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Larvicidal Efficacy Of *Mentha Longifolia* Against *Aedes Aegypti*, *Anopheles Stephensi*, And *Culex Quinquefasciatus*

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Abstract

The present study was carried out to assess the larvicidal potential of the indigenous plant *Mentha longifolia* against the larvae of the *Aedes aegypti* (vector of Dengue, Chikungunya, and Zika), *Anopheles stephensi* (vector of urban malaria), and *Culex quinquefasciatus* (vector of Lymphatic filariasis). The acetone extract of plant leaves was used at 25, 50, 100, 200, 300, and 500 ppm dilutions in bioassays against different larval instars of the three mosquito species. Twenty larvae were exposed to the leaf extract at each concentration in a final volume of 100 ml formulation taken in a 250 ml glass beaker. The mortality data were analysed by the log-probit method, and lethal concentration (LC) values were calculated. The LC_{50} - LC_{90} values of *Mentha longifolia* extract against third instar larvae were calculated as 73.56 – 160.45, 72.49 & 146.57, and 81.23 – 84.69, while fourth instar larvae were calculated as 78.16 – 172.64, 81.17 – 173.47, and 144.24 – 149.26 for *Aedes aegypti*, *Anopheles stephensi,* and *Culex quinquefasciatus,* respectively.

Keywords: mosquito larvae, *Mentha longifolia*, *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*, Plant extract, LC_{50}, LC_{90}.

I. INTRODUCTION

Mosquito-borne diseases such as dengue , chikungunya, Zika virus, malaria, Japanese encephalitis, and leishmaniasis are the serious threats to public health (WHO, 2006)[1]. Vector-borne diseases occur worldwide and cause millions of deaths annually (Ravi Kumar and Rahuman, 2011)[2]. The only way to reduce such disease outbreaks is to control the mosquito population. Mosquito control is often chemical-based, which causes serious health issues, environmental pollution, and vector resistance. Therefore, alternate methods are being regularly discovered, and the plants serve as one of the most important sources of several compounds which possess potential insecticidal properties and are free from harmful environmental effects (Isman, 1995)[4], (Isman, 2006)[3]. Many plant products have been explored against mosquitoes either as larvicides and/or adulticides or as adult repellents (Sukumar *et al.,* 1991)[5]. Medicinal plants have played a key role in human health. These plants are a good source of bioactive insecticidal phytochemicals that can kill mosquito larvae

with high mortality rates (Mdoe et al., 2014)[6]. The chemicals play a role by inducing changes in the development, midgut epithelium, mutating the DNA, and producing reactive oxygen species in the larvae (Arjunan et al., 2012)[7]. However, the phytochemicals are highly specific, rapidly biodegradable, eco-friendly, and less toxic to human health (Ghosh et al., 2012)[8]. Organophosphorus compounds such as fenthion, temephos, and chlorpyrifos, are commonly used as larvicides against mosquitoes and other aquatic insects. However, because of the known hazardous effects of chemical insecticides (Din *et al.,* 2011)[9], major consideration has been shifted steadily on the use of plant-based products as larvicides or adulticides which can provide an alternative to the synthetic chemicals (Junwei *et al.,* 2006)[10]. In the present study we explored the larvicidal potential of indigenous plants i.e. *Mentha longifolia* against larvae of the different mosquito species.

II. MATERIALS AND METHODS

2.1 Rearing of Larvae

To maintain a healthy colony of *Anopheles stephensi* was maintained under laboratory conditions for research, surveillance, or bioassay studies. The temperature was set at 26°C ± 2°C and the relative humidity at 75% ± 5% respectively. Temperature and humidity controls are probably the most important factors in the successful rearing of mosquitoes. Climate-controlled room is required with a photoperiod of 12 h of light and 12 h of darkness, allowing the best and most uniform development.

2.2 Larvicidal testing

Standard methods for testing the susceptibility of mosquito larvae to insecticides were followed in all the experiments with slight modifications. The acetone extract of plant leaves was used at 25, 50, 100, 200, 300, and 500 ppm dilutions in bioassays against different larval instars of the three mosquito species. Twenty larvae were exposed to the leaf extract at each concentration in a final volume of 100 ml formulation taken in a 250 ml glass beaker. Four replicates for each concentration and the control (with acetone and emulsifier) were used for larval bio-efficacy. The larval mortality at different concentrations and in the control was recorded after 24h of continuous exposure. The mortality data were analysed by the log-probit method, and lethal concentration (LC) values (50 and 90) were calculated. The mortality rates were calculated using the World Health Organization (WHO, 2006)[1] bioassay protocol, with slight modifications.

$$\textbf{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

The corrected mortality between 5% and 20% necessitated that the mortalities of treated groups be corrected according to Abbott's formula wherever required (Abbott, 1925)[11].

$$\textbf{Corrected mortality} = \frac{\text{Observed mortality in treatment - Observed mortality in Control}}{\text{100 – Control Mortality}} \times 100$$

2.3 Statistical analysis

Probit regression analysis revealed the LC_{50} and LC_{90} values, along with their respective Lower and Upper Fiducial Limits (LFL, UFL), R^2 (Coefficient of determination), and P values against 3rd and 4th instar larvae of *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* treated with various concentrations of the leaf extract are presented in the results. The 95% confidence interval values, degrees of freedom, $\chi 2$ goodness of fit tests, and regression equations were recorded. Whenever $\chi 2$ value was found significant ($p < 0.05$). A heterogeneity correction factor was used in the calculation of confidence limits.

III. RESULTS

3.1 Effects of *Mentha longifolia* extract on the developmental rate of *Anopheles*, *Aedes,* and *Culex*.

Standard methods for testing the susceptibility of mosquito larvae to insecticides were followed in all the experiments. Four replicates for each concentration and the control were tested for larval bio-efficacy. Larval mortality at different concentrations as 25, 50, 75, 100, 125, and 150 ppm, and in the control at 0 ppm, were recorded after 24 hours of continuous exposure. The percent mortality values for III and IV instar larvae of *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* treated with various concentrations of the leaf extract are presented in the results.

3.1.1 Effects of *Mentha longifolia* extract on the developmental rate of *Anopheles stephensi*

Table 1a: Larvicidal activity of *Mentha longifolia* extracts on the 3rd and 4th instar larvae of *Anopheles stephensi*

Observed mortality in percentage after 24 hours						
Concentration	**25 ppm**	**50 ppm**	**75 ppm**	**100 ppm**	**125 ppm**	**150 ppm**
Mean % Mortality in 3rd Instar larvae	5	18	42	66	83	96
Mean % Mortality in 4th Instar larvae	4	15	38	59	76	91

Note; 0 % mortality was recorded in control. Conc. = Concentration, ppm = Parts per million

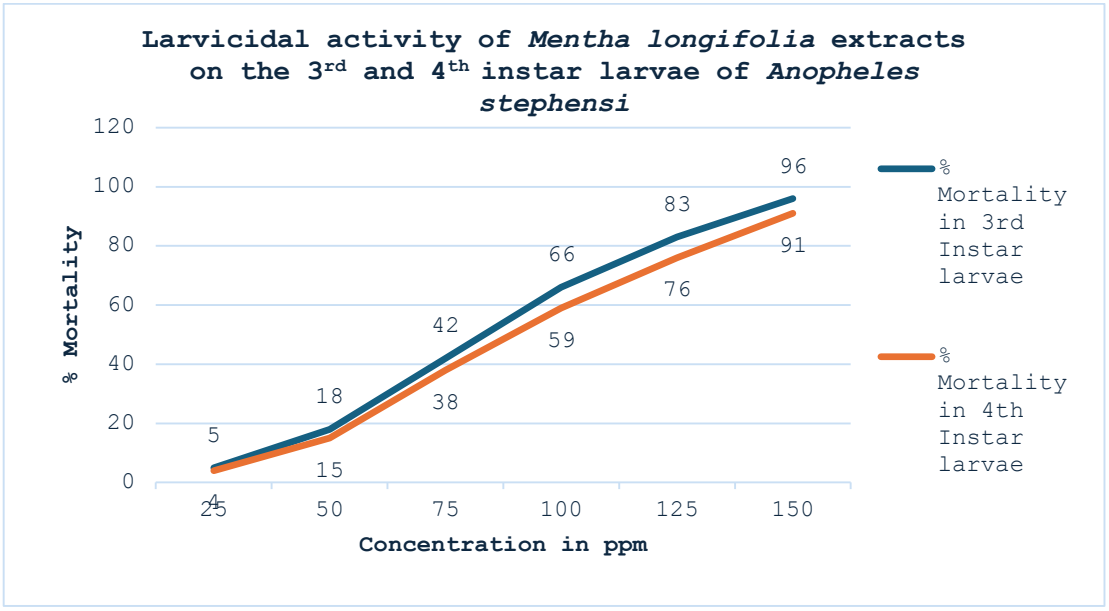

Fig. 1

Dose-dependent mortality was observed against the III[rd] and IV[th] instar larvae of the *Anopheles stephensi.* After 24 hours of exposure, 6 different concentrations of 25, 50, 75, 100, 125, and 150 ppm were tested. *Mentha longifolia extracts* at these mentioned concentrations produced the mean % mortality as 5, 18, 42, 66, 83, 96% and 4, 15, 38, 59, 76, 91% in the III[rd] and IV[th] instar larvae, respectively (Table 1a, Fig.1).

Table 1b: LC50 and LC90 with fiducial limits (95%) of tested *Mentha longifolia* extracts against 3[rd] and 4[th] instar larvae of *Anopheles stephensi*

Larval Stages	LC_{50} **(in ppm)**	LC_{90} **(in ppm)**	**LFL (Confidence level 95%)**	**UFL (Confidence level 95%)**	**R^2 (R-squared value)**	**INOVA p-value**
Mean % Mortality in 3[rd] Instar larvae	72.49	146.57	2.77	5.60	0.943	0.045
Mean % Mortality in 4[th] Instar larvae	81.17	173.47	2.81	4.95	0.962	0.029

LC50 =Lethal concentration 50 at which 50% of the target population died. LC90 = Lethal concentration 90 at which 90% of the target population died. LFL = Lower fiducial limit UFC = Upper fiducial limit, R^2= coefficient of determination, p value = Level of significance $p \leq 0.05$

LC_{50} and LC_{90} values against 3rd instar larvae were calculated as 72.49 ppm and 146.57 ppm, while 4th instar larvae were calculated as 81.17 ppm and 173.43 ppm, respectively. LFL and UFL values against 3rd and 4th instar larvae found as 2.77, 5.6 ppm, and 2.81, 4.95 ppm, respectively. The value for R-squared can range from 0 to 1. A value of 0 indicates that the response variable cannot be explained by the predictor variable at

all. A value of 1 indicates that the response variable can be perfectly explained without error by the predictor variable. The R^2 values of 0.943 and 0.962 indicate that the response variables are perfectly explained without error. Similarly, p-values 0.045 and 0.029, i.e., $p \leq 0.05$, show that the P value is statistically significant, supporting the effectiveness of *Mentha longifolia* as a larvicide for *Anopheles stephensi* mosquitoes.

3.1.2 Effects of *Mentha longifolia* extract on the developmental rate of *Aedes aegypti*

Table 2a: Larvicidal activity of *Mentha longifolia* extracts on the 3rd and 4th instar larvae of *Aedes aegypti*

Observed mortality in percentage after 24 hours						
Concentration	**25 ppm**	**50 ppm**	**75 ppm**	**100 ppm**	**125 ppm**	**150 ppm**
Mean % Mortality in 3rd Instar larvae	6	19	48	64	79	93
Mean % Mortality in 4th Instar larvae	5	17	44	62	75	91

Note; 0 % mortality was recorded in control. Conc. = Concentration, ppm = Parts per million

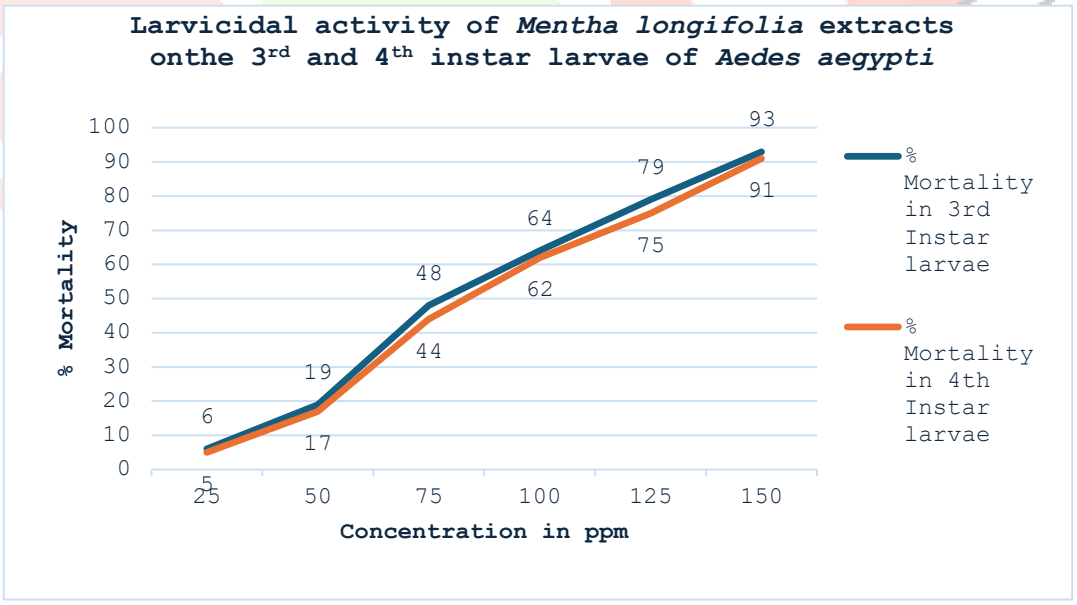

Fig. 2

Dose-dependent Mortality was observed against the III[rd] and IV[th] instar larvae of the ***Aedes aegypti.*** After 24 hours of exposure, 6 different concentrations of 25, 50, 75, 100, 125, and 150 ppm were tested. *Mentha longifolia* extracts at these mentioned concentrations produced 6, 19, 48, 64, 79, 93% and 5, 17, 44, 62, 75, 91% larval mortality in the III[rd] and IV[th] instar, respectively (Table 2a, Fig.2).

Table 2b: LC50 and LC90 with fiducial limits (95%) of tested *Mentha longifolia* extracts against 3rd and 4th instar larvae of *Aedes aegypti*

Larval Stages	LC_{50} (in ppm)	LC_{90} (in ppm)	LFL (Confidence level 95%)	UFL (Confidence level 95%)	R^2 (R-squared value)	INOVA p-value
Mean % Mortality in 3rd Instar larvae	73.56	160.45	2.73	4.83	0.961	0.046
Mean % Mortality in 4th Instar larvae	78.16	172.64	2.76	4.68	0.967	0.035

LC50 =Lethal concentration 50 at which 50% of the target population died. LC90 = Lethal concentration 90 at which 90% of the target population died. LFL = Lower fiducial limit UFC = Upper fiducial limit, R^2= coefficient of determination, p value = Level of significance $p \leq 0.05$

LC_{50} and LC_{90} values against 3rd instar larvae were calculated as 73.56 ppm and 160.45 ppm, while 4th instar larvae were calculated as 78.16 ppm and 172.64 ppm, respectively. LFL and UFL values against 3rd and 4th instar larvae found as 2.73, 4.83 ppm, and 2.76, 4.68 ppm, respectively. The value for R-squared can range from 0 to 1. A value of 0 indicates that the response variable cannot be explained by the predictor variable at all. A value of 1 indicates that the response variable can be perfectly explained without error by the predictor variable. The R^2 values of 0.961 and 0.967 indicate that the response variables are perfectly explained without error. Similarly, p-values 0.046 and 0.035, i.e., $p \leq 0.05$, show that the P value is statistically significant, supporting the effectiveness of *Mentha longifolia* as a larvicide for *Aedes aegypti*.

3.1.3 Effects of *Mentha longifolia* extract on the developmental rate of *Culex quinquefasciatus*

Table 3a: Larvicidal activity of *Mentha longifolia* extracts on the 3rd and 4th instar larvae of *Culex quinquefasciatus*

Observed mortality in percentage after 24 hours						
Concentration	**25 ppm**	**50 ppm**	**75 ppm**	**100 ppm**	**125 ppm**	**150 ppm**
Mean % Mortality in 3rd Instar larvae	8	14	46	62	84	92
Mean % Mortality in 4th Instar larvae	6	12	42	58	82	91

Note; 0 % mortality was recorded in control. Conc. = Concentration, ppm = Parts per million

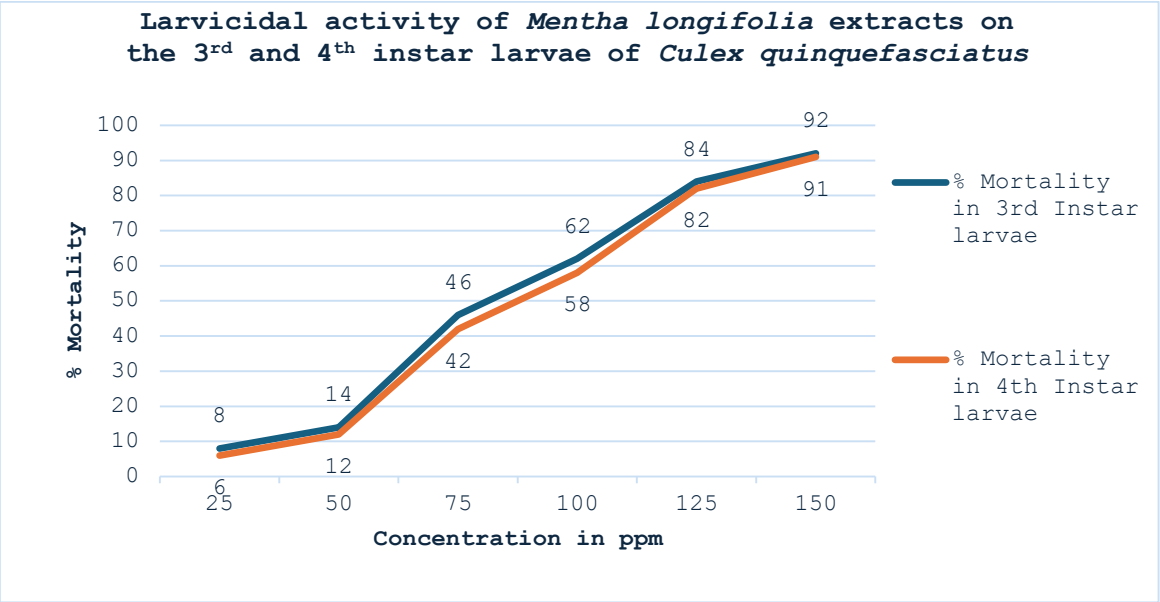

Fig. 3

Dose-dependent Mortality was observed against the III[rd] and IV[th] instar larvae of the *Culex quinquefasciatus.* After 24 hours of exposure, 6 different concentrations of 25, 50, 75, 100, 125, and 150 ppm were tested. *Mentha longifolia* extracts at these mentioned concentrations produced 8, 14, 46, 62, 84, 92% and 6, 12, 42, 58, 82, 91% larval mortality in the III[rd] and IV[th] instar, respectively (Table 3a, Fig.3).

Table 3b: LC50 and LC90 with fiducial limits (95%) of tested *Mentha longifolia* extracts against 3[rd] and 4[th] instar larvae of *Culex quinquefasciatus*

Larval Stages	LC_{50} **(in ppm)**	LC_{90} **(in ppm)**	**LFL (Confidence level 95%)**	**UFL (Confidence level 95%)**	**R^2 (R-squared value)**	**INOVA p-value**
Mean % Mortality in 3[rd] Instar larvae	81.23	84.69	4.26	6.01	0.991	0.003
Mean % Mortality in 4[th] Instar larvae	144.24	149.26	4.22	6.18	0.989	0.003

LC50 =Lethal concentration 50 at which 50% of the target population died. LC90 = Lethal concentration 90 at which 90% of the target population died. LFL = Lower fiducial limit UFC = Upper fiducial limit, R^2= coefficient of determination, p value = Level of significance $p \leq 0.05$

LC_{50} and LC_{90} values against 3rd instar larvae were calculated as 81.23 ppm and 84.69 ppm, while 4th instar larvae were calculated as 144.24 ppm and 149.26 ppm, respectively. LFL and UFL values against 3rd and 4th instar larvae found as 4.26, 6.01 ppm, and 4.22, 6.18 ppm, respectively. The value for R-squared can range from 0 to 1. A value of 0 indicates that the response variable cannot be explained by the predictor variable at all. A value of 1 indicates that the response variable can be perfectly explained without error by the predictor variable. The R^2 values of 0.991 and 0.989 indicate that the response variables are highly explained, with minimal error. Similarly, p-values 0.003 and 0.003, i.e., $p \leq 0.05$, show that the P value is statistically

significant, supporting the effectiveness of *Mentha longifolia* as a larvicide for *Culex quinquefasciatus* mosquitoes.

V. DISCUSSION

The extensive use of synthetic organic chemical insecticides results in environmental hazards and resistance in major species and this has necessitated the need to develop more potent and environmentally safe insecticides. The results from the study showed that this gum exhibited larvicidal activity. Storax showed the larvicidal effect against the 3rd and 4th instar larvae with LC50 value of 194.93 ppm and an LC90 value, 397.33 ppm (Hashmat Imam et. al., 2013)[12]. The dose effect curve indicates that the mortality increased with increased concentration ($P< 0.05$). This confirms the report of Shadia et al.[13] that there is a positive correlation between concentration and the percentage of mortality. A considerable number of plant derivatives have shown to be effective against mosquitoes in a safe manner. Though several plant species from different families have been reported for mosquitocidal activity, only a few botanicals have moved from laboratory to field use, which might be due to the presence of phytochemicals when compared to synthetic insecticides (Green M, Singer JM et.al., 1991)[14]. Plants belonging to different family have been extensively screened/studied for their larvicidal activity ever since the discovery of the larvicidal potential of the extract of *Chrysanthemum cinerariaefolium* (Omena MC et.al. 2007)[15]. Chakkaravarthy et al. reported the larvicidal efficacy of *Azadiracta indica* and *Datura metal* (linn.) leaf extracts against the third instar larva of *Culex quinquefasciatus* (Diptera: Culicidae). The hexane and chloroform extract showed LC50 values as 246.38, 198.82, 709.96 and 562.07 ppm, respectively (Chakkaravarthy VM, Ambrose T et.al. 2011)[16]. Kovendan K et al. studied on *Orthosiphon thymiflorus*, the LC50 values of hexane, chloroform, ethyl acetate, acetone and methanol extract of *Orthosiphon thymiflorus* on third instar larvae of *Anopheles stephensi* were LC50= 201.39, 178.76, 158.06, 139.22 and 118.74 ppm; *Culex quinquefasciatus* were LC50=228.13, 209.72, 183.35, 163.55 and 149.96 ppm and Aedes aegypti were LC50=215.65, 197.91, 175.05, 154.80 and 137.26 ppm respectively (Kovendan K, Murugan K, Vincent S, Barnard. 2012)[17]. Maheshwaran et al. reported solvent extracts of chloroform, ethanol, and hexane, leaf extract of *Leucas aspera* against *Culex. quinquefasciatus* than *Aedes aegypti* 4th instar larvae. The LC50values were 518.88, 1059.13, 193.43 and 588.76, 1565.95, 199.72 ppm, respectively (Maheshwaran R, Sathish S, Ignacimuthu S. 2008)[17]. The larvicidal activity of storax may be due to the presence of the major chemical compound, which contains Benzyl alcohol, styrene, and cinnamic acid. These effects may potentially contribute to the process of larvicidal effect (Kenneth CL.1975)[18].

Therefore, the results of this study are supposed to encourage further studies on the identification of the active compounds involved and their mode of action. The field trials are needed to recommend *Mentha longifolia* as an anti-mosquito product used to combat mosquitoes in mosquito control and public health programs.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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