



## Mosquito larvicidal potential of *Piper longum* L. leaf extract and fractions

N R Padma Priya<sup>1\*</sup>, R D Stevens Jones<sup>2</sup>

<sup>1</sup> Research Scholar, Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil, Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India

<sup>2</sup> Associate Professor, Department of Zoology and Research Centre, Scott Christian College (Autonomous), Nagercoil, Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India

### Abstract

To evaluate the larvicidal efficacy of crude and fractionated extracts of *Piper longum* leaf against *Aedes aegypti*, and *Culex quinquefasciatus* mosquitoes. The larvicidal activity was carried out by World Health Organization protocols. The larvicidal activity was exposed to various concentrations of *P. longum* crude and three fractionated extracts (CC-CE- 4, 6, 8) against third instar mosquitoes. The mortality of larvae was observed after 24 and 48h of exposure. Probit analysis was done for LC<sub>50</sub> and LC<sub>90</sub> values. The crude extract has 24 and 48h LC<sub>50</sub> and LC<sub>90</sub> values for *Ae.aegypti* 28.08; 48.39, 23.51; 44.56 µg/dl, and *Cx. quinquefasciatus* has values 33.32; 58.64, 26.58; 52.72 µg/dl respectively. The three fractionated extracts have values for *Ae. aegypti* 17.72; 29.80, 13.52; 23.23 µg/dl, 15.01; 27.02, 12.44; 23.77 µg/dl, 12; 25, 47, 9; 21.02 µg/dl, and for *Cx. quinquefasciatus* 19.30; 37.40, 15.36; 29.69 µg/dl, and 18.96; 36.74, 15.06; 28.6 µg/dl, and 15.98;35.21, 12; 28 and 18.96; 36.74, 15.06; 28.6 µg/dl respectively. The fractionated extracts are effective than crude extract against the third instar larvae of mosquitoes. Further studies to determine the active components of effective extracts.

**Keywords:** crude extract, fractionated extracts, larvicidal potential, mosquitoes, medicinal plant

### Introduction

Mosquito-borne diseases are considered major public health diseases affecting millions of people every year [1]. Mosquitoes transmit viruses, nematodes, and protozoan parasites from one organism to another. World Health Organization has announced mosquitoes as 'Public Enemy Number One' [2]. The day-biting mosquito *Aedes* transmits dengue, yellow fever, chikungunya viruses, and the night-biting mosquito *Culex* transmits filarial nematode, [3, 4]. Apart from this, mosquitoes cause nuisance, and their bites cause skin allergy and loss of sleep [5]. Unplanned urbanization, climate change, and enormous solid wastes are major issues associated with mosquito breeding and their population increase [6].

Targeting the immature stages is a key factor in controlling vector mosquitoes [7]. To kill larvae, chemical larvicides such as organophosphates are being used; but several disadvantages have been reported due to harmful effects to humans and other associated organisms [8, 13]. Particularly, the development of resistance to synthetic insecticides leads to a greater increase of vector mosquitoes. Synthetic chemical pesticides are causing many side effects on man, the environment, and non-target organisms. Plant extracts are good alternatives to chemical insecticides to control the mosquito population [14]. Many earlier reports confirm the bioactivity of several plants extracts [15, 17]. In the present study the *Piper longum* leaf extract screened *Aedes aegypti* and *Culex quinquefasciatus* mosquito larvae.

*P. longum* was reported to possess antifungal, antibacterial, antitumor, antiasthmatic, insecticidal, and mosquito larvicidal activities [18]. *P.longum* extracts possess larvicidal activity on mosquitoes [19, 21]. Plant extracts and its phytochemicals have been assessed as an alternative to

toxic pesticides conventionally used for insect pests control. The biodegradability into nontoxic products of natural products makes them appealing to find safer, effective, and sustainable new insecticides that can be integrated into management programs, especially because of their commercial acceptance [22]. Thus we aimed to assess the larvicidal activity of crude and fractionated extracts of *P. longum* against medically important vectors *Ae. aegypti* and *Cx. quinquefasciatus*.

### Materials and Methods

#### Collection of plant material

Fresh *P.longum* leaves were plucked from plants and cleaned thoroughly with tap water and shade dried at room temperature and the dried samples were powdered using an electric blender and the fine powder was transferred to airtight containers for further use.

#### Preparation of crude extracts

A quantity of 20g of dried leaf powder was extracted in 200ml of ethanol using the soxhlet apparatus to obtain the extract. The final extract of *P. longum* collected was concentrated under a rotary vacuum evaporator and preserved in air-tight bottles for conducting further experiments.

#### Fractionation of *P. longum*

The crude extract obtained from ethanol, which is to be fractionated by column chromatography (silica gel 60-120 mesh). The column was initially packed and compacted using petroleum ether. The column was thoroughly checked for close packing silica gel. The extract dissolved in ethanol was mixed with a non-polar solvent, chloroform in the

following proportions. Chloroform was mixed with ethanol in the ratio, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. The different fractions were collected separately in numbered 50 ml beakers.

Each fraction was checked for the presence of active ingredients using thin-layer chromatography (TLC). TLC plates were prepared using 40- micron size silica gel slurry spread on glass plates and activating in a hot air oven at 100°C for about 1 hour. The plates were cooled and about 1µl of each chromatic fraction was loaded at the base of the plate using a micropipette. The plates were placed inside a chamber containing about 50 ml of a solvent mixture containing 5:5 ethanol and chloroform. The chamber was closed with a glass lid and the TLC plate was taken out when the solvent reaches the top portion of the plate. The movement of the solute was followed by developing the plate inside another chamber concentrated with iodine vapors.

### Test mosquitoes

Egg cards of *Ae. aegypti* and *Cx. quinquefasciatus* were bought from the Centre for Research in Medical Entomology (CRME), ICMR, Madurai. The eggs were incubated in the laboratory in two separate trays containing tap water. The hatched out larvae were fed with powdered dog biscuits and yeast in the ratio of 3:1.

### Exposure of mosquito larvae to *P. longum* leaf extract

The third instar *Ae. aegypti* and *Cx. quinquefasciatus* were raised in the laboratory and removed for the experiments at the appropriate time. Crude extract prepared using ethanol and extracts prepared from three specific fractions (CC-CE 4 -6:4, CC-CE 6-4:6, CC-CE 8-2:8) were tested against third instar mosquito larvae. The larvae were exposed to the toxicants in clean 100 ml glass beakers. For the crude extract concentrations ranging from 5-50 (µg/dl) were tested against *Ae. aegypti* and 6-60(µg/dl) were tested against *Cx. quinquefasciatus* larvae. Similarly analyzing the mortality of mosquito larvae after 48hr of exposure, the mortality of *Ae. aegypti* and *Cx. quinquefasciatus* was assessed for the earlier concentration levels. For CC-CE 4, CC-CE 6, CC-CE

8 concentrations ranging from 3-30 (µg/dl) were tested against *Ae. aegypti* and 4-40 (µg/dl) were tested against *Cx. quinquefasciatus*. Similarly analyzing the mortality of mosquito larvae after 48hr of exposure, the mortality of *Ae. aegypti* and *Cx. quinquefasciatus* was assessed for the earlier concentration levels. In each concentration four replicates were maintained. The exposed larvae were continuously monitored, but mortality was recorded after 24h and 48h. Mortality was recorded for a maximum period of 48h, as the larvae molted into the fourth instar any time after 48h. The molting was speeded up when the larvae were transferred to the toxicant solutions.

### Data Analysis

The mortality data of larvae were subjected to probit analysis for calculating LC<sub>50</sub>, LC<sub>90</sub>, and other statistics at 95% confidence limits of the upper confidence limit (UCL) and lower Confidence limits (LCL), were calculated by using the dose-effect probit analysis [23]. ANOVA and Tukey analyses were carried out to understand the impact of the different types of extracts on both *Ae. aegypti* and *Cx. quinquefasciatus*.

### Results

From the crude extract, nine eluted fractions were produced by column chromatography fractionation. All fractions were preliminarily screened for larvicidal activity against fourth instar larvae of *Ae. aegypti* at various concentrations(7-56 µg/dl). Among the nine fractions CC-CE 8 has the highest larvicidal activity, and CC-CE 6, CC-CE-4 has moderate activity. LC<sub>50</sub> and LC<sub>90</sub> values were 23.54; 49.49, 38.38; 63.38, and 39.13; 66.53 µg/dl respectively. For that reason, CC-CE-8, CC-CE-6, and CC-CE-4 were selected for the larvicidal bioassay. The larvicidal activities of *P. longum* leaf extract against *Ae. aegypti*, and *Cx. quinquefasciatus* were presented in table1. The 24 and 48h LC<sub>50</sub> and LC<sub>90</sub> values are highest for crude extracts, and CC-CE-8 has least values. The Tukey comparison between the mosquito species indicated significantly differences between crude and fraction LC<sub>50</sub> and values (Table 2).

**Table 1:** n hr. LC<sub>50</sub>, LC<sub>90</sub> values and their confidence intervals for third instar of mosquito larvae exposed to crude and fractionated leaf extracts of *P. longum*

Extract	Mosquito	Hours of observe	Concentration (µg/dl)					
			LC <sub>50</sub>	LCL	UCL	LC <sub>90</sub>	LCL	UCL
Crude	<i>Ae. aegypti</i>	24	28.08	12.95	43.21	48.39	33.26	63.52
		48	23.51	8.38	38.64	44.56	39.23	49.89
	<i>Cx. quinquefasciatus</i>	24	33.32	15.16	51.48	58.64	40.48	76.8
		48	26.58	8.42	44.74	52.72	34.56	70.88
CC-CE4	<i>Ae. aegypti</i>	24	17.72	8.64	26.8	29.80	20.72	38.88
		48	13.52	6.18	20.86	23.23	15.89	30.57
	<i>Cx. quinquefasciatus</i>	24	19.30	7.19	31.41	37.40	25.29	49.51
		48	15.36	8.22	22.5	29.69	22.55	36.83
CC-CE6	<i>Ae. aegypti</i>	24	15.01	5.93	24.09	27.02	17.94	36.1
		48	12.44	5.10	19.78	23.77	16.43	31.11
	<i>Cx. quinquefasciatus</i>	24	18.96	6.85	31.07	36.74	24.63	48.85
		48	15.06	5.27	24.85	28.6	18.88	38.46
CC-CE8	<i>Ae. aegypti</i>	24	12.18	3.10	21.26	25.47	12.14	38.8
		48	9	1.66	16.34	21.02	13.68	28.36
	<i>Cx. quinquefasciatus</i>	24	15.98	3.60	28.36	35.21	22.83	47.59
		48	12	2.21	21.79	28	18.21	37.79

CC-CE – Column Chromatography-Chloroform Ethanol fraction, LC<sub>50</sub> – lethal concentration that kills 50% of the exposed larvae, LC<sub>90</sub> – lethal concentration that kills 90% of the exposed larvae, LCL- lower confidence limit (95%), UCL- upper confidence limit (95%).

**Table 2:** Mean n hr LC<sub>50</sub> of *P. longum* leaf extracts to third instar mosquito larvae

Sl.No.	Extract	Mosquito Species			
		<i>Ae. aegypti</i>		<i>Cx. quinquefasciatus</i>	
		LC <sub>50</sub> Time interval(in h)			
		24	48	24	48
1	Crude	<sup>a</sup> 28.08 <sup>w</sup> ±2.52	<sup>a</sup> 23.51 <sup>y</sup> ±2.13 <sup>y</sup>	<sup>a</sup> 33.52 <sup>x</sup> ±2.2 <sup>x</sup>	<sup>a</sup> 26.58 <sup>y</sup> ±2.45 <sup>y</sup>
2	CC-CE 4	<sup>b</sup> 17.72 <sup>w</sup> ±2.34 <sup>x</sup>	<sup>b</sup> 13.52 <sup>y</sup> ±2.35	<sup>b</sup> 19.3 <sup>w</sup> ±2.23 <sup>x</sup>	<sup>b</sup> 15.36 <sup>y</sup> ±2.15
3	CC-CE 6	<sup>bc</sup> 15.01 <sup>w</sup> ±2.44 <sup>x</sup>	<sup>b</sup> 12.44 <sup>y</sup> ±2.23	<sup>b</sup> 18.96 <sup>w</sup> ±2.28 <sup>x</sup>	<sup>b</sup> 15.06 <sup>y</sup> ±2.42
4	CC-CE 8	<sup>b</sup> 12.18 <sup>w</sup> ±2.19 <sup>x</sup>	<sup>b</sup> 9.0 <sup>y</sup> ±2.39	<sup>b</sup> 15.98 <sup>w</sup> ±2.2 <sup>x</sup>	<sup>b</sup> 12.0 <sup>y</sup> ±2.1

**Note:** means in a column prefixed with the same alphabets are not significantly different row-wise comparison of means suffixed with w and x- 24 h; y and z-48 h-means not significantly different when suffixed with the same alphabets.

When the 24 and 48h LC<sub>50</sub> values of *Ae. aegypti* and *Cx. quinquefasciatus* were compared (within rows) for both crude extracts and fractions, values significantly varied between the two-time interval whereas species-level comparison indicated significant variation only between 24h LC<sub>50</sub> values of the crude extracts against *Ae. aegypti* and *Cx. quinquefasciatus*. A similar comparison of LC<sub>90</sub> values

(Table 3) indicated significant variations between the crude extracts and the three fractions extracted from the crude extract. When the two mosquito species were compared, both the 24 and 48h LC<sub>90</sub> values significantly varied between the two species for the crude as well as three extracts.

**Table 3:** Mean n hr LC<sub>90</sub> of *P. longum* leaf extracts to third instar mosquito larvae

Sl.no.	Extract	Mosquito Species			
		<i>Ae. aegypti</i>		<i>Cx. quinquefasciatus</i>	
		LC <sub>90</sub> Time interval(in h)			
		24	48	24	48
1	Crude	<sup>a</sup> 48.39 <sup>w</sup> ± 2.21	<sup>a</sup> 44.56 <sup>y</sup> ±2.28	<sup>a</sup> 58.64 <sup>x</sup> ±2.76	<sup>a</sup> 52.72 <sup>z</sup> ±2.06
2	CC-CE 4	<sup>b</sup> 29.80 <sup>w</sup> ± 2.47	<sup>b</sup> 23.23 <sup>y</sup> ±2.31	<sup>b</sup> 37.40 <sup>x</sup> ±2.24	<sup>b</sup> 29.69 <sup>z</sup> ±2.28
3	CC-CE 6	<sup>b</sup> 27.02 <sup>w</sup> ± 2.37	<sup>b</sup> 23.77 <sup>y</sup> ±2.26	<sup>b</sup> 36.74 <sup>x</sup> ±2.29	<sup>b</sup> 28.67 <sup>z</sup> ±2.22
4	CC-CE 8	<sup>b</sup> 25.47 <sup>w</sup> ± 2.27	<sup>b</sup> 21.02 <sup>y</sup> ±2.4	<sup>b</sup> 35.21 <sup>x</sup> ±2.31	<sup>b</sup> 28.0 <sup>z</sup> ±2.37

**Note:** means in a column prefixed with the same alphabets are not significantly different row-wise comparison of means suffixed with w and x- 24 h; y and z-48 h-means not significantly different when suffixed with the same alphabets.

**Discussion**

Plant extracts contain bioactive compounds that can kill larvae and other life stages genetically modify, kindle deterrence, inactivate the eggs, act as phytosterilants, instill sterility or prevent normal development. Such varied targeted action cannot be expected when chemicals are used. Chemical pesticides act according to the all or none kit and go principle and are not functionally target specific. In spite of the handicaps related to administering plant-based pesticides, they are very powerful, causing the least havoc to the environment [24, 26]. *P. longum* is a medicinal plant that grows luxuriantly in the areas of Western Ghats. The leaves of this plant may be picked even from the wild. The plant contains several bioactive compounds and is insecticidal. If only these compounds are properly isolated and incorporated into mosquitocidal formulations, mosquitoes could be intercepted at different levels of development [27, 28].

In this study *P. longum* crude extract is less toxic compare to the fractions obtained from the extracts CC-CE 8 recorded the highest toxicity with a 48h LC<sub>50</sub> and LC<sub>90</sub> of 9.0±2.39; 21.02±2.4 for *Ae. aegypti*, and 12.0±2.1; 28.0±2.37 for *Cx. quinquefasciatus*. Based on Tukey analysis, the LC<sub>50</sub> of the three fractions did not vary among themselves but significantly varied from the crude extract. The crude extract is less toxic compared to the three fractions. The crude extract contains many bioactive compounds which interact with one another, reducing the toxicity of individual components. The LC<sub>50</sub> and LC<sub>90</sub> values of CC-CE 8 are the least indicating that CC-CE 8 is highly toxic to both *Ae. aegypti* and *Cx. quinquefasciatus*. But Turkey’s analysis indicated that the LC<sub>50</sub> and LC<sub>90</sub>

fractions were not significantly different among themselves.

**Conclusion**

In conclusion, the bioassay result of this study indicated the larvicidal property of *P. longum* leaf crude and fractionated extracts against *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. From the results, the fractionated groups showed significantly higher activity than the crude. Future research to the characterization of a pure compound of the active fractionated group should be explored to find a new highly efficient larvicidal substance.

**Acknowledgement**

The authors acknowledge the immense help received from the Emeritus Professor S. Sam Manohar Das, Department of Zoology and Research Centre, Scott Christian College (Autonomous), for his support in conducting the study. The authors are also grateful to authors/editors/publishers of all those articles, journals, and books from where the literature for this article has been reviewed and discussed.

**References**

1. Aregawi M, Cibulskis R, Otten M, Williams R, Dye C. World Malaria Report,2008, 190.
2. WHO. The world health report - conquering suffering, enriching humanity. Geneva, 1997.
3. Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA, Elango G. Efficacy of the larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitology Research*,2009;104:1365-1372.
4. Borah R, Kalita MC, Kar A, Talukdar AK. Larvicidal

- efficacy of *Toddalia asiatica* (Linn.) Lam against two mosquito vector *Aedes aegypti* and *Culex quinquefasciatus*. *African Journal of Biotechnology*,2010;9:2527-2530.
5. Peng Z, Yang J, Wang H, Simons FE. Production and characterization of monoclonal antibodies to two new mosquitoes *Aedes aegypti* salivary proteins. *Insect Biochemistry and Molecular Biology*,1999;29:901-914.
  6. Karunamoorthy K, Ilango K, Murugan K. Laboratory evaluation of traditionally use plant-based insect repellents against the malaria vector *Anopheles arabiensis* Patton. *Parasitology Research*,2010;106:1217-1223.
  7. Zahran HE, Abdelgaleil SA. Insecticidal and developmental inhibitory properties of monoterpenes on *Culex pipiens* L. (Diptera: Culicidae). *Journal of Asia-Pacific Entomology*,2011;14(1):46-51.
  8. Sutthanont N, Choochote W, Tuetun B, Junkum A, Jitpakdi A, Chaithong U, et. al. Chemical composition and larvicidal activity of edible plant derived essential oils against the Pyrethroid-susceptible and resistant strains of *Aedes aegypti* (Diptera: Culicidae). *Journal of Vector Ecology*,2010;35:106-115.
  9. Bayen S, Occurrence, bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems-a review. *Environment International*,2012;48:84-101.
  10. Mulyatno KC, Yamanaka A, Ngadino Konishi E. Resistance of *Aedes aegypti* (L.) larvae to temephos in Surabaya, Indonesia. *The Southeast Asian Journal of Tropical Medicine and Public Health*,2012;43:29-33.
  11. Grisales N, Poupardin R, Gomez S, Gonzalez IF, Ranson H, Lenhart A. Temephos resistance in *Aedes aegypti* in Colombia compromises dengue vector control. *PLOS Neglected Tropical Diseases*,2013;7:e2438.
  12. Chen CD, Nazni WA, Lee HL, Rashid YN, Lardizabal ML, Azirun MS. Temephos resistance in field *Aedes (Stegomyia) albopictus* (Skuse) from Selangor, Malaysia. *Tropical Biomedicine*,2013;30:220-230.
  13. Chavshin AR, Dabiri F, Vatandoost H, Bavani MM. Susceptibility of *Anopheles maculipennis* to different classes of insecticides in West Azarbaijan Province, Northwestern Iran. *Asian Pacific Journal of Tropical Biomedicine*,2015;5(5):403-406.
  14. Guerrero RR, Pérez MAR, Campos MN. Toxicity of Mexican native plant extracts against larvae of *Aedes aegypti* (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine*,2015;5(4):287-291.
  15. Hayatie L, Biworo A, Suhartono E. Aqueous extracts of seed and peel of *Carica papaya* against *Aedes aegypti*. *Journal of Medical and Biological Engineering*,2015;4(5):417-421.
  16. Pavela R, Benelli G. Ethnobotanical knowledge on botanical repellents employed in the African region against mosquito vectors – a review. *Experimental Parasitology*,2016;167C:103-108.
  17. Subashini K, Sivakami R, Jeyasankar A. Larvicidal activity of *Scutellaria violacea* (Lamiaceae) leaf extracts against three important human vector mosquitoes: *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae) from Tamil Nadu, India. *International Journal of Mosquito Research*,2017;4(2):108-110.
  18. Singh A, Navneet. Critical review on various ethnomedicinal and pharmacological aspects
  19. of *Piper longum* Linn. (Long pepper or pippali) *International Journal of Innovative Pharmaceutical Sciences and Research*,2018;6(1):48-60.
  20. Lee HS. Pesticidal constituents derived from Piperaceae fruits. *Agricultural Chemistry and Biotechnology*,2005;48(2):65-74.
  21. Chaithong U, Choochote W, Kamsuk K, Jitpakdi A, Tippawangkosol P, Chaityasit D et al. Larvicidal effect of pepper plants on *Aedes aegypti* (L.) (Diptera: Culicidae). *Journal of the Society for Vector Ecology*,2006;31(1):138-144.
  22. Choochote W, chaithong U, Kamsuk K, Rattanachanpichai E, Jitpakdi A, Tippawangkosol P et al, Adulticidal activity against *Stegomyia aegypti* (Diptera: Culicidae) of three *Piper* spp. *Revista do Instituto de Medicina Tropical de São Paulo*,2006;48(1):33-37.
  23. Rimando A, Duke SO. Natural Products for Pest Management. Chapter 01. ACS Symposium Series; American Chemical Society: Washington, DC, 2006.
  24. Finney DJ. In: Probit Analysis. London, Cambridge University Press, 1971, 68-72.
  25. Ansari MA, Vasudevan MP, Tandon, Razdan RK. Larvicidal and mosquito repellent action of peppermint (*Mentha piperata*) oil. *Bioresource Technology*,2000;71:267.
  26. Duke JA. Dr. Duke's. Phytochemical and Ethnobotanical Databases. 2000. Retrieved from the World Wide Web. Hyperlink <http://www.arsgrin.gov/cgi-bin/duke/>
  27. Silverio MRS, Espindola LS, Lopes P, Vieira PC. Plant Natural Products for the Control of *Aedes aegypti*: The Main Vector of Important Arboviruses. *Molecules*,2020;25(15):3484.
  28. Pavela R, Benelli G. Ethnobotanical knowledge on botanical repellents employed in the African region against mosquito vectors – a review. *Experimental Parasitology*, 2016;167C:103–108.
  29. Dey P, Goyary D, Chattopadhyay P, Kishor S, Karmakar S, Verma AG. Evaluation of larvicidal activity of *Piper longum* against the dengue vector, *Aedes aegypti*, malarial vector, *Anopheles stephensi* and filariasis vector, *Culex quinquefasciatus*. *South African Journal of Botany*,2020;132:482-490.