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Insecticidal activities and comparative efficacy of essential oils of three *Citrus* species (Sapindales: Rutaceae) against *Anopheles gambiae* mosquitoes

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Abstract

Essential oils (EOs) from insecticidal plants are becoming prominent again because of their effectiveness in managing disease vectors as well as their eco-friendly nature. The present study evaluated the insecticidal activity of three *Citrus* species, namely *Citrus limon*, *C. sinensis,* and *C. aurantifolia,* against larvae and adults of *Anopheles gambiae*. These fully mature *Citrus* species collected from Ilorin, Nigeria, were extracted, and the chemical components were identified using gas chromatography-mass spectrometry. The EOs extracted were then assessed for toxicity efficacy against larvae and adults of *An. gambiae* using WHO protocols. Citral was a major component identified in *C. aurantifolia*, D-limonene in *C. limon* and Myrtenol in *C. sinensis*. The highest percentage knockdown was recorded in 0.3 ml of *C. limon* (95.6±1.15 mosquitoes) against *An. gambiae*, while the lowest KDT_{50} and KDT_{90} were found in 0.5ml of *C. aurantifolia* (5.79 mins) and *C. limon* (19.61 mins), respectively. A 100% mortality rate was observed in adult female *An. gambiae* at concentrations of 0.1 ml of *C. limon* and 0.3 ml of both *C. limon* and *C. aurantifolia*. This result was not significantly different from the 99% mortality rate observed with the control, deltamethrin. Additionally, 100% mortality was recorded in *An. gambiae* larvae at 0.3 ml of both *C. aurantifolia* and *C. limon* after 48 hours of exposure. The lowest LT_{50} and LT_{90} values were recorded at 0.5 ml of *C. limon*, with times of 3.86 hours and 9.49 hours, respectively. The three citrus oils evaluated demonstrated significant adulticidal and larvicidal bioactivities, indicating promising outcomes that can be further developed for *An. gambiae* management.

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1. Introduction

The critical need to investigate and adopt better protective measures against insect-borne diseases stems from the incessant human health hazards and burdens associated with such diseases and the available protective measures (Adelaja et al., 2021). Malaria, an insect-transmitted disease, is the second leading cause of infectious disease-related mortality in Africa after tuberculosis, and it is highest among children below the age of five on a global scale (WMR, 2023). In this same context, Nigeria accounts for a significant 27% of world malaria cases, and with a 5 million increase in cases from 2021 to 2022, Nigeria is the second-largest contributor after Pakistan, accounting for 26%. (WMR, 2023). The primary insect vector responsible for malaria transmission in Nigeria is *Anopheles gambiae* (Diptera: Culicidae) (Akpan et al., 2018; Ande et al., 2022). This vector transmits *Plasmodium falciparum*, the causative agent of malaria (Nigeria Malaria Operational Plan, 2021; Oduola et al., 2022).

The preferred protective measures, i.e., targeted deployment of synthetic larvicides, indoor residual sprays, and insecticide-treated nets, have demonstrated notable efficacy in reducing the burden of mosquito-borne illnesses (WHO, 2012), but the surge in resistance development caused by overreliance has become a significant burden. A notable case in point is that the malaria mosquito vector *An. gambiae* in Nigeria has been reported to be resistant to all common insecticides throughout the country (Oduola et al., 2019; Omotayo et al., 2021), which has necessitated supplementary and alternative measures for integrated management. This unfortunate and ongoing situation has prompted the search for better protective measures for humans.

Scientific exploration of plant sources for insect protective agents has unveiled novel, bio-rational, cost-effective solutions that have rejuvenated traditional practices used against insect-borne illnesses (Dube et al., 2011; Adelaja et al., 2021). Consequently, plant species with inherent insecticidal properties are poised to regain prominence in the effective management of insect vectors, as they possess a reservoir of active components that can be harnessed; moreover, they have ready applications in Africa (Karunamoorthi et al., 2009; Adelaja et al., 2023).

Citrus species (Sapindales: Rutaceae), a plant source commonly consumed and renowned as a food-flavouring agent, offers substantial industrial by-products rich in valuable compounds like essential oils (EOs). These EOs, easily extracted from fruit peels through distillation or cold pressure, have received extensive attention for their toxic activities against the life stages of several mosquito species (Al-Seenia et al., 2018). EOs have a long history of being used for their insecticidal effects, as substantiated in the literature (Oshaghi et al., 2002; Soonwera, 2015; Gomes et al., 2019; Sharma et al., 2022). Murugan et al. (2012) studied the larvicidal and adulticidal efficacy of *C. sinensis* (L.) Osbeck essential oil against *An. stephensi, Aedes aegypti,* and *Culex quinquefasciatus*. Sanei-Dehkordi et al. (2016) studied the larvicidal bioactivity of *C. aurantium* essential oil against *An. stephensi* and characterized their active constituents through GC-MS analysis. Sharma et al. (2022) studied the larvicidal efficacy of *C. limon* (L.) Osbeck and *C. reticulata* essential oils against *An. stephensi* and characterized their major components through GC-MS analysis. The concept of green larvicides refers to all types of profitable and nature-orientated materials that can contribute to the depletion of mosquito populations (Sharma et al., 2022).

In light of the urgent demand for novel solutions to combat vector-borne diseases and protect vulnerable populations, this research undertakes a pivotal role. There is a notable gap in understanding the toxicity profiles and comparative effectiveness of essential oils derived from *Citrus* species against *An. gambiae*, the primary vector of malaria. Consequently, this investigation endeavours to provide first-of-its-kind critical foundational insights into the toxicity attributes and relative efficacies of EOs sourced from three distinct *Citrus* species (*C. sinensis*, *C. aurantifolia* (Christm.), and *C. limon*) against *An. gambiae*, thereby advancing our knowledge frontier and potentially informing innovative disease vector control strategies.

2. Materials and Methodology

2.1 Plant Resource and Handling

The fruit peels of fully mature *Citrus* species (Sapindales: Rutaceae), namely, sweet orange (*C. sinensis*), lime (*C. aurantifolia*), and lemon (*C. limon*), were collected individually from multiple fresh growing plants around Ilorin (Latitude: 8º 44' to 8º 46’ N and Longitude: 4º 55’ to 4º 57'). The collected plant materials were identified at the herbarium of the Department of Plant Biology, University of Ilorin, using accessory details.

2.2 Oil Extraction Process

The oils were extracted following the method suggested by Ganjewala (2009). The collected fruit peels of the *Citrus* species were thoroughly washed with water and air-dried for one week in the shade at room temperature. The dried plant materials were each pulverized, and then the essential oils were extracted by hydro distilling the dried pulverized fruit peels using a Clevenger apparatus for 3 hours at 100 °C. Each isolated oil was dried over anhydrous sodium sulphate and stored in amber-coloured vials at 4 °C until required for subsequent assays.

2.3 Preparation of Serial Dilutions

The stock solution was prepared by dissolving 1 ml of the extracted plant oils in 100 ml of acetone. Serial dilutions of each citrus plant oil were prepared by diluting the stock solution in the solvent (i.e., for 0.1 ml, 10% of the stock solution of plant oil was diluted in 90% of the solvent (1:9)). Three concentrations were prepared for each plant oil (viz: 10% (1:9) – 0.1 ml, 30% (3:7) – 0.3 ml, and 50% (5:5) – 0.5 ml). These concentrations were determined based on previously collected data (unpublished data).

2.4 Mosquito Sourcing and Rearing

The Ethical Committee, Faculty of Life Science, University of Ilorin, granted ethical clearance for mosquito sourcing and rearing (UERC/ASN/2017/898). The larvae and adults of *An. gambiae* mosquitoes were obtained from an existing colony that was known to be susceptible to deltamethrin. The mosquito colony was housed within the Entomological Research Laboratory at the University of Ilorin and was maintained at a temperature of 27±10 ºC and a relative humidity of 85±5%. The larvae were fed non-fatty biscuits and yeast (3:1) while the adults received a 10% sugar solution (Adelaja et al., 2023). Before conducting the bioassays, adult mosquitoes were meticulously categorized into male and female groups using the morphological keys by Coetzee (2020).

2.5 Gas Chromatography-Mass Spectrometry Analysis

The extracted essential oils were analysed using gas chromatography-mass spectrometry (GC-MS) on a Hewlett Packard 5890 II gas chromatograph, interfaced to a single quadrupole mass selective detector (Model 5972). The column was an HP-5 MS capillary column (30 × 0.25 mm, film thickness 0.25 mm). Helium was the carrier gas, set at a flow rate of 0.6 ml/min. Injector and MS transfer line temperatures were set at 220 and 250 °C, respectively. The oven program temperature was held at 35 °C for 5 mins and then 4 °C/min to 150 °C for 2 mins and finally 20 °C/min to 250 °C for 5 mins. Diluted samples (10:100 in CH_2C_{l2}, v/v) of 1 µL were injected manually using a split mode (1:100 split ratio). The components were identified by comparing their relative retention indices, calculated using n-alkanes (C8-C20) as GC-MS standards, with existing databases for compound identification. Additionally, mass spectra were analysed against those of standards from literature and those supplemented by the National Institute of Standards and Technology (NIST), provided by Hewlett Packard with the GC/MS control and data processing software (Joshi, 2017).

2.6 Adulticidal Bioassay

Adult female mosquitoes were used in accordance with the World Health Organization standard procedure (WHO, 2013), with slight modifications. Whatman papers measuring 12 × 15 cm were each inundated uniformly with 2.5 ml of the desired concentration of essential oil following the methodology outlined by Adelaja et al. (2023). The impregnated Whatman papers were air-dried for 5 minutes and the interior of the WHO exposure tube was lined by a paper. A cohort of twenty-five (25) adult female mosquitoes, aged between 2 and 3 days, was introduced into the holding chamber and allowed to acclimatize for 1 h. Thereafter, members of the cohort were carefully eased into the exposure chamber lined with Whatman paper impregnated with varying concentrations of each essential oil and the controls (0.1 ml, 0.3 ml, 0.5 ml, 0.05% deltamethrin and Acetone). Cohort members were subsequently monitored closely for 60 minutes at 10-minute intervals for knockdowns, and the percentage knockdown was calculated.

$$\%\,Knockdown = \frac{Total\ number\ of\ knockdown\ mosquitoes\ after\ 60\ minutes}{Total\ number\ of\ exposed\ mosquitoes} \times 100$$

All the mosquitoes were carefully relocated back into the holding chamber for 24 hours to facilitate recovery. Glucose solution (10%) was offered in cotton wool to recovering individuals and survivors. The number of dead mosquitoes was recorded at the end of the 24-hour recovery period, enabling computation of the percentage of mortality.

$$\%\,Mortality = \frac{Total\ number\ of\ dead\ adult\ mosquitoes\ after\ 24\ hrs}{Total\ number\ of\ exposed\ adult\ mosquitoes} \times 100$$

The experimental procedure was carried out in four replicates (25 per replicate). Where the mortality rate for the negative control group (Acetone) was between 5% to 20%, the mortalities of the treated groups were adjusted using the Abbott's formula (Abbott, 1925).

2.7 Larvicidal Bioassay

The larvicidal efficacy of the various concentrations of the three essential oils (0.1 ml, 0.3 ml, 0.5 ml) and control (Acetone) were assessed following the guidelines outlined by the World Health Organization (WHO, 2005). Twenty-five (25) third instar larvae of *An. gambiae* mosquitoes were placed into test cups (200 ml) filled with 100 ml of distilled water. One hour was designated for acclimatization. Subsequently, 2 ml aliquots of various plant oil concentrations were introduced, as previously described. Distilled water with and without acetone were used as positive and negative controls, respectively. Each quadruplet set up per concentration was monitored after 24 hrs, 48 hrs, and 72 hrs for larval mortality, which was confirmed by a lack of movement when pricked at the siphon with a needle. The calculated percentage mean mortality was employed as an indicator of the effectiveness of the respective treatments.

$$\%\,Mortality = \frac{Total\ number\ of\ dead\ larvae\ after\ 24\ hrs}{Total\ number\ of\ exposed\ larvae} \times 100$$

In instances where the mortality rate of the negative control ranged from 5% to 20%, adjustments were made to the mortalities observed in the treated groups. This adjustment followed the established formula by Abbott (1925).

2.8 Data Analysis

One-way Analysis of Variance (ANOVA) was used for the assessment of larval and adult mortalities following various exposure ranges. To discern the distinctions among means (P<0.05), Tukey's Honestly Significant Difference (HSD) post hoc test was deployed. A two-way ANOVA was employed to ascertain statistical variances between citrus oils at different concentrations and exposure durations. The knockdown time (KdT_{50} and KdT_{95}) and lethal time (LT_{50} and LT_{90}) values for successive plant oil concentrations were determined using probit analysis, as described by Finney (1971). All statistical analyses were performed utilizing the GraphPad Prism 8 software.

3. Results

3.1 GC-MS Analysis

Table 1 shows the gas chromatography-mass spectrometry (GC-MS) results of major chemical constituents found in EOs of *C. limon*, *C. sinensis*, and *C. aurantifolia*. The results revealed that Cyclotrisiloxane, hexamethyl is the major constituent of *C. limon* (20.97%), *C. sinensis* (16.46%), and *C. aurantifolia* (21.72%) EOs. The analysis also detected several other key constituents in the EOs of all three species: Myrtenol was present in both *C. limon* (0.23%) and *C. sinensis* (0.05%) EOs; *C. aurantifolia* EO contained Pentane, 1-Chloro (2.94%), Citral (0.39%), Terpinen-4-ol (0.25%), and Propanenitrile, 2-hydroxy (0.24%) (Table 1. and Fig. 1a); *C. sinensis* EO contained 1-Heptanol (5.85%) and Benzonthiophene-3-carbohydzrazide (0.08%) (Table 1. and Fig. 1c); D-limonene (0.28%), D-carvone (0.28%), 1, 5 Cyclooctadiene (0.41%), and Cyclohexanone, 2-methyl (0.57%) were present in *C. limon* EO (Table 1. and Fig. 1b).

3.2 Adulticidal Activity

Fig. 2 depicts the mean percentage of adult female *An. gambiae* knockdowns by EOs extracted from the fruit peels of *C. limon*, *C. aurantifolia,* and *C. sinensis* fruits. After 60 mins, there were no significant knockdown effects (P=0.2845-0.9999) between all concentrations of *C. sinensis* (0.1, 0.3, 0.5 ml) and 0.1 ml of *C. aurantifolia* and *C. limon*, and the negative control (acetone). However, 0.3 and 0.5 ml of *C. aurantifolia* and *C. limon* showed significant knockdown effects (P<0.0001) compared to the negative control. The highest percentage knockdown was recorded in 0.3 ml of *C. limon* (95.6±1.15 mosquitoes), followed by 0.3 and 0.5 ml of *C. aurantifolia* (91.1±2.31 and 84.4±4.04 mosquitoes) and 0.5 ml of *C. limon* (91.1±2.31 mosquitoes), which was significantly higher (P<0.05) than the percentage knockdown observed in deltamethrin (71±2.10 mosquitoes) after 60 mins post-exposure. However, all concentrations of *C. sinensis* displayed significantly lower (P<0.05) knockdown values (0.00±0.00 to 4.4±0.58 mosquitoes) in comparison to *C. aurantifolia* and *C. limon* and deltamethrin. Meanwhile, 0.1 ml concentrations of *C. limon* (4.4±1.15 mosquitoes) and *C. aurantifolia* (31.1±1.53 mosquitoes) displayed significantly lower (p<0.05) percentage knockdown compared to deltamethrin. *C. limon* exhibited the best knockdown effect, followed by *C. aurantifolia* and *C. sinensis*.

Table 1: Major chemical constituents of *Citrus* species plant oils analysed by GC-MS.

	Citrus limon			***Citrus aurantifolia***			***Citrus sinensis***		
N/S	**Compound**	**Area%**	**RT**	**Compound**	**Area%**	**RT**	**Compound**	**Area%**	**RT**
1	1,4-Cyclohexadiene, 1-methyl-	0.03	3.4	Propanenitrile, 2-hydroxy-	0.74	4.1	1-Heptanol	5.85	4.1
2	3,7-Octadien-2-ol, 2-methyl-6-methylene	0.04	6.5	Pentane, 1-chloro-	2.94	4.4	Benzothiophene-3-carbohydrazide, 4 ,5,6,7-tetrahydro-2-acetylamino-	0.08	9.2
3	D-Limonene	0.28	6.9	α-Methyl-3,4-(methylenedioxy)phenethylamine hydrochloride	0.11	7.6	Myrtenol	0.05	9.8
4	6-Hexadecen-4-yne, (E)-	0.07	7.6	Terpinen-4-ol	0.75	9.0	Cyclotrisiloxane, hexamethyl-	16.46	14.3
5	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, trans	0.08	8.3	Citral	0.39	9.8			
6	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans	0.57	9.1	Cyclotrisiloxane, hexamethyl-	21.72	14.3			

7	D-Carvone	0.28	9.6
8	1,5-Cyclooctadiene, 3-(1-methyl-2-propenyl)-	0.41	10.9
9	Cyclotrisiloxane, hexamethyl-	20.97	13.7
10	Myrtenol	0.23	10.1

RT – Retention Time

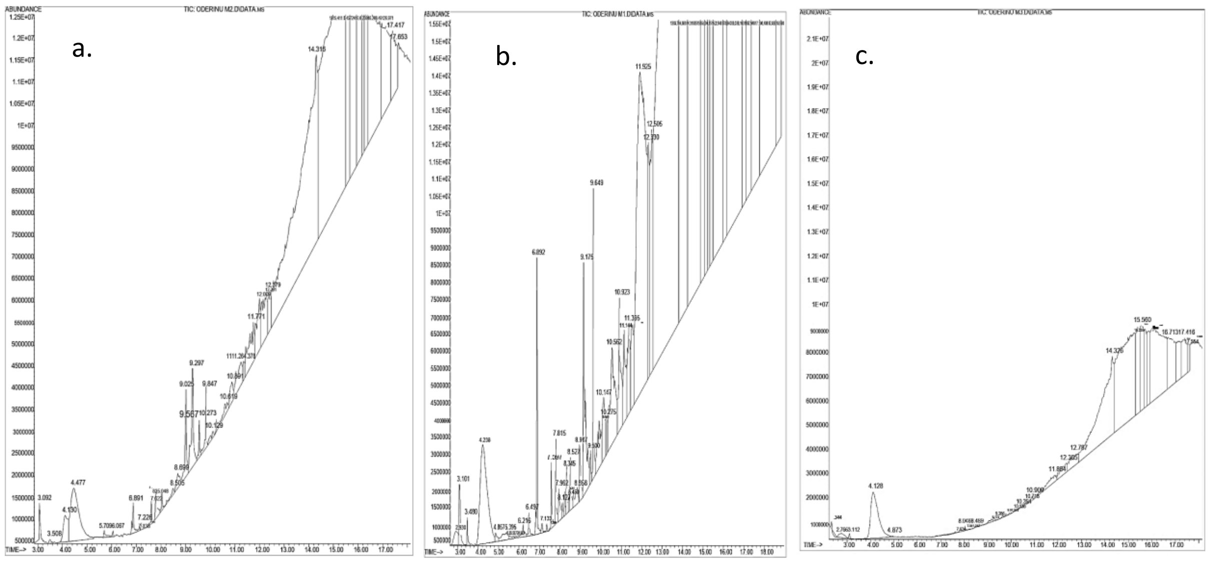

Figure 1: GC-MS analysis a. *C. aurantifolia* b. *C. limon* c. *C. sinensis*

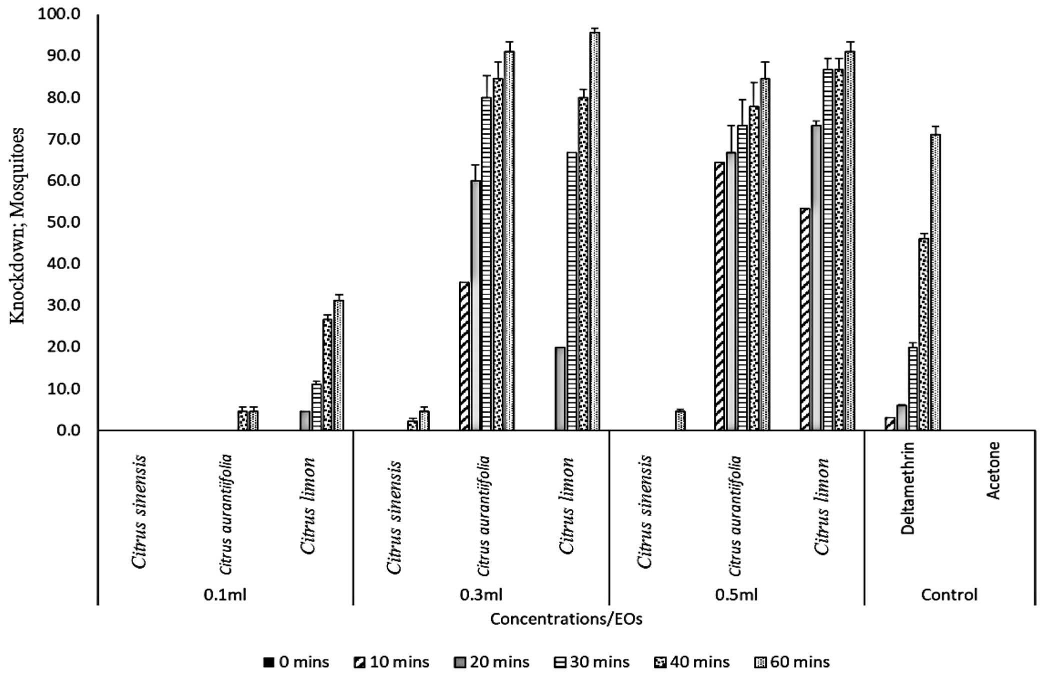

Figure 2: Mean progressive percentages of female adult *An. gambiae* knockdowns recorded on exposure to 0.1, 0.3. and 0.5 ml of three citrus oil types and deltamethrin.

The knockdown time (KDT_{50} and KDT_{90}) for different concentrations of the EOs from the three citrus species evaluated is presented in Table 2. *C. aurantifolia* had the lowest KDT_{50} at 0.5 ml (5.79 mins), followed by *C. limon* at 0.5 ml (8.60 mins). The highest KDT_{50} was recorded with *C. aurantifolia* at 0.1 ml (36.74 mins), followed by *C. limon* at 0.1 ml (32.95 mins). Meanwhile, the lowest KDT_{90} was recorded with *C. limon* at 0.5 ml (19.61 mins), while the highest KDT_{90} was recorded with *C. limon* at 0.1 ml (56.98 mins).

Table 2: Knockdown time (KDT_{50} and KDT_{90}) values of female adult *An. gambiae* on exposure to varying concentrations of three citrus oil types.

Concentration (ml)	**KDT_{50} Values (95%CI) mins**			**KDT_{90} Values (95%CI) mins**		
	0.1	**0.3**	**0.5**	**0.1**	**0.3**	**0.5**
C. sinensis	NA	45 (20.88-933.4)[a]	51.61 (NA)[a]	NA	NA	NA
C. aurantifolia	36.74 (NA)[a]	11.81 (6.08-16.87)[b]	5.79 (1.22-13.60)[b]	37.15 (NA)[a]	29.05 (NA)[b]	NA
C. limon	32.95 (27.04-37.62)[a]	23.63 (21.09-26.40)[b]	8.60 (5.07-10.86)[b]	56.98 (NA)[a]	48.68 (32.25-105)[b]	19.61 (NA)[b]
Deltamethrin (0.05%)		37.24 (35.13-39.35)[c]			174.2 (125.8-293.0)[c]	

KDT_{50} and KDT_{90} values followed by different superscripts are significantly different (*P<0.05*) across varying concentrations, *NA* not applicable, *CI* confidence interval, n=100

Fig. 3 shows the percentage mortality of adult female *An. gambiae* after exposure to EOs from *C. limon*, *C. aurantifolia,* and *C. sinensis*. There was a significant difference (*P*<0.05) in mortality rates between all concentrations of the three *Citrus* species evaluated and the negative control (acetone). The percentage mortality observed for different concentrations of EOs (84.4 - 100%) did not differ significantly (*P*>0.05) compared to deltamethrin (99%). A 100% mortality was recorded in 0.1 ml *C. limon* and 0.3 ml concentrations of *C. limon* and *C. aurantifolia*. The lowest percentage mortality was recorded for *C. aurantifolia* at 0.1 ml concentration (84%). The same percentage mortality (97.8%) was observed for 0.1 ml *C. sinensis* and 0.5 ml *C. aurantifolia.* Similarly, 0.3 ml *C. sinensis* and 0.5 ml *C. limon* resulted in 91.1% mortality (Fig. 3). *Citrus limon* elicited the greatest mortality activity, followed by *C. aurantifolia* and *C. sinensis*.

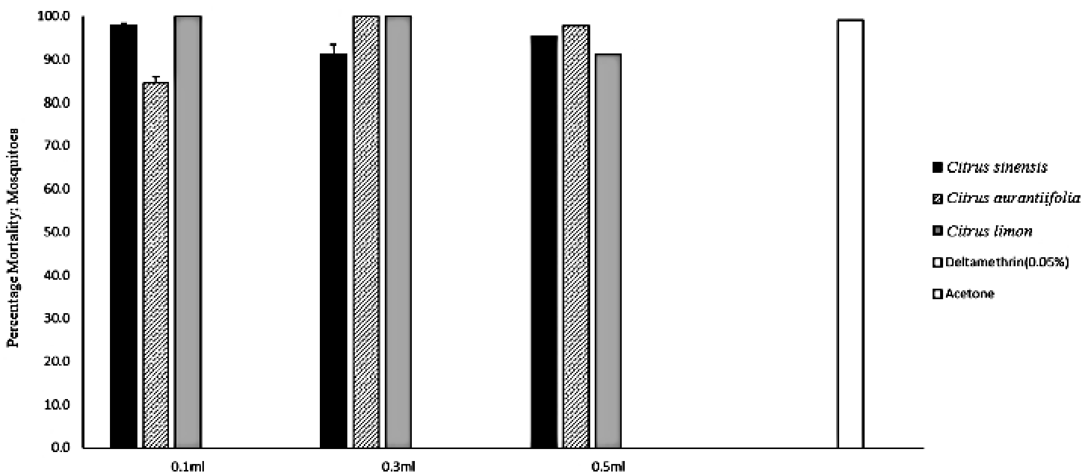

Figure 3. Percentage mortality of female adult *An. gambiae* recorded on exposure to 0.1, 0.3. and 0.5 ml of three citrus oil types and deltamethrin.

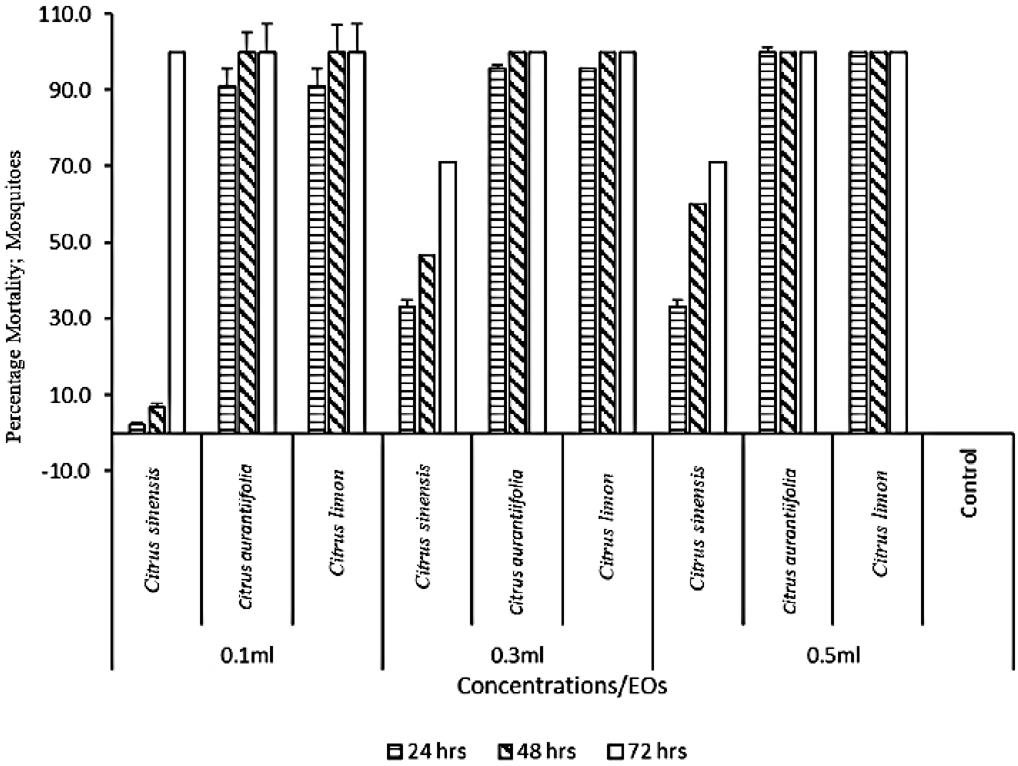

Figure 4. Percentage mortality of *An. gambiae* larvae recorded on exposure to 0.1, 0.3. and 0.5 ml of three citrus oil types.

3.3 Larvicidal Activity

The percentage mortality of *An. gambiae* larvae when exposed to EOs of *C. limon*, *C. aurantifolia*, and *C. sinensis* is presented in Fig. 4. Even at the lowest concentration of 0.1 ml, all EOs tested displayed appreciable activity as a toxicant against *An. gambiae* third instar larvae, resulting in significantly higher mortalities (P<0.05) than the negative control. The toxicity intensity was shown to be concentration dependent, i.e., when concentration of EOs increased, mortality rates also increased.

After 24 hours of post-exposure to EOs of *C. limon*, *C. aurantifolia*, and *C. sinensis*, there was a significant difference (P <0.0001) between the larval mortalities of *An. gambiae* at 0.1 ml concentration compared to 0.3 and 0.5 ml concentrations. However, there was no significant difference (0.059 < P > 0.9999) between 0.3 and 0.5 ml concentrations. Moreover, *An. gambiae* larvae exposed to *C. limon* (91.1%, 95.6%, 100%) and *C. aurantifolia* (91.1%, 95.6%, 100%) exhibited significantly higher (P<0.05) mortality rates compared to *C. sinensis* (2.2%, 33.3%, 33.3%) at the three concentrations (Fig. 4).

After 48 hours of post-exposure, there was a significant difference (P<0.0001) in the larval mortalities of *An. gambiae* in *C. sinensis* at 0.1 ml concentration compared to 0.3 and 0.5 ml concentrations. However, there was no significant difference (P= 0.0566) between 0.3 and 0.5 ml concentrations. Also, the mean larval mortalities of *An. gambiae* mosquitoes exposed to *C. limon* (100%) and *C. aurantifolia* (100%) were significantly higher (P<0.05) compared to *C. sinensis* (6.7%, 46.7%, 60%) at the three concentrations (Fig. 4).

After 72 hours of post-exposure, no significant difference (P>0.9999) was observed between the larval mortalities of *An. gambiae* in *C. limon* (100%), *C. aurantifolia* (100%), and *C. sinensis* (100%, 71.1%, 71.1%) at the three concentrations (Fig. 4).

Table 3 shows the lethal median time (LT_{50} and LT_{90}) of *C. limon*, *C. aurantifolia*, and *C. sinensis* EOs against *An. gambiae* larvae. The LT_{50} values ranged from 19.75 to 24.66 hrs for *C. sinensis*, 7.40 to 11.8 hrs for *C. aurantifolia*, and 3.86 to 6.41 hrs for *C. limon*. The lowest LT_{50} values were recorded with *C. limon* at 0.5 ml concentration (3.86 hrs), followed by 0.3 and 0.1 ml (4.35, 6.41 hrs) of the same citrus oil. Meanwhile, the highest LT_{50} values were recorded with *C. sinensis* at 0.3 ml concentration (24.66 hrs), followed by 0.5 ml (19.75 hrs) of the same citrus oil (Table 3).

The LT_{90} values varied from 630 to 1808 hrs for *C. sinensis*, 15.85 to 31.46 hrs for *C. aurantifolia*, and 9.49 to 38.48 hrs for *C. limon*. The lowest LT_{90} values were recorded with *C. limon* at 0.5ml (9.49 hrs), followed by 0.3 ml concentration (13.92 hrs) of the same citrus oil. Meanwhile, the highest LT_{90} values were recorded with *C. sinensis* at 0.3 ml (1808 hrs), followed by 0.5 ml (630 hrs) of the same citrus oil (Table 3).

Table 3: Lethal median time (LT_{50} and LT_{90}) values of *An. gambiae* larvae exposed to varying concentrations of three citrus oil types.

Concentrations (ml)	LT_{50} values (95%CI) h			LT_{90} values (95%CI) h		
	0.1	**0.3**	**0.5**	**0.1**	**0.3**	**0.5**
C. sinensis	NA	24.66 [a] (21.47-28.25)	19.75 (16.82-23.07)[a]	NA	1808 (NA)[a]	630 (NA)[a]
C. aurantiifolia	11.8 (10.45-13.23)[a]	7.57 (7.16-7.99)[b]	7.40 (6.53-8.28)[b]	31.46 (22.17-52.63)[a]	15.85 (13.47-19.11)[b]	25.13 (18.01-42.77)[b]
C. limon	6.41 (5.11-7.77)[a]	4.35 (3.80-4.91)[b]	3.86 (3.47-4.31)[b]	38.48 (16.56-31.05)[a]	13.92 (10.27-19.52)[b]	9.49 (3.66-10.94)[b]

LT_{50} and LT_{90} values followed by different superscripts are significantly different (*P<0.05*) across varying concentrations *NA* not applicable *CI* confidence interval

4. Discussion

The emergence of insecticide resistance in Nigeria's predominant malaria vector across multiple regions of the country has highlighted the importance of exploring and implementing alternative and complementary strategies to ensure the efficacious management of malaria vectors (Oduola et al., 2019; Omotayo et al., 2021). The present study provides compelling insights on the intricate relationship between EOs extracted from different *Citrus* species (*C. sinensis*, *C. limon*, and *C. aurantifolia*) and the toxicity exhibited against both the larval and female adult stages of *An. gambiae* mosquitoes, the major vector of malaria. This has implications for both vector management and ecological considerations.

The chemical constituents of insecticidal plant EOs greatly influence their bioactivity against different disease vectors (Pandey et al., 2014). This study investigated the major chemical constituents of the three *Citrus* species under consideration and their bioactivity. The GC-MS analysis revealed that Citral is one of the major chemical constituents in *C. aurantifolia,* while D-limonene is among the major constituents in *C. limon*. Similarly, Mahanta et al. (2020) and Sarma et al. (2019) also reported citral among the major components found in EOs extracted from the peels of *C. aurantifolia*. Likewise, Kaskoos (2019) and Paw et al. (2020) identified limonene as a main constituent of *C. limon*.

Citrus EOs are composed of secondary metabolites that are used by the plant for protection from microbes, insect pests, and herbivorous animals (Kabera et al., 2014). Humans can leverage this to protect themselves from insect vectors that carry diseases. In this research, the knockdown of *An. gambiae* after 60 minutes of exposure to *C. aurantifolia* and *C. limon* oils outperformed deltamethrin, a synthetic insecticide used for bed nets in malaria vector management, with the lowest KDT_{50} and KDT_{90}. This observation is noteworthy in the context of sustainable vector control methods, as relying on chemical insecticides in the form of aerosols raises concerns about their environmental impacts and the development of insecticide resistance. Because of their eco-friendly nature, the use of natural products for managing insect vectors is a highly regarded strategy to reduce disease burden (Brah et al., 2023).

All of the citrus species evaluated displayed significant toxicity (KD and mortality) against adult females of *An. gambiae,* which was comparable to deltamethrin, a commonly used public health insecticide. Also, *C. aurantifolia* and *C. limon* showed 100% mortality, which was similar to that observed in deltamethrin. The current study's results are consistent with those of Soonwera (2015) and Athira and Pushpalatha (2021), who found that *C. limon* caused a high knockdown and mortality activity on adult female *Aedes aegypti* and *Culex quinquefasciatus* after 60 minutes post-exposure. Similarly, Oshaghi et al. (2002) reported that *C. limon* displayed excellent toxicity against *An. stephensi,* while Roekmi-ati et al. (2008) and Sharma et al. (2022) elucidated excellent insecticidal effects of EOs from *C. aurantifolia* on *Aedes aegypti*. The results of this research confirm the potent knockdown and toxicity properties of *C. limon* and *C. aurantifolia* oils on adult female *An. gambiae*, indicating their potential usefulness and applicability in malaria vector management. Importantly, the efficacy of these natural EOs in comparison to a synthetic insecticide like deltamethrin demonstrates the potential of botanical extracts as effective mosquito control agents.

All the *Citrus* species evaluated displayed significant toxicity activity against *An. gambiae* larvae as well. Similarly, Adelaja et al. (2023) showed that oils from *C. sinensis* exhibited good larvicidal activity against *An.*

gambiae larvae. Also, Sharma et al. (2022) reported that EOs from some *Citrus* species showed significant larvicidal activity on *An. stephensi*. Oils from *C. limon* and *C. aurantifolia* displayed excellent larvicidal activity at the highest concentration after 24 hrs of exposure. Meanwhile, 72 hrs post-exposure, the lowest concentration resulted in 100% mortality of *An. gambiae* larvae. The best LT_{50} and LT_{90} were observed in *C. limon* against *An. gambiae* larvae, which were the lowest among the *Citrus* species evaluated. Similarly, oil from *C. limon* has been reported to inhibit the larval development of *Aedes aegypti* (Gomes et al., 2019), *Culex quinquefasciatus*, and *An. stephensi* (Sharma et al., 2022).

5. Conclusion

The EOs extracted from the peels of *C. sinensis*, *C. limon*, and *C. aurantifolia* demonstrated notable efficacy against both the larval and adult stages of *An. gambiae*. Particularly noteworthy was the remarkable adulticidal activity exhibited by the oils from *C. limon* and *C. aurantifolia*, rivalling the effectiveness of deltamethrin, a widely utilized insecticide in public health interventions. Moreover, the larvicidal potency of these oils evidenced exceptionally high mortality rates among *An. gambiae* larvae. Citral was identified in *C. aurantifolia*, D-limonene in *C. limon*, and myrtenol in *C. sinensis*. However, further investigation into these major components is needed to confirm their presence and determine their precise mode of action against *An. gambiae*, which is a critical step in the development of botanical insecticides for malaria vector control. These findings hold significant promise for the practical application of citrus-derived essential oils as potent agents in the fight against malaria transmission.

Declaration of Competing Interest

The authors reported no potential conflict of interest.

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