

Original Research Article

Evaluation of larvicidal effects of five different tropical plant extracts against filarial vector *Culex quinquefasciatus*

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Received: 16 November 2025

Revised: 16 December 2025

Accepted: 19 December 2025

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ABSTRACT

Background: The aim of the study was to assess the larvicidal activity of leaf extracts of *Sphaegneticola trilobata*, *Syzygium cumini*, *Averrhoa carambola*, *Tamarindus indica* and *Citrus maxima*.

Methods: The 3rd and 4th instar larva of *Culex quinquefasciatus* were tested to evaluate larval mortality after 24-hour exposure to different plant leaf extracts. Larvicidal activity of *S. trilobata* leaf extract, *S. cumini*, *A. carambola*, *T. indica*, *Culex quinquefasciatus*, and *C. maxima* leaf was observed at different concentrations of distilled water (1250-10000 ppm), acetone (250-850 ppm), methanol (2000-3500 ppm) and 50% ethanol (3000-5000 ppm).

Results: Larval mortality was checked after 24h of exposure and lethal concentrations at LC50 of *S. trilobata* were 4183.866 ppm, 486.109 ppm, 2478.026 ppm, 3541.842 ppm respectively and at LC90 were 11717.896 ppm, 908.933 ppm, 3321.521 ppm, 4892.513 ppm respectively. Lethal concentrations of *S. cumini* at LC50 were 9045.301 ppm, 476.714 ppm, 1797.028 ppm, 2825.363 ppm respectively and at LC90 were 14411.664 ppm, 1070.723 ppm, 2433.824 ppm, 4211.212 ppm respectively. And, for *A. carambola* lethal concentrations at LC50 were 8405.722 ppm, 927.011 ppm, 2080.519 ppm, 4977.002 ppm respectively and at LC90 were 13438.102 ppm, 1455.087 ppm, 3020.974 ppm, 6165.633 ppm respectively. For *T. indica*, application dosage was 7038.103 ppm, 565.073 ppm, 1739.942 ppm, and 3876.449 ppm. Again, for *Culex quinquefasciatus* larvae, the larvicidal LC50 values of *C. maxima* leaf extract at the application dosage are 2931.936 ppm, 633.329 ppm, 1306.784 ppm, and 1710.784 ppm respectively.

Conclusions: These results indicate the potential of the phytochemicals derived from experimental plants.

Keywords: *Culex quinquefasciatus*, *Sphaegneticola trilobata*, *Syzygium cumini*, *Averrhoa carambola*, *Tamarindus indica*, *Citrus maxima*

INTRODUCTION

Human lymphatic filariasis acts as one of the most threatening diseases across the world by *Culex* mosquitoes.¹ *Culex* mosquitoes are able to transmit deadly diseases like St. Louis encephalitis, West Nile viral fever and Japanese encephalitis. The best way to control these

diseases is to control the vector species first. Indiscriminate use of synthetic chemicals as mosquito control measure has given rise to some drastic consequences such as toxicity to non-target organisms, human health and threat to global environment.² The widespread use of these chemicals also caused resistance in the vector species.³ Chemicals derived from plant parts have been proved to be efficient tool in mosquito control

programme, since they can act as toxicants, repellent, growth inhibitors, reproductive inhibitors and oviposition deterrent.⁴ In addition, these repellents derived from plants are rapidly biodegradable, eco-friendly, comparatively cheaper and also less harmful to non-target organisms of ecosystem. Although the use of phytochemicals has been on practice since 1920, the discovery of synthetic chemicals alternated them in mosquito control programme.⁵

Phytochemicals may be extracted from various parts of plants like fruits, leaves, stems, barks, roots etc. Plants from families- Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Rutaceae show larvicidal, adulticidal or repellent activities against different mosquito species. Variation of larvicidal potential of the same plant changes with different solvents use.⁶ The essential oil extracted from leaves of *Sphaegneticola trilobata* act as antioxidant, antibacterial hepatoprotective, cough relieving agent, antifungal, anti-inflammatory, analgesic agent. The phenolics found in the plant extracts of *Syzygium cumini* also show significant antioxidant activity.⁷ The leaves are enriched with flavonol glycosides quercetin, myricitin, triterpenoids, galloyl carboxylase.^{8,9}

The raw leaves of *Averrhoa carambola* also have an acidic flavour and used as a sorrel substitute. Phytochemicals found in different parts of *Citrus maxima* plant include different chemical classes such as alkaloids, saponins, carbohydrates, flavonoids, glycosides, phenols, carotenoids, amino acids, terpenoids, anthraquinone.¹⁰ Phytochemical experiment on *Tamarindus indica* revealed the presence various types of active ingredients such as L-(-) malic acid tartaric acid, arabinose, xylose galactose, uronic acid, phenolic compounds cardiac glycosides.¹¹⁻¹³ Some notable research works evaluating the toxic effects of plant leaf extracts against the filarial vector *Culex quinquefasciatus* larvae have been reported all over the world.¹⁴⁻³² The present study investigates the larvicidal potential of five indigenous plant leaf extracts as an environmentally safe measure to control filarial vector *Culex quinquefasciatus*.

METHODS

The study was conducted from March 2022 to November 2022. The study was conducted in the Entomological Research Laboratory of Department of Zoology, University of Chittagong. The University of Chittagong is situated in mouza jangal Paschim-patty of Fatehpur Union under Hat hazari Upazila with 1754 acres of campus area. Latitudes of the campus area is between 20°27'30" N and 20°29'0" N and the longitude is between 90°46'30" E and 91°47'46" E. The area comprises of hilly area, plains lands and valleys. Both the hilly area and plain lands are covered with different types of plants such as trees, herbs, shrubs. Trees like Neem, Mahagony, Jarul, Hijol, Shegun, Krishnachura can be found here. Besides, different types of shrubs such as Dhutra, Bishkathali, Wedelia, Lantana,

Vat, Tagar, Hatirshor etc. are also found to be naturally grown in the open lands of the campus.

The fresh leaves of *Sphaegneticola trilobata*, *Syzygium cumini*, *Averrhoa carambola*, *Tamarindus indica* and *Citrus maxima* were collected from the campus area. The larvae of the experimental mosquito *Culex quinquefasciatus* Say were also collected from the natural breeding ground of mosquito across the campus area. The solvents used for extraction of phytochemicals were provided from departmental store. To evaluate the toxicity of leaf extracts of five indigenous plant species: *Sphaegneticola trilobata*, *Syzygium cumini*, *Averrhoa carambola*, *Tamarindus indica* and *Citrus maxima* on *Culex quinquefasciatus* larvae, short term bioassays were conducted in the entomological research laboratory of Department of Zoology, University of Chittagong. The time period required for the bioassay was between March 2022 to November 2022.

Collection of the test organism

The larvae of *Culex quinquefasciatus* were collected from stagnant water sites in Chittagong University campus area by dipping method. Larvae were identified according to the key of Corbel et al (2007).¹⁴ After collecting the larvae, they were transferred into plastic containers along with the dirty water of the breeding site and then the container was covered with net. The container containing mosquito larvae was carried towards the laboratory for bioassay. In the laboratory 3rd and 4th instar larvae were separated by means of dropper and kept in petri dishes for further experiment.

Collection of the plant parts

The leaves of *Sphaegneticola trilobata*, *Syzygium cumini*, *Averrhoa carambola*, *Tamarindus indica* and *Citrus maxima* were collected from the Chittagong University campus area. All the plant parts were examined carefully and washed with tap water. The fresh and uninfested plant parts were taken for the experiment. Then they were dried in shade and kept in oven at 40°C for five to six hours before grinding. After that the plant parts were grinded in electric grinder separately and sieved using a 60-mesh sieve to obtain fine powder.

Extraction preparation

The powder of dry leaves was weighted in balance and added to the solvent as follow-

10 g of leaf powder in 100 ml of solvents such as distilled water, methanol, acetone and 50% ethanol. The required amount of dry leaf powder was placed in a 500 ml conical flask and required amount of solvent was poured into the conical flask and then plugged with a cork stopper. The flask was shaken vigorously for mixing the powder with the solvents perfectly and then kept for three hours to ensure maximum extraction of toxic components of the

leaf powder. After three hours, the resultant solution was filtered through a muslin cloth. This filtered resultant solution is the stock solution of the experimental leaf. The stock solution preparation was done on the experimental day.

Bioassays

Prior to the final experiments several preliminary bioassay were conducted in the laboratory at room temperature to final out 1-99% mortality of different extracts obtained from the leaf of *Sphaegneticola trilobata*, *Syzygium cumini*, *Averrhoa carambola*, *Tamarindus indica* and *Citrus maxima* against the mosquito larvae of *Culex quinquefasciatus* say.

In each beaker, 10 larvae were released through dropper. For each concentration three replicates were conducted to check the mortality and a control (tap water) was also included with each concentration. Larvae were considered dead if they showed no sign of movement even after being touched with a glass rod. After 24 hours the larval mortality was recorded. The percentage of larval mortality was calculated using the following formula.

$$\text{Mortality} = \frac{\text{No. of dead larvae}}{\text{No. of larvae introduced}} \times 100$$

Statistical analysis

The LC₅₀ and LC₉₀ values were estimated by using probit analysis. The regression equation was deducted from empiric probit, working probit, weighting probit was calculated from the respective empirical probit.

The values of Chi-square (X²) were determined by using experimental data such as expected number of mosquito larvae killed and observed number of mosquito larvae killed and then compared with the tables of the statistics for (n-1) degrees of freedom at 5% and 1% level of significance.

The relative potency (a reciprocal of the equitoxic doses) was obtained by taking the highest LC₅₀ value as unity and dividing the highest LC₅₀ value of toxicant with the respective LC₅₀ values of each toxicant for each mosquito larvae.

Authentication of the plant materials

The plant species used in this study were authenticated based on their morphological characteristics using standard taxonomic keys and regional floristic literature. Fresh and healthy leaves were collected from the Chittagong University campus and cross-verified with available botanical descriptions to ensure correct species identification. Only correctly identified, mature, and disease-free plant materials were selected for extraction to maintain consistency and reliability of the experimental results.

RESULTS

The different aqueous extract of (distilled water, acetone, methanol and 50% ethanol) of dry leaves of *Sphaegneticola trilobata*, *Syzygium cumini*, *Averrhoa carambola*, *Tamarindus indica* and *Citrus maxima* exhibited prominent larvicidal activity of different levels against the larvae of *Culex quinquefasciatus*. The effectiveness of these extracts upon mosquito larvae was assessed at various concentrations under normal laboratory conditions.

The percentages of larval mortality after 24-hour exposures to the five concentrations of this aqueous plant leaf extract is presented in Table 1. Mortalities increased with an increase in the concentration of the aqueous plant leaf extracts. There was no mortality in control. The regression equation, P value of Chi-square of the leaf extracts are given in Table 1. The graphically representation of probit mortality lines for the leaf extracts of *S. trilobata*, *S. cumini*, *A. carambola*, *T. indica* and *C. maxmima* are shown in the Figure 1. Effects of the extracts of *S. trilobata*, *S. cumini*, *A. carambola*, *T. indica* and *C. maxmima* leaf extracts on *Culex quinquefasciatus* larvae.

The mortality ranges of *Culex quinquefasciatus* laevae varied from 6.67-96.67%, 10.00-96.67%, 10.00-96.67, 10.00- 96.67% and 10.00-96.67% for *S. trilobata*, *S. cumini*, *A. carambola*, *T. indica* and *C. maxmima* respectively. The Chi-square values of acetone, methanol and 50% ethanol extracts of *S. trilobata* showed insignificance at 0.01 and 0.05 levels which indicate observed mortality was same as expected mortality, whereas Chi-square value of distilled water extract showed significance at 0.05 level and insignificance at 0.01 level.

The Chi-square values of distilled water, methanol and 50% ethanol extracts of *S. cumini* leaf showed insignificance at 0.01 and 0.05 levels whereas the acetone extracts showed significance at 0.01 and 0.05 levels. In case of *A. carambola* leaf, the values of Chi-square of distilled water, acetone, methanol, 50% ethanol extracts showed insignificance at 0.01 and 0.05 levels which means all the extracts of *A. carambola* leaf showed observed mortality, Chi-square values of distilled water, methanol, acetone extracts of *C. maxmima* leaf showed insignificance at 0.05 levels.

In case of *T. indica* leaf Chi-square values of distilled water, methanol, 50% ethanol extracts showed significance at 0.01 and 0.05 levels, whereas acetone extracts showed significance at 0.05% level but insignificance at 0.01% level. During the experimental period, larvicidal activity of dry leaf powder of *S. trilobata*, *S. cumini*, *A. carambola*, *T. indica* and *C. maxmima* of different solvents such as distilled water, acetone, methanol and 50% ethanol were tested against the 3rd and 4th inster larvae of *Culex quinquefasciatus*. The larvicidal activity of the leaf extracts varied with the doses and solvents also.

Table 1: Toxicity of distilled water, methanol, acetone and 50% ethanol extracts of *S. trilobata*, *S. cumini*, *A. carambola*, *T. indica* and *C. maxima* leaves on *Culex quinquefasciatus* larvae exposed for 24 hours.

Extracts	Solvent	Dose range (ppm)	Mortality range (%)	Regression equation	χ^2 value
<i>Sphaegneticula trilobata</i>	Distilled water	1250-10000	6.67-93.33	3.044x-6.044	10.088 0.01<p<0.05
	Acetone	250-850	10.00-93.33	4.449x-6.958	6.305 p>0.01>0.05
	Methanol	2000-3500	13.33-93.33	10.936x-32.112	4.681 p>0.01>0.05
	50% ethanol	3000-5000	10.00-96.67	11.909x-37.695	2.145 p>0.01>0.05
<i>Syzygium cumini</i>	Distilled water	6000-14000	13.33-93.33	16.131x-19.288	5.381 p>0.01>0.05
	Acetone	200-1000	13.33-96.67	3.770x-5.101	13.454 P<0.01<0.05
	Methanol	1300-2500	10.00-96.67	10.902x-30.476	9.23 p>0.01>0.05
	50% ethanol	2000-4000	16.67-96.67	7.771x-21.774	8.716 p>0.01>0.05
<i>Averrhoa carambola</i>	Distilled water	5000-12000	10.00-93.33	6.096x-18.938	4.348 p>0.01>0.05
	Acetone	600-1400	13.33-93.33	6.816x-15.268	5.399 p>0.01>0.05
	Methanol	1000-2000	20.00-96.67	7.958x-19.846	4.22 P>0.01>0.05
	50% ethanol	1000-3000	16.67-93.33	4.771x-10.444	5.716 P>0.01>0.05
<i>Tamarindus indica</i>	Distilled water	4000-12000	13.33-96.67	5.676x-16.773	22.17 P<0.01<0.05
	Acetone	400-800	16.67-93.33	10.708x-24.501	46.598 P<0.01<0.05
	Methanol	1000-3000	13.33-96.67	6.601x-16.265	36.603 P<0.01<0.05
	50% ethanol	3000-5000	10.00-96.67	14.181x-45.862	7.12 P>0.01>0.05
<i>Citrus maxima</i>	Distilled water	2000-4000	10.00-90.00	8.50x-24.45	2.131 P>0.01>0.05
	Acetone	400-1000	13.33-96.67	6.749x-13.955	11.684 0.01<P<0.05
	Methanol	1000-2000	20.00-96.67	7.958x-19.846	4.22 P>0.01>0.05
	50% ethanol	1000-3000	16.67-93.33	4.771x-10.444	5.716 P>0.01>0.05

Table 2: The LC50 and LC90 values along with their confidence limits for leaf extracts of *S. trilobata*, *S. cumini*, *A. carambola*, *T. indica* and *C. maxmima* on *Culex quinquefasciatus* larvae.

Extracts	Extract	LC ₅₀ (ppm)	Confidence limit	
			Lower	Upper
<i>Sphaegneticula trilobata</i>	Distilled water	4183.866	3418.799	5056.931
	Acetone	2478.026	2342.290	2614.334
	Methanol	486.109	429.049	544.068
	50% ethanol	3541.842	3661.684	4017.341
<i>Syzygium cumini</i>	Distilled water	9045.301	8241.021	3825.149
	Acetone	1797.028	1690.887	1900.984

Continued.

Extracts	Extract	LC ₅₀ (ppm)	Confidence limit	
			Lower	Upper
	Methanol	476.714	402.511	551.389
	50% ethanol	2825.363	2615.535	3032.221
<i>Averrhoa carambola</i>	Distilled water	8405.722	7665.115	9158.360
	Acetone	2080.519	1933.407	2227.422
	Methanol	927.011	848.669	1005.098
	50% ethanol	4977.002	4782.428	5177.202
<i>Tamarindus indica</i>	Distilled water	7038.103	6297.873	7770.696
	Acetone	1739.942	1550.184	1926.577
	Methanol	565.073	523.107	606.444
	50% ethanol	3876.449	3698.916	4051.779
<i>Citrus maxima</i>	Distilled water	2931.936	2740.388	3130.407
	Acetone	1306.784	1206.080	1396.353
	Methanol	633.321	577.408	687.725
	50% ethanol	1710.784	1505.888	1910.202

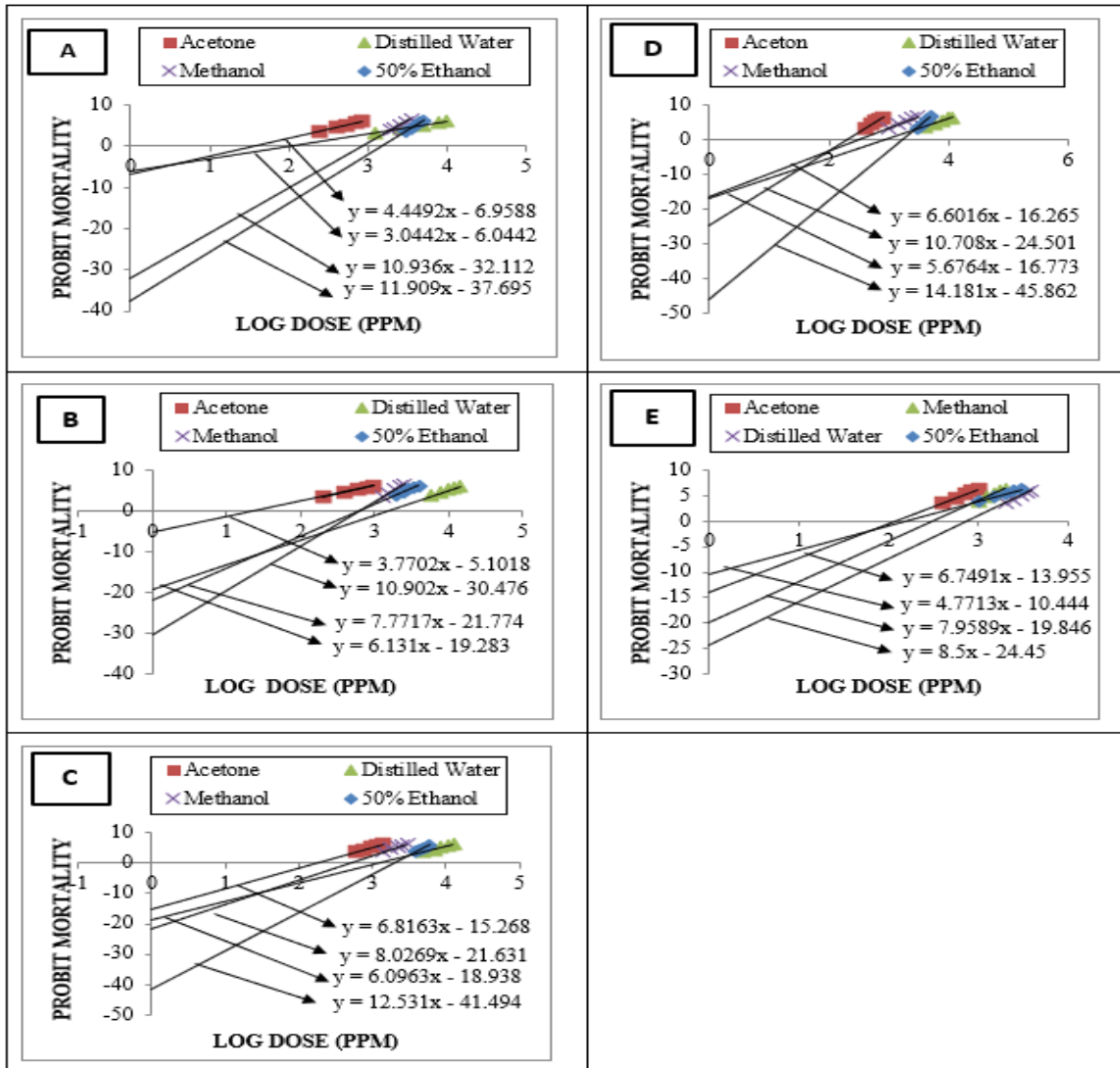


Figure 1: Regression lines for determining the LC₅₀ of distilled water, acetone, methanol and 50% ethanol extracts of the leaf of *S. trilobata* (A), *S. cumini* (B), *A. carambola* (C), *T. indica* (D) and *C. maxima* (E) respectively on *Culex quinquefasciatus* larvae after 24 hours of exposure.

DISCUSSION

The present research work found that the distilled water leaf extract of *S. cumini* was least toxic with highest LC₅₀ value (9045.301 ppm) and acetone leaf extract of *S. cumini* was the most toxic among the all-leaf extracts with lowest LC₅₀ value (476.714 ppm). Evaluating the all five experimental plants the order of toxicity of the four solvents was: acetone extract of *S. cumini* leaf > acetone extract of *S. trilobata* leaf > acetone extract of *T. indica* leaf > acetone extract of *C. maxima* leaf > acetone extract of *A. carambola* leaf > methanol extract of *C. maxima* leaf > 50% ethanol extract of *C. maxima* leaf > methanol extract of *T. indica* leaf > methanol extract of *S. cumini* leaf > methanol extract of *A. carambola* leaf > methanol extract of *S. trilobata* leaf > 50% ethanol extract of *S. cumini* leaf > distilled water extract of *C. maxima* leaf > 50% ethanol extract of *S. trilobata* leaf > 50% ethanol extract of *T. indica* leaf > distilled water extract of *S. trilobata* leaf > 50% ethanol extract of *A. carambola* leaf > distilled water extract of *A. carambola* leaf > distilled water extract of *S. cumini* leaf. Although the larvicidal activity depends on the active ingredients present on the plant leaves, the solubilizing capacity of the experimental solvents makes a lot of difference. The LC₅₀ value of distilled water leaf extracts of *S. trilobata* was 4183.866 ppm which was closely related to the LC₅₀ (4150.342 ppm) of distilled water leaf extract of *Pongamia pinnata*.^{14,33} The LC₅₀ of acetone leaf extract of *S. trilobata* can be compared with the LC₅₀ of chloroform leaf extract of *Momordica charantia* (465.85 ppm).^{15,34} The LC₅₀ value of methanol leaf extract of *S. trilobata* was 2478.028 ppm with methanol leaf extract on *Chromoleana odorata* (LC₅₀ 2381.00 ppm).

In case of 50% ethanol leaf extract of *S. trilobata*, the LC₅₀ value (3541.842 ppm) was very much similar with the LC₅₀ value of distilled water leaf extract of *Aegele mermelos* (3281.914 ppm) studied by Nasiruddin *et al.* (2018).^{14,35} LC₅₀ value of distilled water leaf extract of *S. cumini* was 9045.301 ppm which disagreed with the LC₅₀ of distilled water leaf extract of *Nerium oleander* (48200 ppm). Again, Prabakar and Jabanessan (2004) studied the effectiveness of chloroform leaf extract of *Trichosanthes anguina* with LC₅₀ of 567.81 ppm which was very close to the LC₅₀ value of acetone leaf extract of *S. cumini* (LC₅₀ 476.714 ppm). Whereas LC₅₀ of methanol leaf extract of *S. cumini* (1797.028 ppm) was almost similar to the LC₅₀ of chloroform leaf extract of *Citnullus vulgaris* (1636.04 ppm).^{15,36} LC₅₀ of 50% ethanol leaf extract of *S. cumini* was 2825.363 ppm which agreed with the findings Siam *et al.* (2021) with methanol leaf extract of *Swietenia mahagoni* (LC₅₀ 2831.023).

The LC₅₀ of distilled water extract of a *A. carambola* leaf was 8405.722 ppm which disagreed with the LC₅₀ of distilled water extract of *Mallotus nudiflorus* leaf (27644.562 ppm).^{16,37} Acetone leaf extract of *A. carambola* having LC₅₀ value of 927.011 ppm showed very close similarity with the LC₅₀ of Chloroform leaf

extract of *Luffa acutangola* (839.81 ppm).^{15,32} The LC₅₀ of methanol extract of *A. carambola* leaf was 2080.519 ppm which was quite similar with the LC₅₀ of methanol extract of *Cinnamomum tamala* leaf (2169.021 ppm).^{17,34} The 50% ethanol leaf extract of *A. carambola* showed LC₅₀ value of 4977.002 ppm which disagreed with the LC₅₀ of alcoholic leaf extract of *Eucalyptus camaldulensis* (736.00 ppm).¹⁸ The LC₅₀ of distilled water leaf extract of *T. indica* was 7038.103 ppm which disagreed with the LC₅₀ of distilled water leaf extract of *Aphanamixis polystachya* (25547.949 ppm).¹⁶ The acetone leaf extract of *T. Indica* having the LC₅₀ value 565.073 ppm was almost related to the methanol leaf extract of *Annona squamosa* (566.964 ppm).¹⁷

The methanol extract of *T. indica* showed LC₅₀ value of 1739.942 ppm which was almost similar to the LC₅₀ value of methanol leaf extract of *Lantana camera* (1775.666 ppm) of Tusslin (2016).¹⁷ The 50% ethanol leaf extract of *T. indica* showed LC₅₀ value of 3876.449 ppm which disagreed with the 50% ethanol leaf extract of *Pinus caribea* (713.00 ppm).²⁰ LC₅₀ of distilled water leaf extract of *C. maxima* was 2931.936 ppm which was very close to the LC₅₀ of *Swietenia mahagoni* 50% ethanolic leaf extract (2749.526 ppm) as studied by Siam *et al.* (2021).¹⁶ Qureshi *et al.* (2017) observed the toxic potential of *Ocimum basilicum* and *Mentha piperita* against *Culex quinquefasciatus* larvae.¹⁹ The LC₅₀ value of *O. basilicum* leaf with distilled water extract against 3rd instar larvae were 512.22 ppm which indicates similarities with the LC₅₀ value of acetone extract of *T. indica* leaf (565.073 ppm) of the current study. The LC₅₀ of methanol leaf extract of *C. maxima* (1306.784 ppm) was somewhat similar with the chloroform leaf extract of *Benineasa cerifera* (1189.30).¹⁵ The 50% ethanol leaf extract of *C. maxima* showed LC₅₀ value of 1710.784 ppm having close relation with the methanol leaf extract of *Eucalyptus globules* (1634.808 ppm).^{17,38}

This study was conducted under controlled laboratory conditions using short-term (24-hour) bioassays, which may not fully reflect the effectiveness of plant extracts under natural field conditions. Additionally, the active phytochemical constituents responsible for larvicidal activity were not isolated or characterized, and non-target organism toxicity was not assessed, limiting ecological generalizability.

CONCLUSION

The co-existence and evolution of plant along with insects made them capable of producing a plethora of chemical defences, which may be used as eco-friendly insecticides. As plants have been screened for their insecticidal potential against mosquitoes and some of them have been found to be promising. *Culex quinquefasciatus* is considered as an invasive species as it shows significant detrimental impacts on the indigenous species or native eco-system along with human or vertebrate animal health. The leaf extracts of *Sphaegneticola trilobata*, *Syzygium*

cumini, *Averrhoa carambola*, *Tamarindus indica* and *Citrus maxima* have revealed to be in possession of active ingredients that if properly utilized can serve as an alternative for the control of mosquito larvae. Among all the solvents acetone showed the highest solubility capability as all the acetone leaf extracts were highly toxic. Again, all four extracts of *C. maxima* leaf powder were highly toxic whereas the acetone leaf extract of *S. cumini* was the highest toxic among all of twenty types of extracts. So, the present study suggests the further evaluation of the *C. maxima* leaf powder in broad sense and in case of solvent acetone can be suggested because of its high solubility power of botanicals.

ACKNOWLEDGEMENTS

Authors would like to acknowledge all the person associated with this study.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Islam MR, Akter S, Nasiruddin M, Awal MA, Haider MM. Evaluation of larvicidal effects of five different tropical plant extracts against filarial vector *Culex quinquefasciatus*. Int J Res Med Sci 2026;14:42-9.