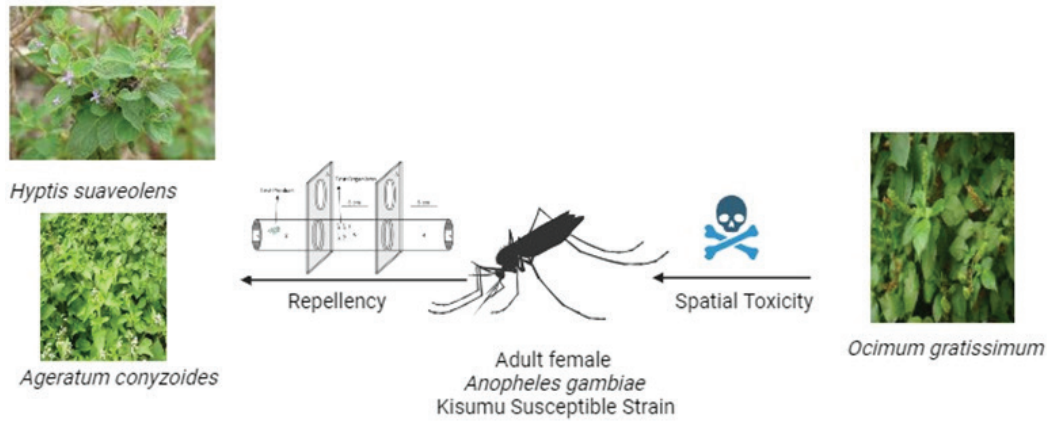


RESEARCH ARTICLE

Repellency and Toxicity Effects of Selected Plant Essential Oils Against *Anopheles gambiae*

O.J. Adelaja*, A.O. Oduola, A.I. Omotayo, A.O. Adeogun, A.T. Ande and A. Obembe



Highlights

- Essential oils from five plant species showed strong potential as eco-friendly mosquito control agents.
- *Hyptis suaveolens* and *Ageratum conyzoides* repelled *Anopheles gambiae* more effectively than DEET.
- Oils from *Citrus sinensis*, *Ocimum gratissimum*, and *Nicotiana tabacum* caused knockdown and mortality similar to deltamethrin.
- *Ocimum gratissimum* showed the fastest knockdown, highlighting its potential for vector control.

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Repellency and Toxicity Effects of Selected Plant Essential Oils Against *Anopheles gambiae*

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Abstract: Plant-derived essential oils show significant potential as eco-friendly agents in controlling disease vectors, including mosquitoes and other arthropods. This study assessed the repellent properties and spatial toxicity of selected plant essential oils against the *Anopheles gambiae* Kisumu Susceptible Strain (KSS). Essential oils were extracted from *Hyptis suaveolens* (Bush mint), *Ocimum gratissimum* (Scent leaf), *Nicotiana tabacum* (tobacco), *Ageratum conyzoides* (goat weed), and *Citrus sinensis* (sweet orange) via steam distillation. The repellency and spatial toxicity against *Anopheles gambiae* KSS were evaluated using the WHO tube and Peet-Grady Chamber methods, respectively, using WHO guidelines, with N, N-diethyl-meta-tolamide (DEET, 10% w/v) and Deltamethrin (0.05% w/v) as controls. The data obtained were analysed using ANOVA with Tukey's HSD and probit. *Hyptis suaveolens* and *Ageratum conyzoides* exhibited strong repellent effects 30 minutes after exposure, with effectiveness ranging from 96.5% to 100%, outperforming DEET, which showed 86.67% repellency. The tested plant essential oils demonstrated statistically significant repellent properties ($p < 0.05$), ranging from 96% to 100% at concentrations of 0.3 and 0.5 mg/mL, outperforming DEET. Essential oils from *C. sinensis*, *O. gratissimum*, and *N. tabacum* showed 100% knockdown at a concentration of 0.5 mg/mL, demonstrating comparable efficacy with deltamethrin. At 0.3 mg/mL, *O. gratissimum* showed 100% mortality of *Anopheles gambiae* KSS within 60 minutes post-exposure, indicating strong knockdown potential. All oils at 0.3 and 0.5 mg/mL achieved 100% mortality after 24 hours post-exposure. *Ocimum gratissimum* at 0.5 mg/mL showed the most expedient knockdown times (KDT_{50} and KDT_{95}). These results highlight the repellent and toxicity effects of the tested plant essential oils against *An. gambiae* KSS, suggesting their potential application as alternatives to synthetic insecticides for *An. gambiae* management strategies.

Keywords: *Anopheles gambiae*; Knock Down; Plant essential oils; Repellency; Spatial toxicity

INTRODUCTION

Insect-borne diseases and agricultural pests continue to impose a significant burden on human societies. In 2023, malaria caused an estimated 597,000 deaths globally, with 95% of these fatalities occurring in the WHO African Region (WHO, 2024). The highest burden was concentrated in Nigeria (30.9%), the Democratic Republic of the Congo (11.3%), Niger (5.9%), and Tanzania (4.3%) (WHO, 2024). In Nigeria, malaria accounts for 30% of childhood

mortality and 11% of maternal deaths (NMEP, 2022), with *Anopheles gambiae* the primary vector responsible for ~90% of transmission due to its anthropophilic behaviour and widespread resistance to pyrethroid-treated bed nets (NMEP, 2022; Hemingway et al., 2016). The current reliance on synthetic insecticides, such as indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs), is increasingly unsustainable: 78% of Nigerian *Anopheles* populations now exhibit KDR mutations conferring pyrethroid resistance (Oduola et al., 2019), while residual toxicity from organophosphates raises ecological and public health concerns (Tusting et al., 2019). These challenges underscore the urgent need for novel, resistance-breaking alternatives rooted in sustainable practices.

Throughout time, plants have consistently offered robust and dependable agents, maintaining their bio-efficacy. Ethnobotanical studies across Africa have contributed to understanding traditional practices in the control of insect vectors. For example, Karunamoorthi (2009) explored the traditional use of mosquito repellent plants among the Ethiopian Oromo ethnic group, and Youmsi et al. (2017) conducted a similar inquiry in Cameroon. Edwin-Wosu et al. (2013) conducted an ethnobotanical survey in Nigeria's Eastern region, documenting various insecticidal plant species. Adelaja et al. (2021) extended this exploration, conducting an ethnobotanical inquiry into insecticidal plant materials across diverse ethnic groups in Nigeria's North-Central geopolitical zone.

Historically, various cultures have utilised whole plants, either dried or freshly harvested, as natural insect repellents by strategically placing them in homes, granaries, and storage spaces. For example, plants like lavender, neem, or chrysanthemums were hung, scattered, or stored in bundles to deter pests such as moths, beetles, and weevils, leveraging their inherent bioactive compounds that repel or disrupt insect behaviour (Rants'o et al., 2023). Moreover, research by Adelaja et al. (2021) asserts that plants continue to serve as a prolific source of insecticidal compounds. African historical records, for instance, detail plants and their constituents known for repelling, deterring feeding, and displaying toxic properties on insects (Ezekwesili-Ofili & Okaka-Antoinette, 2019). The

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isolation and synthesis of organic compounds from diverse plants, such as pyrethroids, azadirachtin, rotenones, and others, have been extensively utilised in managing insect pest activities over time (Souto et al., 2021). However, the dominance of inorganic synthetics, although more potent, has overshadowed these efforts, leading to environmental concerns and insect resistance (David et al., 2018).

Mosquitoes, among the many African insect vectors, have attracted significant attention due to their pervasive presence in households and their role in disease transmission. Mosquitoes serve as critical vectors for numerous tropical diseases, significantly impacting public health (Cooper et al., 2025; David et al., 2018; Egunyomi et al., 2010). Malaria, a mosquito-borne disease, is a leading cause of infectious illness-related mortality in Africa and is also the primary cause of death among Nigerian children under five, accounting for 27% of global cases (NDHS, 2018; WHO, 2019; 2024). Notably, *Anopheles gambiae* Giles 1920 (Diptera: Culicidae) assumes the role of the principal malaria vector in Nigeria (NDHS, 2018). Initial success in reducing malaria through synthetic insecticides deployed via indoor residual spraying (IRS) and insecticide-treated bed nets (ITNs), is now facing critical challenges due to widespread insecticide resistance in *Anopheles gambiae* and related vectors (Oduola et al., 2019). Resistance mechanisms, including metabolic detoxification (e.g., cytochrome P450 upregulation) and target-site mutations (e.g., *kdr* alleles)—have rendered pyrethroids, the primary class of insecticides used in ITNs, increasingly ineffective across Africa (Boussougou-Sambe et al., 2024; Kabula et al., 2024; Oruni et al., 2024). For example, recent studies in Uganda, Tanzania, and the Democratic Republic of Congo (DRC) reveal escalating resistance to pyrethroids, carbamates, and organophosphates, threatening malaria control progress (Boussougou-Sambe et al., 2024; Dennis et al., 2024; Oruni et al., 2024).

As observed by Dube et al. (2011), botanical oils and extracts offer innovative and promising bio-rational solutions for personal protection, revitalising traditional insect control methods. Noutcha et al. (2016) emphasise the richness of plant essential oils as sources of physiologically active components and insect repellents. In the pursuit of a natural, non-resistant, and ecologically sustainable approach to malaria vector control, the investigation into plants harbouring bioactive compounds (Adelaja et al., 2021) becomes paramount. The use of botanical insecticides for mosquito control has gained renewed interest due to the ecological and resistance-related challenges posed by synthetic chemicals. Historically, plants like *Azadirachta indica* (neem) and *Chrysanthemum cinerariifolium* (pyrethrum) were widely utilised for their bioactive compounds, such as azadirachtin and pyrethrins, which disrupt insect nervous systems (Shalan et al., 2005).

This study bridges this gap by evaluating the repellent and toxic effects of *Hyptis suaveolens*, *Ocimum gratissimum*, *Nicotiana tabacum*, *Ageratum conyzoides*, and *Citrus sinensis* selected for their (1) historical use in Nigerian vector control, (2) rich essential oil profiles, and (3) preliminary evidence of bioactivity against other Diptera

(Adelaja et al., 2021; Edwin-Wosu et al., 2013; Zhang et al., 2020). By testing these species against the Kisumu Susceptible Strain (KSS) of *An. gambiae*, we establish baseline efficacy metrics while providing a comparative framework for future studies on resistant populations. Our findings aim to revitalize ethnobotanical knowledge through evidence-based validation, offering scalable templates for bio-rational insecticide development in regions disproportionately affected by both malaria and pesticide resistance.

MATERIAL AND METHODS

Collection of Plant Materials

The botanical specimens used in this study, namely the leaves of *Ocimum gratissimum* (Lamiaceae), *Hyptis suaveolens* (Lamiaceae), *Nicotiana tabacum* (Solanaceae), *Ageratum conyzoides* (Asteraceae), and the fruit peel of *Citrus sinensis* (Rutaceae), have been previously identified as prominent insecticidal plant sources. These selections were based on findings from an ethnobotanical investigation conducted among local communities in various villages within North-central Nigeria, as reported by Adelaja et al. (2021).

To ensure the accuracy and authenticity of our study materials, each botanical specimen was individually sourced from thriving plants within the vicinity of Ilorin (Latitude: 8° 44' to 8° 46' N, Longitude: 4° 55' to 4° 57'). Following the collection, rigorous taxonomic identification was conducted at the herbarium of the Department of Plant Biology. Accompanying descriptive information further facilitated the identification process, resulting in the precise verification of each specimen as follows: *Hyptis suaveolens* (UILH/003/931), *Ocimum gratissimum* (UILH/012/984), *Nicotiana tabacum* (UILH/005/504), *Ageratum conyzoides* (UILH/013/140), and *Citrus sinensis* (UILH/010/159). These herbarium specimens were deposited as voucher specimen collections at the University of Ilorin Herbarium.

Preparation of Plant Materials

Fresh leaves of *Hyptis suaveolens* (Bush mint), *Ocimum gratissimum* (Scent leaf), *Nicotiana tabacum* (tobacco), *Ageratum conyzoides* (goat weed), and *Citrus sinensis* (sweet orange) were collected from mature, disease-free plants in Ilorin, Nigeria (8.5373° N, 4.5444° E) during the dry season (January–March 2021). For each species, 500 g of freshly harvested leaves were uniformly spread on sterile mesh trays in a shaded, well-ventilated room maintained at 25 ± 2°C and 45–50% relative humidity for 7 days. After drying, the leaves exhibited a mean moisture loss of 68.3 ± 2.1%, resulting in 150 ± 5 g of dried material per species.

The dried leaves were ground into a fine powder (particle size ≤ 1 mm) using an industrial grinder. Essential oils were extracted via hydro-distillation using a 2 L Clevenger apparatus: 100 g of powdered leaves were mixed with 400 mL of distilled water and heated at 100°C for 4 hours until oil separation ceased. The oils were collected, dried over 5 g of anhydrous sodium sulphate (per 10 mL of oil) for 24 hours to remove residual moisture, and filtered through Whatman No. 1 filter paper. Yield percentages

were calculated as (oil volume / initial dried plant mass) $\times$ 100, with *O. gratissimum* yielding the highest ($1.8 \pm 0.2\%$) and *C. sinensis* the lowest ($0.6 \pm 0.1\%$). The oils were transferred into 2 mL amber glass vials, labelled, and stored at 4°C for ≤ 30 days before bioassays.

Preparation of stock solutions and test concentrations

Stock solutions of each essential oil were prepared by dissolving 1.0 mL of oil (assumed density ≈ 1.0 g/mL) in 99.0 mL of HPLC-grade acetone, yielding a 10 mg/mL stock concentration (1% w/v). Serial dilutions were performed to generate three test concentrations: 0.1 mg/mL, 0.3 mg/mL, and 0.5 mg/mL. For the 0.1 mg/mL solution, 1.0 mL of stock was mixed with 9.0 mL of acetone (1:10 dilution). The 0.3 mg/mL concentration was prepared by combining 3.0 mL of stock with 7.0 mL of acetone (3:10 ratio), while the 0.5 mg/mL solution used a 5.0 mL stock + 5.0 mL acetone (1:1 ratio). All dilutions were validated using UV-Vis spectrophotometry at $\lambda = 254$ nm to confirm accuracy ($\pm 2\%$ margin of error).

Range-finding tests were conducted to determine optimal concentrations. Preliminary trials tested 0.05–2.0 mg/mL in increments of 0.05 mg/mL ($n = 50$ mosquitoes per concentration) to identify the minimum effective dose (MED) and lethal concentration (LC_{50}). Data from these trials (unpublished) revealed that 0.1–0.5 mg/mL elicited dose-dependent repellency (20–95%) without causing solvent toxicity, aligning with OECD Guideline No. 214 for arthropod testing.

Mosquito collection and rearing

Female adult *An. gambiae* mosquitoes of the KSS strain were procured from the insectary facilities of the Nigerian Institute of Medical Research (NIMR) located in Lagos. The maintenance of this mosquito population adhered strictly to established protocols and standards (WHO, 2005). The environmental conditions within the insectary were maintained (Constant temperature of $27 \pm 2^\circ\text{C}$ and a Relative Humidity of $85 \pm 5\%$). For the rearing of the larval stages, plastic trays measuring 24 cm $\times$ 18 cm $\times$ 4 cm were employed, each containing 500 mL of distilled water. The larval nutrition consisted of a mixture of non-fatty biscuits and yeast in a ratio of 3:1. On the other hand, the adult mosquitoes were raised within a mosquito cage measuring 50 cm $\times$ 50 cm $\times$ 50 cm, and they were provided unrestricted access to a 10% sugar solution for sustenance.

Repellent activity assays

The experimental approach described in Deletre et al. (2015) involving the two-choice behavioural response testing technique was implemented. The experimental setup consisted of a specialised apparatus composed of two interconnected tubes, following the configuration of the WHO insecticide susceptibility test kit. This apparatus was divided into three distinct chambers: treated, untreated, and neutral (Figure 1). To conduct the trials, specific concentrations of plant essential oils (0.1 mg/mL, 0.3 mg/mL, and 0.5 mg/mL) were impregnated onto Whatman paper (110 mm diameter) by applying 1.0 mL of each solution per paper. This resulted in a final application rate of ~ 0.0105 mg/cm² (total mass per paper divided by its area).

The impregnated paper was positioned at the centre of the treatment chamber, adhering to the methodology outlined by Adelaja et al. (2023). Additionally, human-worn socks, serving as a natural attractant following Deletre et al. (2015), were placed at the opposite end of the chamber to ensure separation from the treated paper and to replicate host-seeking behaviour.

One of the objectives of the study was to assess the repellent response of *An. gambiae* KSS to insecticidal plant essential oils. To achieve this, four treatments were evaluated by introducing: (i) Natural attractant (human-worn socks), (ii) Solvent (acetone) + natural attractant, (iii) Test plant oil + natural attractant, and (iv) N, N-diethyl-meta-toluamide (DEET) + natural attractant. After the introduction of *An. gambiae* KSS aged 2–4 days old into the neutral chamber, following a one-minute acclimatization period, the sliding doors that demarcated the three chambers were simultaneously opened for 30 minutes. During this interval, readings were taken at specified time points (after 10-minute intervals). After the 30-minute testing period, the chambers were closed, and the count of adult female *An. gambiae* KSS within the treatment chamber was recorded. This process was repeated ten times for each of the experimental designs with a new batch of *Anopheles* mosquitoes, to ensure comprehensive data collection and analysis.

Toxicity assays

The experiment was conducted within a Peet-Grady Chamber measuring 180 $\times$ 180 $\times$ 180 cm, adhering to established standards. One hundred adult female *An. gambiae* KSS aged 2–4 days were introduced into the chamber through designated release ports, following the procedures outlined in WHO (2009) guidelines.

To apply the plant essential oils, a 110 mm diameter Whatman filter paper was employed as the substrate. Using a micropipette, 1.0 mL of each plant essential oil concentration (0.1 mg/mL, 0.3 mg/mL, and 0.5 mg/mL) was meticulously administered onto a 110 mm diameter Whatman filter paper, following the methodology detailed by Adelaja et al. (2023). This resulted in an application rate of 0.00105 mg/cm², 0.00316 mg/cm², and 0.00526 mg/cm², respectively (calculated as [concentration $\times$ volume] divided by the paper's surface area, 95 cm² for a 110 mm diameter). The treated paper was then centrally positioned within the chamber, and the air circulation system (facilitated by a fan) was activated. The ensuing observations encompassed periodic assessments (10-minute intervals) of mosquito knockdown for 60 minutes.

Following the designated time frame, all mosquitoes were systematically collected and placed within clearly labelled paper cups. In these cups, the mosquitoes were provided with a 10% sugar solution. Mortality rates were subsequently assessed after a 24-hour recovery period from the treatment application. The experimental conditions were maintained at a controlled temperature of $27 \pm 2^\circ\text{C}$ and a relative humidity of $80 \pm 10\%$, upholding consistency in the testing environment.

For comparative purposes, a control utilizing deltamethrin

was employed, adhering to guidelines from the Centers for Disease Control. The deltamethrin solution, characterized by a stock concentration of 10 mg/mL and a diagnostic dose of 12.5 µg/BT, was employed as the benchmark for assessment. The experiment was executed with precision, and each treatment condition was subjected to replication four times, aligning with the protocol outlined in WHO (2009).

Data analysis

Percentage knockdown and percentage repellency were calculated using the formulas below:

% Knock Down

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

Percentage Repellency

$$= \frac{\text{Number of mosquitoes in Treated} - \text{Number of mosquitoes in Control}}{\text{Number of mosquitoes in Treated} + \text{Number of mosquitoes in Control}} \times 100$$

(Kain et al., 2013)

The statistical analysis encompassed various methodologies to uphold rigour. For evaluating the mean mosquito counts within treated chambers and mortalities across a spectrum of exposures, a one-way ANOVA was selected. To ascertain potential variations between different plant essential oils, a two-way ANOVA was conducted. Tukey's HSD post hoc test was employed to segregate means with a significance level of $p=0.05$.

The Knockdown Times (KdT_{50} and KdT_{95}) for successive concentrations of plant essential oils were determined via probit analysis, relying on mortality data as outlined in Finney (1971). In instances where the control group exhibited a mortality rate exceeding 5%, the treatment groups underwent adjustments through Abbott's methodology. All statistical analyses were performed utilising the GraphPad Prism 8 software, ensuring precision and reliability throughout the investigation.

Ethical statement

The study was conducted after obtaining certified ethical approval from the University of Ilorin Ethical Review

Committee with UERC Approval Number UERC/ASN/2017/898.

RESULTS

Repellency activity of plant essential oils

The results depict the average number of *An. gambiae* KSS observed within both the treated and control chambers during the evaluation of the designated plant essential oils, as illustrated in Figure 2. Notably, an observation was made that across all experimental arrangements except for the negative controls involving Natural attractant (Human odour) and solvent + natural attractant, the control chamber exhibited statistically significantly higher mean counts of *An. gambiae* KSS ($P<0.05$).

All concentrations of the selected plant essential oils (*H. suaveolens*, *O. gratissimum*, *A. conyzoides*, *N. tabacum*, and *C. sinensis*) exhibited a compelling repellent efficacy of 100% (20.00 ± 0.00 mosquitoes) against *An. gambiae* KSS, even in the presence of a natural attractant. A minor exception was observed in the case of 0.3 mg/mL concentration of *H. suaveolens*, where a repellent efficacy of 96.5% (19.33 ± 1.15 mosquitoes) was recorded. No statistically significant variation ($P=0.0968$; $F=8.552$; $DF=4$) was discernible among the diverse concentrations of the evaluated plant essential oils, as depicted in Figure 2a-e. There was a statistically significant difference ($P<0.05$) between the repellent effectiveness noted for *An. gambiae* KSS across all assessed plant essential oils (96.5 – 100%) and the positive control, DEET (76.5 – 80%), in the presence of a natural attractant.

Toxicity activity of plant essential oils

The results on the knockdown effect, mortality, and knockdown time of selected plant essential oils on *An. gambiae* KSS, as assessed within a controlled Peet-Grady chamber setting, are succinctly presented in Figure 3 and Table 1.

Following a 60-minute exposure of *An. gambiae* KSS to varying concentrations of the plant essential oils, the percentage of knockdown ranged between 58.3% and 66.7%, 90% and 96.7%, and 95% and 98.3% for concentrations of 0.1 mg/mL, 0.3 mg/mL, and 0.5 mg/mL, respectively, of oils obtained from *H. suaveolens* and *A. conyzoides*. Notably, these percentages were lower

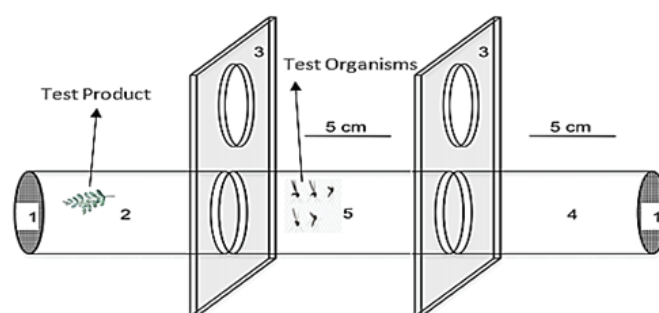


Figure 1: Set up for the repellent activity assay using a WHO diagnostic test kit following Deletre et al. (2015): 1 - end cap covered by a net, 2 - treatment chamber, 3 - sliding door, 4 - untreated chamber, and 5 - chamber where mosquitoes were introduced

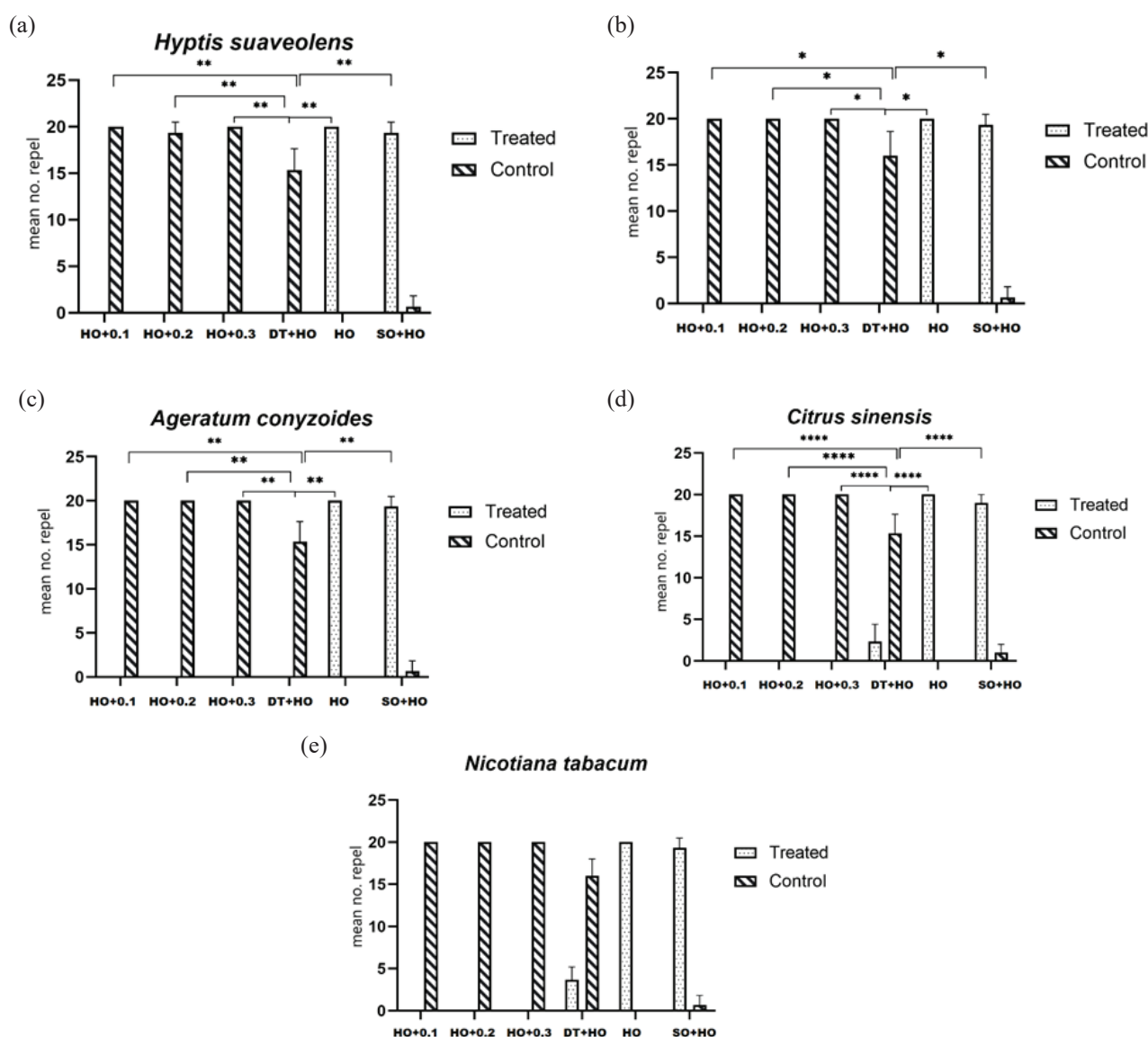


Figure 2: Mean number of *An. gambiae* KSS in both treated and control chambers when plant essential oils from (a) *H. suaveolens*, (b) *O. gratissimum*, (c) *A. conyzoides*, (d) *C. sinensis* and (e) *N. tabacum* were assayed (mean no. repel – mean number of mosquitoes repel, HO – Human Odour, DT – DEET, SO – Solvent, concentrations are expressed in mg/mL)

than the recorded knockdown rate of the positive control, deltamethrin (100%), following the same 60-minute exposure period (depicted in Figure 3 a and e). In contrast, the oil from *O. gratissimum*, at a concentration of 0.3 mg/mL, achieved a knockdown effect equivalent to the positive control (Fig. 3 c), while concentrations of 0.1 mg/mL and 0.3 mg/mL of oils from *C. sinensis* and *N. tabacum* displayed a comparatively lower knockdown percentage (ranging from 68.3% -71.7% and 90% -96.7%, respectively) on *An. gambiae* KSS (depicted in Figure 3 b and d).

Strikingly, concentrations of 0.5 mg/mL of oils derived from *C. sinensis* and *N. tabacum* yielded a significant effect ($P>0.05$) analogous to the positive control deltamethrin, resulting in a hundred percent knockdown rate after 60 minutes post-exposure. Intriguingly, no significant disparity ($P>0.05$) was observed in the percentage knockdown on *An. gambiae* KSS for concentrations of 0.5 mg/mL of oils from *H. suaveolens*, *C. sinensis*, *O. gratissimum*, *N. tabacum*,

and *A. conyzoides*, when compared to the positive control deltamethrin (Figure 3 a-e).

After a 24-hour interval following exposure to varying concentrations of plant essential oils on *An. gambiae* KSS, concentrations of 0.3 mg/mL and 0.5 mg/mL of all five evaluated plant essential oils achieved a complete mortality rate of 100%, aligning with the mortality rate recorded for the positive control, deltamethrin (Table 1). Notably, no significant difference ($P>0.05$; $F=5.137/7.405$; $DF=30$) was observed between the mortality rates resulting from deltamethrin and the concentrations of 0.3 mg/mL and 0.5 mg/mL for the assessed plant essential oils. In contrast, concentrations of 0.1 mg/mL of all five plant essential oils yielded mortality rates ranging from 68% to 85%, failing to achieve complete mortality (Table 1). A significant distinction was observed between the mortality rates of 0.1 mg/mL concentrations of the five plant essential oils and deltamethrin (Table 1).

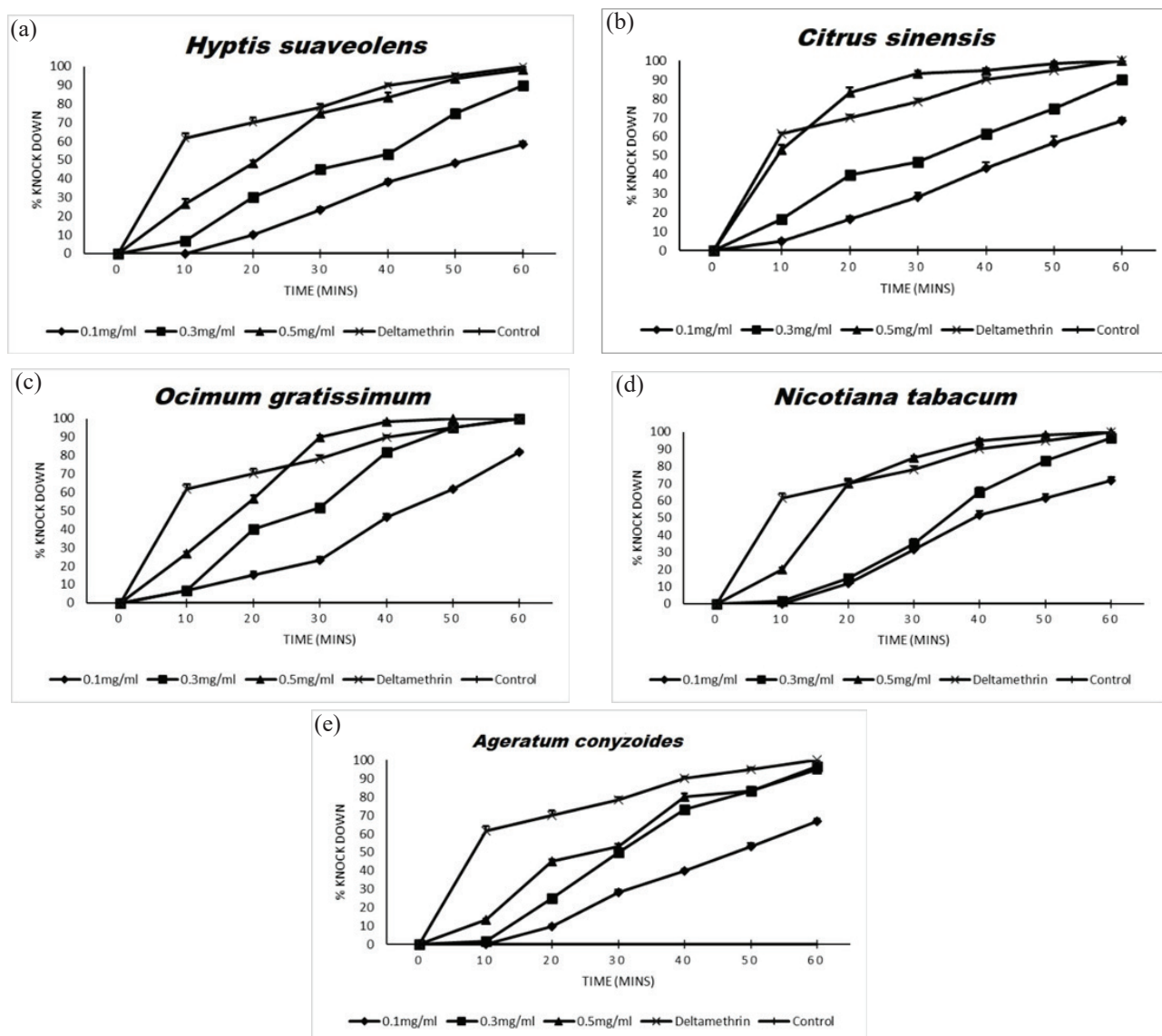


Figure 3: Percentage knockdown after 60 minutes of *An. gambiae* KSS exposed to oils from (a) *H. suaveolens* (b) *C. sinensis* (c) *O. gratissimum* (d) *N. tabacum* and (e) *A. conyzoides* (% Knockdown – Percentage Knockdown of mosquitoes; MINS - Minutes)

Regarding the knockdown time metrics, the lowest KdT_{50} was observed at a concentration of 0.5 mg/mL for *O. gratissimum* (21.32 mins), succeeded by 0.5 mg/mL of *H. suaveolens* (25.18 mins), 0.3 mg/mL of *A. conyzoides* (33.47 mins), and 0.1 mg/mL of *N. tabacum* (34.8 mins). These values were inferior to the KdT_{50} recorded for the positive control, deltamethrin (36.10 mins). Notably, the highest KdT_{50} values were documented for 0.1 mg/mL concentrations of *C. sinensis* (73.08 mins) and *A. conyzoides* (66.27 mins).

Conversely, the lowest KdT_{95} was ascertained at a concentration of 0.5 mg/mL for *O. gratissimum* (37.05 mins), trailed by 0.5 mg/mL of *H. suaveolens* (73.49 mins), and concentrations of 0.1 mg/mL and 0.3 mg/mL for *N. tabacum* (92.51 mins and 95.58 mins, respectively). These values were notably lower than the KdT_{95} exhibited by the positive control, Deltamethrin (114.9 mins). Conversely, the most extended KdT_{95} values were observed for 0.1 mg/mL of *A. conyzoides* (372.6 mins) followed by 0.3 mg/mL of *O. gratissimum* (271.7 mins) (as elaborated in Table 1).

DISCUSSION

Plant secondary metabolites, such as alkaloids, terpenoids, and phenolic compounds, exhibit targeted bioactivity, including insecticidal and antimicrobial properties, as part of their natural defence mechanisms against herbivores and pathogens (Jahan et al., 2025). This phenomenon stems from the presence of toxins and secondary metabolites functioning as either toxicants or repellents against hematophagous insects, as highlighted by Benelli et al. (2012) and Zhang et al. (2020). The present study involved the careful selection and collection of plant materials initially profiled by Adelaja et al. (2021). Essential oils were extracted from these plants and underwent a thorough assessment for both repellent and toxic activity against *An. gambiae* KSS.

Under ecologically relevant conditions (i.e., in the presence of a natural mosquito attractant), plant essential oils from *A. conyzoides* and *N. tabacum* demonstrated previously unreported repellent efficacy against *An. gambiae* KSS

Table 1: Knockdown times (KdT₅₀ and KdT₉₅) and percentage mortality of *Anopheles gambiae* KSS mosquitoes exposed to different concentrations (0.1–0.5 mg/mL) of plant essential oils compared with deltamethrin (10 mg/mL) and solvent control

Plant essential oils	Conc. (mg/mL)	KdT ₅₀ (95% CI) (Mins)	KdT ₉₅ (95% CI) (Mins)	% Mortality after 24 hrs
<i>Hyptis suaveolens</i>	0.1	44.48 (18.73-105.6)	163.2 (17.78-1498)	68 ^a
	0.3	NA	NA	100 ^b
	0.5	25.18 (17.89-35.43)	73.49 (17.27-312.8)	100 ^b
<i>Citrus sinensis</i>	0.1	73.08 (0.218-24497)	403.2 (NA)	85 ^a
	0.3	NA	NA	100 ^b
	0.5	NA	NA	100 ^b
<i>Ocimum gratissimum</i>	0.1	58.72 (18.33-188.1)	172.2 (18.42-1610)	83 ^a
	0.3	36.68 (13.25-101.5)	271.7 (5.693-12970)	100 ^b
	0.5	21.32 (19.99-22.74)	37.05 (29.94-45.85)	100 ^b
<i>Nicotiana tabacum</i>	0.1	34.8 (21.83-55.48)	92.51 (19.84-431.4)	82 ^a
	0.3	38.7 (28.29-52.95)	95.58 (37.71-242.3)	100 ^b
	0.5	NA	NA	100 ^b
<i>Ageratum conyzoides</i>	0.1	66.27 (5.297-829.2)	372.6 (2.568-54055)	77 ^a
	0.3	33.47 (24.30-46.12)	127.6 (39.08-416.5)	100 ^b
	0.5	NA	NA	100 ^b
Deltamethrin	10	36.1 (13.55-96.19)	114.9 (4.899-2697)	100 ^b
Solvent	NA	NA	NA	0 ^c

Conc.- Concentration, KdT – knockdown time, NA – Not Applicable, percentage mortality values followed by different subscripts differ significantly ($P < 0.05$) between plant essential oils and varying concentrations ($n=100$)

strain. These oils exhibited repellency comparable to or exceeding that of established botanical repellents (*H. suaveolens*, *O. gratissimum*, and *C. sinensis*), whose repellent properties against *Anopheles* mosquitoes were previously documented (Adelaja et al., 2023; Afolabi, 2016; Benelli et al., 2012; Gbolade, 2001). This study highlights *A. conyzoides* and *N. tabacum* as novel candidates for integrated vector management strategies requiring repellent activity under attractant-challenged conditions.

The plant essential oils evaluated in this study exhibit remarkable potential as eco-friendly alternatives to synthetic insecticides for malaria vector control. Notably, *Ocimum gratissimum*, *Hyptis suaveolens*, *Ageratum conyzoides*, *Nicotiana tabacum*, and *Citrus sinensis* oils demonstrated 96.5–100% repellency against *Anopheles gambiae* KSS across concentrations of 0.1–0.5 mg/mL, significantly outperforming DEET (76.5–80% repellency; $P < 0.05$) under attractant-challenged conditions. This high efficacy, achieved at far lower concentrations than conventional botanical repellents (e.g., citronella at 5% w/v; Yoon et al., 2015), underscores their potential for sustainable deployment in resource-limited settings.

A striking finding was the rapid knockdown effect of *O. gratissimum* oil, which achieved 100% knockdown at 0.5 mg/mL within 21.32 minutes (KdT₅₀), surpassing deltamethrin's KdT₅₀ of 36.10 mins. This performance exceeds prior reports for *O. gratissimum* (KdT₅₀: 42.5 mins at 0.5 mg/mL; Ileke & Adeshina, 2019) and contrasts with the slower action of *C. sinensis* oil in earlier studies, which required double the concentration (1.0 mg/mL) for 80%

knockdown (Manimaran et al., 2012). Furthermore, all oils at 0.3–0.5 mg/mL induced complete mortality (100%) within 24 hours, matching deltamethrin's efficacy while operating at a fraction of its concentration (10 mg/mL). These results highlight a critical advantage: the tested oils achieve lethal effects at doses orders of magnitude lower than many synthetic counterparts, reducing environmental and operational burdens.

The potency of *N. tabacum* oil is particularly noteworthy. At 0.5 mg/mL, it elicited 100% knockdown and mortality within 60 minutes, outperforming earlier findings that required 1.2 mg/mL for 85% efficacy (Ileke et al., 2015). This aligns with field observations by Obembe et al. (2018), where tobacco smoke reduced mosquito presence, but our study quantifies its insecticidal activity at concentrations feasible for practical formulations. Similarly, *H. suaveolens* and *A. conyzoides* oils demonstrated rapid knockdown (KdT₅₀: 25.18 and 33.47 mins, respectively) at 0.5 mg/mL, suggesting their utility in regions where pyrethroid resistance threatens current interventions.

However, non-target effects on beneficial insects (e.g., pollinators) or human health remain unassessed, a critical limitation given the broad-spectrum nature of plant essential oils. Additionally, this study was conducted under controlled laboratory conditions; field efficacy, longevity, and environmental degradation of these oils in real-world settings (e.g., sunlight exposure, humidity) were not evaluated. The use of a susceptible *An. gambiae* strain (KSS) also limits extrapolation to wild, resistant populations. Future studies should assess non-

target impacts on pollinators and aquatic ecosystems, validate EO formulations (e.g., nanoemulsions) in field conditions, explore synergies with existing insecticides to curb resistance, and confirm sustained efficacy through behavioural assays in natural settings.

CONCLUSION

This study highlights the strong repellent and insecticidal potential of essential oils from *H. suaveolens*, *A. conyzoides*, *N. tabacum*, and *O. gratissimum* against *Anopheles gambiae* KSS. Notably, repellency rates reached up to 100%, exceeding that of DEET under attractant-challenged conditions. *O. gratissimum* achieved complete knockdown at low concentrations with significantly faster KdT₅₀ than deltamethrin, and all tested oils induced 100% mortality within 24 hours. The rapid action of *O. gratissimum* and *H. suaveolens* compared to synthetic pyrethroids underscores their potential as effective alternatives, particularly in areas with documented pyrethroid resistance. These plant-derived oils, biodegradable and less irritating than DEET, could enhance malaria control when integrated into indoor residual sprays (IRS) at 0.3 mg/mL or insecticide-treated nets (ITNs). However, mode-of-action studies are needed to optimise formulations and assess synergies with existing tools. Future work must validate field efficacy, environmental stability (e.g., via nanoemulsions), and non-target impacts to ensure scalable, resistance-proof interventions.

DECLARATION OF CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare.

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AUTHOR CONTRIBUTION

Conceptualisation: **O.J.A., A.O.O.**; Data Collection: **O.J.A., A.I.O., A.O.A.**; Statistical Analysis: **O.J.A.**; Data Interpretation: **O.J.A.**; Writing the First draft: **O.J.A.**; Manuscript editing and reviewing: **A.O.O., A.T.A., A.O.**; Project administration: **O.J.A., A.O.O.**

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