

Original Research

Insecticidal effect of Essential Oils of *Citrus limon*, *Cymbopogon citratus* and *Syzygium aromaticum* and their Synergistic Combinations Against *Anopheles* Mosquitoes

Tunde Ayobami Owolabi^{1*}, Philippine Chigozie Okubor², James Danga³, Issa Onimisi Bello⁴

Abstract

While synthetic insecticides offer effective pest control, their many side effects necessitate exploring alternatives, this study investigates the insecticidal potential of essential oils from lemon (*Citrus limon*), lemongrass (*Cymbopogon citratus*), and clove (*Syzygium aromaticum*) against *Anopheles* mosquitoes, a prime vector of malaria, a deadly global disease. The oils were extracted via hydro-distillation, Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze the chemical profiles. The insecticidal activity was tested using a susceptibility test to evaluate the knockdown, mortality rates and time against adult mosquitoes following standard protocols. The extraction procedure yields of 0.5, 0.5, and 0.2% were obtained for lemon, lemongrass, and clove respectively. GC-MS showed limonene as the dominant compound in lemon oil (66.4%), while citral a (48.3%) and citral b (39.9%) were most abundant in lemongrass oil, and that of Clove is eugenol (69.0%). Adulticidal efficacy results put clove oil to have the best activity with a knockdown rate of 75 ± 0.75 at 14 minutes, a mortality rate of 45 ± 0.74 at 25 minutes, lemon oil exhibited the least activity with 30 ± 0.866 knockdown rate at 26 minutes, a mortality rate of 15 ± 0.47 at 42 minutes. However, the combination of Lemongrass and Clove (FM2) demonstrated an enhanced overall insecticidal efficacy beyond the capabilities of individual oils with a knockdown rate of 85 ± 0.25 at 7 minutes and a mortality rate of 60 ± 0.37 at 16 minutes. Despite mosquitoes' resistance to the individual oil, these findings suggest that these oils can be optimized for more effective insect control when combined, consequently, this research has contributed to the quest for a better, cheaper, safer and eco-friendly for mosquito control.

Keywords

Natural insecticides; Essential Oils; Mosquitoes; Gas Chromatography-Mass Spectrometry.

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Insekticidni učinek eteričnih olj *Citrus limon*, *Cymbopogon citratus* in *Syzygium aromaticum* ter njihovih sinergijskih kombinacij proti komarjem *Anopheles*

Izvleček

Sintetični insekticidi zagotavljajo učinkovit nadzor nad škodljivci, vendar imajo tudi številne nezaželene stranske učinke. S tem namenom smo v raziskavi testirali nekatere njihove naravne alternative. V študiji smo raziskali insekticidni potencial eteričnih olj limone (*Citrus limon*), limonske trave (*Cymbopogon citratus*) in klinčkov (*Syzygium aromaticum*) proti komarjem *Anopheles*, glavnemu prenašalcu smrtonosne svetovne razširjene malarije. Olja so bila ekstrahirana s hidrodestilacijo, za analizo kemijskih profilov pa je bila uporabljena plinska kromatografija z masno spektrometrijo (GC-MS). Insekticidno aktivnost smo testirali s testom občutljivosti, da bi po standardnih protokolih ocenili izločanje, stopnjo smrtnosti in čas delovanja na odrasle komarje. S postopkom ekstrakcije je bilo pridobljeni 0,5 %, 0,5 % in 0,2 % ekstrakti eteričnih olj za limono, limonsko travo in nageljnovke žbice. GC-MS je pokazala, da je limonen prevladujoča spojina v limoninem olju (66,4 %), medtem ko je bilo v olju limonske trave največ citrala a (48,3 %) in citrala b (39,9 %), v olju nageljnove žbice pa evgenola (69,0 %). Rezultati testov učinkovitosti delovanja na odrasle komarje so pokazali, da je imelo olje nageljnove žbice najboljšo aktivnost, saj je bila stopnja izločanja $75 \pm 0,75$ v 14 minutah, stopnja smrtnosti $45 \pm 0,74$ v 25 minutah, limonino olje pa je imelo najmanjšo aktivnost, saj je bila stopnja izločanja $30 \pm 0,866$ v 26 minutah, stopnja smrtnosti pa $15 \pm 0,47$ v 42 minutah. Vendar je kombinacija limonske trave in nageljnove žbice (FM2) pokazala večjo splošno insekticidno učinkovitost, ki presega zmogljivosti posameznih olj, s stopnjo izločanja $85 \pm 0,25$ v 7 minutah in stopnjo smrtnosti $60 \pm 0,37$ v 16 minutah. Kljub odpornosti komarjev na posamezno olje te ugotovitve kažejo, da je mogoče ta olja v kombinaciji optimizirati za učinkovitejše zatiranje insektov, zato je ta raziskava prispevala k iskanju boljšega, cenejšega, varnejšega in okolju prijaznega sredstva za zatiranje komarjev.

Ključne besede

Naravni insekticidi; eterična olja; komarji; plinska kromatografija - masna spektrometrija.

Introduction

Plants have long served as a source of natural remedies, and essential oils, extracted from various plant parts, represent a concentrated form of these bioactive compounds. These oils exhibit various properties including insecticidal, antibacterial, and antioxidant effects, as documented in research (Petrovska et al., 2012).

The environmental impact of synthetic pesticides is a growing concern. While these chemicals offer effective pest control, their persistence in the environment and potential toxicity to non-target organisms necessitates the exploration of alternative solutions (Park and Tak, 2016). Essential oils from renewable resources present a potentially more sustainable approach. Their mode of action often involves repelling insects rather than eradicating them, minimizing disruption to ecological balance.

Essential oils are plant products derived through

hydro-distillation or other processes that retain the original fragrance and flavor of their source (Lutz et al., 2014). They are a diverse group of plant oils that are highly fragrant. The most typical places to find them are peels, seeds, bulbs, rhizomes, bark, wood, flowers, buds, leaves, twigs, and resinous materials. According to reports (Park and Tak, 2016; Ayvaz et al., 2010), many plants that possess essential oils can be used as pesticides or repellents. Within the higher plant kingdom, there are 17,500 species of aromatic plants.

Cymbopogon citratus, also known as lemongrass, has become widely naturalized and invasive in various regions. This versatile herb is renowned for its medicinal properties, traditionally employed to treat a range of ailments including arthritis, headaches, dyspnea, common wounds, and bacterial infections (Oniha et al., 2023).

Citrus limon, commonly known as lemon, belongs to the Rutaceae family and is native to tropical and subtropical regions. This citrus tree is characterized by its glossy,

evergreen leaves, fragrant white flowers, and yellow fruit with a sour taste. Lemon is widely utilized for its medicinal and culinary purposes (Moshood, 2023).

Clove, scientifically known as *Syzygium aromaticum*, is an aromatic spice native to the Maluku Islands in Indonesia and widely cultivated in tropical regions. Belonging to the Myrtaceae family, clove is renowned as a culinary and medicinal herb. It is particularly rich in essential oils, notably eugenol, which gives it its characteristic fragrance and therapeutic properties (Cortés-Rojas et al., 2014).

Mosquito is the principal carrier of malaria. Malaria, a potentially fatal disease, is transmitted through the bite of an infected female *Anopheles* mosquito carrying protozoan parasites from the genus *Plasmodium*. These parasites enter the bloodstream during mosquito feeding and migrate to the liver where they mature and multiply rapidly. Upon release back into the bloodstream, infected red blood cells cause recurrent bouts of fever and other symptoms. Diseases transmitted by adult female *Anopheles* mosquitoes that feed on humans are collectively termed mosquito-borne diseases. Given the millions of lives at risk annually from these illnesses, they pose a significant global

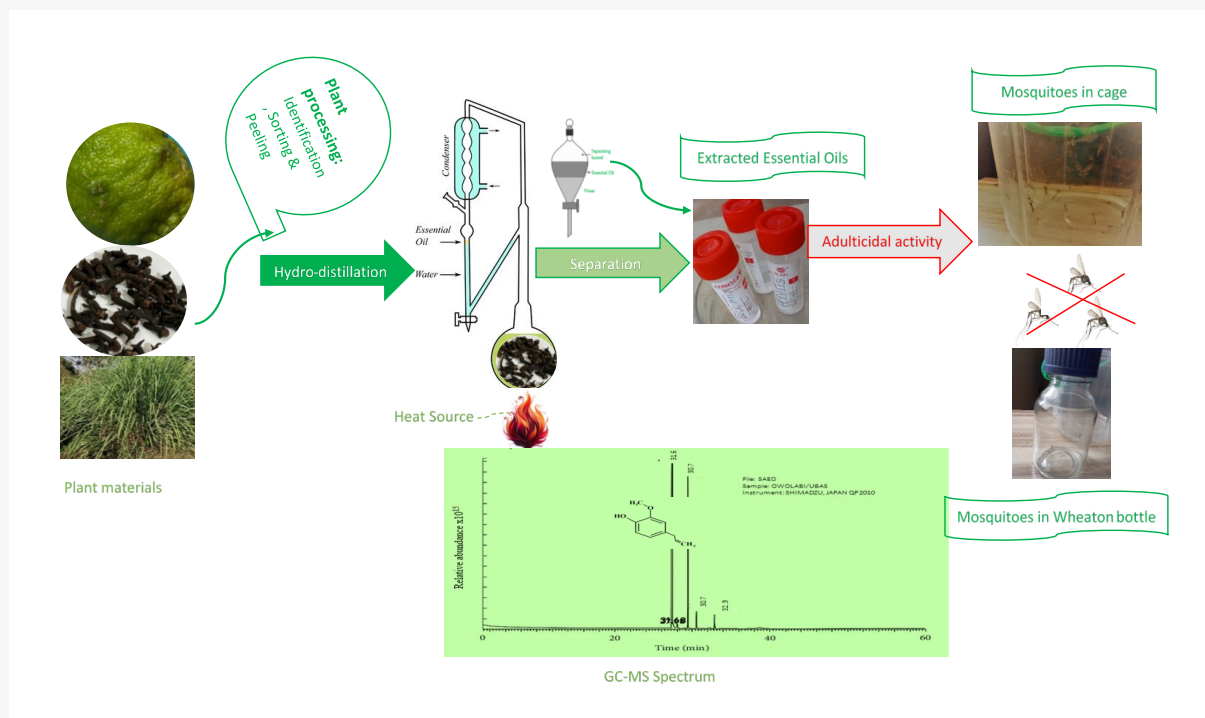
health threat. Malaria is caused by various species of the *Plasmodium* parasite (Foster and Walker, 2019).

The objective of this study was to investigate the adulticidal potential of several combinations of *Citrus limon*, *Cymbopogon citratus* and *Syzygium aromaticum* against female adults of *Anopheles* mosquitoes.

Materials and Methods

Plant collection and identification

In November 2023, the aerial parts of *Cymbopogon citratus* (lemongrass), and the fruit of *Citrus limon* (Lemon) were collected from Okada settlements, except for the dried fruits of *Syzygium aromaticum* (clove) which were purchased from Okada market in Ovia-Northeast LGA, Edo State, Nigeria. The plants were identified and verified in the Dora Akunyili College of Pharmacy herbarium, herbarium numbers; IUO/16/205, IUO/11/104, IUO/19/391 for *Cymbopogon citratus*, *Citrus limon* and *Syzygium aromaticum* and respectively and voucher specimens deposited in the herbarium.



Extraction of the essential oils

The fresh parts of each plant (250 g) were individually subjected to steam distillation using a distillation setup comprising an electric heating mantle, a 2000 mL round-bottomed flask, a Clevenger, a condenser, and a chiller. The plant material was placed in the flask along with 1200 mL of distilled water. The mixture was heated using the electric mantle to approximately 100°C and maintained at this temperature for at least 3 hours until the distillation process was completed and no further essential oil (EO) could be extracted.

The collected distillate, consisting of a mixture of essential oil and water, was transferred to a glass-separating funnel for phase separation based on their differing densities. Sodium sulfate was added for the complete removal of the remaining water. The percentage yield of the essential oils extracted was determined using the formula;

$$\% \text{Yield} = \frac{\text{Volume of extracted Oil}}{\text{Weight of original plant material}} \times 100$$

Gas Chromatography-Mass Spectroscopy (GC-MS)

The essential oils were subjected to GC-MS analysis on a GC-MS instrument (SHIMADZU, JAPAN QP2010) with Elite – DB- 5M column and the GC-MS solution version 2.53 software. Initially, the oven temperature was maintained at 60°C

for 3 minutes, and the temperature was gradually increased up to 250°C at 10.0/24.0 min and 4.0 µL of samples were separately injected for analysis. Helium gas 99.995% of purity was used as a carrier gas as well as an eluent. The flow rate of helium gas was set to 0.99 mL/min. The sample injector temperature was maintained at 250°C and the split ratio is 40 throughout the experiment. The ionization mass spectroscopic analysis was done with 70 eV. The mass spectra were recorded for the mass range 50-600 m/z for about 24 minutes. The identification of components was based on a comparison of their mass spectra with that of the library. As the compounds separated on elution through the column, they were detected in electronic signals. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization detector where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments were charged ions with a certain mass. The m/z ratio obtained was calibrated from the graph obtained which was called as the mass spectrum graph which is the fingerprint of the molecule. The identification of compounds was based on the comparisons of their mass spectra with NIST Library 2021 WILEY8, FAME. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

Biological activity and synergism of essential oil samples

Essential oil combinations

Table 1. Individual essential oils and their combinations.

Tabela 1. Posamezna eterična olja in njihove kombinacije.

EOs and their combinations	Plant	Code	Formulation
Individual Eos	<i>Citrus limon</i>	CLEO	10% C. limon EO + 90% ethyl alcohol
	<i>Syzygium aromaticum</i>	SAEO	10% S. aromaticum EO + 90% ethyl alcohol
	<i>Cymbopogon citratus</i>	CCEO	10% C. citratus EO + 90% ethyl alcohol
Combinations		FM1	10%CLEO+ 10% SAEO + 80% ethyl alcohol
		FM2	10%SAEO+ 10% CCEO + 80% ethyl alcohol
		FM3	5% CLEO + 5% SAEO + 5% CCEO + 75% ethyl alcohol
	Positive control	LDT	10% LDT + 90% ethyl alcohol
	Negative control	ETAL	100% Ethyl alcohol

EO- Essential oil, CCEO- C. citratus essential oil, CLEO- Citrus limon essential oil, SAEO- Syzygium aromaticum essential oil, FM1- Formula 1, FM2- Formula 2, FM3- Formula 3

Mosquito Rearing

The eggs of the Anopheles Mosquitoes were collected within the Okada communities. The mosquito colony was kept under standard laboratory conditions and photoperiod of 12-h light and 12-h dark. The eggs of the mosquito were brought to hatch in a plastic tray containing 1000 mL of clean water. The larvae were fed with fish food pellets for 12–15 days until they pupated. The pupae were not fed with any food. One hundred new pupae were collected in a 300-ml plastic cup containing 200 ml of clean water, transferred into an insect cage (the size of 30 × 30 × 30 cm³), and left lying until developed into adults. Mosquito adults were provided with a 5% glucose solution as food, soaked in cotton sheets. Two-day-old female adults (not yet fed with blood meal) were collected as subjects for a World Health Organization susceptibility test (W.H.O, 2023).

World Health Organization Susceptibility Test

Five mosquitoes were taken to the Zoology laboratory for identification. Twenty-five 2-day-old female mosquitoes (not yet fed with blood meal) were exposed to 2 mL of each formulation, which was dropped onto a filter paper (Whatman® No.1) the size of 12 × 15 cm² for 1 hr in a treatment tube (44 mm in diameter and 125 mm in length) then transferred to a non-treatment tube. The knockdown rate was recorded at 30 minutes, and the mortality rate was recorded at 1 hour after exposure. Each treatment was performed in three replicates. Ten percent (w/v) Lambda-cyhalothrin and 90% v/v ethyl alcohol were used as positive control and negative control, respectively. The criterion for knockdown and mortality was no movement of any of the mosquito bodies. The distinction between knockdown and mortality was that knockdown was an occurrence recorded at 30 min after exposure while mortality was an occurrence recorded at 1 hr after exposure (W.H.O, 2023). Knockdown (KR%) and mortality rates (MR%) were calculated by the following formula:

$$\text{Knock down Rate (KR\%)} = \frac{\text{NK}}{\text{NT}} \times 100$$

$$\text{Mortality Rate (MR\%)} = \frac{\text{ND}}{\text{NT}} \times 100$$

where NK is the total number of knocked-down adults; ND is the total number of dead adults, and NT is the total

number of treated adults. The means of these rates were analyzed and compared by analysis of variance (ANOVA). Susceptibility levels were classified according to WHO criteria: Susceptible (S) means 98–100% of mosquito mortality, Possible Resistant (PR) means 80–97% of mosquito mortality, and Resistant (R) means less than 80% of mosquito/housefly mortality.

Results

Percentage yield of Essential oil from the three selected plants

Essential oil obtained from several rounds of extractions of a total of 2kg of freshly collected plants for the individual selected plants is presented in Table 2 below. An average quantity of 1.2-1.3, 1.8-1.9, 0.8-1.0 mLs were separately extracted equivalent to average yields of 0.4-0.5, 0.1-0.2, and 0.5-0.6% respectively for *Cymbopogon citratus*, *Synzygium aromaticum* and *Citrus limon*. The colors oils are yellow, and white liquids respectively.

Chemical compositions of the evaluated Essential Oils

GC-MS studies on the chemical constituents of *Citrus limon*, *Cymbopogon citratus*, and *Synzygium aromaticum* oils revealed the presence of 19, 25, and 21 compounds, constituting 97.9, 91.1 and 97.4% of all the compositions, respectively (Table 3 – 5).

Results of GC-MS analysis of essential oil from *Citrus limon*

Table 3 below shows the mass spectrum of essential oil of *Citrus limon* peels obtained by GC-MS, it revealed that the oil contains nineteen (19) compounds. The most abundant compound is limonene with a peak area of 66.4% and retention time of 6.93. This is followed by γ-terpinene, with a retention time of 7.73, geranial with a peak area of 4.0% and retention time of 15.98, while trans-carveol is the least abundant compound with a peak of 0.1% and a retention time of 13.64. Table 3 shows the breakdown, while figure 2 shows the spectrum of the entire components in the essential oil from *Citrus limon* peels.

Table 2. Physical characteristics and percentage yield of the essential oils (EO).

Tabela 2. Fizikalne lastnosti in odstotni izkoristek eteričnih olj (EO).

Plant name	Family	Common name	Parts extracted	Colour of EO	Yield %
<i>Cymbopogon citratus</i>	Poaceae	Lemon grass	leaves	yellow	0.48
<i>Citrus limon</i>	Rutaceae	Lemon	Peel	white	0.5
<i>Syzygium aromaticum</i>	Myrtaceae	Clove	Dried buds	white	0.2

Table 3. GC-MS analysis of volatile oil composition of *Citrus limon*.Tabela 3. GC-MS analiza sestave hlapnih olj *Citrus limon*.

No.	Retention Time (min)	Compound	% Concentration
1	4.25	α -pinene	2.09
2	4.83	sabinene	0.59
3	5.32	β -pinene	3.90
4	5.58	myrcene	2.5
5	5.84	n-octanal	0.14
6	5.94	α -phellandrene	0.13
7	6.30	α -terpinene	0.23
8	6.93	limonene	66.4
9	7.29	(E)- β -ocimene	0.12
10	7.73	γ -terpinene	6.97
11	8.65	terpinolene	0.55
12	9.03	linalool	0.97
13	12.03	terpinen-4-ol	2.01
14	12.18	iso-verbanol	0.61
15	12.71	α -terpineol	2.06
16	13.64	trans-carveol	0.1
17	15.24	geraniol	0.24
18	15.98	geranial	4.0
19	20.64	geranyl acetate	0.41
		Unidentified	5.98
		Total	100

Results of GC-MS analysis of essential oil from *Cymbopogon citratus*

Table 4 shows the mass spectra of *Cymbopogon citratus* (Lemon Grass) obtained by GC-MS analysis it revealed twenty-five (25) compounds found in the essential oil from the plant. The most abundant compound is Citral a with a peak area of 48.26% and a retention time of 20.41.

This is followed by Citral b with a peak area of 39.9% and retention time of 18.92, while Geranyl Butyrate, Dihydroisocaryophyllene epoxide, trans- β -Ocimene are the least abundant compounds with peaks of 0.02% each and retention time of 31.98, 27.09, 10.01 respectively. Table 4 and figure 3 shows the entire components and the spectrum of the essential oil from *Cymbopogon citratus* (Lemon grass).

Table 4. GC-MS Analysis of volatile oil composition of *Cymbopogon citratus*.

Tabela 4. GC-MS analiza sestave hlapnih olj *Cymbopogon citratus*.

No.	Retention Time (min)	Compound	% Concentration
1	5.716	α -Pinene	0.05
2	8.412	δ -3-Carene	0.05
3	9.161	Limonene	1.70
4	10.006	trans- β -Ocimene	0.02
5	12.266	Linalool	0.31
6	13.133	cis-Verbenol	0.05
7	14.261	γ -Geranial	0.24
8	14.427	trans-Chrysanthemol	0.32
9	15.171	Isogeranial	0.82
10	16.004	Isogeranial	1.43
11	16.529	Lilac aldehyde	0.60
12	18.922	Citral b	39.85
13	20.405	Citral a	48.26
14	22.962	2-Hexenyl laurate	0.17
15	23.751	Eugenol	0.08
16	24.878	Geranyl acetate	0.42
17	26.193	trans-Caryophyllene	0.32
18	26.341	Chrysanthenone	0.10
19	27.086	Dihydroisocaryophyllene epoxide	0.02
20	27.596	α -Humulene	0.06
21	29.721	Cuparene	0.07
22	30.078	γ -Muurolene	0.52
23	30.795	cis- γ -Bisabolene	0.07
24	31.981	Geranyl Butyrate	0.02
25	32.742	Caryophyllene oxide	1.07
		Unidentified	3.38
		Total	100

Results of GC-MS analysis of essential oil from *Syzygium aromaticum*

The spectroscopy analysis of *Syzygium aromaticum* (Clove) GC-MS revealed the essential oil from the plant contains twenty-one (21) compounds (Table 5). The most abundant and major compound is Eugenol with a peak area of 69.0% and a retention time of 27.04, followed by Eugenyl

acetate with a peak area of 13.5% and a retention time of 33.66, while α -Muurolene, Cubenol, Isocaryophyllene, and 2-Heptyl acetate are the least abundant compound with a peak of 0.02% and retention times of 32.47, 38.01, 30.88 and 12.23 respectively. Table 5 shows the breakdown of the entire components, while Figure 4 shows the spectrum of the essential oil from *Syzygium aromaticum* commonly called clove.

Table 5. GC-MS analysis of the volatile composition of *Syzygium aromaticum*.

Tabela 5. Analiza hlapne sestave *Syzygium aromaticum* z GC-MS.

No.	Retention time (min)	Compound	% Concentration
1	22.48	Chavicol	0.25
2	12.23	2-Heptyl acetate	0.02
3	19.13	Methyl salicylate	0.06
4	26.10	α -Cubebene	0.42
5	27.50	α -Copaene	0.28
6	29.47	β -Caryophyllene	13.23
7	27.04	Eugenol	68.98
8	30.45	α -Cubebene	0.05
9	30.88	Isocaryophyllene	0.02
10	31.39	β -Cadinene	0.06
11	31.51	γ -Muurolene	0.07
12	30.75	α -Humulene	1.50
13	31.74	D-Germacrene	0.11
14	32.16	β -Copaene	0.05
15	38.01	Cubenol	0.02
16	32.78	α -Farnesene	0.27
17	33.66	Eugenyl acetate	13.48
18	35.73	Caryophyllene oxide	0.27
19	32.47	α -Muurolene	0.02
20	36.76	Humuladienone	0.03
21	37.47	Epicubenol	0.05
		Unidentified	0.76
		Total	100

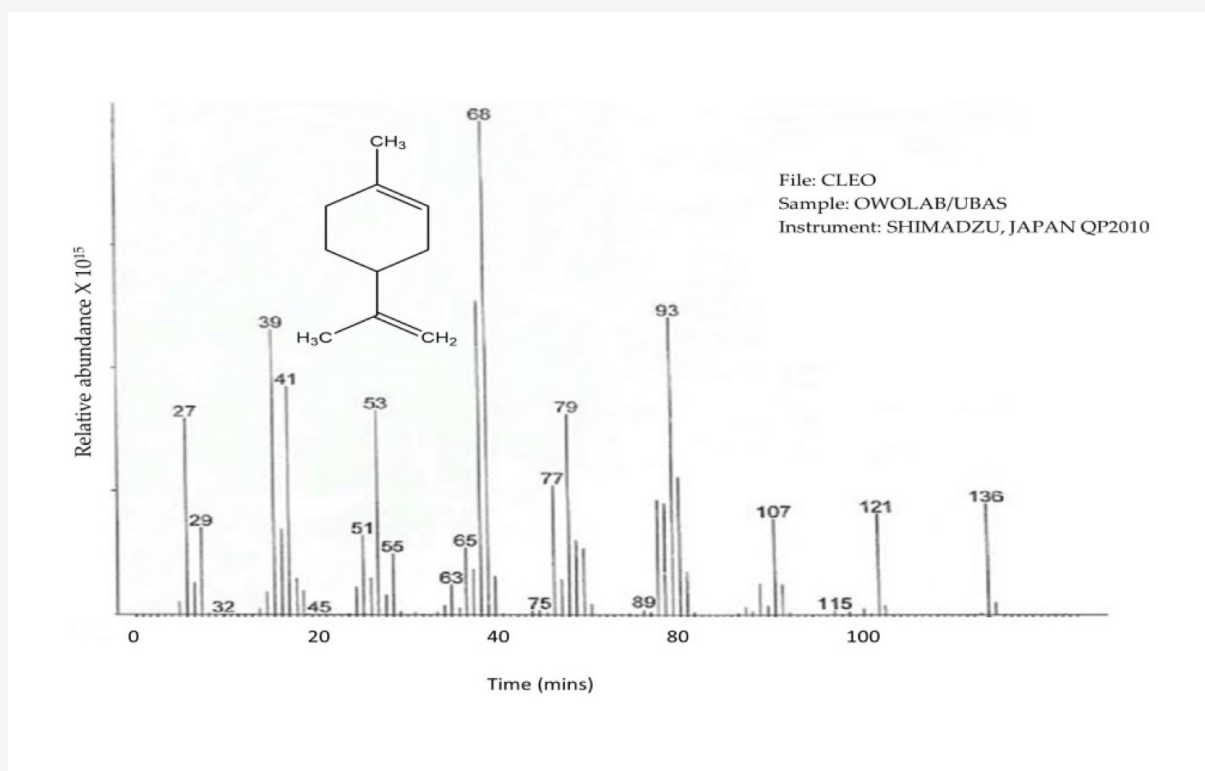


Figure 2. GC-MS Spectrum of *Citrus limon* essential oil.

Slika 2. GC-MS spekter eteričnega olja *Citrus limon*.

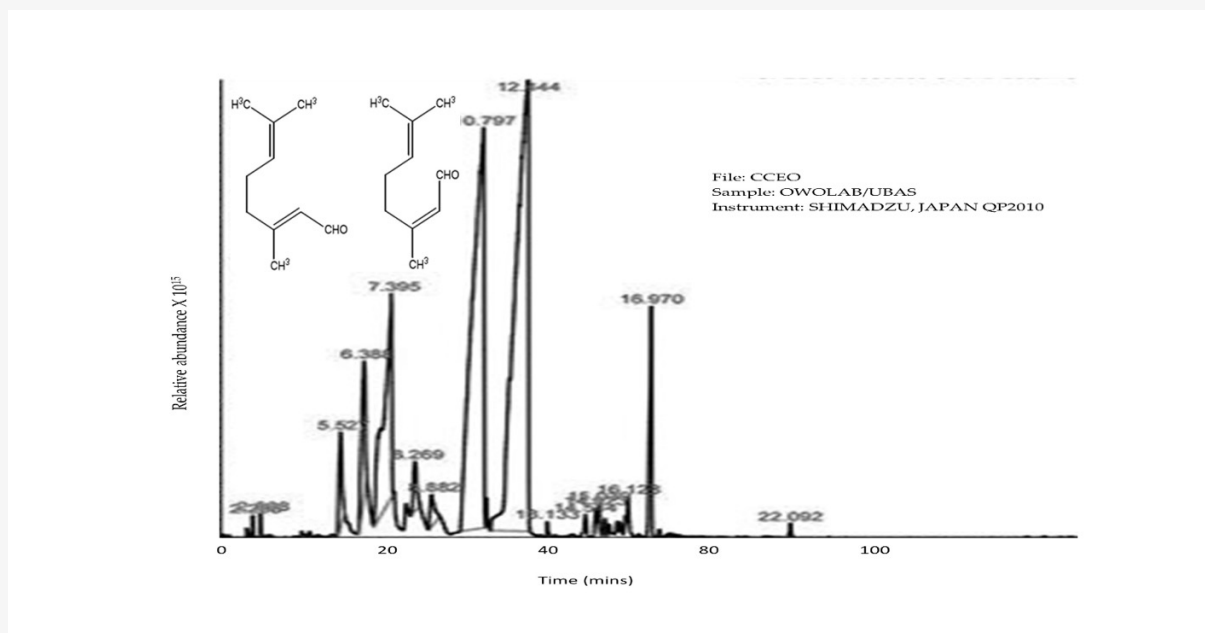


Figure 3. GC-MS Spectrum of *Cymbopogon citratus* essential oil.

Slika 3. GC-MS spekter eteričnega olja *Cymbopogon citratus*.

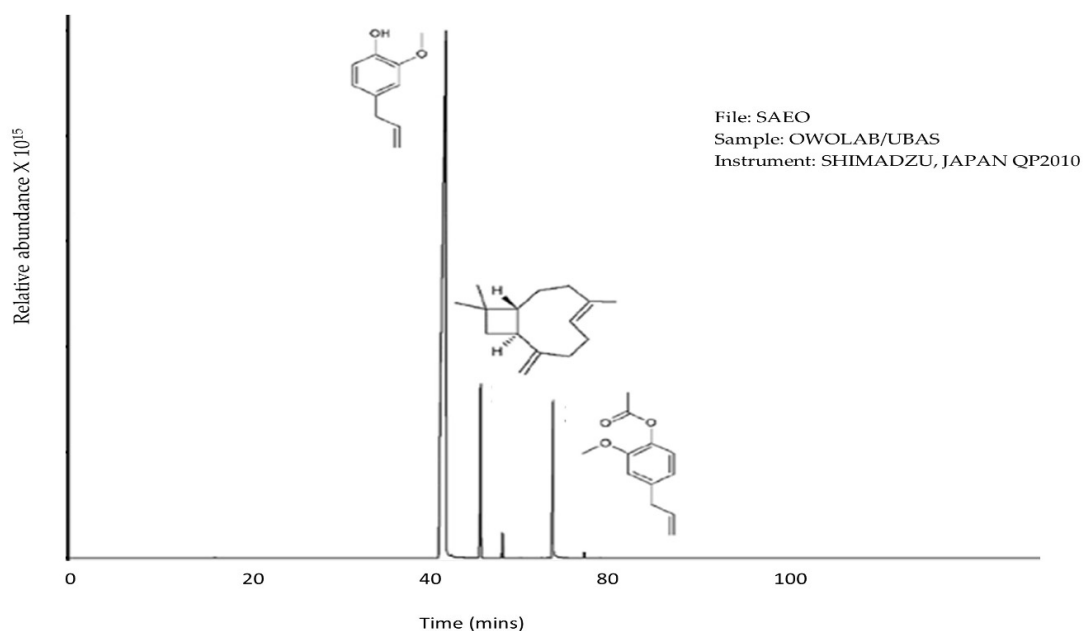


Figure 4. GGC-MS Spectrum of *Syzygium aromaticum* essential oil.

Slika 4. GC-MS spekter eteričnega olja *Syzygium aromaticum*.

Results of Knockdown, mortality, and time efficacy of EOs on *Anopheles* mosquitoes

Table 6 presents the knockdown rates and times of various essential oils (EOs) and their combinations on *Anopheles* mosquitoes. *Cymbopogon citratus*, *Syzygium aromaticum*, and *Citrus limon* oils were tested both individually and in combinations, among the individual oils, *Syzygium aromaticum* exhibited the shortest knockdown time at 14 minutes and knockdown rate of 75%, while *Citrus limon* showed the longest times at 26 minutes for knockdown time and 30% of the mosquitoes were knocked down. Combinations of these oils generally reduced both knockdown times and rates compared to individual oils, with the combination of *Cymbopogon citratus*, and *Syzygium aromaticum* (FM2) demonstrating the most rapid effects with a knockdown time of 7 minutes and rates of 85%. These findings suggest synergistic effects of the oil combinations in enhancing their effectiveness against *Anopheles* mosquitoes.

Discussion

While synthetic insecticides are effective, their widespread use raises environmental and health concerns. They not only harm target insects but also pose risks to animals, humans, and beneficial insects crucial for ecosystem health (Chrustek *et al.*, 2018; Khazri *et al.*, 2015; Liu, 2015). This current study aligned with the efforts to combat mosquito-related issues emphasizing the exploring natural insecticides against mosquitoes.

The GC-MS analysis of essential oils from *Citrus limon*, *Cymbopogon citratus*, and *Syzygium aromaticum* revealed diverse and significant chemical compositions that contribute to the observed biological activities. *Citrus limon* oil is predominantly composed of limonene (66.4%), followed by γ -terpinene (7.0%) and geranial (4.0%). *Cymbopogon citratus* oil primarily contains citral a (48.3%) and citral b (39.9%), with minor compounds contributing to its profile. *Syzygium aromaticum* oil is rich in eugenol (69.0%) and eugenyl acetate (13.5%). These results are closely similar to the previous

Table 6. Percentage mortality rates (MR), mortality time (MT), susceptibility status (S), knockdown rates (KR), and knockdown time (KT) of individual essential oil and their combinations against females *Anopheles* mosquito at 1 hour after exposure.

Tabela 6. Odstotki smrtnosti (MR), čas smrtnosti (MT), stanje občutljivosti (S), stopnja otrpnosti (KR) in čas otrpnosti (KT) posameznih eteričnih olj in njihovih kombinacij proti samicam komarja *Anopheles* 1 uro po izpostavljenosti.

Essential oils	Knockdown Time (minutes)	Knockdown Rates (%)	MR%	Status	MT (min)
ETAL	∞	0d	0d	R	∞
LDT	4	100d	100d	S	7
CCEO	18	60±0.866	30±0.25	R	33
SAEO	14	75±0.75	45±0.74	R	25
CLEO	26	30±0.866	15±0.47	R	42
FM1	9	45±0.66	60±0.5	R	16
FM2	7	85±0.25	50±0.37	R	29
FM3	17	65±0.5	57±0.5	R	13

LDT: Lambda-cyhalothrin, ETAL: Ethyl alcohol, SAEO: *Syzygium aromaticum* Essential oil, CCEO: *Cymbopogon citratus* Essential oil, CLEO *Citrus limon* Essential oil, F1 = 10%CLEO+ 10% SAEO + 80% ethyl alcohol, F2 = 10%SAEO+ 10% CCEO + 80% ethyl alcohol, F3 = 5% CLEO + 5% SAEO + 5% CCEO + 75% ethyl alcohol, S = Susceptible is defined as 98–100% mortality, PR = Possible Resistant is defined as 80–97% mortality, R = Resistant is defined as < 80% mortality. Data represent mean ± SD, (n = 25); *p < 0.05

reports on the chemical composition of these oils with slight variations in the percentage concentrations but with complete deviations from physical characteristics such as color, and percentage yield (Kaur *et al.*, 2019; Dangol *et al.*, 2023; El Aboubi *et al.*, 2023; Valková *et al.*, 2022; Haro-González *et al.*, 2021; Khan *et al.*, 2023), for instance, in 2019, Kaur *et al.* reported 69.68 % of eugenol from *Syzygium aromaticum* obtained from Punjab in India with a pale yellow oil and a yield of 12.3 % (w/v) as opposed our findings which revealed 68.98% eugenol, with white oil and a yield of 0.2%. Some literatures (Ben Hsouna *et al.*, 2017; Mortazavi *et al.*, 2010) also reported a yield of 3 % from *Citrus limon* peel and others reported it to contained a yellow color oil chiefly composed of limonene (60.7 %) (Kačániová *et al.*, 2024) as opposed to ours with white colored oil and yield of 0.5%, although, with similar limonene concentration of 66.4%. The color of essential oils can vary considerably based on factors such as the plant species, extraction method, growing conditions, and processing techniques (Hüsnü *et al.*, 2007; Karalija *et al.*, 2022). Citrus limon oil is typically pale yellow or colorless due to its high limonene content, which is a colorless compound. Our research shows that the limonene content in our samples is higher than most previously reported ones (Ben Hsouna *et al.*, 2017; Mortazavi *et al.*, 2010) with pale yellow color, thereby resulting in an oil with a white colored oil we obtained. Additionally, essential oils

from plants harvested at different growth stages (e.g., early bloom versus full maturity) may exhibit subtle color differences (de Sousa *et al.*, 2023). Furthermore, the composition of the soil plays a role, with nutrient-rich soils leading to oils with more intense colors, particularly if they contain higher levels of pigment-producing compounds (Hüsnü *et al.*, 2007). However, many researchers have postulated similar reports on the chemical compositions of *Citrus limon* (Gbolade *et al.*, 2011; El Khoury *et al.*, 2017; de Lima *et al.*, 2024; Khan *et al.*, 2024). Similarly, the results of the chemical composition of *Cymbopogon citratus* are in line with the reports of many researchers (Bicchi *et al.*, 2008; Nurmansyah *et al.*, 2022; de Lima *et al.*, 2024). These noted variations are mainly due to geographical, meteorological, and soil circumstances, as well as the plant's maturity during harvest time (Karalija *et al.*, 2022).

The insecticidal efficacy tests demonstrated that these essential oils, both individually and in combination, are effective against *Anopheles* mosquitoes. Clove oil exhibited the quickest knockdown and mortality, while a synergistic blend of the oils of *Cymbopogon citratus*, and *Syzygium aromaticum* significantly enhanced these effects. An old study by Núñez and Aquino supports this phenomenon (Núñez and Aquino, 2012) with a more recent study by Uçkun and Karakoyun, and Owolabi *et al.* which testified to the efficacy of essential oils in combating pests like mos-

quitoes (Uçkun and Karakoyun, 2023; Owolabi et al., 2024). The efficacy of Clove essential oil against insects has been elaborately reported by many researchers (Saini et al., 2024; Khan et al., 2023), good number of literature have reported the pesticidal activities of Lemon grass (Moustafa et al., 2021; Sharma et al., 2022), while other studies have also justified the efficacies of *Citrus limon* against pests (Zhang et al., 2023; Almeida et al., 2022; Ben Hsouna et al., 2017). Synergism in pharmacological activities refers to the phenomenon where the combined effect of two or more substances is greater than the sum of their individual effects. This is a common occurrence in plant constituents including essential oil, where multiple compounds work together to enhance therapeutic outcomes or reduce side effects. As noted in this current study, where combined oils resulted in better activity, many literatures have also demonstrated this phenomenon (Pezzani et al., 2019; Ye et al., 2011; Nagaprashantha et al., 2011).

Conclusions

In conclusion, this research demonstrates that the combination of essential oils (Lemongrass and Clove) significantly enhances their effectiveness against *Anopheles* mosquitoes, exhibiting both faster knockdown and mortality, despite the mosquitoes in the study area have developed resistance to the individual essential oil analyzed. The synergistic action of the combined oils was found to improve mosquito knockdown and mortality rates compared to individual oils, suggesting that these blends may offer a more

potent alternative to conventional insecticides. The results also highlight the potential for developing environmentally friendly and sustainable mosquito control strategies, using essential oils as a natural remedy. Further studies are recommended to optimize the oil combinations, determine the most effective application methods, and assess their long-term efficacy and safety for both humans and the environment.

Author Contributions

Conceptualization, O.T.; Methodology, O.T., O.P.; Software, D.J., O.T.; Investigation, O.T., D.J.; Resources, O.P., O.T., I.O.; Data curation, O.T.; Writing—original draft preparation, O.T.; writing—review and editing, O.P., D.J., and I.O.; Supervision, O.T. All authors have read and agreed to the published version of the manuscript.

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Data Availability

Other data are available and can be accessed by mailing the corresponding author via owolabitunde1@gmail.com..

Conflicts of Interest

The authors declare no conflict of interest.

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