.24)	652.07 (572.12–782.85)	0.94*
	48	Y= –0.391 +0.003X	153.86 (57.25–183.19)	572.73 (497.68–702.07)	4.78*
Third Instars	24	Y= –0.839 +0.003X	290.05 (248.39–330.18)	733.03 (631.18–910.47)	0.10*
	48	Y= –0.624 +0.004X	171.95 (122.24–208.60)	585.09 (468.08–613.31)	0.58*
Fourth instars	24	Y= –0.843 +0.003X	316.33 (273.16–362.51)	797.09 (675.55–1020.08)	0.09*
	48	Y= –0.601 +0.003X	195.05 (140.49–235.03)	611.06 (533.64–740.88)	0.52*
Pupae	24	Y= –0.887 +0.002X	358.11 (312.56–416.39)	875.25 (729.23–1157.86)	0.21*
	48	Y= –0.640 +0.003X	218.22 (165.80–258.22)	655.17 (567.58–806.07)	0.32*

LC_{50} – Lethal concentration that kills 50% of the exposed larvae and pupae, LC_{90} – Lethal concentration that kills 90% of the exposed larvae and pupae, LFL = Lower fiducidal limit, UFL = Upper fiducidal limit, x^2 – Chi–square value, df – degrees of freedom, *Significant at P<0.05 level.

Table 5
Larval and pupal toxicity effect of methanol leaf extract of *M. citrifolia* against *An. stephensi* at 24 & 48 hrs

Conc. (ppm)	Hours	First instars	Second Instars	Third Instars	Fourth instars	Pupae
100	24	41.3±1.70e	39.2±1.75e	38.5±1.21e	37.4±1.55e	36.9±1.82e
	48	49.2±1.10d	45.7±1.21d	45.6±1.38d	43.2±1.12d	41.3±1.12d
200	24	62.8±1.33de	58.6±1.08de	53.1±1.16de	60.1±1.08de	57.5±1.50de
	48	67.6±1.08cd	63.8±1.26d	60.5±1.50cd	59.3±1.82cd	58.5±1.20cd
300	24	80.4±1.19c	76.2±1.15c	73.2±1.75c	70.7±1.64c	67.6±1.08c
	48	92.0±0.00a	84.5±1.64ab	80.7±1.41bc	79.8±1.58c	75.7±1.14c
400	24	100.0±0.00a	94.0±0.00a	90.4±1.56a	84.9±1.70bc	81.1±1.42b
	48	100.0±0.00a	100.0±0.00a	99.0±0.00a	92.7±1.34ab	89.7±1.45bc
500	24	100.0±0.00a	100.0±0.00a	100.0±0.00a	96.0±0.00a	88.2 ±1.58ab
	48	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	94.7±1.70a

Control–Nil mortality; Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT Each (Mean± SD) value of five replicates.

Table 6

Lethal concentration values of methanol leaf extract of *M. citrifolia* against *An. stephensi* at 24 & 48 hrs

Mosquito larval instars and pupae	Exposure hours	Regression equation	LC_{50} values (LFL–UFL) (ppm)	LC_{90} values (UFL–UFL) (ppm)	x^2 (df = 4)
First instars	24	Y= –1.064 +0.007X	146.08 (39.14–201.11)	322.12 (261.28–470.71)	8.62*
	48	Y= –0.924 +0.008X	117.83 (88.12–140.45)	281.22 (256.84–314.34)	4.59*
Second Instars	24	Y= –0.997 +0.006X	159.07 (130.03–182.69)	363.48 (334.52–402.12)	3.98*
	48	Y= –0.963 +0.007X	133.07 (32.38–184.75)	310.16 (254.14–436.06)	7.02*
Third Instars	24	Y= –1.020+0.006X	172.16 (94.41–221.62)	388.56 (328.53–507.12)	5.46*
	48	Y= –0.939 +0.007X	139.44 (21.03–197.20)	329.70 (266.76–482.45)	8.23*
Fourth instars	24	Y= –0.779 +0.005X	185.08 (126.85–194.85)	436.51 (397.55–491.27)	1.81*
	48	Y= –0.868 +0.006X	146.04 (113.31–171.82)	361.74 (331.72–402.12)	3.36*
Pupae	24	Y= –0.649 +0.004X	202.68 (124.97–208.25)	513.56 (459.71–595.47)	0.83*
	48	Y= –0.711+ 0.005X	149.58 (108.96–180.57)	419.19 (381.54–471.83)	0.31*

LC_{50} – Lethal concentration that kills 50% of the exposed larvae and pupae, LC_{90} – Lethal concentration that kills 90% of the exposed larvae and pupae, LFL = Lower fiducidal limit, UFL = Upper fiducidal limit, x^2 – Chi-square value, df – degrees of freedom, *Significant at P<0.05 level.

at 24h; 117.83, 133.07, 139.44, 146.04 and 149.58 ppm at 48; and LC_{90}=322.12, 363.48, 388.56, 436.51 and 513.56 ppm at 24; 281.22, 310.16, 329.70, 361.74 and 419.19 ppm at 48 h, respectively (Table 2). The *Ae. aegypti* had values LC_{50}=181.27, 210.40, 229.80, 256.73 and 292.01 ppm at 24h; 122.49, 138.22, 165.23, 177.38 and 197.47 ppm at 48; The LC_{90} values of 407.99, 485.65, 534.14 621.46 and 756.79 ppm at 24; 368.41, 409.22, 436.17, 520.48 and 604.08 ppm at 48 h, respectively (Table 4) The *Cx. quinquefasciatus* had values of LC_{50}=226.70, 256.97, 290.05, 316.33 and 358.11 ppm at 24h; 139.30, 153.86, 171.95, 195.05 and 218.22 ppm at 48; and LC_{90}=560.35, 652.07, 733.03 797.09 and 875.25 ppm at 24; 388.58, 572.12, 585.09, 611.06 and 655.17 ppm at 48 h, respectively (Table 6).

4. Discussion

Mosquitoes are blood feeding insects and serve as vectors for spreading human diseases such as malaria, dengue fever, yellowfever, encephalitis, West Nile fever, lymphatic filariasis, etc. and therefore, they continue to pose a serious public health problem throughout the world. Since prevention is better than cure, control of growing mosquito population is an urgent and immediate demand by the society. Hence, there has been an increasing interest in the development of alternative methods of mosquito control which are less hazardous to humans and other living organisms. In this regard, plantderived compounds have emerged as good candidates, not only as new effective tools in vector management but also as environmentally safer agents [19-23]. Furthermore, the crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors [24].

Earlier authors reported that the methanol extract of Cassia fistula exhibited LC_{50} values of 17.97 and 20.57 mg/L, *An. stephensi* and *Cx. quinquefasciatus*, respectively [25]. The neem formulation, Neem Azal, produced an overall mortality or inhibition of emergence of 90 % (EI90, when third-instar larvae were treated) at 0.046, 0.208, and 0.866 ppm in *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti*, respectively [26]. The effect of three citrus species and enantiomers of α – and β –pipenes were also studied against third instar larvae of culex pipenes [27].These studies were based on plant extract aginst mosquito larvae. In the present results, *M. citrifolia* against *An. stephensi* the LC_{50} and LC_{90} values of first to fourth-instars larvae and pupae were LC_{50} values of 146.08, 159.07, 172.16, 185.08 and 202.68 ppm at 24h; 117.83, 133.07, 139.44, 146.04 and 149.58 ppm at 48; The LC_{90} values of 322.12, 363.48, 388.56, 436.51 and 513.56 ppm at 24; 281.22, 310.16, 329.70, 361.74 and 419.19 ppm at 48 h, respectively. Earlier instars were more susceptible to the extracts compared to the late instars. Similar differences in responses of the various larval instars of *Ae. aegypti*, *An. stephensi*, and *Cx. pipiens* molestus exposed to crude extracts of Millingtonia hortensis, Melia volkensii, and Melia azaderach were recorded by other researchers [28, 29] who reported the dose-dependent increase in mortality of first, second, third, and fourth instar larvae of *An. subpictus* on exposure of Solanum villosum extracts at 200 ppm with 100% mortality.

Larvicidal studies were carried out against *C. quinquefasciatus* and the results were compared with bulk permethrin. The LC_{50}

of nanopermethrin and bulk permethrin to *C. quinquefasciatus* was 0.117 and 0.715 mg/L respectively [30]. Sakulku et al [31] have reported the low release rate of nanoemulsion with large droplet size that resulted in prolonged mosquito repellant activity compared to the nanoemulsion with small droplet size. The corresponding LC_{50} value of leaf acetone, absolute alcohol, petroleum ether, chloroform/methanol (1:1, v/v), benzene and ethyl acetate extracts of Solanum nigrum were 72.91, 59.81, 54.11, 32.69, 27.95 and 17.04 ppm, respectively, after 24 h of exposure period against *C. quinquefasciatus* [32]. Changbunjong et al [33] reported that the ethanolic crude extract from Solanum xanthocarpum was investigated for its mosquito larvicidal activity; the LC_{50} against the larvae of *C. quinquefasciatus* was 573.20 mg/l while the LC_{90} was 1,066.93 mg/l.

Mathew *et al* [34] reported that leaf chloroform extracts of Nyctanthes arbortristis showed lethal values (LC_{50}=526.3. 780.6 ppm (24 h) and LC_{50}=303.2, 518.2 ppm (48 h)) against *Ae. aegypti* and *An. stephensi*, respectively. Flower methanol extracts of the above plants showed lethal values (LC_{50}=679.4, 244.4 ppm; LC_{90}=1071.3, 433.7 ppm) against *An. stephensi* after 24 and 48 h, respectively. The LC_{50} values of hexane, chloroform, ethyl acetate, acetone and methanol extract of *O. thymiflorus* third instar larvae of *An. stephensi* were LC_{50}= 201.39, 178.76, 158.06, 139.22 and 118.74 ppm; *Cx. quinquefasciatus* were LC_{50}=228.13, 209.72, 183.35, 163.55 and 149.96 ppm and *Ae. aegypti* were LC_{50}=215.65, 197.91, 175.05, 154.80 and 137.26 ppm, respectively [35].

Clitoria ternatea leaf methanol extract showed dose-dependent larvicidal activity against *An. stephensi* with LC_{50} values of 555.6 (24 h) and 867.3 (48 h) ppm, also the root extracts with LC_{50} value of 340 ppm (48 h). Seed extract showed larvicidal activity (LC_{50}=116.8, 195 ppm) after 24 h and (LC_{50}=65.2, 154.5 ppm) after 48 h treatment against *An. stephensi* and *Ae. aegypti*, respectively. Larvicidal activity of flower methanol extract showed LC_{50} values 233 and 302.5 ppm against *An. stephensi* and *Ae. aegypti*, respectively, after 48 h treatment. Methanol extract showed lowest LD values against several inster of larvae and 50 adult (121.59, 142.73, 146.84, 202.98, 290.65, 358.42 and 300.03 $\mu g/cm^2$, respectively) which indicates highest toxicity or insecticidal activity [36]. In the present results, *M. citrifolia* against *Ae. aegypti* the LC_{50} and LC_{90} values of first to fourth-instars larvae and pupae were LC_{50} values of 181.27, 210.40, 229.80, 256.73 and 292.01 ppm at 24h; 122.49, second 138.22, 165.23, 177.38 and 197.47 ppm at 48; The LC_{90} values of 407.99, 485.65, 534.14 621.46 and 756.79 ppm at 24; 368.41, 409.22, 436.17, 520.48 and 604.08 ppm at 48 h, respectively.

Ghosh *et al* [37] isolated a phytosteroid compound from Cestrum diurnum which exhibited remarkable biocontrol potentiality against larval mosquitoes. The ethanolic water extract (10% concentration) from the seeds and leaf parts of Myristica fragrans displayed an LC_{50} of 2.22 ppm against the 3rd instar larvae of *An. stephensi* [38]. Previous reports on extracts of *Psammaplysilla purpurea* and Haliclona cribricutis showed LC_{50} values of < 50 ppm against *Ae. aegypti* [39], whereas fucoidan derived from Undaria pinnatifida seaweed showed LC_{50} values of 9.17 $\mu g\ ml^{-1}$ against P. falciparum [40]. Recent studies on the larval and pupal mortality of *An. stephensi* after the treatment of methanolic extract of Clerodendrone inerme leaf extract showed 22% mortality at I instar larvae as a result of treatment at 20 ppm; in contrast, it was increased to 81% at 100 ppm of *C. inerme* leaf extract of larval and pupal mortality of *An. stephensi* (I to IV instars) after the treatment of methanolic extract of Acanthus ilicifolius at different concentrations (20 to 100 ppm). A 23% mortality was noted at I instar larvae by the treatment of *A. ilicifolius* at 20 ppm, whereas it was increased to 89% at 100 ppm of *A. ilicifolius* leaf extract treatment [41]. Kovendan et al [42] have reported that the leaf extract of methanol L. aspera leaf extract against *An. stephensi*, respectively.

Khanna *et al* [43] have reported that the larvicidal crude leaf extract of Gymnema sylvestre showed the highest mortality in the concentration of 1,000 ppm against the larvae of *Ae. subpictus* (LC_{50}=166.28 ppm) and against the larvae of *Cx. quinquefasciatus* (LC_{50}=186.55 ppm), and the maximum efficacy was observed in gymnemagenol compound isolated from petroleum ether leaf extract of *G. sylvestre* with LC_{50} values against the larvae of *Ae. subpictus* at 22.99 ppm and against *Cx. quinquefasciatus* at 15.92 ppm. Santhoshkumar et al [44] reported that the maximum efficacy was observed in crude methanol and aqueous leaf extracts of *Nelumbo nucifera* against the larvae of *Ae. subpictus* (LC_{50}=8.89 and 11.82 ppm, and LC_{90}=28.65 and 36.06 ppm) respectively and against the larvae of *Cx. quinquefasciatus* (LC_{50}=9.51 and 13.65 ppm, and LC_{90}=28.13 and 35.83 ppm) respectively. The methanol leaf extract of *C. gigantea* against *Cx. quinquefasciatus* the LC_{50} value of 104.66, 127.71, 173.75, and 251.65 ppm, respectively. The LC_{90} value of 268.67, 323.50, 432.11 and 581.66 ppm, respectively. The LC_{50} value of pupae was 314.70 ppm, and the LC_{90} value of pupae was 665.04 ppm, respectively [45].

Calotropis procera against *An. stephensi* we observed >95% mortality after 24 h from 256 ppm. Tests with latex showed 99% mortality at 64 ppm for *An. stephensi*, only 44% mortality against *Cx. quinquefasciatus* and a maximum of 67% in 256 ppm, respectively [46]. The leaf extract of *A. alnifolia* with different solvents - hexane, chloroform, ethyl acetate, acetone and methanol were tested for larvicidal activity of against mosquito vectors. The early fourth instar larvae of *An. stephensi* had values of LC_{50}=197.37, 178.75, 164.34, 149.90 and 125.73 ppm and LC_{90}=477.60, 459.21, 435.07, 416.20, and 395.50 ppm, respectively. The *Ae. aegypti* had values of LC_{50}=202.15, 182.58, 160.35, 146.07, and 128.55 ppm and LC_{90}=476.57, 460.83, 440.78, 415.38, and 381.67 ppm, respectively. The *Cx. quinquefasciatus* had values of LC_{50}=198.79, 172.48, 151.06, 140.69, and 127.98 ppm and LC_{90}=458.73, 430.66, 418.78, 408.83, and 386.26 ppm, respectively. The results of the leaf extract of A. alnifloia are promising as good larvicidal activity against the mosquito vector, *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* [47]. The larval and pupal mortality was found in the leaf extract of methanol

Carica papaya against the first to fourth instar larvae and pupae of values LC_{50} 51.76, 61.87, 74.07, 82.18 and 440.65 ppm, respectively [48]. In the present results, *M. citrifolia* against *Cx. quinquefasciatus*, the LC_{50} and LC_{90} values of first to fourth-instars larvae and pupae were LC_{50} values of 226.70, 256.97, 290.05, 316.33 and 358.11 ppm at 24h; 139.30, 153.86, 171.95, 195.05 and 218.22 ppm at 48; The LC_{90} values of 560.35, 652.07, 733.03 797.09 and 875.25 ppm at 24; 388.58, 572.12, 585.09, 611.06 and 655.17 ppm at 48 h, respectively.

In conclusion, we sought to determine whether a methanol leaf extract from *M. citrifolia* could be used for mosquito control. We observed a functional response by all immature life stages of mosquito vectors, *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* to the natural larvicidal product extracts, the crude extracts of *M. citrifolia*. Therefore, this study provides first report on the mosquitocidal activities against three species of mosquito vectors of this plant extract from India. This is new eco-friendly approaches for the control of mosquito vector as target species.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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