International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2021; 8(4): 27-30

www.dipterajournal.com
Received: 13-05-2021
Accepted: 17-06-2021

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Evaluation of larvicidal efficacy of *Melaleuca styphelioides* Sm. against *Culex quinquefasciatus* larvae

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Abstract
In many developing countries vector-borne diseases are one of the causes of illness and death. The larvae of *Culex quinquefasciatus* are vectors that transmit the parasites and pathogens that cause diseases like filariasis, dengue, yellow fever, malaria, Japanese encephalitis, chikungunya, and so forth, which are considered harmful to the population in tropic and subtropical regions. Botanical insecticides provide an alternative to synthetic insecticides because they are generally considered safe, are biodegradable, and can often be obtained from local sources. The larvicidal effects of *Melaleuca styphelioides* using essential oil and leaf powder extracts against *Culex quinquefasciatus* larvae were conducted. A higher concentration of essential oil was found to be effective in causing the death of the larvae after one-hour treatment with an LC50 and LC90 of 360.33ppm and 684.33 ppm respectively. The percentage of mortality using powder extract was also found to increase with an increase in concentration and time. The time interval of 72 hours was found to be more effective with leaf powder with LC50 (245.89ppm) and LC90 (534.76ppm). The leaf extracts of *M. styphelioides* are effective in killing mosquito larvae and can be developed as an eco-friendly approach for the control of mosquitoes.

Keywords: *Melaleuca styphelioides*, *Culex quinquefasciatus*, larvicidal

Introduction
Mosquitoes are vectors transmitting threatening diseases like malaria, filariasis, dengue fever, Japanese encephalitis, and chikungunya fever and as there is no specific drug available for the treatment of these diseases; vector control is the best strategy [1]. *Culex quinquefasciatus* is a common house mosquito distributed worldwide in tropic and temperate regions which feed on organic materials in the water. The mosquito larvae are primarily controlled by the application of organophosphates and insect growth regulators [2]. These control measures caused environmental pollution and resistance by the mosquitoes which can be minimized to a great extent by the use of environment-friendly, economical bio degradable, and indigenous control methods [3].
Plants may be alternative sources of mosquito larvicidal agents and there are reports regarding the effective use of plant extracts as larvicides, repellent, oviposition attractants and insect growth hormone regulators [4-6]. The essential oil obtained from aromatic plants is now a day used as natural mosquito repellents [7].
The present work has been done to assess the larvicidal potential of the methanol extract and essential oil from *Melaleuca styphelioides* against the larva of *Culex quinquefasciatus*. The plant belonging to Myrtaceae family is a tree with prickly leaves and spike of creamy- white flowers commonly known as prickly-leaved paper bark.

Materials and Methods
Melaleuca styphelioides Sm. used in this study was authenticated from Botany Department of University College, Thiruvananthapuram, Kerala, India and the voucher specimen of the plant had been deposited in the herbarium for future reference (Figure -1). The fresh leaves were collected and essential oil was extracted using Clevenger apparatus by hydrodistillation. The leaves were also shade dried and pulverized to a coarse powder. Essential oil and dried leaf powder were subjected to the study of larvicidal activity.

Fig 1: The plant had been deposited in the herbarium for future reference

The larvae of *Culex quinquefasciatus* were collected from the breeding sites of highly polluted sewage and stagnant water areas of Thiruvananthapuram district and identified by the Department of Zoology, University College, Thiruvananthapuram. They started to form colony and larvae were kept in a tray containing tap water. They were maintained and the experiments were done at room temperature.

Mosquito Larvicidal Bioassay

The larvicidal bioassay was carried out by the method of standard guidelines of the World Health Organisation [8]. The potential of crude leaf extract as larvicide against the vector, *Culex quinquefasciatus* was evaluated at concentrations of 50 to 1000ppm. Similarly different concentrations of essential oil (50,100,250,750 and 1000ppm) were prepared. About 10 mosquitoes were introduced into the beaker containing 20 ml of the above concentration of the extracts and 20ml of tap water was used as control. Each treatment was conducted in triplicates. The complete setup of powder extract was exposed for 72 hours and after 24, 48, and 72 hours of treatment percentage of mortality were calculated. The mosquito larvicidal activity in essential oil was recorded during every one-hour interval. Larvae were considered dead if they settled and remained motionless at the bottom of the beaker with no response to light stimulus. The LC50 (lethal concentration that kills 50% of the exposed larvae) and LC90 (lethal concentration that kills 90% of the exposed larvae) values were calculated using probit analysis method.

$$\text{Percentage of Mortality} = \frac{\%\text{ of mortality in treated} - \%\text{ of mortality in control}}{100 - \%\text{ of mortality in control}}$$

Statistical Analysis

The probit analysis (IBM SPSS, Statistics 26) was used for calculating the lethal concentrations, LC50 and LC90, and their 95% confidence limit of upper and lower confidence levels [9].

Results and Discussion

The larvicidal activity of extracts was determined on *Culex quinquefasciatus* using different concentrations of extracts (50 ppm, 100ppm, 250 ppm, 500ppm, 750ppm, and 1000 ppm). The larval mortality was first observed after 12 hours of treatment with essential oil and since all larvae were found dead the larval mortality was scored after 1hr, 2hr, 3hr, 4hr, and 5hr, and the results are presented in Table -1. The lower concentration did not exhibit mortality up to 4 hours and only 6.66% mortality was observed after the fifth hour of treatment. After 1 hour all the larvae were found to be dead at higher concentration (750ppm and 1000ppm).

Table 1: Larvicidal activity of essential oil of *Melaleuca styphelioides* against *Culex quinquefasciatus*

Concentration (ppm)	Death of larvae out of 15 and percentage of mortality				
	1hr	2hr	3hr	4hr	5hr
50	0 (0%)	0 (0%)	0 (0%)	0	1(6.66%)
100	0 (0%)	9 (60%)	11 (73.3%)	12 (80%)	15 (100%)
250	5(33.3%)	9 (60%)	12 (80%)	15 (100%)	-
500	8 (53.3%)	12 (80%)	14 (93.3%)	15 (100%)	-
750	15 (100%)	-	-	-	-
1000	15(100%)	-	-	-	-

The output of the probit analysis is used to compare the amount of extract required to create the same response in each of the various extracts used. The median lethal concentration (LC50 and LC90) values were least at 4 hours of treatment indicating the most effective time of treatment. The LC50 and LC90 values after one hour of treatment with essential oil were 360.33ppm and 684.33 ppm. LC50 and LC90 values after 5 hours of treatment, where the death of all larvae has occurred were 142.05ppm and 187.05 ppm, respectively (Table-2). The larvicidal activity was found to be concentration-dependent. From the results it is clear that 4 hours of treatment with essential oil was the most potent mortality time for *Cx. quinquefasciatus* larvae with the least LC50 (83.12ppm) and LC90 (116.98ppm).

Table 2: Relative toxicity of *Melaleuca styphelioides* essential oil against larvae of *Culex quinquefasciatus*

Time (Hours)	LC50	95% Confidence Limits of concentration		LC90	95% Confidence Limits of concentration		Intercept ± SE	Chi-Square
		Lower	Upper		Lower	Upper		
1	360.33	277.93	442.45	684.33	544.05	1033.97	-11.76±2.44	5.88*
2	151.15	24.48	353.47	501.07	239.85	22498.32	-5.36±1.01	10.44*
3	110.19	21.73	237.86	297.95	155.71	8148.30	-6.05±1.23	9.09*
4	83.12	65.50	100.26	116.98	97.63	195.10	-16.57±5.12	0.595
5	142.05	115.16	274.03	187.05	142.87	641.00	-23.08±7.81	0.140
LC_{50}-lethal concentration that kills 50% of the exposed larvae; LC_{90}-lethal concentration that kills 90% of the exposed larvae								
*p≤0.5, level of significance of chi-square values								

Mosquito larvae were exposed to six different concentrations of leaf powder extracts of *M.styphelioides*. Mortality was recorded after 24, 48, and 72 hours. Percentage of mortality after 24 hours ranged from 0% to 66.66% at different test concentrations, after 48houra it ranged from 0% to 93.33%, and after 72 hours mortality increased to 100% at 1000ppm concentration (Table-3). The LC50 values of *M. styphelioides* leaf powder extract after 24 hours (634.20ppm), 48hours (260.90ppm) and 72 hours (245.89ppm), revealed their order of larvicidal activity against *C. quinquefasciatus* larvae (Table -4). The percentage of mortality was found to increase with the increase in concentration and time. The time interval of 72 hours was found to be more effective with lower LC50 (245.89ppm) and LC90 (534.76ppm) values when compared with 24hours and 48 hours of treatment with *M.styphelioides* leaf powder extract.

Table 3: Larvicidal activity of *M.styphelioides* leaf powder extract against *Culex quinquefasciatus*

Concentration (ppm)	Death of larvae out of 15 and percentage of mortality		
	24hr	48hr	72hr
50	0 (0%)	0 (0%)	0 (0%)
100	0	0	0
250	4 (26.66%)	12 (80%)	11(73.33%)
500	6 (40%)	11(73.33%)	12 (80%)
750	8 (53.33%)	13 (86.66%)	14 (93.33%)
1000	10 (66.66%)	14 (93.33%)	15 (100%)

Table 4: Relative toxicity of *Melaleuca styphelioides* leaf powder extract against *Culex quinquefasciatus* larvae

Time (Hours)	LC50	95% Confidence Limits of concentration		LC90	95% Confidence Limits of concentration		Intercept ± SE	Chi-Square
		Lower	Upper		Lower	Upper		
24	634.20	461.62	986.96	2252.90	1310.82	8618.83	-6.52±1.48	1.548
48	260.90	79.22	502.93	705.34	388.65	6519.50	-7.17±1.28	9.35*
72	245.89	180.98	313.95	534.76	410.64	811.51	-9.08±1.69	5.72
LC_{50}-lethal concentration that kills 50% of the exposed larvae; LC_{90}-lethal concentration that kills 90% of the exposed larvae.								
*p≤0.5, level of significance of chi-square values.								

Plant as potential larvicides is considered as the viable and preferred alternative in the control of the mosquito species at the community level. A large number of plant extracts have been reported to have mosquitocidal or repellent activities against mosquito vectors, but few plant products have shown practical utility for mosquito control [10]. The result revealed that, among the two extracts tested for larvicidal property, essential oil from *M.styphelioides* was found to be a more promising larvicide to control mosquito larvae. Essential oils derived from *Baeckea frutescens*, *Callistemon citrinus*, *Melaleuca leucadendra*, and *Syzygium nervosum* of Myrtaceae family have shown larvicidal activities against A*edes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* [11]. A natural product with an LC_{50} between 50 mg/L and 100 mg/L was reported to be moderately active [12]. *M. quinquenervia* oil was found to be active and had significant larvicidal activity (<50 mg/L) for the species *Culex* and *Aedes* [13].

Conclusion

No publicized reports were encountered on the toxicity of *M.styphelioides* on non-target aquatic organisms, suggesting their potential role in the control of disease vector mosquitoes. The use of these plant extracts as larvicides in mosquito control instead of synthetic insecticides could reduce the cost, adverse environmental effects, and pollution.

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