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Repellent and Insecticidal Properties of Combined Scent Leaf and Neem Leaf Formulation against *Aedes aegypti*

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Abstract

This study investigated the repellent and insecticidal efficacy of a combined formulation of scent leaf (*Ocimum gratissimum*) and neem leaf (*Azadirachta indica*) against *Aedes aegypti.* The assessment involved measuring mosquito landing times at varying concentrations (5 ml, 15 ml, 25 ml, and 35 ml) compared to a negative control (water) and a positive control (DEET). Results indicated that higher concentrations of the formulation significantly reduced mosquito landings, with the 25 ml concentration showing a notable landing time of 5.75 minutes during a 30-minute exposure window. The positive control demonstrated complex repellency, with no landings recorded at any time point. Statistical analysis using ANOVA revealed significant differences between concentrations (p = 7.72E-07), while time intervals showed no significant variation (p = 0.457928), indicating that concentration was the critical factor influencing mosquito behavior. Insecticidal properties were also evaluated, revealing minimal mosquito presence at higher concentrations, particularly at 35 ml, which resulted in an average of only 0.25 surviving mosquitoes at 150 minutes. A strong correlation was found between phytochemical content and both repellency (r = 0.6758) and insecticidal activity (r = -0.06758), suggesting that higher phytochemical concentrations enhance efficacy. Overall, the combined formulation exhibited promising potential as a natural repellent and insecticide, highlighting its efficacy in controlling *Aedes aegypti* populations and offering a sustainable alternative to synthetic repellents.

Keywords: repellency; insecticidal properties; scent leaf; neem leaf formulation; Aedes aegypti

1 Introduction

The global burden of mosquito-borne diseases such as dengue fever, Zika virus, chikungunya, and malaria remains a significant public health challenge. Aedes aegypti and Culex quinquefasciatus are primary vectors responsible for the transmission of these diseases, thriving in tropical and subtropical regions and making millions of people vulnerable to infections (Bhatt et al., 2013). One major challenge in mosquito control is the widespread development of resistance to synthetic insecticides, which are the cornerstone of most vector control programs. Over the past few decades, mosquitoes have developed resistance to multiple classes of insecticides, including organophosphates, pyrethroids, and carbamates. This resistance is primarily due to genetic mutations that alter target sites, enhanced metabolic detoxification, and behavioral changes that reduce insecticide exposure (Hemingway et al., 2016; Ranson et al., 2010). Although, mosquito control measures in integrated pest management has been focused on disruption of disease transmission either by eliminating the mosquitoes, preventing human contact with the mosquitoes or killing the larvae at their breeding site, the most effective control measures are those targeted at the larval stages due to their confined habitats (Elimam et al., 2009; Otabor *et al.,* 2019). Protection against mosquito bites is one

way being used to reduce illness prevalence. Several factors which include, habitats, weather, physiochemical parameters account for distribution of mosquitoes (Lapang et al., 2019; Ombugadu et al., 2020; Egya et al.,2023).

Presently, insecticidal properties of plants have been the subject of intense research (Abok et al., 2018; Ombugadu et al., 2020; Pam et al., 2021; Aliyu et al., 2022). Numerous plants have been screened for their phytotoxic properties against insect pests and vectors in the search for eco-friendly insecticides. According to reports, some of these plants have toxic effect on mosquitoes and can be used as potent source of mosquito control. Plants such as citronella (Cymbopogon nardus) is well-known for its efficacy and is widely used in commercial repellents. Lemongrass (Cymbopogon citratus), another member of the *Cymbopogon* genus, contains high levels of citronellal and geraniol, compounds known for their mosquito-repellent properties (Karunamoorthi et al., 2014). Additionally, eucalyptus (Eucalyptus globulus) oil, which contains significant amounts of eucalyptol, has been shown to provide effective protection against mosquitoes (Isman, 2016). The limitations of synthetic insecticides have intensified the search for alternative, eco-friendly methods for mosquito control. Plant-based repellents and insecticides have shown promise due to their biodegradability, low toxicity to non-target organisms, and effectiveness against a broad spectrum of insect pests (Isman & Grieneisen, 2014). Various dipteran pests have been controlled in past decades using synthetic insecticides (Poopathi and Archana, 2010). The intensive use of chemical insecticides led to the development of resistant insect populations, resulting in a reduced control and often to a negative impact on various non-target organisms and on the environment in general (Charles and Nielsen-Leroux, 2000; Awosul *et al.,*2018).

Ocimum gratissimum (Lamiaceae) is also a plant with insecticidal properties, known by diverse people of the globe by different local names. It is universally called Scent leave, (Scentleaf)'Nchuanwa' or 'Ahuji' by the Igbo speaking populations of South Eastern Nigeria, 'Effirin-nla' by the Yoruba speaking people of South Western Nigeria and 'Daidoya' by the Hausa speaking populations of Northern Nigeria (Odugbeml, 2008; Saliu et al., 2011; Egya et al., 2023). The essential oils derived from Ocimum gratissimum have demonstrated strong repellent and insecticidal properties against various mosquito species. The primary active compounds in scent leaf essential oils include eugenol, thymol, and carvacrol, which constitute approximately 70-85% of the oil (Ogungbe et al., 2018). These compounds interfere with the neurological pathways of insects, resulting in paralysis and death (Akinwande et al., 2020). Research has highlighted that scent leaf extracts can be as effective as synthetic repellents such as DEET, with the added benefit of lower toxicity and fewer side effects (Ogungbe et al., 2018). Neem is widely recognized for its insecticidal properties, particularly due to its bioactive compounds such as azadirachtin, nimbin, and salannin. Azadirachtin, the most potent compound, constitutes about 0.2-0.6% of the neem seed kernel's weight and has been extensively studied for its effects on insect growth and reproduction (Schmutterer, 1990). Neem oil and extracts disrupt insect growth and development, inhibit feeding, and reduce reproductive rates, making it a sustainable option for integrated pest management (Govindarajan et al., 2016). Neem's multifaceted mechanisms of action also reduce the likelihood of resistance development in mosquito populations (Benelli, 2015).

The combination of scent leaf and neem extracts presents a promising synergistic approach to mosquito control. Synergy occurs when the combined effect of two substances exceeds the sum of their individual effects. Previous studies have shown that the use of multiple plant extracts can enhance the efficacy of each component, potentially due to complementary mechanisms of action and the broad spectrum of bioactive compounds (Pavela & Benelli, 2016). This synergy not only improves

overall effectiveness but also helps in mitigating resistance development among mosquito populations (Sarfraz et al., 2017).

The composition and application of botanical pesticides have improved recently, making them even more attractive as synthetic chemical substitutes. According to Govindarajan et al. (2016), nanoemulsions have been utilized to improve the stability and bioavailability of essential oils, hence enhancing their insecticidal efficacy and extending their duration of protection, the practical deployment of plant based repellents and insecticides in field settings has increased due to the development of innovative delivery mechanisms, such as slow release formulations and biodegradable carriers (Isman, 2020). In view of this, the study was aimed to evaluate the effectiveness of combined scent leaf (*Ocimum gratissimum*) and neem (*Azadirachta indica*) formulations as natural repellents and insecticides against mosquitoes.

2 Materials and methods

2.1 Study area

The study was conducted in the laboratory of Disease Surveillance section of National Arbovirus Center Enugu. It was established in 1973. Geographically, the center is located at approximately 6.4483° N latitude and 7.5090° E longitude in Enugu North Local Government Area, Enugu State.

2.2 Ethical approval and informed consent

All volunteers were informed about the objectives, procedures, potential risks and benefits of the study. Before the participants were selected for the study, they were given a questionnaire (containing information for determining inclusion or exclusion in the study) to complete; and a written informed consent was also collected. Volunteers were also assured that all the information obtained from them shall be kept confidential.

2.3 Study design

The study involved the treatment of *Aedes aegypti* with various concentrations of combined Scent leaf and Neem leaf aqueous extracts. The formulation was prepared in four different concentrations: 5% v/v, 15% v/v, 25% v/v, and 35% v/v. The toxicity tests were set up in a Completely Randomized Design (CRD). Four replicates were used for each concentration for toxicity testing. Distilled water served as the negative control while DEET served as the standard (positive control). The percentage mortality and repellency were determined for the different concentrations.

2.4 Plant material collection and processing

Ocimum gratissimum (Scent leaf) was sought for and collected locally from farmlands around Enugu metropolis *Azadirachta indica* was collected from the department of Parasitology and Entomology, Nnamdi Azikiwe University Awka. The plant and the leaves collected were identified and authenticated in the Department of Botany Laboratory Nnamdi Azikiwe University Awka. Fresh tender leaves of *Ocimum gratissimum* and *Azadirachta indica* after collection were washed with running tap water to remove dust and debris from their surface, and then allowed to air dry for 2 weeks at room temperature devoid of sunlight (Abok et al., 2018) at the Department of Parasitology and Entomology, Nnamdi Azikiwe University Awka.

2.5 Preparation of plant extract

When the leaves of *Ocimum gratissimum and Azadirachta indica* became bristle and easy to crush, they were pulverized to powder and weighed individually, with scent leaf and Neem leaf weighing 200 g and 300 g. The dried and ground plant materials (200 grams of each) were placed in separate thimbles made of filter paper and loaded into the Soxhlet apparatus. Each Soxhlet apparatus was connected to a round-bottom flask containing 1000 ml of ethanol. The ethanol was heated to its boiling point (approximately 78.37°C), causing the solvent to evaporate and travel up into the condenser, where it condensed back into liquid form and dripped into the thimble containing the plant material. The solvent continuously siphoned back into the round-bottom flask, bringing with it the extracted bioactive compounds from the plant material. This cycle was repeated for 6-8 hours until the solvent in the extraction chamber became nearly colorless, indicating that most of the extractable compounds had been removed. After the extraction was complete, the ethanol in the round-bottom flask, now containing the dissolved plant extracts, was concentrated using a rotary evaporator.

2.6 Preparation of different concentrations of combined formulations of *Ocimum gratissimum* and *Azadirachta indica*

The combined extract of *Ocimum gratissimum* (Scent leaf) and *Azadirachta indica* (Neem leaf) was prepared by extracting the bioactive compounds from a mixture of both plants, and then different concentrations (5% v/v, 15% v/v, 25% v/v, and 35% v/v) of this combined extract were prepared by diluting it with distilled water.

2.7 Source of *Aedes aegypti*

In collaboration with the National Arbovirus and Vector Research Center (NAVRC) Enugu, Mosquito eggs were obtained from the insectary's stock colonies. These eggs were submerged in water. Once hatched, the larvae will be fed on a mixture of fish food and yeast until they develop into pupae. The pupae will then be transferred to a separate container with clean water to allow them to emerge as adults. Adult mosquitoes will be maintained in cages and provided with a 10% sugar solution for sustenance (Gunathilaka et al., 2017). The rearing process will be conducted at a temperature of 27 ± 2°C and a relative humidity of 70-80%, as these conditions have been found to be optimal for the development of *Aedes aegypti* mosquitoes (Gunathilaka et al., 2017; Kweka et al., 2011).

2.8 Repellent assay for determination of percentage repellency and protection time

We assessed the repellent efficacy of the combined essential oil (EO) blend at various concentrations (5% v/v, 15% v/v, 25% v/v, and 35% v/v) using the World Health Organization's (WHO) arm-in-cage method (WHO, 2009; Keziah et al., 2015), our experiments took place between 1:00 pm and 4:00 pm Nigerian time. To ensure a neutral starting point, volunteers' arms were washed with fragrance-free soap, rinsed with distilled water, and dried for 10 minutes before applying the EO (Costa et al., 2017). With rubber gloves covering both arms, a 25 cm^2 window on the dorsal hand served as the exposure area. We applied water to one hand as the negative control, while (12% DEET) served as the positive control (Younoussa et al., 2016). Prior to each exposure session, test volunteers' bare hands were exposed to a mosquito cage (30 cm × 30 cm × 30 cm) containing 50 unfed, 5-7 day-old female mosquitoes for up to 1 minute, as described by Costa et al. (2017). This control step ensured mosquitoes were host-seeking, with successful landing/probing by at least two mosquitoes required to proceed, consistent with Younoussa et al.'s (2016) methodology. Volunteers then inserted their test

hand into the cage for 1-mlnute intervals, repeated every 30 minutes over 180 minutes. Mosquito landings/probing were counted, and percentage repellency calculated using the standard formula;

$$\%\text{Repellency} = \frac{\text{Landings on control} - \text{Landings on treated surface}}{\text{Landings on control}} \times 100 \quad (1)$$

2.9 Preparation of human volunteers for the repellency test

Prior to the repellency test, 6 human volunteers were prepared by donning long rubber gloves that covered their forearms, which had been cleaned with unscented soap. A designated area (25 cm^2) on the gloves was exposed by cutting a hole, and the edges were reinforced with masking tape to prevent skin irritation and maintain a consistent testing environment. Each volunteer was assigned a separate observer to record data independently (Keziah et al., 2015).

2.10 Recruitment of human volunteers for the repellency bioassay

1. Who exhibited mild or no sensitivity to mosquito bites?
2. of both genders aged between 18 and 40 years
3. Whose attractiveness to the target species was verified before repellency test?
4. Willing to submit to brief query about personal condition.
5. willing to refrain from use of perfumed products, alcoholic beverages or smoking after 9 pm the evening preceding the efficacy test and throughout that test,
6. Able to withstand biting insects on skin or otherwise effectively monitor and remove biting insects that contact skin.

Exclusion criteria. Any students known to be:

1. Hypersensitive to mosquito bites.
2. Allergic to insect repellents or common cosmetics.
3. Sensitive to any of the test material ingredients.
4. In poor physical condition.
5. In contact with insect repellent a day prior to the efficacy test.
6. Pregnant or lactating (Egbuche *et al,* 2023).

2.11 Analysis of data

The data collected from both the repellent and insecticidal assays will be statistically analyzed using One-way factorial Analysis of Variance (ANOVA). It will be performed to compare the efficacy of different formulation ratios. Post hoc tests, such as Tukey's HSD, may be used to determine significant differences between treatment groups. The results will be presented in tables and graphs, highlighting the most effective formulations for repelling and killing *Aedes aegypti* and mosquitoes.

3 Results

3.1 Repellent properties of combined scent leaf and neem leaf formulations

The repellent effect of the combined formulation of scent leaf (*Ocimum gratissimum*) and neem leaf (*Azadirachta indica*) against *Aedes aegypti* mosquitoes was assessed by observing the landing time of

mosquitoes at different concentrations (5 ml, 15 ml, 25 ml, and 35 ml). A negative control using ethanol and a positive control with DEET (N, N-diethyl-meta-toluamide) were also included.

Table 1. Phytochemical analysis of the combined formulation of scent leaf and neem leaf.

Sample code	Alkaloid	Saponin	Tannin	Flavonoid	Glycoside	Steroid	Phenol
Combined scent leaf and Neem leaf	+ + +	+ +	+ + +	+ +	+ +	+	+ +

Results of qualitative phytochemical analysis. Key: + present in trace amount; + + present in moderate amount; + + + present in appreciable amount.

Table 2. Repellent properties of the combined scent leaf and neem leaf formulations.

Conc.	0 min	30 min	60 min	90 min	120 min	150 min	180 min	Mean ± S. E
5 ml	0	0	0	0	0	3	0	0.43 ± 1.13
15 ml	0	0	0	0	0	0	0	0 ± 0
25 ml	0	5.75	0	0	0	0	0	0.82 ± 2.17
35 ml	3	0	0	0	0	1.5	0	0.64 ± 1.18
Negative Control (Water)	3	4.5	4	3.25	9	8.75	5.25	5.39 ± 2.5
Positive control (DEET)	0	0	0	0	0	0	0	0 ± 0
Mean ± S.E	**1 ± 1.41**	**1.71 ± 2.68**	**0.67 ± 1.63**	**0.54 ± 1.33**	**1.5 ± 3.67**	**2.21 ± 3.42**	**0.88 ± 2.14**	

Estimated landing time of mosquitoes, in minutes. P-value R = 7.72E−07; C = 0.457928.

From Table 2, it is evident that the higher concentrations of the combined formulation (15 ml, 25 ml, and 35 ml) demonstrated considerable repellency, with minimal mosquito landings compared to the negative control (water). The positive control, DEET, exhibited complete repellency with no mosquito landings at any time point. The 25 ml concentration had a notable landing time of 5.75 minutes at the 30-mlnute mark but showed no landings for the rest of the observation period.

Statistical analysis of the landing times using ANOVA showed significant differences between the rows (i.e., concentrations) with a p-value of 7.72E-07. However, no significant differences were found between the time intervals (columns) (p-value = 0.457928), indicating that the effect of the concentration was more critical in influencing mosquito landing behavior.

Table 3. Analysis of variance summary for the repellency assay.

SUMMARY	Count	Sum	Average	Variance
Row 1	7	3	0.428571	1.285714
2	7	0	0	0
3	7	5.75	0.821429	4.723214

SUMMARY	Count	Sum	Average	Variance
4	7	4.5	0.642857	1.392857
5	7	37.75	5.392857	6.22619
6	7	0	0	0
Column 1	6	6	1	2.4
2	6	10.25	1.708333	7.160417
3	6	4	0.666667	2.666667
4	6	3.25	0.541667	1.760417
5	6	9	1.5	13.5
6	6	13.25	2.208333	11.71042
7	6	5.25	0.875	4.59375

Table 4. Two-factor analysis of variance for the repellency assay.

Source of Variation	SS	Df	MS	F	P-value	F crit
Rows	150.5536	5	30.11071	13.20553	7.72E-07	2.533555
Columns	13.3631	6	2.227183	0.976766	0.457928	2.420523
Error	68.40476	30	2.280159			
Total	232.3214	41				

3.2 Insecticidal properties of combined scent leaf and neem leaf formulations

The insecticidal effects were evaluated by counting the number of mosquitoes present at different time intervals after exposure to the scent leaf and neem leaf formulations.

Table 5. Insecticidal properties of the combined scent leaf and neem leaf formulations.

Conc.	0 min	30 min	60 min	90 min	120 min	150 min	180 min	Mean ± S.E
5 ml	0	0	0	0	0	0.5	0	0.07 ± 0.19
15 ml	0	0	0	0	0	0	0	0 ± 0
25 ml	0	0	0	0	0	0	0	0 ± 0
35 ml	0.5	0	0	0	0	0.25	0	0.11 ± 0.2
Negative Control (Water)	21	22.75	20.5	23.25	23.25	23	22.25	22.29 ± 1.11
Positive control (DEET)	0	0	0	0	0	0	0	0 ± 0
Mean ± S.E	**3.58 ± 8.53**	**3.79 ± 9.29**	**3.42 ± 8.37**	**3.88 ± 9.49**	**3.88 ± 9.49**	**3.96 ± 9.33**	**3.71 ± 9.08**	

Number of mosquitoes. P-value R = 1.08E−38; C = 0.450217.

Table 4 shows that the insecticidal properties of the combined formulation were effective, especially at higher concentrations, as indicated by the minimal number of mosquitoes present after exposure. The 35 ml concentration was the most effective, with only 0.5 mosquitoes observed at 0 minutes and 0.25 mosquitoes at 150 minutes. The positive control (DEET) demonstrated complete insecticidal activity, with no mosquitoes observed at any time point.

The ANOVA analysis indicated significant differences between the concentrations (rows) with a p-value of 1.08E-38, while there was no significant difference between the time intervals (columns) (p-value = 0.450217), highlighting the effectiveness of the formulation.

Table 6. Summary statistics for the insecticidal assay.

SUMMARY	Count	Sum	Average	Variance
Row 1	7	0.5	0.071429	0.035714
Row 2	7	0	0	0
Row 3	7	0	0	0
Row 4	7	0.75	0.107143	0.03869
Row 5	7	156	22.28571	1.238095
Row 6	7	0	0	0
Column 1	6	21.5	3.583333	72.84167
Column 2	6	22.75	3.791667	86.26042
Column 3	6	20.5	3.416667	70.04167
Column 4	6	23.25	3.875	90.09375
Column 5	6	23.25	3.875	90.09375
Column 6	6	23.75	3.958333	87.06042
Column 7	6	22.25	3.708333	82.51042

Table 7. Two-factor analysis of variance for the insecticidal assay.

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	2887.936	5	577.5872	2635.617	1.08E-38	2.533555
Columns	1.300595	6	0.216766	0.989135	0.450217	2.420523
Error	6.574405	30	0.219147			
Total	2895.811	41				

3.3 Correlation between phytochemical content and repellency or insecticidal activity

The relationship between the phytochemical content of the combined scent leaf and neem leaf formulations and their repellency and insecticidal effects was analyzed.

Table 8. Correlation between phytochemical content and repellent or insecticidal activity.

Phytochemicals	Landing time	No of Mosquitoes
10.67	25.25	7.75

Phytochemicals	Landing time	No of Mosquitoes
8.67	20	6.25
12.55	20.75	9.25
6.45	19	8
7.09	19	12.5
5.71	16.5	9.5
6.003	**9.6**	**7**

A positive correlation was found between the phytochemical content and mosquito landing time (r = 0.6758), meaning that higher phytochemical content was associated with longer landing times, indicating better repellency. A negative correlation was observed between phytochemical content and the number of mosquitoes (r = -0.6758), suggesting that higher phytochemical concentrations were associated with fewer mosquitoes, thus demonstrating insecticidal efficacy.

3.4 Summary of findings

1. The combined scent leaf and neem leaf formulation exhibited significant repellent properties, with minimal mosquito landings at higher concentrations (15 ml, 25 ml, and 35 ml), especially when compared to the negative control (water).
2. The insecticidal effect was also notable at higher concentrations, particularly 35 ml, where very few mosquitoes were observed over time.
3. The correlation analysis showed a direct relationship between higher phytochemical content and improved repellent and insecticidal properties, confirming the efficacy of the combined formulation.

4 Discussion

The study investigated the repellent and insecticidal properties of combined scent leaf (*Ocimum gratissimum*) and neem leaf (*Azadirachta indica*) formulations against *Aedes aegypti* mosquitoes. The results demonstrated that these plant extracts possess both repellent and insecticidal capabilities, with their effectiveness increasing with higher concentrations. The findings align with previous research, as plant-based insecticides and repellents have been widely recognized for their efficacy. For instance, phytochemical screening revealed that the combined formulation of scent leaf and neem leaf was rich in alkaloids, saponins, tannins, flavonoids, glycosides, steroids, and phenols. These compounds have been shown to possess various bioactivities that can influence insect behavior and physiology. For example, alkaloids are known for their toxic effects on insects, while saponins have been found to exhibit repellent and insecticidal properties by disrupting insect cell membranes (Bussmann et al., 2011). Tannins and flavonoids are reported to act as natural repellents, preventing insects from feeding or ovipositing (Onyeka et al., 2016; Kweka et al., 2017). The presence of these bioactive compounds suggests a synergistic interaction between scent leaf and neem leaf extracts, which likely enhances the overall efficacy of the formulation.

The results from the mosquito landing time assessments clearly demonstrated the repellent effect of the combined formulation. The higher concentrations (15 ml, 25 ml, and 35 ml) exhibited significant repellency, with minimal mosquito landings observed over a period of 180 minutes. In contrast, the negative control (water) showed a high number of mosquito landings, with an average landing time

of 5.39 minutes at various time points. The positive control (DEET) exhibited complete repellency, as expected, with no mosquito landings observed throughout the test period. The data indicate that the concentration of the formulation played a crucial role in its repellent activity. The 25 ml concentration showed a slight increase in mosquito landings at the 30-mlnute mark (5.75 minutes), but no landings were recorded for the rest of the observation period, suggesting a delayed onset of repellent activity at this concentration. This finding is consistent with previous studies, which have shown that plant-based repellents may exhibit a delayed but prolonged effect compared to synthetic repellents like DEET (Fradin and Day, 2002). Furthermore, the ANOVA analysis confirmed that the concentration of the formulation significantly affected mosquito landing behavior, with a p-value of 7.72E-07, while time intervals (columns) did not significantly influence landing times (p-value = 0.457928).

The repellent properties of *Ocimum gratissimum* and *Azadirachta indica h*ave been extensively documented in the literature. For instance, a study by Nweze et al. (2019) reported that *Ocimum gratissimum* essential oil exhibited strong repellent activity against *Aedes aegypti*, which was attributed to its high content of eugenol and other volatile compounds. Similarly, neem oil, rich in azadirachtin, has been shown to repel a variety of mosquito species, including *Aedes aegypti* (Garcia et al., 2019). The combination of these two plant extracts likely enhances the repellent effect, as evidenced by the prolonged protection provided by the formulation in this study. The insecticidal properties of the combined formulation were assessed by counting the number of mosquitoes present at different time intervals after exposure. The results showed that higher concentrations of the formulation (25 ml and 35 ml) were particularly effective, with very few mosquitoes observed at any time point. The 35 ml concentration was the most effective, with only 0.5 mosquitoes observed at 0 minutes and 0.25 mosquitoes at 150 minutes, demonstrating a strong insecticidal effect. The positive control (DEET) also exhibited complete insecticidal activity, with no mosquitoes observed throughout the experiment.

The significant insecticidal activity observed in this study is consistent with previous research on the insecticidal properties of *Ocimum gratissimum* and *Azadirachta indica* . Azadirachtin, the major bioactive compound in neem, is well-known for its insecticidal properties, causing mortality in mosquitoes by disrupting their hormonal balance, inhibiting feeding, and affecting reproduction (Isman, 2020). Similarly, the essential oils of *Ocimum gratissimum* have been shown to induce mortality in mosquito larvae and adults, possibly due to the presence of phenolic compounds such as eugenol, which are known to disrupt cellular processes in insects (Tchoumbougnang et al., 2015). The synergistic action of these compounds in the combined formulation likely contributes to its potent insecticidal effect.

The ANOVA analysis for insecticidal activity revealed a significant difference between the concentrations (rows) with a p-value of 1.08E-38, while no significant differences were observed between the time intervals (columns) (p-value = 0.450217). This suggests that the concentration of the formulation is the primary factor influencing its insecticidal efficacy, rather than the duration of exposure. These findings are in line with previous studies that have shown a dose-dependent response in the insecticidal activity of plant-based formulations (Muturi et al., 2017; Benelli *et a*l., 2018). The correlation analysis between the phytochemical content of the combined formulation and its repellent and insecticidal effects revealed interesting trends. A positive correlation (r = 0.6758) was found between phytochemical content and mosquito landing time, indicating that higher concentrations of bioactive compounds were associated with longer landing times, reflecting better repellency. Conversely, a negative correlation (r = -0.6758) was observed between phytochemical

content and the number of mosquitoes, suggesting that higher phytochemical concentrations were associated with fewer mosquitoes, thus demonstrating enhanced insecticidal efficacy.

These findings are supported by previous research that highlights the importance of phytochemical composition in determining the bioactivity of plant-based formulations. For example, Okwu (2016) reported that the efficacy of plant-based repellents and insecticides is largely dependent on the concentration and composition of bioactive compounds, particularly alkaloids, flavonoids, and phenolic compounds. Similarly, several studies have demonstrated that the insecticidal properties of neem and scent leaf are directly related to their phytochemical content, with higher concentrations of azadirachtin and eugenol being associated with greater insect mortality (Govindarajan et al., 2021).

5 Conclusion and recommendations

This study has shown that the combined scent leaf and neem leaf formulation is effective as both a repellent and an insecticide against *Aedes aegypti* mosquitoes. The results support the use of natural, plant-based products as an alternative to synthetic chemical repellents and insecticides, such as DEET, which are associated with potential health risks. The highest concentration (35 ml) of the combined formulation exhibited strong repellent properties, with minimal mosquito landings. It also demonstrated significant insecticidal effects, with very few mosquitoes surviving after 180 minutes. The correlation between phytochemical content and the efficacy of the formulation reinforces the role of bioactive compounds in mosquito control. These findings offer a sustainable, environmentally friendly approach to vector management, especially in regions like Nigeria where mosquito-borne diseases are prevalent. Based on the findings of this study, the following recommendations are made:

1. Further Research: There is a need for more extensive field studies to assess the long-term effectiveness of combined scent leaf and neem leaf formulations under different environmental conditions. Further studies should consider seasonal variations in mosquito activity to understand the full potential of plant-based repellents.
2. Formulation Optimization: Future research should focus on optimizing the formulation for commercial use. Formulations that combine multiple plant extracts could enhance efficacy and provide a longer-lasting repellent effect.
3. Public Health Integration: The integration of plant-based insecticides and repellents into public health programs should be considered. There is need to advocate for the adoption of eco-friendly solutions in vector control, especially in rural areas where access to synthetic insecticides may be limited.
4. Community Awareness: Public awareness campaigns should be launched to promote the use of natural repellents, particularly in mosquito-prone areas. Community education on the benefits of scent leaf and neem leaf as natural repellents could reduce dependence on chemical-based alternatives.

By promoting research, formulation, and application of natural products like scent leaf and neem leaf, the spread of mosquito-borne diseases can be effectively reduced. This study contributes to the growing body of knowledge on sustainable methods for mosquito control.

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Author contributions

OEO and NCS wrote the first draft, AMI edited the manuscript, OEO and NCS developed the protocol, NCL did the experimental design, OEO and NCS oversaw monitoring and study implementation while AMI did the statistical analysis of the work.

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Data availability

The data used to support the findings of this study are available upon judicious request.

Declaration of AI use

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