

Mosquitocidal activity of *Naringi crenulata* (Maha vilvam) plant extract against *Culex quinquefasciatus* mosquito vector

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Abstract

Mosquitocidal activity of *Naringi crenulata* (Maha vilvam) was tested against the fourth instar larvae of *C. quinquefasciatus* and plant extracts to prepare different concentrations and against larvicidal activity of *C. quinquefasciatus* mosquito vector. Perusal of the data clearly indicated that maximum larval mortality in ethyl acetate extract was recorded in *C. quinquefasciatus* mosquito at 24hrs. Values were recorded in different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm, the values in the percentage of larval mortality 78.50 ± 2.71 , 59.14 ± 3.79 , 37.37 ± 6.20 and $21.35 \pm 5.35\%$ and then LC_{50} (LCL-UCL), LC_{90} (LCL-UCL) X^2 values for 2.499(2.114-2.912), 6.271(5.423-7.606)0.293 respectively. Then the values in the percentage of pupal mortality in same extract at 71.74 ± 3.45 , 53.34 ± 2.21 , 34.84 ± 3.29 and $20.11 \pm 3.07\%$ and then LC_{50} (LCL-UCL), LC_{90} (LCL-UCL) X^2 values for 2.826 (2.396-3.327), 7.098(6.037-8.865)0.186 and the values in the percentage of ovum mortality in same extract at 73.34 ± 7.45 , 51.47 ± 2.41 , 30.51 ± 3.65 and $21.36 \pm 4.71\%$ and then LC_{50} (LCL-UCL), LC_{90} (LCL-UCL) X^2 values for values in 2.827(2.419-3.299), 6.849(5.877-8.421)0.374 respectively.

Keywords: *Naringi crenulata*, *Culex quinquefasciatus*, mosquitocidal activity, larvicidal activity, pupicidal activity and ovidicidal activity

Introduction

Mosquitoes are responsible for the transmission of many diseases to both humans and animals worldwide. Mosquitoes are the most pervasive insects and spread a greater number of illnesses in humans around the world. Mosquitoes are declared as "Public enemy number one" by WHO. Mosquito vectors (*Aedes*, *Anopheles* and *Culex*) are responsible for number of diseases like malaria, dengue fever, chikungunya, filariasis, Japanese encephalitis and yellow fever. Overall, 40% of human population are in danger of dengue. Synthetic insecticides cause physiological resistance in mosquitoes, in addition to that they affect nontarget life forms and besides it acts as pollutant (Subashini, K *et al.*, 2017) [1]. In recent years, use of many of the former synthetic insecticides in mosquito control programme has been limited.

It is due to lack of novel insecticides, high cost of synthetic insecticides and concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non-biodegradable nature, higher rate of biological magnification through ecosystem, increasing insecticide resistance on a global scale. Nevertheless, the extensive use of chemical pesticides have led to resistance in vectors, pollution, bioaccumulation, and several health and environmental concerns. (Bhatt. R. P *et al.*, 2010) [3]. Mosquitoes are controlled by synthetic pesticides, which have substantial environmental consequences.

Botanicals have historically been advocated as desirable alternatives to synthetic agrochemicals due to their alleged reduced environmental and human health impact in comparison to the latter Bioactive compounds, which have been utilized extensively in complementary and alternative medicine for decades, are prevalent in plants. Previous research has established the critical role of larvicides in mosquito populations at breeding sites.

(A. Anurag. *et al.*, 2019) [2] Kingdom: Animalia; Phylum: Arthropoda; Class: Insect; Order: Diptera; Family: Culicidae; Genus: *Aedes*; Species: *aegypti*. *Culex quinquefasciatus* is an important vector of *Bancroftian filariasis* in tropical and subtropical regions. According to WHO (1984), about 90 million people worldwide were once infected with *Wuchereria bancrofti*. In India alone is 25 million people microfilariasis and 19 million people suffer from filarial diseases (Deepa, J. *et al.*, 2015) [4].

The pupa of *Culex* mosquito is lighter than water and, therefore, floats on the surface. The pupa is the non-feeding stage of development, but pupae are mobile, responding to light changes and moving (tumble) with a flip of their tails towards the bottom or protective areas. When the pupa is about 2 days old, the eyes, antennae, legs and wings are slowly formed that can be visible through the transparent outer covering. The respiratory trumpets of *Culex* mosquito are long and narrow. Adult *C. quinquefasciatus* vary in length. The type of biological form is an efficient vector of malaria in urban areas (Deepalakshmi, S *et al.*, 2017) [5].

(Deepalakshmi and Jeyabalan, 2017) After facing several problems due to injudicious and over application of synthetic insecticides in nature, re-focusing phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activity's wide selection of plants from herbs, shrubs and large trees was used for extraction of mosquito toxins. Plants produce numerous chemicals, many of which have medicinal and pesticidal properties. (Deng. Y., *et al.*, 2021.) [6]

More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest and mosquito control programmes. Members of the plant families- (Foster, W. 2002) [7]. Solanaceae, Asteraceae,

Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae have various plant products available. In the present study *Naringi crenulata* plant leaf was tested against *C. quinquefasciatus*.

Materials and Method

The plant *Naringi crenulata* was identified as botanist, Department botany, Arignar anna government arts college, Namakkal District, Tamil Nadu, India. Fresh *Naringi crenulata* was collected from in and vicinity of our college, Arignar anna government arts college, Namakkal District, Tamil Nadu, India.

Collection of plant materials

Fresh *Naringi crenulata* was collected from in around our college, Arignar anna government arts college, Namakkal District, Tamil Nadu, India. The voucher specimen of (UGZOPH No.1) was prepared and deposited in PG and Research Department of Zoology, Government Arts College, Namakkal, Tamil Nadu, India.

Extraction method

The plant was shade dried under room temperature ($27.0 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH). After drying the whole plant material was powdered by utilizing electric blender. 500g of plant powder was separated by Soxhlet extraction methods with hexane, chloroform and ethyl acetate derivation, consecutively with expanding extremity of solvents and filtered through Whatman's No. 1 filter paper and it was condensed. The concentrates were gathered in clean borosil vials and stored in the refrigerator for experiments against important mosquito vectors.

Mosquito species & Vector rearing

The mosquito species *Culex quinquefasciatus* were rearing in the laboratory. The larvae were kept in plastic trays and filled with tap water. Dog biscuits and yeast were provided once a day initially for development of all stages. The whole setup was maintained at $28 \pm 2^\circ\text{C}$ and 70-80% relative humidity under the 14:10 light and dark cycles (Kamaraj *et al.*, 2009).

Larvicidal activity

The extracts were prepared and tested against the freshly moulted (0-6 hrs) fourth instar larvae of selected mosquito species. For dissolving of plant extracts 2 drop tween 20 was added and then it was diluted with 100ml of dechlorinated water to obtain desired concentrations. The control was prepared using 2 drop Polysorbate 20 (Tween 20) in 100ml of dechlorinated water. 250-ml transparent cups were used for bioassay and five replications were maintained. The tenth of freshly molted fourth instar larvae of mosquitoes were introduced in respective concentrations of plant extracts. The results were observed and recorded after treatment of 24 hours. The LC_{50} value was calculated by using profit analysis (Finney, 1971). The LC_{50} , LC_{90} and other statistics chi-square values were calculated by using the software using statistical package of social science (SPSS) version 16.0 for windows, significance level was set at $p \leq 0.05$.

$$\text{Corrected mortality of larvae} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage of larval mortality} = \frac{\text{Numbere of dead larvae}}{\text{No of larvea introduced}} \times 100$$

Pupicidal bioassay

The pupicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO (2005). Similar test concentrations as stated in the previous experiments were prepared and tested against the pupae of *Culex quinquefasciatus*. Polysorbate 20 (Tween 20) is used as emulsifier and distilled water treated as control. Ten pupae of each mosquito species were introduced in respective concentrations of plant extracts. The pupal mortality was observed and recorded after 24 hours of treatment. For each experiment, five replicates were maintained at a time. The percentage of mortality was calculated by using Abbott's formula (1925) [1].

$$\text{Percentage mortality of Pupae} = \frac{\text{Numbere of dead pupae}}{\text{No of pupae introduced}} \times 100$$

Ovicidal bioassay

The ovicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO (2005). Similar test concentrations as stated in the previous experiments were prepared and tested against the pupae of *Culex quinquefasciatus*. Polysorbate 20 (Tween 20) is used as emulsifier and distilled water treated as control. 100 eggs of each mosquito species were introduced in respective concentrations of plant extracts. The ovum mortality was observed and recorded after 24 hours of treatment. For each experiment, five replicates were maintained at a time. The percentage of mortality was calculated by using Abbott's formula (1925) [1].

$$\text{Percentage mortality of eggs} = \frac{\text{Numbere of dead pupae}}{\text{No of pupae introduced}} \times 100$$

Statistical Analysis

Data analysis was carried out using Microsoft Excel 2007. Based on the mortality of the test organisms recorded in these bioassays, LC_{50} and LC_{90} were calculated along with their fiducial limits at 95% confidence level by profit analysis using SPSS software package 16.00 (Statistical Package of Social Sciences) software. Results with $p < 0.05$ were marked as statistically significant.

Results and Discussion

Larvicidal activity of *Naringi crenulata* (Maha vilvam) against the freshly molted (0-6hrs old) fourth instar larvae of *C. quinquefasciatus*. (Table 1 and figure 1)

In the present study *Naringi crenulata* was tested against the fourth instar larvae of *C. quinquefasciatus* and the data pertaining to the experiments are shown in table 1 and figure 1. Perusal of the data clearly indicated that maximum larval mortality in ethyl acetate extract was recorded in *C. quinquefasciatus* mosquito at 24hrs values was recorded in different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm the values in the percentage of larval mortality of 78.50 ± 2.71 , 59.14 ± 3.79 , 37.37 ± 6.20 and $21.35 \pm 5.35\%$ and then LC_{50} (LCL-UCL), LC_{90} (LCL-UCL) X^2 values for 2.499(2.114-2.912), 6.271(5.423-7.606) 0.293 respectively. Chloroform extract, data clearly indicated that maximum larval mortality in Chloroform extract was recorded in *C*

quinquefasciatus mosquito at 24 hrs values was recorded in different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm the values in the percentage of larval mortality 58.48±3.79, 37.31±2.86, 21.47±4.63 and 16.36±4.75 and then LC₅₀ (LCL-UCL), LC₉₀ (LCL-UCL) X² values for 4.066 (3.502-4.910), 8.806(7.334-11.419)0.651 respectively. Petroleum extract was recorded in *C. quinquefasciatus* mosquito at 24 hrs values was recorded in different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm the values in the percentage of larval mortality in 51.33±6.35, 31.74±7.32, 20.43±4.57 and 12.36±2.47% and then LC₅₀ (LCL-UCL), LC₉₀(LCL-UCL) X² values for 4.715 (4.021-5.866), 9.782(8.011-13.103)0.544 respectively. The use of biologically active plant-based products with insecticidal properties have attracted considerable interest from scientists all over the world. (Anurag. A 2019.) [2] The biodegradable of the plant properties and safer on human beings and non-target organisms. Extensive survey of the flora was undertaken to search for potential plant extracts or compounds which could be used in the management of important human vector mosquitoes Foster, W. 2002 [7]. Similar type of activity was already reported by various authors.?

Mosquito species such *C. quinquefasciatus* tested for its larvicidal and pupicidal activity. The latex of *Naringi crenulata* was extracted using water and tested for mosquitocidal properties against selected mosquito species. Among the latex crude extracts tested *C. tritaeniorhynchus* larvae and pupae. The extract showed significant larvicidal and pupicidal activity. (Hira Amir, 2017) [8]. The highest cumulative mortality was observed at the highest concentration of plant leaf and stem extract (500ppm). The investigation of the larvicidal efficacy of the crude leaf ethyl acetate extract of *T. procumbens* was tested against *C. tritaeniorhynchus* showed promising larvicidal activity (Kamaraj *et al.*, 2011) [11].

Pupicidal activity of *Naringi crenulata* (Maha vilvam) against pupae of *Cx. Quinquefasciatus*. (Table 2 and figure 2)

The pupae of *C. quinquefasciatus* and the data pertaining to the experiments and Perusal of the data clearly indicated that maximum pupal mortality in ethyl acetate extract was recorded in *C. quinquefasciatus* 24hrs values was recorded in different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm the values in the percentage of pupal mortality in 71.74±3.45, 53.34±2.21, 34.84±3.29 and 20.11±3.07% and then LC₅₀ (LCL-UCL), LC₉₀(LCL-UCL) X² values for 2.826 (2.396-3.327), 7.098(6.037-8.865)0.186 and Pupae mortality in Chloroform extract was tested at 24 hrs. values was recorded in pupae mortality of 50.37±1.91, 31.41±3.90, 20.45±4.65 and 11.41±3.71% and then LC₅₀ (LCL-UCL), LC₉₀(LCL-UCL) X² values in 4.789 (4.082-5.972), 9.864(8.070-13.238)0.424 respectively. Petroleum ether extract was recorded in *C. quinquefasciatus* at 24 hrs. Values was recorded in percentage of pupae mortality in 37.41±4.43, 24.89±6.23, 18.77±4.31, and 10.10±1.77% and then LC₅₀ (LCL-UCL), LC₉₀(LCL-UCL) X² values for 6.505(5.180-9.587), 13.327(10.050-21.453) 0.395 respectively.

The result suggested that the ethyl acetate of *M. azedarach* leaf extract was an excellent larvicidal potential in controlling mosquito vectors. (Kamaraj, C., *et al.*, 2010) [10]. (A. Jeyasankar *et al.*, 2016) [9] have reported that the ethyl acetate extract of *Phyllanthus Emblica* Linn. Exhibited more than 90% larval mortality at 250ppm on *C. quinquefasciatus*.

Plants belonging to the family Lamiaceae have been screened and studied for their larvicidal activity against mosquitoes. Maia. M F *et al.*, 2011 [12]. Plants that showed promising larvicidal activity were ethanolic aerial extracts of *Teucrium divaricatum* (LC₅₀ 18.6ppm), *Mentha longifolia* (LC₅₀ 26.8ppm), *Melissa officinalis* (LC₅₀ 39.1ppm), *Salvia sclarea* (LC₅₀ 62.7ppm) and *Mentha pulegium* (LC₅₀ 81.0ppm) Rathy *et al.* (2015) have reported that the aqueous extracts of *Ocimum gratissimum*, *Phyllanthus emblica*, *Terminalia chebula*, *Aegle marmelos* and *Lantana camara* exhibited more than 90% larval mortality at 1.08 to 9.12 mg/ml on *A. aegypti*. Kamaraj *et al.* (2010) [10].

Ovicidal activity of *Naringi crenulata* plant leaf extract against of *Cx. quinquefasciatus*(Table 3 and figure 3)

Naringi crenulata plant leaf was tested against the eggs of *C. quinquefasciatus* and the data pertaining to the experiments. Perusal of the data clearly indicated that maximum eggs mortality in ethyl acetate extract was recorded in *C. quinquefasciatus* mosquito at 24 hrs values was recorded in different concentrations of 500ppm, 250ppm, 125ppm, and 62.5ppm the values in the percentage of eggs mortality values in 73.34±7.45, 51.47±2.41, 30.51±3.65 and 21.36±4.71% and then LC₅₀ (LCL-UCL), LC₉₀(LCL-UCL) X² values for values in 2.827(2.419-3.299), 6.849(5.877-8.421)0.374 respectively.

The chloroform extract in same plant of ovum mortality values in 59.40±1.71, 33.76±3.45, 21.43±5.21 and 11.37±6.51% and then LC₅₀ (LCL-UCL), LC₉₀(LCL-UCL) X² values for 4.099 (3.585-4.834), 8.298 (7.051-10.380)0.484 respectively. Petroleum ether extract in 45.31±3.21, 28.47±2.72, 19.51±7.32, 10.12±2.42 and then LC₅₀ (LCL-UCL), LC₉₀(LCL-UCL) X² values for 5.857 (6.461-6.886), 10.806(8.669-15.111) 0.360 respectively. Ovicidal activity of ethyl acetate, butanol, and petroleum ether extracts of five species of Euphorbiaceae plants, *Jatropha curcas*, *Pedilanthus tithymaloides*, *Phyllanthus amarus*, *Euphorbia hirta*, and *Euphorbia tirucalli*, were tested against the early fourth instar larvae of *Aedes aegypti*. The ethyl acetate leaf extract of the experimental plant has also showed ovicidal activity of mosquito species. (Manish. K D *et al.*, 2022) [13].

The ovicidal activity by ethyl acetate, aqueous solution, ethanol leaf extract of *Nerium oleander* against *A. stephensi* at 100, 150, 200, 250, and 300ppm were considered. With each extract at a concentration of 100ppm, the take of hatchability was very high, and nil hatchability was recorded as the concentration of extract was better to 300ppm in the case of aqueous and ethanol extract Singh. (K V *et al.*, 2003) [14]. The LC₅₀ values of *A. stephensi*, *Cx. Quinquefasciatus* and *A. aegypti* and Chi-square values were significant at P<0.05 four solvents for ethyl acetate, benzene, petroleum ether and methanol extract were tested Sinka. (M.E., *et al.*, 2011) [15].

(Subashini, K *et al.*, 2017) [16] ovicidal activity of *L. camara latex* extract in 14% larval mortality was recorded in 98% absorbing, adults emerged from the surviving pupal as they have raised in the plant extract. (Sukumaran., 2020) [18]. ovicidal activity of *Melaleuca leucadendra* leaves extract with ethanol against *C. quinquefasciatus* the mortality is observed after 24 hours the highest mortality (47.5%) reached by concentration 4%. The LOGIT test showed that the number of LC₅₀ was 3.7(37600 mg/l) with 95% significance the extract less effect to kill so it causes lethal effect of *A. aegypti* (Manis kumar., *et al.*, 2022).

Table 1: Larvicidal activity of *Naringi crenulata* (Maha vilvam) (L) plant leaf extracted against mosquitoes

Crude extract	Concentration (%)	Percentage of larval mortality (%)	LC ₅₀ and LC ₉₀		X ²
			LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)	
Ethyl acetate	5%	78.50±2.71	2.499 (2.114-2.912)	6.271 (5.423-7.606)	0.293
	2.5%	59.14±3.79			
	1.25%	37.37±6.20			
	0.625%	21.35±5.35			
chloroform	5%	58.48±3.79	4.066 (3.502-4.910)	8.806 (7.334-11.419)	0.651
	2.5%	37.31±2.86			
	1.25%	21.47±4.63			
	0.625%	16.36±4.75			
Petroleum ether	5%	51.33±6.35	4.715 (4.021-5.866)	9.782 (8.011-13.103)	0.544
	2.5%	31.74±7.32			
	1.25%	20.43±4.57			
	0.625%	12.36±2.47			

The value represents mean ± SD of five replications. LC₅₀ = Lethal Concentration brings out 50% Mortality and LC₉₀ = Lethal Concentration brings out 90% Mortality. LCL-UCL (Lower Confidence Limit- Upper Confidence Limit)

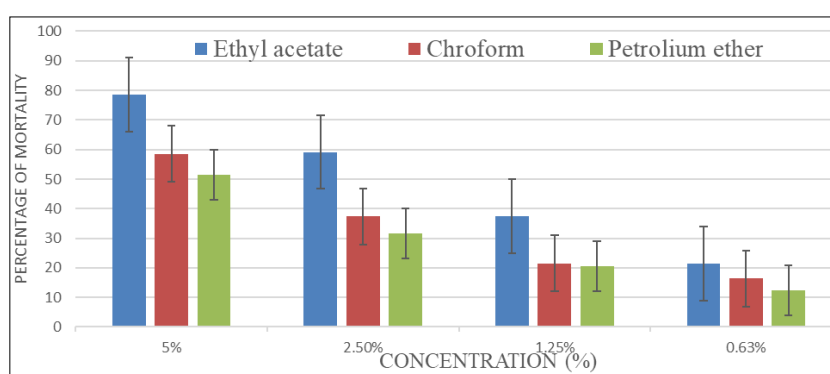


Fig 1: Larvicidal activity of *Naringi crenulata* (Maha vilvam) (L) Corr plant laef extract against mosquitoes

Table 2: Pupicidal activity of *Naringi crenulata* (Maha vilvam) (L) plant leaf extract against mosquitoes

Crude extract	Concentration (%)	Percentage of larval mortality (%)	LC ₅₀ and LC ₉₀		X ²
			LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)	
Ethyl acetate	5%	71.74±3.45	2.826 (2.396-3.327)	7.098 (6.037-8.865)	0.186
	2.5%	53.34±2.21			
	1.25%	34.84±3.29			
	0.625%	20.11±3.07			
chloroform	5%	50.37±1.91	4.789 (4.082-5.972)	9.864 (8.070-13.238)	0.424
	2.5%	31.41±3.90			
	1.25%	20.45±4.65			
	0.625%	11.41±3.71			
Petroleum ether	5%	37.41±4.43	6.505 (5.180-9.587)	13.327 (10.050-21.453)	0.395
	2.5%	24.89±6.23			
	1.25%	18.77±4.31			
	0.625%	10.10±1.77			

The value represents mean ± SD of five replications. LC₅₀ = Lethal Concentration brings out 50% Mortality and LC₉₀ = Lethal Concentration brings out 90% Mortality. LCL-UCL (Lower Confidence Limit- Upper Confidence Limit)

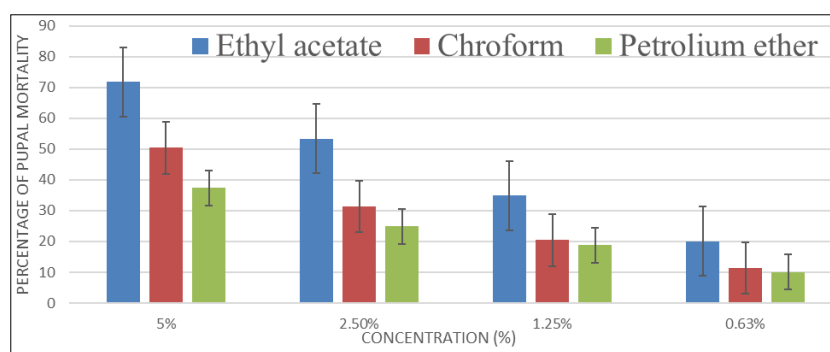


Fig 2: Pupicidal activity of *Naringi crenulata* (Maha vilvam) (L) Corr plant life extract against mosquitoes

Table 3: Ovicidal activity of *Naringi crenulata* (Maha vilvam) (L) plant leaf extract against mosquitoes

Crude extract	Concentration (%)	Percentage of larval mortality (%)	LC ₅₀ and LC ₉₀		X ²
			LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)	
Ethyl acetate	5%	73.34±7.45	2.827 (2.419-3.299)	6.849 (5.877-8.421)	0.374
	2.5%	51.47±2.41			
	1.25%	30.51±3.65			
	0.625%	21.36±4.71			
chloroform	5%	59.40±1.71	4.099 (3.585-4.834)	8.298 (7.051-10.380)	0.484
	2.5%	33.76±3.45			
	1.25%	21.43±5.21			
	0.625%	11.37±6.51			
Petroleum ether	5%	45.31±3.21	5.857 (6.461-6.886)	10.806 (8.669-15.111)	0.360
	2.5%	28.47±2.72			
	1.25%	19.51±7.32			
	0.625%	10.12±2.42			

The value represents mean \pm SD of five replications. LC₅₀ = Lethal Concentration brings out 50% Mortality and LC₉₀ = Lethal Concentration brings out 90% Mortality. LCL-UCL (Lower Confidence Limit- Upper Confidence Limit)

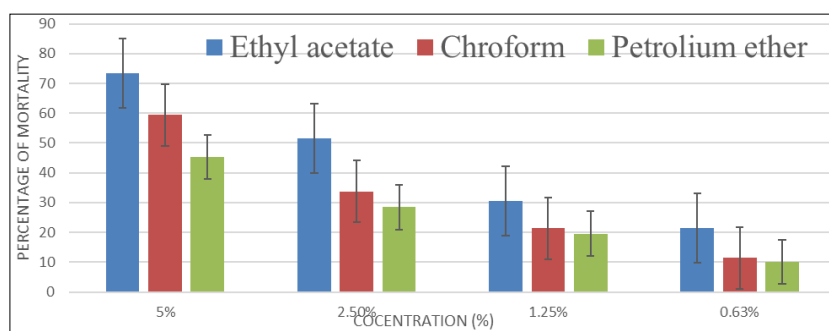


Fig 3: Ovicidal activity of *Naringi crenulata* (Maha vilvam) (L) plant leaf extract against mosquitoes

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