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Original Article

Susceptibility of Adult *Anopheles gambiae* to *Cymbopogon citratus* and *Ocimum gratissimum* Essential Oils Using Contact and Fumigant Methods

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Abstract:

Background: The rise in resistance of *Anopheles gambiae* resistance to recommended insecticides necessitates effective bioactive alternatives in their control. Mosquitocidal activities of the essential oils of *Cymbopogon citratus (lemon grass)* and *Ocimum gratissimum* (scent leaf), in single and combined forms, were evaluated using fumigant and contact exposure techniques. Time-mortality was recorded, and Lethal Times (LT) computed for 50% using probit analysis.
Methods: Adult *Anopheles gambiae*mosquitoes were exposed to 1 %, 2 %, 3 %, 4 %, and 5 % concentrations of *C. citratus* and *O. gratissimum* essential oils, in single and combined trials adopting standard protocols by World Health Organization (WHO).
Results: Best concentration (5%) of *C. citrates demonstrated complete* mortality (100% for contact and 78% for fumigant), whereas *O. gratissimum* at 5% exhibited 93% mortality for contact and 67% for fumigant compared to other concentrations for contact and fumigant exposures ($p < 0.05$). Notably, the combined oils produced complete mortality at all exposure periods (100%), and concentrations, indicating enhance deffectness over the single dose of oils.
Conclusion: It is imperative to adopt the combined oils in vector management trials for adults, following that the findings of this study align with emerging evidence supporting multi-component botanical mixtures to enhance insecticidal efficacy through complementary chemical interactions. Further studies aimed at formulation development are warranted.

Keywords*:* *Anopheles gambiae*, *Cymbopogon citratus*, *Ocimum gratissimum*, *Insecticide resistance*, *Mortality assays*, *Essential oil.*

1. Introduction

Malaria remains one of the most significant vector-borne diseases globally, with *Anopheles gambiae* mosquitoes in various complexes serving as the primary vectors across sub-Saharan Africa [1–3]. Despite considerable progress in malaria control over recent decades, widespread reliance on synthetic insecticides for indoor residual spraying and long-lasting insecticidal nets has contributed to escalating insecticide resistance in *An. gambiae* populations [4].

This growing resistance threatens the effectiveness of recommended vector-control tools and highlights the urgent need for alternative, environmentally sustainable, and locally accessible mosquito control strategies [5, 6]. Botanical insecticides derived from plant essential oils have gained increasing scientific attention as potential substitutes or complements to synthetic chemicals in integrated vector management [7, 8]. Plant essential oils typically comprise diverse blends of bioactive terpenoids and phenolic compounds that exhibit insecticidal, repellent, and oviposition-deterrent effects [9]. Since these compounds often act through multiple physiological targets, they may reduce the risk of resistance development and can offer safer environmental profiles compared with conventional insecticides [10, 11].

Cymbopogon citratus and *Ocimum gratissimum* are aromatic plants widely cultivated and traditionally used in West Africa for medicinal and pest-control purposes [12, 13]. Their essential oils have been reported to possess notable insecticidal properties, attributed largely to constituents such as citral in *C. citratus* and eugenol, thymol, and other phenolics in *O. gratissimum* [14, 15]. However, gaps remain regarding their comparative toxicity, optimal modes of application, and the likelihood for synergistic interactions of the oils. Moreover, the efficacy of these oils may vary depending on the exposure method, with contact toxicity and fumigant toxicity offering distinct mechanisms and operational advantages in vector control settings [16], so investigating the comparative potency and possible synergistic effects of *C. citratus* and *O. gratissimum* essential oils against *An. gambiae* using both contact and fumigant bioassays is, therefore, crucial for advancing plant-based insecticide research. Such studies not only broaden our understanding of plant-derived toxicants but also contribute to the development of affordable, culturally acceptable, and eco-friendly alternatives for malaria vector management.

2. Materials and Methods

2.1 Study Location and Period

This researchwas carried out at Delta State University (DELSU), Abraka, Ethiope East Local Government Area of Delta State, located on Latitude: 5.8 °N and Longitude: 6.1° E on the equator. The climate of the study area is humid tropical with two seasons: rainy season (March - October) and dry season (November - February). Soil type: predominantly sandy loam, during the rainy season between July and September 2024.

2.2 Experimental mosquitoes

Mosquito larvae were acquired from a stagnant pool of water within Abraka, Ajanomi, Urhuovie, and Ebede communities of Delta State. The water containing the larvae was poured into white transparent containers and was transported to the Insectary, Department of Animal and Environmental Biology, Delta State University, Abraka. The larvae were identified and separated into different genera by an Entomologist at the Department of Animal and Environmental Biology, Faculty of Sciences, Delta State University, Abraka, Nigeria. Of the different genera, *Anopheles* mosquito larvae were reared in the laboratory in a cage designed with a mosquito net to prevent the escape of the adult mosquitoes during emergence. During the period of rearing, the larvae were fed with yeast. After emergence, cotton pads soaked in a 10 % glucose solution were used as a food source for adult *Anopheles* mosquitoes [17]. The emerged adult *Anopheles* mosquitoes, a total of 1800, were used for the experiment.

2.3 Collection, identification and authentication of plants

Fresh leaves of *Ocimum gratissimum* and *Cymbopogon citratus* were collected from Abraka, Delta State, Nigeria. Abraka is located at geographical coordinates 5.7902 °N latitude and 6.0987 °E longitude. The study area has a humid tropical climate with two distinct seasons: the rainy season (April to October) and the dry season (November to March). Plant collection was carried out during the rainy season, between May and July 2025. The plants were initially identified and authenticated on 17th September, 2025, by Mr O. E. Michael at the Herbarium Unit, Department of Botany, Faculty of Sciences, Delta State University, Abraka. Voucher specimens were deposited in the herbarium under voucher numbers DELSUH 196 (*O. gratissimum*) and DELSUH 244 (*C. citratus*). Freshly harvested leaves were washed thoroughly with running tap water to remove dust and debris, then air-dried at room temperature (23-29 °C) in the laboratory of the Department of Medical Biochemistry, Delta State University, Abraka, for 21 days until constant weight was obtained following the procedure of Odeghe *et al.* [18]. The dried leaves were subsequently pulverised into fine powder using a mechanical grinder and stored in airtight containers at 4 °C until extraction.

2.4 Extraction of essential oils by hydrodistillation

Essential oils were extracted from the powdered leaves of *Ocimum gratissimum* and *Cymbopogon citratus* using hydrodistillation in a Clevenger-type apparatus, following the method of Odeghe *et al*. [19]. The plant material was directly immersed in distilled water in a round-bottom flask, and the mixture was heated under atmospheric pressure until boiling. Volatile components of the plant material evaporated with the steam generated by the boiling water. The resulting azeotropic mixture was condensed, and the essential oil phase was separated from the aqueous layer due to density differences and immiscibility, as described by Machado *et al.* [20]. Extraction conditions were optimised by varying the distillation time (60, 120, 180, and 240 minutes), temperature (75, 80, 85, 90, 95, and 100 °C), and solid-to-solvent ratios (1:5, 1:10, 1:15, and 1:20 g/mL). For the main extraction, the process was maintained for 3 hours, beginning from the first drop of distillate until no further increase in oil yield was observed. The extracted essential oils were collected, dried over anhydrous sodium sulfate to remove residual moisture, and stored in amber vials at 4 °C until analysis [21].

2.5 Experimental design

A total of 1,800 adult *Anopheles* mosquitoes were randomized into six treatment groups (n = 300 per group). Prior to exposure, all mosquitoes were maintained on 10% glucose. Each group was exposed to essential oils at concentrations of 1%, 2%, 3%, 4%, and 5% under two delivery methods: fumigation and contact. Group I received *Cymbopogon citratus* oil via fumigation, and Group II via contact. Group III and IV received *Ocimum gratissimum* oil via fumigation and contact, respectively. Groups V and VI were treated with a combination of both oils, administered by fumigation and contact, respectively. The experiment assessed the effects of these essential oils on adult mosquito survival [22, 23]. Unexposed mosquitoes served as control of the experiment.

2.6 Preparation of essential oil test solutions

Essential oils extracted from *Cymbopogon citratus* and *Ocimum gratissimum* were prepared as test solutions for bioassays against *Anopheles gambiae*. Test solutions were formulated at concentrations of 1 %, 2 %, 3 %, 4 %, and 5 % (v/v) by dissolving the oils in ethanol as the solvent. Solutions were prepared for each oil individually and in combination (equal parts of both oils) to assess single and combined effects. For each concentration, four replicates were established. Unexposed mosquitoes served as control for the experiment. The test solutions were applied in contact and fumigant bioassays with mortality recorded at 5, 10, 15, 20, 30, 40, 50, and 60 minutes. Each replicate consisted of 25 adult female *Anopheles gambiae* mosquitoes [24, 25].

2.7 Toxicological Bioassays

The selected approaches for insecticide toxicity investigation in this study were fumigant toxicity and contact toxicity. These methods were employed to evaluate the insecticidal effects of essential oils from *Ocimum gratissimum* and *Cymbopogon citratus* on *Anopheles gambiae* through contact and fumigant exposure, using time-mortality response analysis. Concentration-dependent mortality rates were determined for the oils individually and in combination (1:1 v/v ratio mixtures). Twenty-five (25) female of *A. gambiae* mosquitoes (2-5 days old) were used per replicate, with four replicates per treatment. All tests were conducted at room temperature (23-25 °C) and relative humidity of 70-80 %. Mortality was recorded as the cumulative number of dead mosquitoes (those unable to move or fly) at specified time intervals. Survivors after 60 minutes were considered resistant to the oil, and collected for potential further analysis of underlyisng mechanisms of resistance.

2.8 Fumigant exposure

The principle of fumigant exposure testing involves exposing mosquitoes to volatile compounds in a controlled environment and measuring the resultant mortality rates. This method leverages the high volatility of essential oils, which allows them to evaporate and diffuse through the air, contacting and penetrating the respiratory system of mosquitoes. The primary mechanism of action for many essential oils is neurotoxicity, which affects the nervous system of mosquitoes, leading to paralysis and death [26]. The fumigant effect of the essential oils against *A. gambiae* was evaluated using a modified vapour exposure technique. Cotton buds were impregnated with five (5) different concentrations (1 %, 2 %, 3 %, 4 %, 5 % v/v) of the plants' oils (individually and in 1:1 combinations) dissolved in ethanol. The impregnated cotton bud was placed on top of a net-covered glass container (approximately 170 cm^3) to allow vapours to diffuse through the net without direct physical contact. Twenty-five (25) female *Anopheles* mosquitoes were transferred into the container. Containers with cotton buds treated with water served as controls. Three replicates were used for each treatment and control. Mortality was recorded at 5, 10, 15, 20, 30, 40, 50, and 60 minutes from the commencement of exposure. Mortality was calculated using Abbott's formula [27].

2.9 Contact Exposure

The principle of contact exposure involves assessing the lethal effects of a substance when it comes into direct contact with the insect's body. In the context of evaluating essential oils from *Ocimum gratissimum* and *Cymbopogon citratus,* contact toxicity testing was designed to determine the potency of these oils in killing mosquitoes upon direct application. This method is crucial for identifying compounds that can penetrate the insect cuticle, disrupt physiological processes, and ultimately lead to mortality [28, 29]. Cotton buds were impregnated with the five (5) formulated concentrations (1 %, 2 %, 3 %, 4 %, 5 % v/v) of essential oil stock solution (individually and in 1:1 combinations) dissolved in ethanol. The impregnated cotton bud was placed inside a glass container (approximately 170 cm^3) with a net cover to allow direct contact by the mosquitoes. Twenty-five (25) *Anopheles* mosquitoes were transferred into the container after a 1-hour acclimatization period. Containers treated with water only served as controls. Four replicates were used for treatments and two replicates were used for controls. Mortality was recorded at 5, 10, 15, 20, 30, 40, 50, and 60 minutes from the commencement of exposure. Mortality was calculated using Abbott's formula [27].

2.10 Data Analysis

Mosquito mortality data across the different treatments were analysed for statistical significance using one-way ANOVA. Time-to-mortality records were calculated for each treatment group. Lethal concentrations required to achieve 50% mortality (LC_{50}) were estimated using Finney's probit analysis model [30]. All statistical analyses were performed using GraphPad Prism version 10.4.2.

3. Results

3.1 Time-dependent mortality of Anopheles gambiae exposed to *C. citrates* and *O. gratissimum* essential oils via contact and fumigant methods

The mortality of *Anopheles gambiae* increases with time across all treatments (Figures 1 and 2), with notable differences in effectiveness between *C. citratus*, *O. gratissimum* and their combinations. It was revealed that *C. citrates* consistently produced a gradual, time-dependent increase in mortality, reaching moderate efficacy by 60 minutes. In contrast, *O. gratissimum* produced minimal mortality throughout the exposure period, demonstrating relatively weak standalone action. Notably, the combined treatment of *C. citratus* and *O. gratissimum* elicited a markedly enhanced and rapid lethality, achieving steep mortality increases from 10 minutes onward and reaching 100% by 60 minutes (Figure 1), suggesting that combined exposure of the essential oils produced a markedly greater mosquitocidal effect than either extract alone.

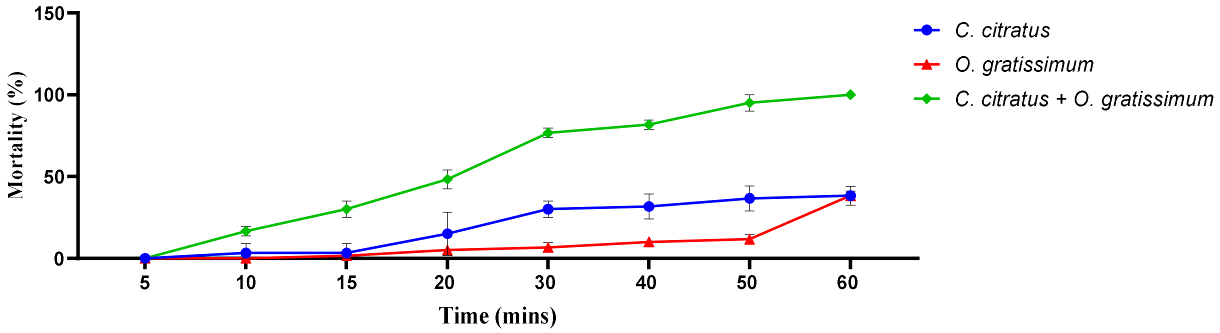

Figure 1. Time mortality of Anopheles gambiae adults exposed to essential oil of C. citratus and O. gratissimum and C. citratus + O. gratissimum using the fumigant method. ($F_{ANOVA} = 31.88$, $p < 0.0001$).

Furthermore, *C. citratus* induced a steady, time-dependent increase in mortality, reaching moderate lethality by 60 minutes. In the contact method (Figure 2). *Ocimum gratissimum* produced comparatively lower mortality across all time points, demonstrating weaker standalone activity. In contrast, the combined treatment of *C. citratus* and *O. gratissimum* demonstrated the highest and most rapid mortality response, reaching 100 % by the end of the exposure period. This enhanced performance possibly suggests a synergistic interaction between the oils, resulting in substantially greater mosquitocidal potency than either extract alone.

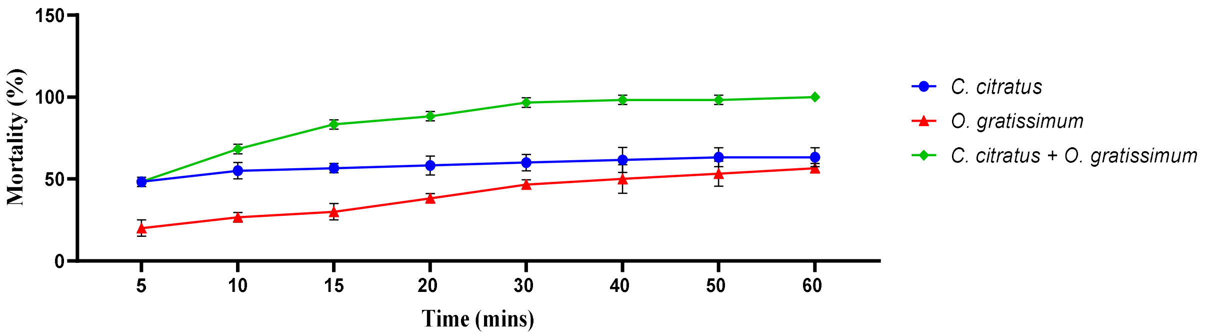

Figure 2. Time mortality of Anopheles gambiae adults exposed to essential oil of C. citratus and O. gratissimum and C. citratus + O. gratissimum using the contact method. ($F_{ANOVA} = 7.674$, $p < 0.0001$).

3.2 Percentage mortality and susceptibility status of Anopheles gambiae following exposure to essential oils of *O. gratissimum, C. citratus*, and their combination

The percentage mortality of *Anopheles gambiae* after 60 minutes of exposure to different concentrations of essential oils from *O. gratissimum*, *C. citratus*, and their combination, using both contact and fumigant methods (Table 1). For *O. gratissimum*, the contact method produced higher mortality than fumigant at all concentrations, yet susceptibility was only observed at the highest concentration (5 %), while fumigant remained ineffective, showing resistance throughout. *C. citrates* also demonstrated stronger activity through contact compared with fumigant, achieving complete mortality at 5 % concentration and becoming effective from 4 % upwards, whereas the fumigant form remained less potent, and mosquitoes were resistant at all concentrations. In contrast, the combination of *O. gratissimum*and *C. citratus* proved highly potent, with both contact and fumigant methods causing 100 % mortality at all concentrations tested, indicating full susceptibility and suggesting a possible synergistic effect when the oils are combined.

Table 1: Percentage mortality of *Anopheles gambiae* exposed to varying concentrations of essential oils from *O. gratissimum*, *C. citratus*, and their combination.

Plant	Methods	Conc. (%)	Percentage Mean Mortality after 60 mins (%)	Status
O. gratissimum	C	1	57	Low mortality
	F		38	Low mortality
	C	2	77	Moderate mortality
	F		40	Low mortality
	C	3	82	Moderate mortality
	F		53	Low mortality
	C	4	85	Moderate mortality
	F		55	Low mortality
	C	5	93	Moderate mortality
	F		67	Low mortality
C. citrates	C	1	63	Low mortality
	F		38	Low mortality
	C	2	75	Moderate mortality
	F		43	Low mortality
	C	3	85	Moderate mortality
	F		68	Low mortality
	C	4	90	Moderate mortality
	F		85	Moderate mortality
	C	5	100	High mortality
	F		78	Moderate mortality
O. gratissimum+	C	1	100	High mortality
C. citrates	F		100	High mortality
	C	2	100	High mortality
	F		100	High mortality
	C	3	100	High mortality
	F		100	High mortality
	C	4	100	High mortality
	F		100	High mortality
	C	5	100	High mortality
	F		100	High mortality

Note: *C= Contact, F = Fumigant, High mortality: 98-100, moderate mortality: 70-97, low mortality: 0-69.*

3.3 Concentration-response Relationship (Probit Analysis) of A. gambiae Adults Mortality Exposed to *C. citratus, O. gratissimum* and *C. citrates Plus O. gratissimum.*

The concentration-response relationship (LC_{50}) demonstrated distinct potency trends using the treatments (Tables 2 and 3). For *C. citratus*, mortality increased progressively with concentration, rising from 63 % at 1 % of the dose to complete mortality (100 %) at 5 %, accompanied by a corresponding shift in (LC_{50}) values from 0.19 to 2.58. *Ocimum gratissimum* exhibited a similar but slightly weaker dose-response pattern, achieving 93 % mortality at the highest concentration (5 %) and lower (LC_{50}) values, indicating reduced lethality compared with *C. citratus*. In contrast, the combined treatment of *C. citratus* and *O. gratissimum* produced 100 % mortality across all tested concentrations, with consistently high (LC_{50}) values (2.58), demonstrating immediate and concentration-independent lethality. This pattern suggests a potent synergistic interaction between the oils, markedly enhancing mosquitocidal activity beyond the effects observed for each extract individually (Table 2).

Table 2: Concentration-dependent mortality and Lethal Concentration (LC_{50}) for *C. citratus*, *O. gratissimum*, and their combination using the contact method.

Plant	Conc. (%)	Log (Conc)	% Mortality (Mean)	LC50
C. citrates	1	0.00	63	0.19
	2	0.30	75	0.74
	3	0.48	85	0.92
	4	0.60	90	1.04
	5	0.70	100.0	2.58
O. gratissimum	1	0.00	57.0	0.18
	2	0.30	77.0	0.74
	3	0.48	82.0	0.92
	4	0.60	85.0	1.04
	5	0.70	93.0	1.48
C. citratus + O. gratissimum	1	0.00	100.0	2.58
	2	0.30	100.0	2.58
	3	0.48	100.0	2.58
	4	0.60	100.0	2.58
	5	0.70	100.0	2.58

Note: LC means lethal concentration.

Moreover, *C. citratus* showed a concentration-dependent increase in mortality, rising from 38% at 1% to a peak of 85% at 4%, before slightly declining to 78% at 5%. The corresponding (LC_{50}) values ranged from -0.31 to 1.04, indicating moderate efficacy with a typical sigmoidal response pattern. However, it was demonstrated that *O. gratissimum* exhibited a weaker dose-response, with mortality increasing modestly from 38% at 1% to 67% at 5 % and lower (LC_{50}) values (-0.31 to 0.44), confirming its comparatively lower standalone potency. In contrast, the combined *C. citratus + O. gratissimum* treatment achieved 100% mortality at all tested concentrations, with consistently high (LC_{50}) values (2.58). This concentration-independent lethality suggests a strong synergistic interaction between the oils, resulting in markedly enhanced mosquitocidal activity that surpasses the effects of each extract applied individually (Table 3).

Table 3: Concentration-dependent mortality and Lethal Concentration (LC_{50}) for *C. citratus*, *O. gratissimum*, and their combination using the fumigant method.

Plant	Conc. (%)	Log (Conc)	% Mortality (Mean)	LC_{50}
C. citrates	1	0.00	38	-0.31
	2	0.30	43	-0.18
	3	0.48	68	0.47
	4	0.60	85	1.04
	5	0.70	78.0	0.77
O. gratissimum	1	0.00	38.0	-0.31
	2	0.30	40.0	-0.25
	3	0.48	53.0	0.08
	4	0.60	55.0	0.13
	5	0.70	67.0	0.44
C. citratus + O. gratissimum	1	0.00	100.0	2.58
	2	0.30	100.0	2.58
	3	0.48	100.0	2.58
	4	0.60	100.0	2.58
	5	0.70	100.0	2.58

Note: LC means lethal concentration.

4. Discussion

Mosquito control remains a central component of vector-borne disease management, particularly in regions where *Anopheles gambiae* persists as the dominant malaria vector [31]. With the growing challenges of insecticide resistance, plant-derived essential oils have gained attention as promising alternatives due to their biodegradability, multiple modes of action, and synergistic potential. The present study evaluated the mosquitocidal activities of *Cymbopogon citratus*, *Ocimum gratissimum*, and their combination using time-mortality bioassays, contact and fumigant exposures, and probit-based dose-response analyses. The findings reveal clear differences in potency among the treatments and provide evidence that suggest strongly perceived synergistic interactions when the oils are combined.

The time-dependent mortality patterns observed in Figures 1 and 2 demonstrate that *C. citratus* consistently induced a steady increase in mortality of *An. gambiae* across both fumigant and contact exposures. This agrees with previous studies that identified citral-rich *C. citratus* oil as a potent insecticidal agent with rapid knockdown effects on mosquitoes and other arthropods [32, 33]. In contrast, *O. gratissimum* exhibited limited standalone activity, producing relatively low mortality across time points. Similar findings were reported by Ileke *et al.* [34], who noted that eugenol-containing *O. gratissimum* oil displayed moderate toxicity on *Anopheles* species unless applied at high concentrations. A key outcome of the present study is the markedly enhanced lethality observed when *C. citratus* and *O. gratissimum* were combined. The mixture produced steep mortality increases from early time points and consistently achieved 100% mortality across all time intervals, in line with earlier reports suggesting that blending essential oils can improve volatility, cuticular penetration, and target-site interactions [35, 36]. The synergistic activity observed here parallels findings by Celestine *et al.* [37], who demonstrated that combining botanical extracts significantly increased toxicity against *An. gambiae* compared with individual components.

Also, results from the percentage mortality of *An. gambiae* after 60 minutes of exposure to different concentrations of essential oils from *O. gratissimum*, *C. citratus*, and their combination, using both contact and fumigant methods, further highlights these potency differences. In both the fumigant and contact methods, *C. citratus* showed stronger activity compared with *O. gratissimum*, with the latter exhibiting resistance in the fumigant form except at the highest concentration. Previous studies similarly reported higher susceptibility of mosquito vectors to *C. citrates* oil than to *O. gratissimum* oil under comparable bioassay conditions [38, 39]. The exceptional performance of the combined oils across all concentrations in both methods, achieving complete mortality, strongly supports the presence of synergistic interactions. This aligns with earlier reports that combinations of essential oils may enhance mortality through complementary chemical profiles and multi-target action [40].

More so, the concentration-response analyses provide further substantiation. For *C. citratus*, mortality increased progressively with concentration and displayed typical probit trends, confirming concentration-dependent lethality. *O. gratissimum*, however, demonstrated weaker dose-response behaviour with lower probit values, consistent with previous reports on its moderate insecticidal performance [41]. Remarkably, the combined treatment produced 100% mortality at all concentrations with uniformly high probit values, exhibiting concentration-independent lethality. Such a pattern is indicative of strong synergy, a phenomenon documented in other botanical mixtures such as *Ocimum-Eucalyptus* and *Cymbopogon-Azadirachta* combinations [42]. This study's findings demonstrate that *C. citratus* exhibited potent insecticidal activity, while *O. gratissimum* showed limited efficacy alone. The marked increase in effectiveness when the oils were combined suggests a strong synergistic effect, supporting the potential of multi-component botanical mixtures as a promising alternative to synthetic insecticides for controlling resistant *Anopheles* populations.

5. Conclusion

The present study demonstrates that *C. citratus* exhibited strong insecticidal activity against *An. gambiae*, whereas *O. gratissimum* showed limited efficacy when applied alone. Notably, the combination of these oils produced markedly enhanced lethality, achieving complete mortality across all concentrations and exposure methods, is suggestive of a robust synergistic effect. These findings support the potential of multi-component botanical formulations as effective alternatives to conventional synthetic insecticides, particularly in regions where mosquito populations have developed resistance. The integration of plant-derived essential oils into vector management strategies is recommended as a sustainable approach to malaria control.

Declarations

Consent for publication: All the authors approved the manuscript for publication.
Consent to Participate: Not applicable.
Data availability statement: All datasets used or generated in this study are accessible from the corresponding author upon reasonable request.
Competing Interests: The authors declare that they have no competing interests.
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