

**Larvicidal Effect of Kaffir Lime (*Citrus hystrix* DC) Leaves
Extract against *Aedes aegypti* Larvae**

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Attempt had been made to develop vector control using natural plant product. For this purpose Kaffir lime (*Citrus hystrix* DC) leaves were collected from Mawlamyine Township, Mon State. One hundred grams of dried leaves powder were extracted with 95% ethanol 1:5 wt/v by cold percolation method in laboratory for 7 days. The resulting 8 gm of viscous material was obtained from 100 gm of dried leaves. Different concentrations of ethanolic leaves extract of *Citrus hystrix* DC as 2.4%, 2.1%, 1.8%, 1.5% and 1.2% were prepared freshly in 100 ml each of distilled water in 150 ml plastic cups. Laboratory-reared Dagon Township strain of *Aedes* mosquito larvae were used for testing larvicidal properties of ethanolic leaves extract of *Citrus hystrix* DC Larvae were exposed 24 hours for each replication in different concentrations in laboratory. Result revealed that the highest dose 2.4% produced 99.5 % mortality followed by 2.1% crude extract with 85.5% mortality of 3rd and 4th instars *Aedes* larvae. And, 1.8% concentration of ethanolic leaves crude extract found 62.5% mortality of *Aedes* larvae within 24 hours. The effective lethal concentrations of LC₅₀ for 50% mortality and LC₉₀ for 90% mortality were found to be 1.73% and 2.08% crude ethanolic leaf extract of *Citrus hystrix*. Beta-citronellal (66.85%) was the most abundance compound in the leaf of *Citrus hystrix*, which might be toxic to the tested mosquito larvae. In conclusion, the ethanolic leaves extract of *Citrus hystrix* exhibited strong larvicidal activity against *Aedes* larvae which could be considered as a potentially alternative source for developing novel larvicides to be used in controlling vectors of mosquito-borne disease.

Key words: *Citrus hystrix*, *Aedes* larvae, Concentration, Mortality, Ethanolic extract

INTRODUCTION

Mosquito-borne diseases such as dengue haemorrhagic fever, malaria, filariasis are harmful to human and they are a public health problem in Myanmar.¹ Billions of people, primarily in tropical countries, are at risk from such diseases which are transmitted by three different genera of mosquitoes as *Aedes*, *Culex* and *Anopheles* species.² To control mosquitoes and mosquito-borne diseases, which have a worldwide health and economic impacts, synthetic insecticide-based interventions are still necessary, particularly in situations of epidemic out-

break and sudden increases of adult mosquitoes.³ However, The indiscriminate use of conventional insecticides is fostering multifarious problems 85 to 90% of applied pesticides never reach target organisms and they disperse through the air, soil and water, creating widespread development of insecticide resistance, toxic hazards to mammals, undesirable effects on non-target organisms, and environmental pollution.⁴⁻⁶ Frequent application of large amount of insecticides leads to increased dangers for humans and

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other organisms and progressively greater environmental damage.

Although plants and their preparations were the only pest management agents available before the advent of synthetic organic chemical, only a few insecticides of plant origin are now commercially available. Some plant extracts from the families of *Asteraceae*, *Piperaceae*, *Annonaceae*, *Lamiaceae* and *Rutaceae* were demonstrated to produce various biological controls such as antifeedant, repellent, and insecticidal properties to many insect species. They have been proved non-toxic to human and environment.⁷ Among the plants investigated to date, one showing enormous potential is the *Citrus* family.⁸ *Citrus* plants such as *Citrus hystrix* DC and *C. reticulata* Blanco are familiar through use of their fruit for culinary purposes and traditional medicine.^{9, 10}

The protective effects of the ethanolic extracts of three edible aromatic plants, mintweed *Hyptis suaveolens*, kitchen mint *Mentha cordifolia* and Kaffir lime *Citrus hystrix*, against rice weevil *Sitophilus oryzae* infestation in stored, milled rice were investigated and found the highest repellent efficacy of ethanolic extracts from kaffir lime within 24 hours.¹¹ The methanolic extract of leaves of kaffir lime is known to inhibit the herpes virus¹² and also used as mosquito repellent.¹³ It is necessary to find out more potential natural products in plants pest. Mosquito control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides. Synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water and air.¹⁴

Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. The increased use of chemical insecticide may inter into the food chain and thereby the internal organs of man and animals may be irreversibly damaged and they even result in mutation of genes. In larval mosquito control, application of

insecticides in ponds, wells and other water bodies may cause health hazards to human and larvivorous fishes. Therefore, there is a need to find out the alternative ways for environmental safety, biodegradable, cost effective and indigenous methods for vector control. The present study was conducted to determine the effects of *Citrus hystrix* ethanolic leaves extract on *Aedes aegypti* larvae.

MATERIALS AND METHODS

Mosquito larvae

Laboratory-reared Dagon Township strain of *Aedes* mosquito larvae were used for testing insecticidal properties of ethanolic leaves extract of *Citrus hystrix* DC.

Collection and preparation of Citrus hystrix leaves extraction

Kaffir lime (*Citrus hystrix* DC.) leaves were collected from Mawlamyine Township, Mon State. A total of 3 kilograms of *Citrus hystrix* leaves were cleaned and dried in shed place at room temperature for 30 days. One hundred grams of dried leaves powder were extracted with 95% ethanol 1:5 wt/v by cold percolation method in laboratory for 7 days. Complete removal of the solvent from the extract was accomplished in glass rotary evaporator. The resulting 8 gm of viscous material was obtained from 100 gm of dried leaves. The viscous material was stored at 4°C until use. The extraction was done in Central Research and Development Center (CRDC).

Larvicidal testing procedure

Based on preliminary tests, further dilutions were prepared with distilled water. Different concentrations of ethanolic leaves extract of *Citrus hystrix* DC as 2.4%, 2.1%, 1.8%, 1.5% and 1.2% were prepared freshly in 100 ml each of distilled water in 150 ml plastic cups. Fifty each 3rd and 4th instars *Aedes aegypti* larvae were put into different concentrations and also negative control test was done simultaneously. Detail testing was done according to method described by

WHO, 1981.¹⁵ Larvae were exposed 24 hours for each replication in different concentrations in laboratory at 27-29°C and 70 to 80% relative humidity. Four replicates were carried out and mortality was checked and recorded after 24 hours of exposure period. Dead larvae were identified when the larvae failed to move after probing with a needle in the thorax region of the body. Lethal concentration LC₅₀ and LC₉₀ values for 95% confidential limits were calculated by following formula.¹⁶

$$X^2 = (O-E)^2 / Ex(100-E)$$

(X²=Chi square, O=Observed value, E=Expected value, O-E=Observed minus expected)

Data analysis

Data entry and processing was made using Microsoft Excel software. The lethal concentrations of LC₅₀ and LC₉₀ values were calculated after 24 hours using dose-effect probit analysis.¹⁶

RESULTS

The ethanolic leaves crude extract of *Citrus hystrix* exhibited strong larvicidal activity against *Aedes* larvae.

Table 1. Larvicidal effect of ethanolic crude extract of *Citrus hystrix* DC leaves against 3rd and 4th instars *Aedes aegypti* larvae

Ethanolic extracts concentration (%)	Mortality					Total tested larvae	Mortality	%
	Test larvae	Test 1	Test 2	Test 3	Test 4			
2.4	50x4	50	49	50	50	200	199	99.5
2.1	50x4	44	45	44	46	200	179	85.5
1.8	50x4	32	34	31	28	200	125	62.5
1.5	50x4	15	12	12	14	200	53	26.5
1.2	50x4	0	1	3	0	200	4	2
Control	50x4	0	0	0	0	200	0	0

Table 1 shows that the highest dose 2.4% produced 99.5% mortality followed by 2.1% crude extract found 85.5% mortality of 3rd and 4th instars *Aedes* larvae. And, 1.8% concentration of ethanolic leaves crude extract found 62.5% mortality of *Aedes* larvae within 24 hours.

Table 2. Effective concentration of *Citrus hystrix* leaves extract on 3rd and 4th instar immature stages of *Aedes aegypti* mosquito

Lethal concentration	95% Upper confidence limit	95% Lower confidence limit	Corrected limit
LC ₅₀	1.76%	1.69%	1.73%
LC ₉₀	2.136%	2.03%	2.08%

$$X^2 = 0.0445, p=0.05, df=4$$

Table 2 shows that the 95% confidence corrected effective lethal concentrations LC₅₀ for 50% mortality and LC₉₀ for 90% mortality were found to be 1.73% and 2.08% crude ethanolic leaf extract of *Citrus hystrix*, respectively.

DISCUSSION

It was found that 2.4% (2400 ppm) of ethanolic leaves extract produced the most potent larvicidal activity with nearly 100% mortality within 24 hours and over 60% mortality at 1.8% concentration within 24 hours. These findings are consistent with that of a study¹⁷ which reported that the water extract of *Citrus* seed showed LC₅₀ values of 135,319.40 and 127,411.88 ppm against the larvae of *Aedes aegypti* and *Culex quinquefasciatus*.¹⁷

Another study revealed that the ethanol plant extract of Kaffir lime (*Citrus hystrix*) 6.4% effected with a high of 90% mortality.¹¹ Another study observed that the leaf oil of *Citrus hystrix* 29.254 and 26.748 µl/g affected 50% mortality at 24 hours and 48 hours after treatment, respectively, and beta-citronellal 66.65% was the most abundant compound in the leaf oil of *Citrus hystrix*.¹⁸ *Citrus hystrix* leaves essential oil showed good repellence properties against *S. litera* larvae. Repellency of *Citrus hystrix* essential oil might be due to the presence of β-citronellal compound which has high bioactive activity.¹⁸ The highest oil content was found in *Citrus hystrix* (3.36%) followed by *S. aromaticum* (1.50%) according to dry weight and high larvicidal efficacy on both pyrethroid-susceptible and -resistant strains of *Aedes aegypti* were dose dependent. When exposed

to the higher oil concentrations, more larvae showed toxic symptoms that led to an increase in mortality values.¹⁹ Other researchers observed that the *C. reticulata* was the highest potential larvicidal efficacy followed by *C. hystrix*, *Z. xerumbet*, *K. galangal* and *S. aromaticum* plant extracts with LC₅₀ of 15.42, 30.07, 48.88, 53.64, and 143.89 ppm, respectively, against the pyrethroid susceptible strain of *Aedes* larvae.¹⁹ These findings agree with the larvicidal activity finding of present study.

The present study revealed that LC₅₀ and LC₉₀ values of 1.73% and 2.08%, respectively, were found to be very effective larvicidal properties against *Aedes* larvae. According to dose effect analysis, 1730 ppm field dose for 50% mortality and 2080 ppm field dose for 90% mortality will be needed to control *Aedes* larvae in field and also 100% mortality was found at 2400 ppm dosage will be the estimated field dosage based on the present study. Residual effect was found 5 days for 100% larval mortality in laboratory.

Citrus hystrix plants are available in many parts of the country although high numbers of plants are grown in coastal areas at Mon State and Thaninthayi Region. High amount of leaves extract was needed for 200 liters of water. Therefore, further analysis is needed to isolate the active compound for larval control, field trials and acceptability.

More studies are needed to elucidate the ovicidal activity against a wide range of mosquito species and the active compound responsible for repellent activity should be identified, which could be used to control different mosquito species in the future.

Conclusion

The finding demonstrates that the leaves extract of *Citrus hystrix* is very effective larvicidal agent against *Aedes* mosquito larvae. It may serve as suitable alternative to synthetic insecticides in future as it is relatively safe and inexpensive and readily available in many areas of the country.

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