





Mosquito larvicidal property of Citrus species

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Abstract

Mosquitoes and their larvae have several detrimental effects on humans, animals and the environment. Their bites cause itching, allergic reactions and skin irritation. Mosquito larvae thrive in stagnant water, polluting water sources and creating breeding grounds for further infestations. Large mosquito populations negatively impact agriculture and livestock by transmitting diseases to animals. Additionally, their presence reduces outdoor activities, affecting tourism and economic productivity in affected regions. The review focuses on the Culicidae mosquito genera *Anopheles, Aedes* and *Culex*, including many species in each. The papers show that Clevenger and Soxhlet apparatus methods maintain high-quality and quantity oils because of their unique properties. These methods are cost-effective and environmentally friendly since chloroform, carbon tetrafluoride and other similar pollutants are not used, which causes severe health issues. Future research will examine how oil release from plant parts varies with age and how this relates to mosquito mortality. Different plant parts may yield varying quantities of oil at different stages, which can be considered as a point of discussion. The present findings support the efficiency of certain *Citrus* species in the Rutaceae family to eradicate mosquitoes and its larvae.

Keywords: Citrus species; LC₅₀; mosquito larvicide; rutaceae

Introduction

Mosquitoes are the primary carriers of different diseases with high mortality rates. According to recent studies, vector-borne diseases cause an intimidating effect that causes more than 700000 deaths annually (1). Each year, over 0.5 billion and 0.96 billion individuals contract malaria and dengue, respectively, with mortality rates exceeding 4 lakh and 0.25 lakh, while lymphatic filariasis affects 0.1 billion people annually (2). The range and severity caused by mosquitoes continue to grow and thus can be considered a global issue. The insecticides used to eliminate them are causing additional problems, such as heavy metal deposition, which later leads to complications like cancer, reproductive disorders and respiratory and neurological conditions (3). Moreover, insecticide-resistant mosquitoes are an alarming issue that can't be controlled (4).

In this instance, substitutes to control mosquitoes have been developed with an almost 100 % death rate. Essential oils are a class of secondary metabolites produced by plants and can be extracted from their leaves, peels, bark and seeds (5). These aromatic oils are 100 % cost-effective, environmentally friendly and have mosquito larvicidal properties. It is proven that the plants from the rutaceae family have aromatic oils which can be extracted from different parts and act as an efficient mosquito larvicide (5). Due to the strong demand for *Citrus* fruits, which produce a significant amount of essential oils, their waste can be

repurposed into valuable products such as insecticides or animal feed (6, 7). In this manner, the least expensive and most efficient insecticide to eradicate mosquitoes can be designed. These essential oils interact with gaba and octopamine receptors, preventing insect resistance development, which is an added advantage (8) (Table 1).

Materials and Methods

Collection and isolation of essential oils

The plant parts were usually collected in the mornings to maximize the oil content, which can be reduced due to stress caused by sunlight, humidity and temperature. These can adversely affect the oil since diurnal variations influence the quality.

This present study found that the Clevenger and Soxhlet apparatus methods were widely used. While both are similar, they differ in the quality of essential oils produced-Soxhlet yields a higher quantity but lower quality, whereas Clevenger provides superior quality with a lower yield. The fresh peels were subjected to hydro-distillation using Clevenger apparatus: 50g (9) or 200g (10) at 70 - 100 °C for 3 hr (6). Anhydrous sodium sulfate was added to absorb the moisture content; thus, it can be stored in cold conditions for further usage (9, 10). Different solvents were also used in the soxhlet extraction method. Other than this, the process remains the same. The plant part was washed and dried in an

ANAMIKA ET AL

Table 1. Plants and their affected mosquito species

S.No	Plant species	Common name	Species
1	Citrus aurantifolia	Lime	An. labranchiae, Cx. quinquefasciatus, A. albopictus, A. aegypti
2	Citrus aurantium	Bitter orange	An. stephensi, Cx. pipiens
3	Citrus hystrix	Kaffir lime	A. aegypti
4	Citrus limetta	Musambi/ sweet lemon	Cx. mimulus, A. albopictus, An. maculates
5	Citrus limon	Lemon	An. stephensi, Cx. pipiens, A. albopictus
6	Citrus paradisi	Grape fruit	An. stephensi, A. aegypti, A. albopictus
7	Citrus reticulata	Mandarin orange	A. albopictus, A. aegypti, Cx. pipiens
8	Citrus sinensis	Sweet orange	A. albopictus, Cx. tritaeniorhynchus, An. stephensi, An. labranchiae, A. aegypti, An. subpictus, Cx. pipens, Cx. quinquefasciatus
9	Citrus sinensis	Valencia orange	A. aegypti, Cx. pipiens
10	Citrus reticulata	Mandarine	A. aegypti, Cx. pipiens
11	Clausena excavate	Pink lime berry	A. aegypti, A. albopictus
12	Feronia limonia	Wood apple	A. aegypti, An. stephensi, Cx. quinquefasciatus
13	Chloroxylon swietenia	Ceylon satin wood	A. aegypti, An. stephensi
14	Ruta chalepensis	Fringed rue	A. aegypti, An. quadrimaculatus, A. albopictus
15	Swinglea glutinosa	Tabog	A. aegypti
16	Toddalia asiatica	Wild orange tree	A. albopictus
17	Zanthoxylum armatum	Prickly ash	A. aegypti, An. stephensi, Cx. quinquefasciatus
18	Zanthoxylum avicennae	Prickly ash	A. albopictus
19	Zanthoxylum limonella	Prickly ash	A. aegypti, An. dirus
20	Zanthoxylum oxyphyllum	Prickly ash	A. aegypti

oven at 60 °C for 48 hr and then these dried parts were made to fine powder using an electric blender. This powder was loaded in the thimble of the Soxhlet apparatus for steam distillation using different solvents such as Di-ethyl ether (11, 12), ethanol (5) and methanol (13). The specifications include 4-5 hr cycle time and an extractor ID of 38 mm. The oil can be condensed by keeping it at room temperature (11) or using a rotary vacuum evaporator (13). Another method for oil extraction is the cold press method (8, 12, 14).

Calculation of LC₅₀

 LC_{50} is the concentration required to kill 50 % of the test organisms and LD_{50} is the dose at which it kills 50 % of the test organisms. The percentage mortality was calculated using the formula:

(No. of dead larvae * 100)/(No. of larvae used) ...(Eqn.1)

The mosquito larvae were collected and used for the test by being introduced to different essential oil concentrations and maintaining control for the cross-check. The number of deaths was noted in 24 hr, i.e., 24 and 48 hr. The larvae were not fed during the study and five replicates were conducted to verify the results (11).

Description

Since 21 components were separated from the leaves, 94.8 % are served by dl-limonene. The peel of *Citrus aurantium* included dl-limonene, β -myrcene and α -piene, while *C. paradisi* displays 35.7 ppm LC50, *C. aurantium* displays 31.2

Table 3. Lethal concentration of *Citrus* extracts affecting *A. albopictus*

ppm, *Anopheles stephensi's* LC₉₀ readings were 35.71 ppm and 70.23 ppm, respectively (9). (Table 2, 3)

In contrast to *C. bergamia*, which contains 31.9 % of linally acetate, *C. limon, C. reticulata* and *C. sinensis* contain 52.8 %, 59.2 % and 88.8 % of limonene. In contrast to *C. sinensis*, which contains 96.1% monoterpene hydrocarbons, *C. bergamia* has 46.7 % monoterpene hydrocarbons and 50.9 % oxygenated chemicals such esters, aldehydes and alcohols. As mentioned earlier, the composition of linalool, c-terpinene, limonene, linally acetate and β -pinene varies among the species (8).

The mosquito larvicidal activity by the *Citrus* species is due to the presence of nomilin in varying concentrations; *C. sinensis* has 377 ppm and 21.2 ppm of limonin and nomilin, respectively, whereas *C. reticulata* have 5.3 ppm and 3.9 ppm of the same. Due to the toxicity of nomilin, the LC₅₀ value is 121.04 ppm; that of limonin is 382.2 ppm after 72-hr exposure (11). (Table 4-6)

Table 2. Plants with affected mosquito species and their lethal concentration

	species	concentration (LC₅₀)
. aurantium	An. stephensi	31.20 ppm
C. paradisi	An. stephensi	73.83 ppm
C. paradisi	A. aegypti	47.3 ppm
C. paradisi	A. albopictus	85.1 ppm
	. aurantium C. paradisi C. paradisi C. paradisi	C. paradisi An. stephensi An. stephensi An. stephensi A. aegypti

S. No	Plant species	Mosquito species	Lethal concentration (LC ₅₀)	Percentage mortality	Lt50 value
1.	C. sinensis	A. albopictus	297 ppm	97%	18.5 hr
2.	C. reticulata	A. albopictus	377.4 ppm	88%	31 hr

Table 4. Citrus extracts with solvents affected mosquitoes and lethal concentration

S. No	Plant species peel and extracted solvent	Mosquito species	Lethal concentration
1	C. sinensis + chloroform	An. Stephensi	LC50 = 58.3 ppm
2	C. sinensis + chloroform	An. Stephensi	LC90 = 298.3 ppm
3	C. sinensis + methanol	Cx. tritaeniorhynchus	LC50 = 38.2 ppm
4	C. sinensis + methanol	Cx. tritaeniorhynchus	LC90 = 184.7 ppm

Table 5. Plants with affected mosquito species and their LC₅₀ and LC₉₀ values

S. No	Mosquito species	Plant species	Lethal concentration (LC90)	Lethal concentration (LC ₅₀)
1	An. labranchiae	C. aurantium	83.8 ppm	160 ppm
2	Cx. quinquefasciatus	C. aurantium	351 ppm	179.8 ppm
3	A. albopictus	C. aurantium	-	322.4 ppm
4	A. aegypti	C. hystrix	-	30.1 ppm
5	An. stephensi	C. limon	138.9 ppm	35.95 ppm
6	A. aegypti	C. reticulata	-	15.4 ppm
7	An. labranchiae	C. sinensis	351.4 ppm	640 ppm
8	An. subpictus	C. sinensis	298.3 ppm	58.3 ppm
9	An. stephensi	C. sinensis	138.9 ppm	35.95 ppm
10	A. aegypti	C. sinensis	1371 ppm	85.9 ppm
11	Cx. pipens	C. sinensis	-	20-160 ppm

Table 6. A. albopictus with different citrus plants, specific parts and time of exposure

S. No	Macauita anacias	Diantenesies	Pla	int part	Time of avecause (by)
5. NO	Mosquito species	Plant species	Seed	Peel	— Time of exposure (hr)
1	A. albopictus	C. limon	395.6 ppm	468.7 ppm	24
2	A. albopictus	C. limon	247.2 ppm	392.2 ppm	48
3	A. albopictus	C. sinensis	906 ppm	1009.4 ppm	24
4	A. albopictus	C. sinensis	759.8 ppm	1041.5 ppm	48

The oil from savage citrange has great efficacy against Aedes albopictuseven after 72 hr, while Cassa grande and Fairchild had the lowest efficiency, according to Faizal et al. Nomilin showed more toxicity than limonin. However, Fairchild and Carrizo citrange exhibited reduced efficiency after 48 hrs an Cassa grande and Carrizocitrange exhibited minimal efficiency after 72 hr (5) (Table 7).

Maximum efficacy was achieved in C. sinensis when 45 0 ppm of ethanol extract was placed; this provided total prote ction lasting for 150-180 min (14) (Table 8). Compared to Aedes aegypti, C. aurantifolia showed LC50 values of 128.8 ppm and 106.8 ppm for the peel and 188.6 ppm and 107.4 ppm for the leaf at 24 and 72 hr, respectively. They demonstrated 5.3 ppm and 17.7 ppm for peel and leaf against A. aegypti as an ovicidal property. The primary components of the leaf of C. aurantifolia were limonene and citral, while the peel included palatinol-1 c and limonene. 26 and 31 compounds were recovered from the peel and leaf, but the peel and leaf were packed with limonene and farnesol (15) (Table 9). At 43.3 %, 51. 5% and 35 % in C. reticulata, C. reticulata var chinese and C. sinensis, respectively, limonene was a vital factor (16). C. sinensis, C. limon and C. paradisi contain the most dangerous amount of y-terpinene, LC₅₀ 202 ppm. of these three species, C. sinensis has the highest larvicidal properties, at 28.7 ppm, followed by C. limon and C. paradisi, at 25 ppm and 37 ppm, respectively (6). z- β -ocimene, cisnaphthalenedecahydro and β -pinene are the main elements of *C. medica, C. grandis* and *C. sinensis*, respectively; α -pinene, which is found in all three, confers the larvicidal activity. with LC₅₀ and LC₉₅ values of 137 ppm and 342.5 ppm for larvae and 78.4 ppm and 126.1 ppm for nymph, respectively, *C. sinensis* has great efficacy in these, resulting in a good nymphocide (7). *C. sinensis* (L) *Osbeck* showed 21.5 ppm LC₅₀, or 100 % death (30 ppm), against *A. aegypti* after 24 hr, while r-limonene, one of its main components, showed LC₅₀ of 27 ppm, or 100 % mortality (50 ppm). Paste complex made with ethanol and water displayed 23 ppm LC₅₀ at 50 ppm, or 93 % (17) (Table 10).

A lethal ester molecule called corynan-17-1,18,19-didehydro-10-methoxy-acelate is accountable for the larvicidal activity in *C. limetta*. The leaf methanolic extracts showed LC₅₀ values of 15560 ppm, 13720 ppm and 11450 ppm, respectively. These values demonstrated greater larvicidal properties against *Cx. mimulus, An. maculates* and *A. aegypti* were determined at 79720 ppm, 86490 ppm and 88210 ppm. *A. albopictus, An. maculates* and *Cx. mimulus* had LC₅₀s for newly molted third instars of 15560 ppm, 13720 ppm and 11450 ppm, respectively; the peel hexane extract of *C. limetta* showed the most potent activity against *An. stephensi* (13, 18).

Table 7. Mosquito species with reference to instar stages, their pupicidal and larvicidal values

S. No	Mosquito species & Instar stages	Larvicidal (LC50)	Larvicidal (LC90)	Pupicidal	
1	An. stephensi; 1 st instar	182.2 ppm	452.4 ppm	LCE0 = 400 0 nnm	
2	2 nd instar	227.9 ppm	544.7 ppm	LC50 = 490.8 ppm	
3	3 rd instar	291.7 ppm	659.3 ppm	1 000- 007 2	
4	4 th instar	398 ppm	858.9 ppm	LC90= 987.3 ppm	
5	A. aegypti; 1 st instar	204.9 ppm	509.7 ppm	1050 - 407 4	
6	2 nd instar	264.3 ppm	607 ppm	LC50 = 497.4 ppm	
7	3 rd instar	342.5 ppm	735 ppm	1 000 - 020 1	
8	4 th instar	436.9 ppm	891.6 ppm	LC90 = 938.1 ppm	
9	Cx. quinquefasciatus; 1st instar	244.7 ppm	567 ppm	L CEO - E21	
10	2 nd instar	324 ppm	729.8 ppm	LC50 = 531 ppm	
11	3 rd instar	385.3 ppm	806.6 ppm	1 600 - 067 2	
12	4 th instar	452.8 ppm	890.1 ppm	LC90 = 967.2 ppm	

ANAMIKA ET AL 4

Table 8. Plant parts, phytochemical composition, mosquitoes affected with lethal concentration

S. No	Plant species	Plant parts	Components	Mosquito species	LC ₅₀ (ppm)	LC90 (ppm)
1	C. aurantium	Fruits	Limonene	Cx. pipiens	39	79
2	C. hystrix	Peel	terpinene-4-ol, D – limonene, β – pinene	A. aegypti	30	51
3	C. limon	Fruits	Limonene	Cx. pipiens	30	78
4	C. reticulata	Peel	γ- terpinene, D – limonene	A. aegypti	15	36
5	C. sinensis	Aorial parte	Limonono	A. aegypti	20	99
6	C. SITIETISIS	Aerial parts	Limonene	Cx. pipiens	51	73
7	Clausona oveavato	Loavos	Torningland	A. aegypti	37	110
8	Clausena excavate	Leaves	Terpinolene	A. albopictus	41	116
9				An. stephensi	15	36
10	Feronia limonia	Leaves	β – pinene, estragole	A. aegypti	11	42
11				Cx. quinquefasciatus	22	60
12	Chloroxylon swietenia	Leaves	Limonene, geijerene,	A. aegypti	16	14
13	Chloroxylon swietenia	Leaves	germacrene D	An. stephensi	28	22
14				A. aegypti	22	36
15	Ruta chalepensis	Aerial parts	2- nonanone, 2- undecanone	An. quadrimaculatus	15	42
16				A. albopictus	35	67
17	Swinglea glutinosa	Aerial parts	Piperitenone, α — pinene, β - pinene	A. aegypti	65	151
18	Toddalia asiatica	Roots	D- limonene, geraniol, isopimpinellin, 4- vinylguaiacol, α — gurjunene	A. albopictus	69	110
19				A. aegypti	54	171
20	Zanthoxylum armatum	Seeds	Limonene, linalool	An. stephensi	58	183
21				Cx. quinquefasciatus	49	146
22	Zanthoxylum avicennae	Aerial parts	1, 8- cineole	A. albopictus	48	141
23	Zanthoxylum limonella	Fruits	Terpinene-4-ol, D- limonene	A. aegypti	24	55
24	Zananoxytam umonetta	riuits	rerpinene-4-ot, D- timonene	An. dirus	57	76
25	Zanthoxylum oxyphyllum	Leaves	Methyl nonyl ketone, methyl heptyl ketone	A. aegypti	7	Not determined

Table 9. Mosquitoes with different citrus plants, time of exposure and lethal concentration

S. No	Mosquito species	Plant species	Time of exposure (Hours)	Lethal concentration (LC50)
1.		C. reticulata	24	32.8 ppm
2.		C. reticulata	48	20.5 ppm
3.	Cu minima	C. reticulata chinase	24	16.1 ppm
4.	Cx. pipiens	C. reticulata chinase	48	13.2 ppm
5.		C. sinensis	24	15.4 ppm
6.		C. sinensis	48	12.5 ppm

 Table 10.
 Mosquitoes with different Citrus plants, specific parts and lethal concentration

S. No	Plant species	Plant part	Mosquito species	Lethal concentration (LC ₅₀)
1	C. hystrix	Fruit	A. aegypti	30.1 ppm
2	C. reticulata	Fruit	A. aegypti	15.4 ppm
3	C. sinensis	Fruit	A. aegypti	11.9 ppm
4	Clausena excavate	Twig	A. albopictus	41.1 ppm
5	Clausena excavate	Leaf	A. albopictus	41.2 ppm
6	Ruta chalepensis	Leaf	A. albopictus	33.2 ppm
7	Toddalia asiatica	Root	A. albopictus	69.1 ppm

Results and Discussion

The Citrus family is one of the most important and widely distributed families in Asian countries and it has high mosquito larvicidal activity. It was understood that a high amount of Citrus fruit waste is dumped worldwide, since it is highly used for juices, canned drinks, wines and much more. These wastes can be reused in such a way that it can be helpful for human welfare and society. Mosquitoes are an important and devastating problem all over the globe, especially in developing countries. Hence, reusing these waste products is an efficient mechanism to minimize the cost-effectiveness and healthiness of mosquitoes. Anopheles, which is a malarial vector, Aedes, the dengue vector; and the Culex, which is a Japanese encephalitis vector, are included in this review. These three genera fall under the Diptera order and the Culicidae family. Aedes mosquito widely spread yellow fever, dengue and chikungunya, but the efficient discovery of the yellow fever vaccine made the scenario less problematic. In this review, 20 *Citrus* species are taken into account.

The literature showed that *C. sinensis* is mostly studied and used because the nomilin, a good larvicide agent, is present in high amounts in this plant. Limonene is another important compound found in almost all *Citrus* species in relatively higher amounts. Corynan-17-1,18,19-didehydro-10-methoxy-acelate is an ester molecule known for its larvicidal activities against *Anopheles, Aedes* and *Culex,* which is found in *C. limetta*. The LC50 values of *C. paradisi, C. aurantium, C. sinensis, C. limon, Feronia limonia* (L.) Swingle, *Chloroxylon swietenia* DC, *Ruta chalepensis L, Zanthoxylum armatum DC* and *Zanthoxylum limonella Alston* against *Anopheles* species are 73.8 ppm, 31.2 ppm, 58.3 ppm, 36 ppm, 15 ppm, 28 ppm, 15 ppm, 58 ppm, 57 ppm respectively.

The LC₅₀ values of *C. paradisi, C. limon, C. reticulata, C. sinensis, C. aurantium, C. hystrix, C. reticulata Blanco, C. sinensis Osbeck, Clausena excavate Burm, Feronia limonia* (L) Swingle, *Chloroxylon swietenia* DC, *Ruta chalepensis* L, *Swinglea glutinosa* (Blanco) Merr, *Toddalia asiatica* (L) Lam, *Zanthoxylum armatum* DC, *Zanthoxylum avicennae* (Lam.) DC, *Zanthoxylum limonella Alston* and *Zanthoxylum oxyphyllum Edgew* against *Aedes* species are 85 ppm, 145 ppm, 318 ppm, 297 ppm, 322 ppm, 30 ppm, 15 ppm, 20 ppm, 41 ppm, 11 ppm, 16 ppm, 22 ppm, 65 ppm, 69 ppm, 54 ppm, 48 ppm, 24 ppm and 7 ppm respectively.

The LC₅₀ values of *C. sinensis, C. aurantium, C. limon, Citrus sinensis* (L.) *Osbeck, Feronia limonia* (L) *Swingle, Zanthoxylum armatum* DC, *C. reticulata and C. reticulata Chinase* against *Culex* species are 38 ppm, 180 ppm, 30 ppm, 51 ppm, 22 ppm, 49 ppm, 33 ppm and 16 ppm respectively. From these values, it is well understood that the *Citrus* species can be used as an efficient mosquito larvicide.

The A. albopictus showed 97 % and 88 % mortality against Citrus sinensis and Citrus reticulata, exhibiting 297 ppm and 377.4 ppm lethal concentrations (LC₅₀). The LT₅₀ values calculated were 18.5 hr and 31 hr for C. sinensis and C. reticulata, respectively. Since Clevenger is used frequently and produces high-quality oils, temperature control is a constraint; however, Soxhlet produces a higher yield with less amount, raising concerns about time consumption. There was no data regarding the age of the plant part collected for the study. Since the age of the plant part is related to the chemicals produced, their concentration and intensity vary depending on the part and the age. Regarding the disparity in mortality between males and females, no data exist. Since males feed on plant sap and females usually spread illness, there may be differences in the likelihood of killing a certain gender.

Conclusion

In conclusion, *Citrus* species (Rutaceae) exhibit significant mosquito larvicidal properties, highlighting their potential as eco-friendly alternatives to synthetic insecticides. Their bioactive compounds effectively target mosquito larvae, reducing vector populations. Further research on formulation, safety and field efficacy is essential to develop sustainable, plant-based larvicides for mosquito control programs.

Compliance with ethical standards

Conflict of interest: No conflict of interest among authors. **Ethical issues:** None

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ANAMIKA ET AL 6

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