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

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**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, 534.51, 624.16 and 756.79 ppm, respectively. The *Cx. quinquefasciatus* had values of LC\(_{50}\)=226.70, 256.97, 290.05, 316.33 and 358.25 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 peri-domestic 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...
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 saponnins, 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, octaeanonic 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, ursoic 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, epiderhydromethoxygaertneroside, 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 °C, 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 Taxonomist, Department of Botany, University of Madras, Chennai, Tamil Nadu. The voucher specimen has been deposited Department of Zoology, Bharathiar University, Coimbatore. *M. citrifolia* leaves were washed with tap water and shade dried at room temperature (28±2 °C) for 10 to 20 days. The air-dried plant materials (leaves) were powdered by an electrical blender. From the leaf, 1 kg powder 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 vacuum evaporator and stored at a temperature of 4 °C. 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 introduced 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 LC50 and LC90 were calculated from toxicity data by using probit analysis [18].

3. Results

The result shows that mortality effects of methanol leaf extract of *M. citrifolia* against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* at 24 and 48 h, respectively (Table 1, 3, 5).

| 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±1.29ke   | 39.6±1.11de |
| 48        |       | 65.6±1.70bc  | 63.2±1.16cd   | 57.7±1.42c   | 54.7±1.95cd   | 51.2±1.41cd |
| 300       | 24    | 69.5±1.77b   | 60.7±1.62c    | 58.3±1.98c   | 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±1.29b   | 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.

| Mosquito larval instars and pupae | Exposure hours | Regression equation | LC50 values (LFL–UFL) (ppm) | LC90 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 (115.79–228.85) | 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 (523.37–727.72) | 0.60*                     |

LC50 – Lethal concentration that kills 50% of the exposed larvae and pupae, LC90 – Lethal concentration that kills 90% of the exposed larvae and pupae, LFL = Lower fiducial limit, UFL = Upper fiducial 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    | 90.0±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 | $L_{50}$ values (LFL–UFL) (ppm) | $L_{90}$ 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*         |

$L_{50}$ – Lethal concentration that kills 50% of the exposed larvae and pupae; $L_{90}$ – 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     | 45.3±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    | 100.0±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 | LC₅₀ values (UFL–LFL) (ppm) | LC₉₀ values (UFL–LFL) (ppm) | x² (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             |                     | 117.83 (88.12–140.45)       | 281.22 (256.84–314.34)      | 4.59*       |
|                                  |                |                     | 159.07 (130.03–182.69)      | 363.48 (334.52–402.12)      | 3.98*       |
| Second instars                  | 24             | Y = -0.997 +0.006X  | 133.07 (32.38–184.75)       | 310.16 (254.14–436.06)      | 7.02*       |
|                                  | 48             |                     | 130.03 (94.41–221.62)       | 388.56 (328.53–507.12)      | 5.46*       |
| Third instars                   | 24             | Y = -1.020+0.006X   | 172.16 (126.85–194.85)      | 436.51 (397.55–491.27)      | 1.81*       |
|                                  | 48             |                     | 139.44 (113.31–171.82)      | 361.74 (331.72–402.12)      | 3.36*       |
| Fourth instars                  | 24             | Y = -0.779 +0.005X  | 185.08 (126.85–194.85)      | 436.51 (397.55–491.27)      | 1.81*       |
|                                  | 48             |                     | 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             |                     | 149.58 (108.96–180.57)      | 419.19 (381.54–471.83)      | 0.31*       |

LC₅₀ – Lethal concentration that kills 50% of the exposed larvae and pupae; LC₉₀ – Lethal concentration that kills 90% of the exposed larvae and pupae; LFL = Lower fiducial limit, UFL = Upper fiducial limit, x² – 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 LC₅₀=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, respectively (Table 2). The *Ae. aegypti* had values LC₅₀=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 LC₅₀ 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 *C. quinquefasciatus* had values of LC₅₀=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 LC₅₀=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 LC₅₀ 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 0.86 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 pipennes* [27]. These studies were based on plant extract against mosquito larvae. In the present results, *M. citrifolia* against *An. stephensi* the LC₅₀ and LC₉₀ values of first to fourth-instars larvae and pupae were LC₅₀ 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 LC₉₀ 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. pippens* 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 LC₉₀
of nanopermethrin and bulk permethrin to *C. quinquefasciatus* was 0.117 and 0.715 mg/L, respectively [30]. Sakuluki et al. [31] have reported the low release rate of nanoeumulsion with large droplet size that resulted in prolonged mosquito repellent activity compared to the nanoeumulsion 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]. Changhunjong 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$_{50}$ was 1,066.93 mg/l.

Mathew et al. [34] reported that leaf chloroform extracts of Nyctanthes arboristis 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 *A. 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 *A. 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 *A. 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 *A. stephensi* and *Ae. aegypti*, respectively. Larvicidal activity of flower methanol extract showed LC$_{50}$ values 233 and 302.5 ppm against *A. 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 μg/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$_{50}$ 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*. Previous reports on extracts of *Psammaphyllis purpurea* and Haliclama cribicurita 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 μg m$^{-1}$ against *P. falciparum* [40]. Recent studies on the larval and pupal mortality of *A. stephensi* after the treatment of methanolic extract of Clerodendrone inerm 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. inerm* leaf extract of larval and pupal mortality of *A. 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 *A. 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 aequous 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 *C. quinquefasciatus* the LC$_{50}$ value of 104.66, 127.71, 173.75, and 251.65 ppm, respectively. The LC$_{50}$ 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 *A. stephensi* we observed ≥95% mortality after 24 h from 256 ppm. Tests with latex showed 99% mortality at 64 ppm for *A. 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$_{50}$=9.51 and 13.65 ppm, and LC$_{90}$=28.13 and 35.83 ppm respectively. The methanol leaf extract of *C. gigantea* against *C. quinquefasciatus* the LC$_{50}$ value of 104.66, 127.71, 173.75, and 251.65 ppm, respectively. The LC$_{50}$ 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].

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 LC50 51.76, 61.87, 74.07, 82.18 and 440.65 ppm, respectively [48]. In the present results, M. citrifolia against Cx. quinquefasciatus, the LC50 and LC90 values of first to fourth–instars larvae and pupae were LC50 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 LC50 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 larvicide 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.

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