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Bio-efficacy of *Ocimum gratissimum, Azadirachta indica*, and *Mesosphaerum suaveolens* Essential Oils Against Eggs and Larvae of Aedes Mosquito

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ABSTRACT

Mosquito-borne diseases continue to be a major public health concern in Nigeria. The rising challenge of insecticide resistance necessitates the need for sustainable and eco-friendly plant-based alternatives. This study evaluated the bioefficacy of essential oils and extracts of Ocimum gratissimum, Azadirachta indica, and Mesosphaerum suaveolens against eggs and larvae of Aedes mosquitoes. Specifically, it sought to assess the larvicidal activity of different concentrations of each essential oil/extract against Aedes aegypti 4th instar larvae, to determine the ovicidal efficacy of the essential oils/extracts on Aedes eggs, and to compare the relative bioefficacy of the three essential oils in inhibiting egg hatch and larval survival. The study employed a randomized survey design, conducted in the Nnamdi Azikiwe University environment, Awka South Local Government Area, Nigeria, between June and August 2025. Fresh plant materials were collected in Awka, Anambra State. Essential oil was extracted through steam distillation for O. gratissimum, while the crude ethanolic maceration method was used for A. indica and M. suaveolens extraction. Bioassays were conducted using oil concentrations 5-25% against batches of 20 4th instar larvae and 30 eggs per replicate. At regular intervals, the mortality counts of eggs and larvae were monitored. Larvicidal LC_{50}, LC_{90}, LT_{50}, and LT_{90} values were determined through probit analysis. Data collected were analyzed using One-way ANOVA at a significance level of $P< 0.05$. Results from the study showed that A. indica had the highest larvicidal potency (LC_{50}=7.65%, LC_{90}=21.52%; LT_{50}=7.48 h, LT_{90}=76.9h), followed by O. gratissimum (LC_{50} =8.40%, LC_{90}=28.20%; LT_{50}=17.5h, LT_{90}=30.2h), M. suaveolens (LC_{50} =8.50%, LC_{90}=24.50%; LT_{50}=7.13h, LT_{90}=80.3h). For ovicidal activity, O. gratissimum exhibited the greatest effect, reducing egg hatchability to 11% at 5% concentration, while both A. indica and M. suaveolens achieved 25% and 24%, respectively; all extracts completely inhibited hatching at 25%. The study highlights these oils/extracts as biodegradable, target-specific alternatives to synthetic insecticides. This research provides localized evidence for vector control, supporting public health strategies in endemic regions. Further studies are recommended to optimize application methods.

Keywords: *Bioefficacy, Essential Oils, Ocimum gratissimum, Azadirachta indica, Mesosphaerum suaveolens, larvicidal activity.*

Introduction

Mosquitoes are some of the most intensely studied creatures in the planet, and their role in disease transmission and biting nuisance makes them worthy of attention [1]. There are over 3,500 species of mosquitoes on earth, being found everywhere except Antarctica. Yet, from this great diversity only a handful can carry the pathogens that cause diseases, and it is these species that have been studied thoroughly. For the purpose of public health, this substantial body of research has helped us to understand mosquito-borne diseases transmission and informed the development of mosquito and disease control methods [1].

Mosquito-borne diseases have high prevalence in the tropics, and in 2020 alone, malaria accrued over 241 million cases globally with 95% of malaria cases and 96% of malaria deaths occurring in Sub-saharan Africa with Nigeria alone accounting for one –third (31.9%) of mortalities due to malaria [2]. Tropical areas, including Nigeria, however, have the best combination of adequate rainfall, temperature and humidity allowing for the breeding and survival of mosquitoes [3]. Among the major vectors, *Aedes* mosquitoes particularly *Aedes aegypti* and *Aedes albopictus* are notorious for transmitting Dengue fever, Zika virus, Chikungunya and Yellow fever [4].

Aedes aegypti also known as the Yellow fever mosquito is found in urban areas, is active both indoors and outdoors has a preference for human blood as its source of blood meals. *Aedes albopictus* commonly referred to as the Asian tiger mosquito is mostly associated with areas of vegetation and found primarily outdoors will bite both domestic and wild animals, as well as humans. *Aedes aegypti* more likely spread viral diseases than the *A. albopictus* because it lives near and prefers to bite people. An extensive study by [5] in Nigeria, reported that *A. aegypti* and *A. albopictus* has significantly increased in abundance in South-east Nigeria [6]. Another study by [3] in Awka campus of Nnamdi Azikiwe University, showed *Aedes* species as the most abundant biting species. These vectors have adapted to urban environment, breeding predominantly in artificial water containers and thriving in densely populated areas. Nigeria, including the South-eastern region where Awka is located continues to experience the burden of arboviral diseases, necessitating sustainable vector control strategies [7].

Traditional vector control programs have relied heavily on synthetic insecticides such as organophosphates and pyrethroids. While initially effective, over-reliance on these chemicals have led to insecticide resistance, environmental contamination, non-target species harm, and adverse human health effects [8]. Advances in biotechnology, geographic information systems, and remote sensing technologies, as well as environmentally friendly approaches, provide new opportunities for improving vector control programs. Among alternative strategies, the use of plant extract-based pesticides stands out as one of the most promising. These compounds, derived from plant extracts, contain different allele-chemicals,that play fundamental roles in interactions between plant and insects. Thus, these substances present potential as a sustainable alternative for the control of vectors, replacing synthetic insecticides. Among these substances, essential oils form a significant group [9, 10] Notably, it is suggested that essential oils from various plant species may be considered potential larvicides against strains of mosquitoes resistant to current pesticides, offering a clean and safe alternative for control of these vectors. This approach not only contributes to reducing the use of harmful chemicals but also promotes more sustainable practices in public health management [11]. Larvicides obtained from essential oils are biodegradable, sustainable, and more effective, proactive, safe, target-specific, and non-harmful to the environment, making them a viable alternative to synthetic insecticides [12, 13].

Plants of *Lamiaceae* family such as *OOcimum basilicum, Ocimum gratissimum, and Mesosphaerum suaveolens,* are prevalent in regions across Africa, Asia, and South America and are known for their string aromatic scents. The mint family (*Lamiaceae*) of flowering plants, with 236 genera and more than 7000 species, is the largest family of the order *Lamiales* and have worldwide economic importance. Many constituents isolated from species within the *Lamiaceae* family have antioxidant, antibacterial, cytotoxic, anti-inflammatory, repellent, and insecticidal properties [14]. Studies have indicated that the essential oils extracted from the selected *Ocimum* species are found to be efficient in killing the larvae of different mosquitoes. The essential oils (EO's) from *O. gratissimum* and *O. campechianum* were shown to reduce the survival of the larval forms of *A. aeygpti* and *A. albopictus*[15, 16]. It has also been reported that the essential oils of *O. gratissimum* and *O. basilicum* exert antimicrobial properties against different bacteria, virus and fungal strains [17].

Neem-based products are highly potent yet unexplored candidates for mosquito control agents. Neem (*Azadirachta indica* A. Juss) belongs to the mahogany family *Meliaceae* and its known as a fast-growing, long live tree with unpleasant-smelling wood. It has evergreen pinnate leaves and small fragrant yellow-white flowers, followed by green-yellow berries. This plant is known as a plant that could be utilized responsibly for the pesticidal, larvicidal, antifeedant or repellent action on various insects, neem oil-based insecticides have been found effective against a wide range of insects of medical and veterinary importance, including mosquitoes, sparing the economically important ones like bees [18]. It has been observed that emulsified formulations of neem oil exhibit strong larvicidal activity under field settings against the larval stages of many mosquito species, including *Aedes*, *Anopheles* and *Culex* species. Macchioni*et al*., 2020 experimentally demonstrated the larvicidal and pupicidal roles of neem oil (0.3% azadirachtin A) against *A. albopictus*[19]. Neem derivatives are effective against all the life stages of mosquito and therefore provide immense scope for multi-strategic mosquito control [20].Given the rising burden of *Aedes* –borne diseases and the growing resistance of synthetic insecticides, there's an urgent need for sustainable, affordable and environmentally friendly alternatives, particularly in endemic regions like Nigeria. Essential oils derived from indigenous plants such as *Ocimum gratissimum*, *Mesosphaerum suaveolens* and *Azadirachta indica* present a promising avenue for mosquito control due to their natural insecticidal properties and local availability. Awka, the capital of Anambra State in South-eastern Nigeria, experiences tropical climatic conditions and urbanization patterns that create ideal breeding grounds for *Aedes* mosquitoes. Yet, there is limited empirical data on the bioefficacy of plant-based larvicides and ovicides in this context. Conducting this study in Awka not only provides insight into the potential of these essential oils as vector control agents but also contributes to localized evidence that can inform public health strategies within the region.

However, the aim of the study is to evaluate the bioefficacy of *Ocimum gratissimum*, *Azadirachta indica* and *Mesosphaerum suaveolens* essential oils against eggs and larvae of *Aedes* mosquitoes. Specifically, the objectives of the study were to assess the larvicidal activity of different concentrations of each essential oil against *Aedes* larvae under laboratory conditions, assess the ovicidal efficacy of the essential oils on *Aedes* eggs, and lastly compare the relative bioefficacy of the three plant based essential oils/extracts namely: *O. gratissimum*, *A. indica* and *M. suaveolens*.

Materials and Materials

Study Area

Anambra state lies within the humid tropical rainforest zone of Nigeria with a total landmass of 4,844km^2, is located on latitude 6° 16`32.7576 North of equator and Longitude 7°0`24.6204 east of Greenwich, with a daily temperature of about 26°8°C/80.2°F. The official language of the people of Anambra state is Igbo, although English is widely spoken throughout the state as a secondary language. The 2020 projected population of Anambra state is 11,400,000. Anambra state has climatic conditions, the wet season and dry season, with a short spell of harmattan between November and January which is a period of cold weather when the atmosphere is generally mostly [21].

Awka south local Government Area (LGA) is made up of nine communities namely:Awka, Amawbia, Ezinato, Isiagu, Mbaukwu, Nibo, Nise, Okpuno and Umuawulu [22]. Awka south LGA has one ethnic group and the LGA is located on latitude 6°10' North of equator and longitude 7°04' East of Greenwich. Awka North is also a LGA in Anambra state with coordinates of 6°15' North of equator and 7°10' East of Greenwich. Ten communities that make up the local government include; AwbaOfemili, Amansea, Ugbene, Ebenebe, Achalla, Urum, Amanuke, IsuAniocha, Mgbakwu and Ugbenu with its local government headquarters at Achalla. Njikoka is a L.G.A in Anambra state, south - central Nigeria lying between latitude 06°20'58 "N to 06° 21'00"N and longitude 06°52'55" is made up of six towns, which include; Abgana, Enugu-Ukwu, Enugwu-Agidi, Nawfia, Nimo and Abba. The area is characterized by a temperature range of 27°-30°C (and has a double maximal rainfall peak in July and September [23].

Study Location

The study was carried out in Nnamdi Azikiwe University Awka, Anambra State, which is located along Onitsha-Enugu expressway, Awka and has a population of 37,182. It has a relative humidity of 70% reaching 80% during the rainy season and an annual rainfall of about 2000mm [3]. The daily temperature ranges from 26°C- 35°C during the dry season and from 22.1°C- 30°C. The Institution is a co-educational and higher educational institution which offers courses and programmes leading to officially recognized higher educational degrees such as undergraduate certification /diploma, bachelor degrees, master's degree and doctorate degrees in several areas of study. It has a total of 14 faculties and 87 Departments [3].

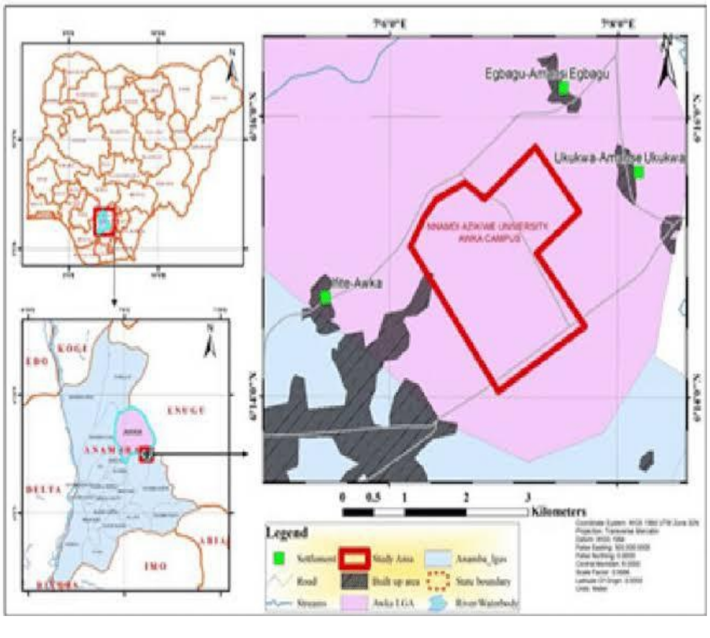


Figure 4: Map of Study area showing Nnamdi Azikiwe University Awka- Nigeria. (Ogbodo, 2020). Source: www.researchgate.com

Study Design

The research followed a completely randomized design (CRD) where five graded concentrations (25%, 20%, 15%, 10%, 5%) of the essential oils/extracts from *Ocimum gratissimum*, *Azadirachta indica,* and *Mesosphaerum suaveolens* and were tested in three replicates to determine ovicidal and larvicidal activity mortality rates after 24-hour exposure period.

Study Population

The study utilized *Aedes* mosquito larvae and eggs purchased from the Department of Parasitology and Entomology, Nnamdi Azikiwe University, and reared to fouth-instar larvae stage in the lab.

Sample Size Determination

Following World Health Organization's (WHO) guidelines, each larvicidal assault treatment (concentration and control) consisted of 20 fouth-instar *Aedes* larvae per replicate, with four replicates. Similarly, each ovicidal assay treatment used 30 *Aedes* eggs per replicate also with three replicates.

Mosquito Collection (Laboratory Rearing and Identification)

Aedes eggs were purchased from the Parasitology and Entomology Laboratory, Nnamdi Azikiwe University. The eggs were placed in a clean bowl containing 500 ml of distilled water supplemented with yeast powder as a food source and maintained at 25-28˚C under a 12:12 light dark-cycle. Hatching was induced by adding a small amount of larval food, and the resulting larvae were reared to the fourth instar stage.

Collection and Processing of Plant Materials

Ocimum gratissimum

Fresh leaves of *Ocimum gratissimum* were purchased from Ifite-Awka in the morning around 8:00am. The leaves were rinsed thoroughly with clean water to remove dust and debris and immediately used for oil extraction.

Mesosphaerum suaveolens

Fresh leaves of *Mesosphaerum suaveolens* were collected along bush paths in Science Village, Nnamdi Azikiwe University, Awka, Anambra State. It was identified and authenticated by a plant taxonomist in the Department of Botany, Nnamdi Azikiwe University with a voucher number NAUH235[A]. The collected leaves were rinsed with clean water, air- dried for a week and used for ethanolic extraction.

Azadirachta indica

Fresh leaves of neem were obtained from the environs of the Parasitology and Entomology Laboratory in Science village, Nnamdi Azikiwe University, Awka. The leaves were air-dried for a week in shade and used for ethanolic extraction.

Extraction of Essential oil of *Ocimum gratissimum*

Hundred gram (100g) of clean fresh scent leaves were cut using a knife, weighed and put in a round bottom flask. Two hundred ml (200ml) of distilled of H_20 was added in the same round bottom flask and subjected to steam distillation using a Clevenger-type apparatus for 1 hour. The volatile oil obtained was collected and stored in air-tight amber-coloured bottles and stored until further analysis and larvicidal testing.

Extraction of Extract from *Mesosphaerum suaveolens*

The dried leaves were crushed into coarse form using a blender and plant extract was obtained through the cold-press maceration method. Hundred gram (100g) of powered leaves was soaked in five hundred mls (500mls) of 95% ethanol for 24 hours at room temperature in an air tight container with intermittent stirring. The resultant suspension was filtered using muslin cloth and concentrated in a water bath at 40˚C. The resulting extracts was stored and labelled until testing.

Extraction of Extract from Azadirachta indica

Ethanolic extracts of neem leaves was prepared through same cold press maceration method as performed by Dachar *et al*., 2016. Hundred gram (100g) of dried neem leaves were crushed into fine powder using a blender and soaked in two hundred mls (200mls) of ethanol for 24 hours in a dark cupboard at room temperature with intermittent stirring and shaking. The mixture was filtered using a Whatman's filter paper and concentrated over a water bath at 60 to 65˚C. The resulting stock solution was stored and labelled in airtight containers until testing to be used as treatment in experiment.

Bioassay Procedure

All bioassays were conducted under controlled insectary conditions. Separate assays were performed for *Aedes* eggs and larvae.

Preparation of Essential Oil/ Extracts Test Solution

Stock solutions of each essential oil and extracts were prepared using acetone as a solvent. Appropriate dilutions were then made with distilled water to achieve target concentration. The dilution formula was applied to calculate the exact volumes of extract and acetone required for each concentration. Care was taken to ensure that the final concentration of acetone in the solution was negligible (<0.5%).

Larvicidal Activity Assay

The larvicidal activity of essential oils/ extracts was evaluated following the World Health Organization standard procedure with slight modifications. Five graded concentrations; 25%, 20%, 15%, 10%, 5% of each oil/extract were prepared using acetone as solvent. Each concentration was tested in four (4) replicates, with a negative control group exposed to acetone only. For each concentration, 20 fourth instar larvae of *Aedes aegypti* were placed in 250 ml of H_2O in clean plastic containers. Larval mortality was recorded after each 3-hour interval for 24 hours of exposure. Dead larvae were counted and removed using pipette, larvae were considered dead if they did not respond to gentle stimulation and touching.

Ovicidal Activity Assay

Aedes eggs from each site on filter paper were exposed to five concentrations of each essential oil/extracts. Eggs were collected and placed in clean plastic containers with 250 ml of distilled water and treated with oil/extract solution for 24 hours. Three replicates were maintained per concentration, with control using acetone only. After 24-hours exposure, egg papers were rinsed, transferred to clean water with yeast, and incubated for 72 hours. The number of hatched eggs were then counted.

Analysis of Data

All data were analyzed correctly and the percentage egg hatch inhibition was calculated. Morality Correction: when control mortality exceeded 5%, observed larval mortality was corrected using Abbot's formula (Abbott, 1925).The median lethal concentration (LC_{50} and LC_{50}) was determined by probit analysis for each essential oil/extract against mosquitoes from each collection site. Difference in mortality/inhibition rates among concentrations, time and essential oils/extracts were assessed using One-way Analysis of variance (ANOVA) with P<0.05 considered statistically significant. For ovicidal data, percentage hatch inhibition was calculated and analyzed using ANOVA.

Results:

Mortality Response to *O. gratissimum* Leaf Oil Extract.

Table 1 shows a clear dose- and time- dependent response on the mortality of 4^{th} instar *Aedes aegypti* larvae exposed to residual applications of *O. gratissimum* leaf oil extract. Mortality increased with both higher concentrations and longer time exposures. At 12 hours, the highest concentration (25%) resulted in 9.33 dead larvae per replicate (93.3% mortality), while the lowest 5% yielded 3.67 dead larvae (36.7% mortality). Mean mortality across exposure times ranged from 2.33 ± 0.40 at 5% to 6.53 ± 1.06 at 25%. Across concentrations, mean mortality increased from 2.46 ± 1.46 at 0 hours to 6.46 ± 1.01 at 12 hours, corresponding to 24.6% to 66.0% mortality.

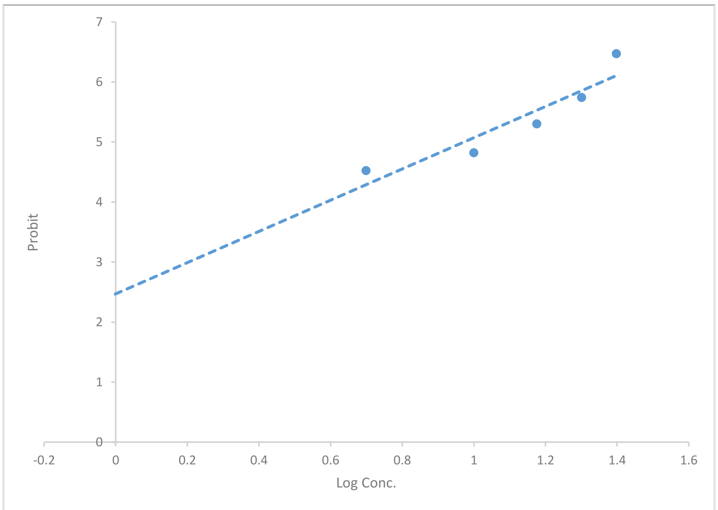
Table 1: Mortality response of Aedes aegypti mosquitoes exposed to residual application of Ocimum gratissimum leaf oil extract

	Exposure time							
Conc (%)	0hrs	3hrs	6hrs	9hrs	12hrs	Mean±s.e	% Mortality	Probit
25	3.33	5.33	6.33	8.33	9.33	6.53±1.06	93.3	6.45
20	3.00	4.00	5.33	7.33	8.00	5.53±0.95	80.0	5.72
15	2.67	3.33	3.67	5.67	6.33	4.33±0.70	63.3	5.29
10	2.00	2.33	3.33	4.00	5.00	3.33±0.54	50.0	4.79
5	1.33	1.67	2.33	2.67	3.67	2.33±0.40	36.7	4.51
Mean±s.e	2.46±1.46	3.33±0.64	4.19±0.71	5.60±1.03	6.46±1.01		-	
% Mortality	24.6	32.0	41.3	56.0	66.0		-	
Control	0.33	0.67	1.33	1.33	1.67	1.06±0.24	10.6	
Probit	3.92	4.32	4.53	5.02	5.21			

Mean of the three replicates (±s.e), Pvc =0.000; Pvt=0.013

Probit Analysis of Mortality against Log Concentration and Time.

Probit analysis of mortality against log concentration revealed a linear relationship described by the equation Y=2.5013x + 2.3702 (R^2=0.8575). The LC_{50} was calculated as 8.4% and LC_{90} was 28.2%. For exposure time, probit against log time gave Y=1.881x + 2.513(R^2=0.9374), with LT_{50} = 17.5 hours and LT_{90}= 30.2 hours. The effects of concentration and time were statistically significant (P=0.000 for concentration; P=0.013 for time).



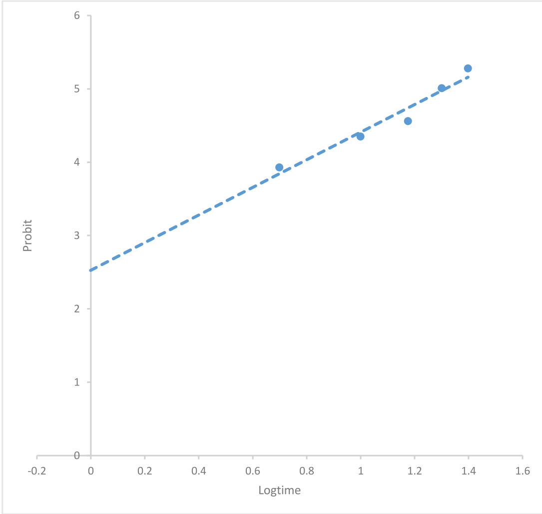


Fig 5: Probit against Log Concentration of Ocimum gratissimum leaf extract on Aedes aegypti
Y=2.5013x + 2.3702
R^2 = 0.8575
LC_{50}=8.4%
LC_{90}=28.2%

Fig 6: Probit against Log Time of Ocimum gratissimum leaf extract on Aedes aegypti
Y=1.881x + 2.513
R^2 = 0.9374
LT_{50}=17.5hrs
LT_{90}=30.2hrs

Mortality Response to *M. suaveolens* Leaf Extract

Table 2 showed similar trends for *Mesosphaerum suaveolens* leaf extract. Mortality rose with concentration and exposure time. At 12 hours, 25% concentration caused 9.00 dead larvae (90.0% mortality), compared to 3.67 deaths (40.00% mortality) at 5%. Mean mortality across times varied from 2.33 ± 0.40 at 5% to 6.33 ± 1.01 at 25%. Across concentrations, mean mortality progressed from 2.39 ± 0.35 at 0 hours (23.9%) to 6.33 ± 0.94 at 12 hours (63.3%).

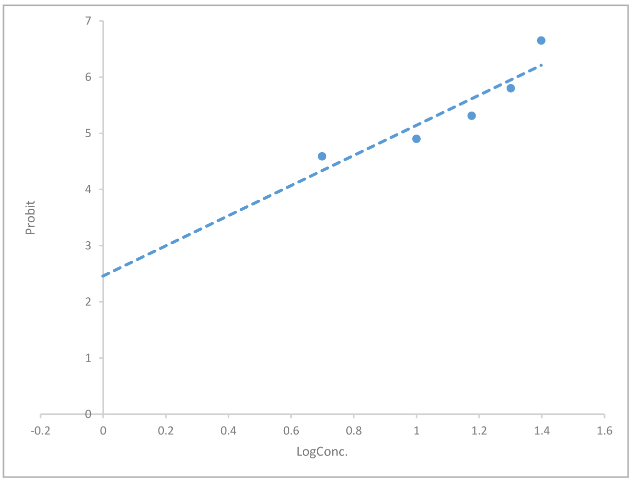
Table 2: Mortality response of Aedes aegypti mosquitoes exposed to residual application of H. suaveolens leaf extract.

	Exposure time							
Conc (%)	0hrs	3hrs	6hrs	9hrs	12hrs	Mean±s.e	% Mortality	Probit
25	3.33	5.00	6.33	8.00	9.00	6.33±1.01	90.0	6.60
20	3.00	3.33	5.33	6.00	7.67	5.06±0.86	76.7	5.78
15	2.33	3.00	3.33	5.33	6.33	4.06±0.75	63.3	5.30
10	2.00	2.33	3.00	4.00	5.00	3.26±0.55	50.0	4.89
5	1.33	1.67	2.33	2.67	3.67	2.33±0.40	40.0	4.59
Mean±s.e	2.39±0.35	3.06±0.56	4.06±0.75	5.20±0.90	6.33±0.94		-	
% Mortality	23.9	30.6	40.6	52.6	63.3		-	
Control	0.33	1.00	1.33	1.33	1.67	1.13±0.22	11.3	
Probit	3.90	4.27	4.56	4.98	5.31			

Mean of the three replicates (±s.e), Pvc =0.001; Pvt=0.005

Probit Analysis of Mortality against Log Concentration and Time

The probit- log concentration regression (Figure 7) was Y=2.5817x + 2.3606 (R^2= 0.825), yielding LC_{50}=8.5% and LC_{90}=24.5%. The probit-log time regression (Figure 8) was Y=1.2321x + 3.6846 (R^2= 0.9224) with LT_{50}=7.13 hours and LT_{90}=80.3 hours.Concentration and time effects were significant (P=0.001 for concentration; P=0.005 for time).



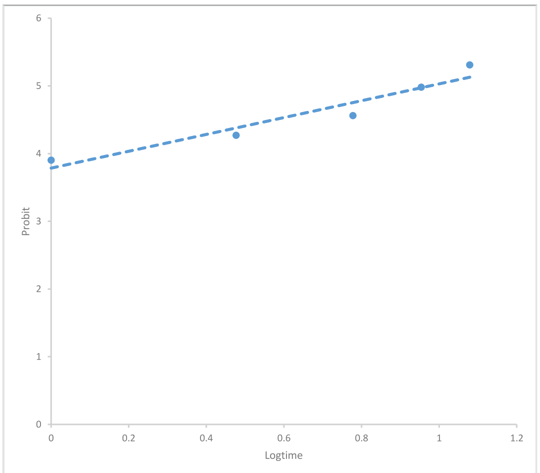


Fig 7: Probit against Log Concentration of H. suaveolens leaf extract on Aedes aegypti
Y=2.5817x + 2.3606
R^2=0.825
LC_{50}=8.5%
LC_{90}=24.5%

Fig 8: Probit against Log Time of H. suaveolens leaf extract on Aedes aegypti
Y=1.2321x + 3.6846
R^2=0.9224
LT_{50}=7.13hrs
LT_{90}=80.3hrs

3.3 Mortality Response to *A. Indica* Leaf Extract

Table 3 shows the mortality response of *A. indica* leaf extract on 4th instar *Aedes aeygpti* larvae. At 12 hours, 25% concentration led to 9.67 death (96.7% mortality), while 5% resulted 4.20 death (42.0% mortality). Mean mortality across times ranged from 2.44 ± 0.49 at 5% to 6.87 ± 1.06 at 25%. Across concentrations, it increased from 2.66 ± 0.40 at 0 hours (2.66%) to 7.37 ± 0.93 at 12 hours (73.7 %).

Table 3: Mortality response of Aedes aegypti mosquitoes exposed to residual application of Azadirachta indica leaf extract

				Exposure time				
Conc (%)	0hrs	3hrs	6hrs	9hrs	12hrs	Mean±s.e	% Mortality	Probit
25	3.67	5.67	6.67	8.67	9.67	6.87±1.06	96.7	6.77
20	3.33	4.33	5.67	7.67	8.67	5.93±0.99	86.7	6.02
15	2.67	3.67	4.33	5.67	7.67	4.80±0.86	76.7	5.62
10	2.33	2.67	3.67	4.33	6.67	3.93±0.77	66.7	5.30
5	1.33	1.67	2.33	2.67	4.20	2.44±0.49	42.0	4.58
Mean±s.e	2.66±0.40	3.60±0.68	4.53±0.75	5.80±1.08	7.37±0.93		-	
% Mortality	26.6	36.0	45.3	58.0	73.7		-	
Control	0.33	1.00	1.33	1.67	1.67	1.20±0.25	12.0	
Probit	4.02	4.38	4.68	5.05	5.52			

Mean of the three replicates (±s.e), Pvc=0.000; Pvt=0.005

Probit Analysis against Concentration and Time.

Probit analysis against log concentration (Figure 9) followed Y=2.8529x + 2.4777 (R^2=0.9326), with LC_{50}=7.65% and LC_{90}=21.52%. Against log time (Figure 10), it was Y=1,2662x + 3.8932 (R^2=0.9021), giving LT_{50}=7.48 hours and LT_{90}=76.9 hours. Both concentration and time had significant effects (P=0.000 for concentration; P=0.005 for time).

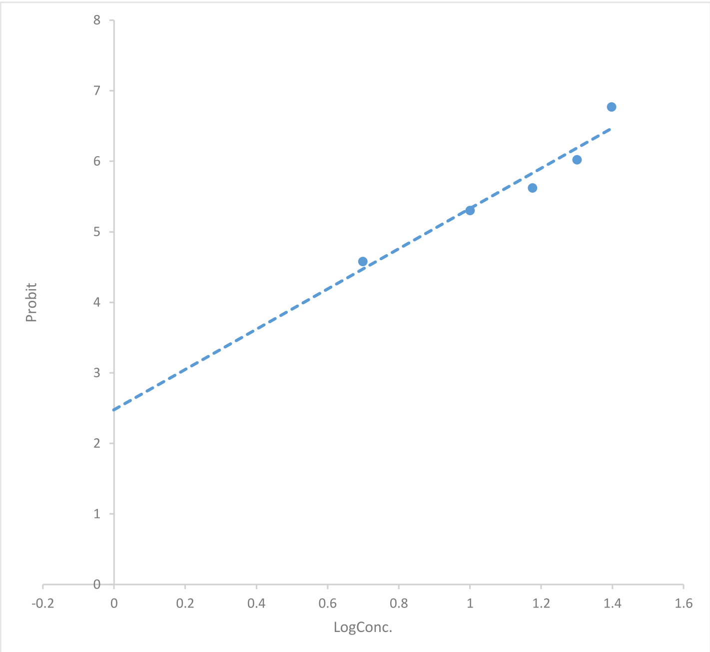


Fig 9: Probit against Log Concentration of A. indica leaf extract on Aedes aegypti
Y=2.8529x + 2.4777
R^2=0.9326
LC_{50}=7.65%
LC_{90}=21.52%

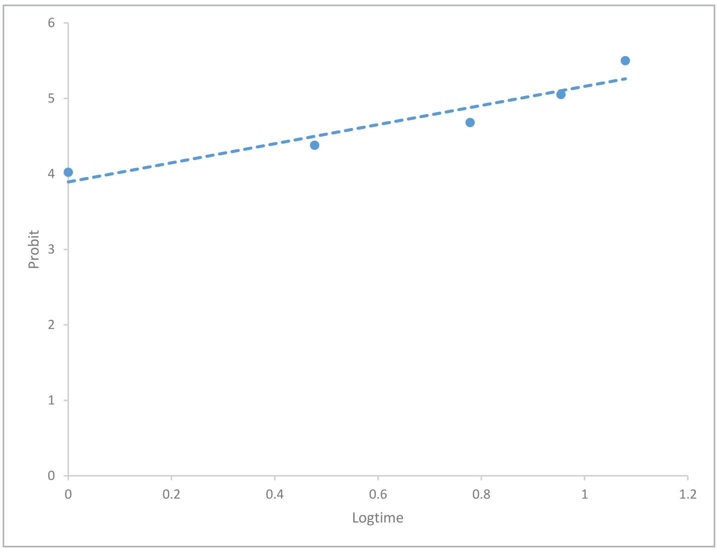


Fig 10: Probit against Log Time of A. indica leaf extract on Aedes aegypti
Y=1.2662x + 3.8932
R^2=0.9021
LT_{50}=7.48hrs
LT_{90}=76.9hrs

Ovicidal Activity

The ovicidal activity of the extracts, measured as the impact of egg hatchability, is summarized in Table 4. Hatch rates decreased with increasing concentrations for all extracts, indicating stronger ovicidal effects at higher doses. At 25%, all extracts completely inhibited hatching (0.00% hatch). At lower concentrations, hatch rates increased: at 5%, *O. gratissimum* allowed 11.00% hatch, *H. suaveolens* 24.00%, and *A. indica* 25.00% (mean 20.00 ± 4.50). Mean hatch rates across concentrations were 2.60 ± 2.13 for *O. gratissimum*, 11.60 ± 4.26 for *H. suaveolens*, and 10.00 ± 4.51 for *A. indica*.

Table 4: Ovicidal activity (Percentage Hatch) of leaf oil extracts from Ocimum gratissimum, Hyptis suaveolens, and Azadirachta indica against Aedes aegypti eggs.

Concentration (%)	*O. grattissimum*	*H. suavulenses*	*A. indica*	Mean (±s.e)
25	0.00	0.00	0.00	0.00±0.00
20	0.00	6.00	3.00	3.00±1.73
15	0.00	10.00	7.00	5.67±2.96
10	2.00	18.00	15.00	11.67±4.91
5	11.00	24.00	25.00	20.00±4.50
Mean (±s.e)	2.60±2.13	11.60±4.26	10.00±4.51	

Discussion

The results demonstrate that all three plant leaf extracts possess significant larvicidal properties against the 4th instar *Aedes aegypti* larvae, with mortality responses that are both concentration- and time-dependent. This aligns with the known bioactive compounds in these plants, such as eugenol in *O. gratissimum* [24], monoterpenes in *M. suaveolens*, and azadirachtin in *A. indica*, which disrupt insect nervous systems, respiration, or cuticular integrity.

Comparing the extracts, *A. indica* was the most potent against 4th instar larvae with the lowest LC_{50} (7.65%), and LC_{90} (21.52%), suggesting its superior efficacy at lower doses. The most important physiological effect of *A. indica* on insects is the growth regulatory effect. It has been proven that the crude or partially purified plant extracts are less expensive and highly effective for control of *Aedes* mosquitoes than the purified extracts [25]. This is consistent with the previous study on the comparative evaluation of larvicidal potential of three plant extracts of *Aedes aeypti* in 2017 where neem extract had the highest toxicity with a LC_{50} of 8.32 mg/ml while scent leafhad a considerably higher LC_{50} of 19.50 mg/ml, indicating lower effectiveness [26]. [27] further confirmed the potency of crude ethanolicneem leave extract on *Aedes aegypti* larvae in Nnamdi Azikiwe University, Awka, Anambra state. The concentration – dependent and time – dependent mortality observed in this study (Table 3) is consistent with the findings of [27], whose Table 1 demonstrated that larvicidal mortality of *Aedes* mosquitoes with both rising concentrations and exposure time when treated with *A. indica* extracts. This further validates the present LC_{50} and LC_{90} values, emphasizing that neem maintains potent larvicidal efficacy even at relatively low concentrations. *O. gratissimum* (LC_{50}= 8.4%, LC_{90} =28.5%) and *M. suaveolens* (LC_{50}=8.5%. LC_{90}=24.5%) were slightly less effective, though still promising. In agreement with these findings of [28] demonstrated a potent larvicidal activity of *O. gratissimum* essential oil on *A. albopictus*, achieving a near-complete mortality (LC_{90} =82.83 ppm) at 24-hour exposure [29]. Notably, this study reached 93.3% mortality within only 12 hours-highlighting the rapid efficacy of the essential oil in the assays. This suggests that *O. gratissimum* may even be more effective when exposure time is extended, but importantly, this result emphasizes its high potency in short durations. Several conclusions like those of [30] have been drawn from research results about scent leaves as an insect-repelling plant [28]. Similarly, to a larvicidal study of the effects of *A. indica*, *O. gratissimum* and *H. suaveolens*by [31] on *Culex* mosquitoes showed that *A. indica*and *O. gratissimum* had higher larvicidal potential than *H. suaveolens*[32]. Lastly, the use of crude ethanolic extraction for *H. suaveolens* in this study aligns with the methodology employed by [33], who reported 100% mortality of *Anopheles gambiae* larvae at 4 mg/L using ethanolic leaf extracts, though *A. indica* achieved the same at 2 mg/L, indicating *M. suaveolens* requires higher doses for complete efficacy [30].

Beyond the lethal concentration values, the lethal time profiles ($LT_{50}$and LT_{90}) provide deeper insights into the mode of action of these extracts. Interpreting these patterns is crucial for understanding not just potency, but also the speed and consistency of their toxicological effects. This study reveals that *A. indica* crude ethanolic extract exhibited the fasted LT_{50} (7.48 hours), followed closely by the *M. suaveolens* LT_{50} (7.13 hours), while *O. gratissimum* essential oil had a considerably slower LT_{50} (17.5 hours). These findings challenge the common perception of neem as primarily a slow-acting compound and provide a nuanced perspective on the toxicological profiles of these biopesticides. The rapid initial knockdown observed with the crude neem extract, which traditionally acts as an antifeedant and growth regulator primarily through the compound azadirachtin, is likely attributed to the nature of a crude ethanolic extraction. Unlike purified azadirachtin, this method captures a complex mixture of secondary metabolites. Recent studies indicate that compounds such as nimbin, and nimboline, present in whole-leaf extracts, may act synergistically or enhance cuticular penetration, leading to a faster knockdown that would be expected from azadirachtin alone [26, 30]. The LT_{90} values provide critical insight and serve to contextualize the initial knockdown times. While the crude neem and *M. suaveolens* extracts showed rapid LT_{50} values, their respective LT_{90} values were significantly higher (76.9 hours and 80.3 hours). This wide disparity suggests a highly variable response within the insect population , where the most susceptible individuals are affected quickly, but a more subset of more resilient insects requires a much longer time to succumb [34, 35].

In contrast, *O. gratissimum* essential oil, despite its slower LT_{50} displayed a far more consistent toxicity profile, indicated by the narrow LT_{50}- LT_{90}gap of just over 12 hours. This suggests that the volatile neurotoxic compounds in the essential oil, such as eugenol, thymol, may target more fundamental, less variable physiological processes in mosquito larvae, resulting in more uniform knockdown across the population [31]. The variability in *O. gratissimum* efficacy compared to other studies is likely due to differences in chemotype, extraction technique and environmental conditions that influence essential oil composition [35].

For ovicidal activity, this study assessed the ovicidal activities of *O. gratissimum* (scent leaf), *A.indica*(neem), *M. suaveolens* (comb bush mint) against *Aedes aegypti* eggs, measured by hatch rates after 72 hours. The results indicated that *O. gratissimum*exhibited the strongest ovicidal effect (11% hatch at 5%), followed by *A. indica* (25% hatch), with *M. suaveolens* showing the least inhibition (24% hatch). Fewer hatches reflects greater ovicidal potency. These results aligns with previous findings by [36], who noted strong concentration-dependent ovicidal effects of *O. gratissimum*[36]. Similarly, Okorie*et al*., 2020 demonstrated that neem extracts showed ovicidal activity but required higher concentrations for significant efficacy, explaining its weaker performance at 5% in this study [37]. The genus of *Ocimum* possess some secondary metabolite content, including alkaloids, saponins, tanins and flavonoids. Alkaloids can inhibit the development of insects by disrupting three main hormones of insects, namely the brain hormones, ecdysone hormones and growth hormones [38].

The superior performance of *O. gratissmum* with previous research highlighting its oviposition deterrent and antimicrobial properties, likely due to eugenol and phenolic compounds that interfere with embryonic development [39]. The extraction method significantly influenced efficacy. *O. gratissimum* essential oil rich in volatile terpeniods and phenylpropanoids penetrated egg chorions more effectively due to its lipophilic nature [40], out-performing ethanolic extracts of *A. indica* and *M. suaveolens* extracts which contains majorly alkaloids, flavonoids, and phenolic compounds that may require higher concentrations to exert ovicidal effects [41]. The moderate effect of *A. indica* can also be attributed to the age of the eggs as eggs were purchased, the 1998 study by [42], found that neem'sazadirachtin is highly effective (nearly 100% mortality) against freshly laid eggs [42]. *M. suaveolens* showed the weakest ovicidal activity, consistent with studies emphasizing its greater efficacy as a larvicide rather than an ovicide [43]. Environmental factors during the ovicidal assay may also have influenced outcomes. Temperature, water pH and dissolved oxygen are known to affect the stability and activity of botanical compounds [44].

Conclusion

To date, many strategies have been explored to control the spread of malaria and mosquito-borne diseases worldwide. This study underscores the remarkable bioefficacy of *O. gratissimum*, *Azadirachta indica* and *Mesosphaerum suaveolens* essential oil/ extracts against *Aedes aeypti* eggs and larvae, offering a significant global benefits. *O. gratissimum* stands out with potent ovicidal action (11% hatch at 5%) and a larvicidal LC_{50} of 8.4%, making it a powerful tool for controlling mosquito breeding. *A. indica*, with an LC_{50} of 7.65% and 25% egg hatch, excels against larvae and remains effective on younger ones, while *M. suaveolens* provides moderate control. These widely available plants, grown in tropical regions, offer a sustainable, cost-effective alternative to synthetic insecticides, reducing environmental harm and resistance risks. Their potency, derived from natural compounds, supports eco-friendly vector control, protecting public health worldwide. The low cost and biodegradability enhance scalability for local communities, promoting global health equity. Future research should prioritize field trails, optimize oil and extract blends for greater efficacy, and expand cultivation to meet demand. These plant extracts promise a sustainable, affordable, and green solution for a healthier world.

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