



Research Article

Production of a Non-Toxic Plant-Based Bio-Remediant to Repel Mosquitoes and Prevent Allergy Caused by the Bite of African malaria mosquito (*Anopheles gambiae*)

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ABSTRACT

Increasingly mosquitoes are becoming more resistant to synthetic repellents particularly pyrethroids and DEET (N,N-Dimethyl-meta toluamide). This calls for development of alternative Bio-repellents that are biodegradable and non-toxic. Therefore, a study was carried out to produce a non-toxic plant-based bio-remediant against the allergy caused by the bite of *Anopheles gambiae*. Mosquitoes were reared under laboratory conditions in wooden cages according to renowned methods. Tests were performed in cages, with a 12:12 (Light: Dark) photoperiod and controlled temperatures of about 27°C. Knockdown effects of the repellent formulations and susceptibility tests were done according to WHO protocols to establish the stability of the repellent cream, sensitivity of human skin to the repellent, and effectiveness of the repellent to prevent mosquito bites. Peppermint oil had the lowest mean value of mosquitoes aligned to the left side of volunteers, with 2.5, and the highest was 21.0 for turmeric oil. ANOVA indicated a significant difference between the means ($p > 0.05$). Peppermint oil had the highest repellency of 95% and the lowest was 21% for turmeric oil. A cream was formulated by mixing the oils of peppermint and ginger, and it gave 4 hours of protection. Comparison of Knockdown times (KdT50 and KdT90), showed that peppermint oil inflicted 99% mortality ($ED_{50} = 0.04$), equivalent to deltamethrin, while ginger oil achieved a high mortality of 80% ($ED_{50} = 0.05$). It is recommended that the cream be utilised as a repellent against mosquito bite allergy and for the control of malaria.

Keywords: Allergy; *Anopheles gambiae*; Bioremediant; Bite; Plant-Based

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INTRODUCTION

Anopheles gambiae is a primary vector of malaria and a common cause of mosquito bites in tropical and subtropical regions. In addition to transmitting malaria, its bites can trigger allergic reactions in sensitive individuals, causing itching, swelling, and skin irritation. Conventional methods of mosquito control, such as synthetic insecticides, pose serious challenges, including environmental pollution, insecticide resistance, and health risks to humans and non-target organisms (Hazarika *et al.*, 2020).

Increasingly mosquito populations are becoming resistant against synthetic repellents particularly

pyrethroids and DEET (N,N-Dimethyl-meta toluamide). This has directed researchers to explore newer alternative repellents. Furthermore, toxicological and environmental concerns have instigated new investigations in recent years (Adeogun *et al.*, 2012). In search of better alternatives to synthetic repellents, the use of botanicals to control household insect-pest is being looked upon as a main source for safer and eco-friendly insecticides. Since, botanicals are more eco-friendly, economical, species specific, biodegradable and have lesser or no harmful effects on non-target organisms including

human beings, they are preferable over synthetic ones (Miot *et al.*, 2004; Hazarika *et al.*, 2020).

Even though, mosquito repellents based on chemicals have remarkable safety profiles, they are toxic against the human skin and nervous system and may cause rashes, swelling and eye irritations (Pandey *et al.*, 2013). Furthermore, DEET causes neurological issues and scarring in adults; picardin is associated with inhalation toxicity and eye irritation. Scourge leads to liver and thyroid problems, anvil boost tumor growth in cancer; IR3535 is toxic in oral and dermal inhalation (Patil *et al.*, 2023). This has therefore necessitated the need for research and development of environmentally safe, biodegradable, low cost, and indigenous methods for vector control. According to past research, the essential oils of the leaves of *Cymbopogon nardus* (Citronella), *Cymbopogon citratus* (Lemongrass), *Cymbopogon winterianus* (Citronella), *Ocimum basilicum* (Sweet Basil), *Ocimum sanctum* (Tulsi), *Ocimum americanum* (Hairy Basil), *Eucalyptus citriodora* (Eucalyptus), *Eucalyptus globulus* (Eucalyptus), *Rosmarinus officinalis*, *Melissa officinalis*, *Curcuma longa* (Turmeric), rhizomes, *Citrus sinensis* (Sweet Orange) peels, *Citrus hystrix* (Kaffir Lime) peels, *Citrus limonum* (Lemon) peels, *Syzygium aromaticum* (Clove) buds and *Pinus roxburghii* resins, have all shown very high mosquito repellent activity. Moreover, the extracts of *Azadirachta indica* (Neem) seeds, leaves of *Alpinia galangal* (Greater Galangale), *Vitex negundo* (S. Nika), and *Tribulus terrestris* (S. Gokatu) have been studied as possible mosquito repellents. The selection of these plants was based on their availability as raw materials, scientific evidence and folkloric use as mosquito repellents (Makhaik *et al.*, 2005; Aremu *et al.*, 2009; Pougjai *et al.*, 2011; Adeogun *et al.*, 2012; Adeniran and Fabiyi, 2012; Pandey *et al.*, 2013).

Essential oils have received attention as potentially controlling vectors of mosquito-borne disease (Sutthanont *et al.*, 2010). Natural products used as insecticides may have less of an environmental impact due to shorter latency, possibly resulting in reduced resistance (Hardin and Jackson, 2009). Sukumar *et al.* (1991) evaluated 344 different plant species to control mosquitoes instead of synthetic chemical insecticides. Some essential oil derived compounds may be used on humans similar to some conventional insecticides, because they are selective and have fewer harmful effects than some conventional insecticides (Mumcuoglu *et al.*, 2002). Recent studies have highlighted the potential of plant-based Bio-remediants as a safer alternative to synthetic chemicals. Certain plant oils possess natural

repellent and insecticidal properties, making them promising candidates for eco-friendly mosquito control. This project aims to develop a non-toxic, plant-based Bio-remediant oil that not only repels or eliminates *Anopheles gambiae* but also minimizes allergic reactions caused by its bites.

MATERIALS AND METHODS

Rearing of *Anopheles gambiae*

The test mosquitoes, *Anopheles gambiae* were reared from field collected and identified eggs, in laboratory conditions in wooden cages. The eggs, larvae and pupae were identified based on the keys of (Service 1984). They were fed with ten percent (10%) w/v sugar solution and rabbit blood. A glass beaker containing 500 mL of water was kept for egg laying. The eggs were collected and transferred to a polypropylene tray containing three litres of water. Brewer's yeast powder was provided as food supplement for the larvae. Collected pupae were kept in small cages (40 cm × 40 cm × 40 cm) for emergence into the adults. Female *Anopheles gambiae* (5-6 days old) were taken from the colony maintained at 27±2°C and 70±5 % RH for repellent assay (Hazarika *et al.*, 2020).

Test mosquitoes

The mosquitoes used for the laboratory repellent bioassay were 3-7 days old, laboratory-bred and starved adult females of *Anopheles gambiae*. Prior to the time of tests, they were starved for 24 hours but provided with only water (Musau, 2024).

Cage tests

Tests were performed in cages according to Innocent *et al.* (2010), with a 12:12 (Light: Dark) photoperiod and controlled temperatures of about 27°C and a relative humidity of 80% maintained by use of an electric fan heater (WHO 1996; 2009). Active female host-seeking *Anopheles gambiae* mosquitoes aged 3-7 days were collected from stock population using an aspirator and starved for the preceding 24 hours. Acetone/petroleum jelly mixture (1:1) was negative control while 20% DEET (N, N-diethyl-m-toluamide) was the positive control. Volunteers who had avoided use of fragrance, any mosquito repellent, perfumed soap or tobacco for 12 hours prior and during the experiment (WHO, 2009; Innocent *et al.*, 2010) were used for the experiments. The forearm, from the elbow to the wrist was rinsed with water and air dried. The oils were applied as evenly and as thinly as possible. The rest of the hand from the wrist to the fingers was covered with latex glove to prevent the mosquitoes from biting (WHO, 2009). Ethical permission for use of volunteers was obtained from

the Ethical Committee, Faculty of Life Sciences, Federal University Dutsin-Ma.

Test timing was between 7 pm and 10 pm since the mosquitoes typically bite at night. Host-seeking behaviour of the mosquitoes was tested prior to the experiment. This was done by placing a pre-cleaned hand in the cage and counting the number of mosquitoes that aligned within 10 seconds. If at least 5 mosquitoes aligned on the hand, the mosquitoes inside the cage were regarded as host-seeking and the repellency experiment was continued. Volunteer's forearm which had been rubbed with 1 ml of the test oil was exposed to the cage and the number of mosquitoes that aligned or biting the arm was recorded in each minute for 5 minutes. Mosquitoes were given over one hour interval and the above procedure was followed for each of the other extracts and essential oils. Each test was repeated thrice to get a mean value of mosquito repellent activity (Ranasinghe *et al.*, 2016).

The Acetone-petroleum jelly mixture was applied on the other forearm that had been prepared as above, and served as negative control. The volunteer's forearm that had been prepared as above and was subsequently inserted into the cage for five (5) minutes. Mosquitoes that landed on or probed on the hand during this period were counted and shaken off before they can imbibe any blood (WHO, 2009; Innocent *et al.*, 2010). Each trial was carried out in duplicate on different days and with different volunteers. The number of mosquito bites during the periods 0 to 2 mins, 2–15 min, and 15–60 min were counted in order to estimate the protection percentage for each interval. Percent protection efficacy (PE) was determined according to Ansari *et al.* (2005) and Phasomkusolsil and Soonwera (2010), as follows:

Percent protection efficacy refers to how much bites are reduced in the protected group versus the unprotected group as expressed by the formula below:

$$(PE) = (C-T)/C \times 100.$$

Where C and T are the mean numbers of mosquitoes that landed on the control and test arm respectively (Musau, 2024).

Determination of knockdown effect of formulated product

The Knockdown effect of the cream formulations (see below) were determined using WHO bioassay (WHO, 2013). Tests were done in triplicates with positive and negative controls. Filter papers were treated with the formulated the cream (see below) and then air dried.

Each was inserted into a chamber. Twenty-five active *Anopheles gambiae* mosquitoes, 3-7 days old, that had not previously fed on blood, were aspirated from the stock populations of adult mosquitoes and used for this test. They were placed in each of the chambers on filter papers impregnated with different concentrations of the formulated products for 1 hour. Untreated filter papers were regarded as negative control while citronella oil at concentrations of 500 mg/m² was positive control. After one hour the mosquitoes were transferred to different holding chambers and fed with 10% sucrose solution. Mortality and recovery within 24 hours were scored and the time taken to knock down 90% of the population (KD90) at 95% confidence interval was determined (Musau, 2024).

Properties of formulated Cream

The cream is a smooth, peach emulsion, with a pleasant odour.

Preparation of Herbal Mosquito Repellent Cream

Preparation of Oil Phase

The oils were added to liquid solution at gentle heating on water bath. Essential oils were added at (45 degree Celsius) just before mixing two phases. Antioxidant moisturizing Agent, coloring agents, and fragrances were subsequently added.

Preparation of Water Phase

Aqueous ingredients such as 10% glycerin and water soluble preservatives were dissolved in sufficient quantity of water (rose water). Then propylene glycol (10%) was added as the last part of aqueous phase.

Mixing Sequence

Aqueous phase and oil phase were added into separate containers before mixing. The W/O emulsion was made by making the amount of oil phase equal to aqueous phases. Aqueous phase was added to oil phase through wall of the container to ensure little loss of the two phases. The emulsion was stirred at constant temperature (65 to 75 degree Celsius) for 45 – 60 min for proper emulsification. Thereafter, heat-sensitive ingredients (color, fragrances) were added at lower temperature (below 40 degree Celsius) (Ranasinghe *et al.*, 2016).

Preparation of the mosquito repellent cream

The gel was prepared by mixing ginger, peppermint and turmeric (Table 1), to make a total active ingredients gel of 16% (V/V%). Distilled water was added to the mixture until the final volume was 400.00 ml and all the constituents were mixed using the mechanical stirrer for one hour. Finally, the mixture was poured into plastic containers and the gel was allowed to set (Ranasinghe *et al.*, 2016).

Table 1: The essential oils utilised in this study

S/N	Botanical Name	Local Name	English Name
1	<i>Zangiber officinale</i> Roscoe	Citta	Ginger
2	<i>Mentha piperita</i> L.	Na'ana'a	Pepper mint
3	<i>Curcuma longa</i> L.	Citta gauni	Turmeric

Purchase of commercially available essential oils

Essential oils of ginger, pepper mint) and turmeric) were purchased from local herbal pharmacies in Katsina.

Preparation of stock solutions and test concentrations

The stock solutions of each essential oil was prepared by dissolving 1.0 mL of oil (assumed density $\approx$ 1.0 g/mL) in 99.0 mL of HPLC-grade acetone, yielding a 10 mg/ mL stock concentration of 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 were tested at 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}) (Adelaja *et al.*, 2025).

WHO susceptibility test

Susceptibility testing was carried out using the standard WHO protocol (1998) using diagnostic kits and impregnated paper. Each tube was marked with a red spot or a green spot, indicating its use for holding insecticide paper or for holding mosquitoes for 1 hour after exposure. Twenty-five mosquitoes were collected from a cage with an aspirator and blown into green spot tubes lined with clean white paper, with a movable slide attached. Filter paper (Whatman® No.1) 12cm x 15cm was cut for the bioassay. The filter paper was treated with essential oils and placed into red spot tubes. These were screwed onto the opposite side of the movable slide; the slide was then opened and the mosquitoes gently blown into the red spot tube after which the slide was closed again. Twenty-five female *Anopheles* mosquitoes were exposed to the impregnated paper

for 1 hour in each tube while the mosquitoes used as controls were exposed to paper without insecticide and then gently blown back into the green spot tube. Each test was performed in five replicates with simultaneous control sets; the negative controls were impregnated with soybean oil only. Knockdown rates were recorded at 10, 30 and 60 minutes during the 1 hour exposure. At the end of the exposure period mosquitoes were transferred to recovery tubes and provided with 5% glucose solution. Mortality was observed after 24 hours.

Dose Response Lines and Effective Dose Tests

The test established and estimated the dose response lines and effective dose of the two individual formulations (8% and 10%) that passed the preliminary test corresponding to 50% (ED_{50}) protection from mosquito landing and/or probing as compared to the effective dose of the standard (Odomos® mosquito repellent cream) containing 12% N, N-diethyl-m-toluamide (DEET) corresponding to 50% (ED_{50}) protection from mosquito landing and/or probing. Furthermore the test estimated the complete protection time of the individual repellent formulation, which is the time between the application of the repellent and the first mosquito landing and / or biting. The experiment was conducted on a separate day for each of the repellent formulations using one hundred and sixty (160) adult female *Anopheles* mosquitoes of age range between 6-10days old and was kept in eight mosquito netted cages with each containing 20 adult female *Anopheles* mosquitoes. Before the test was conducted, mosquitoes were fed with the blood from the animal skin and then starved for 24hs. The test was carried out in the dark in quadruplicates using the human – bait (WHO, 2009). Eight human volunteers were used for this experiment. 1ml of each of the sample was rubbed on the forearm of the four volunteers while 1g of the standard was rubbed on the arm of another four human volunteers. The untreated arms of the volunteers were used as control. The readiness of the mosquitoes was tested by exposing the untreated arm of the volunteers for about 10 secs. The test was then conducted at the interval of 3min, 30min, 1h,

and 2h. The percentage repellency was determined using the method of (Abdelkarim *et al.*, 2006).

Evaluation of Mosquito Repellence

Repellence of mosquitoes was done in 3 steps, i) stability of the repellent cream, ii) sensitivity of human skin to the repellent, and iii) effectivity of the repellent to prevent mosquito bites (Jufri *et al.*, 2016).

$$\% \text{ Repellency} = 100 - \frac{\text{No. of bites on treated area}}{\text{No. of bites on control area}} \times 100$$

Repellent Stability Test

Repellent stability test evaluated the stability of the cream lotion. The lotion was centrifuged at a speed of 3750 rpm for 5 hours or 5000 to 10000 rpm for 30 minutes. The lotion was subsequently observed for any separation of phases in the lotion (Jufri *et al.*, 2016).

Sensitivity Test

This test observed the effect of repellent on human skin. Three percent (3%) of biooil concentration was utilised. It was smeared unto the panelist arm in 5 x 5 cm wide, then a patch was placed on it and observed for 24 hours (Jufri *et al.*, 2016).

Effectivity test

This was done to observe the effectivity of mosquito repellents on 10 volunteers for 3 days, using 25 mosquitoes. One hand of each volunteer was tested for repellence and the other as control for a period of 6 hours using different concentrations to compare their effectiveness. The protection was calculated based on the formula below:

$$\text{Effectivity} = \frac{\text{Total mosquitoes used} - \text{Mosquitoes that land on hand}}{\text{No. of volunteers}} \times 100$$

Data Analysis

Percentage knockdown and percentage repellency were calculated according to Kain *et al.*, 2013 using the formulae below:

% Knock Down =

$$\frac{\text{Total No. of Knockdown mosquitoes after 60 min}}{\text{Total No. of exposed mosquitoes}} \times 100$$

One-way ANOVA was used to determine the mean mosquito counts within treated chambers and mortalities across the spectrum of exposures. To ascertain potential variations between different plant essential oils, a two-way ANOVA was conducted. The Knockdown Times (KdT50 and KdT95) for successive

concentrations of plant essential oils were determined via probit analysis, relying on mortality data as outlined by Finney (1971). In instances where the control group exhibited a mortality rate exceeding 5%, the treatment groups underwent adjustments through Abbott's methodology.

RESULTS

Table 2, shows that Peppermint oil had the lowest mean value of mosquitoes aligned to the left side of volunteers and aligned/Bit with 2.5, followed by ginger oil 6.0 and the highest was 21.0 for turmeric oil. ANOVA has indicated a significant difference between the means ($p > 0.05$).

Table 3 shows that the Daily Bio-efficacy test of repellent cream on day 1 showed that the cream had recorded the best protection indoors and outdoors between 5 – 7 pm with no (0) mosquitoes landing but the control had 29 landings during the same period. Similar findings were recorded in day 2. ANOVA has indicated a significant difference between the days and also between indoor and outdoor protection ($p > 0.05$).

Evaluation of Effects of Repellent on Mosquitoes

The biooils are of peppermint and ginger had very strong repellent activity on *Anopheles* mosquitoes. The higher the concentration of the biooil used, the stronger the odor and the stronger the repellent activity of the oils.

Dose response study

In Table 4, the study indicated that the higher the concentration of the oil the higher the repellence on *Anopheles gambiae*. Peppermint had the highest repellence of 76%, ginger 47% and turmeric had the lowest of 18% at 0.02 mg/cm² concentration of essential oils. However their highest activity were at 0.21 mg/cm² when the highest repellence were 95% for mint and the lowest was 21% for turmeric.

Sensitivity Test

Table 5, indicates that majority of volunteers did not experience hardening, bump, vesicles, swelling, skin discolouration and pain. However, there are very few cases of mild itching, rash and redness of skin.

Table 6, compares Knockdown times (KdT50 and KdT90), where the percentage mortality of *Anopheles gambiae* exposed to various concentrations of essential oils, deltamethrin and solvent control indicated that pepper mint oil inflicted 99% mortality, equivalent to deltamethrin. Ginger oil also imposed a high mortality of 80%.

Table 2: Mean values for the *Anopheles gambiae* aligned/left and aligned/Bit for essential oils

Essential oil	Replicate 1 Total aligned/left aligned/Bit	mosquitoes and	Replicate 2 Total aligned/left aligned/Bit	mosquitoes and	Mean value of mosquitoes aligned/left aligned/Bit
Ginger oil 10% (V/V %)	5		7		6.0
Pepper mint oil 10% (V/V %)	3		2		2.5
Tumeric oil 10% (V/V %)	19		23		21.0

Table 3: Daily Bio-efficacy test of repellent Cream

Time (pm)	Day 1				Day 2			
	Indoor Control	Cream	Outdoor Control	Cream	Indoor Control	Cream	Outdoor Control	Cream
5 – 6	25	0	30	0	30	0	32	0
6 - 7	29	0	28	0	28	0	33	0
7 - 8	22	2	27	3	29	4	36	2
8 - 9	20	4	29	3	25	4	31	4
9 – 10	21	5	26	4	27	3	37	6
10 – 11	23	4	28	2	24	5	34	3

Table 4: Dose response of concentration of essential oils on *Anopheles gambiae*

Essential oil	Conc. 0.02 mg/cm ²			Conc. 0.12 mg/cm ²			Conc. 0.21 mg/cm ²		
	Mean mosquito SD	No. of biting ±	% Repelle ncy	Mean of mosquito biting ± SD	No. Repellency	%	Mean mosquito biting ± SD	No. of Repellen cy	ED ₅₀ (mg/c m ²)
Ginger	3.0 +-1.17		47	2.8 +-1.15	57		2.4 +-1.15	68	0.050
Pepper mint	2.3+-0.35		76	1.7+-1.12	87		1.4 +-1.09	95	0.040
Turmeric	3.8+-1.75		18	3.5+-1.80	21		2.6+-1.73	21	0.075

Table 5: Skin Sensitivity factor test of repellent cream on *Anopheles gambiae*

Volunteer	Pruritis (itching)	Induration (Hardening)	Rash	Papules (Bump)	Bullae (Vesicle)	Erythema (Redness)	Edema (Swelling)	Skin discolouration	Pain
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
5	-	-	+	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-
8	+	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	+	-	-	-

Table 6: Comparison of Knockdown times (KdT50 and KdT90), percentage mortality of *Anopheles gambiae* exposed to various concentrations of essential oils, Deltamethrin and solvent control

Plant essential oils	Con. (mg/mL)	KdT ₅₀ (95% CI) (min)	KdT ₉₅ (95% CI) (min)	% Mortality after 24 hrs
Ginger	0.1	36.02 (17.60 – 106.2)	144.02 (18.50 – 274.5)	77 ^a
	0.3	65.12 (0.642 – 3113)	145.42 (0.672 – 368.2)	79 ^b
	0.5	74.08 (0.04 – 2567)	154.55 (0.20 – 377.7)	80 ^b
Pepper mint	0.1	56.02 (17.70 – 105.7)	188.22 (18.66 – 97 ^a	
	0.3	65.08 (0.672 – 3222)	1890.5)	99 ^b
	0.5	74.10 (0.20 – 2344)	245.42 (6.543 – 99 ^b	
Turmeric			13878.2)	
			96.65 (39.20 – 311.7)	
	0.1	19.10 (15.47 – 34.05)	68.02 (15.50 – 1142.5)	39 ^a
	0.3	19.51 (10.52 – 22.20)	55.42 (10.22 – 1011.3)	40 ^b
	0.5	14.06 (13.12 – 20.34)	45.55 (8.20 – 98.7)	34 ^b
Deltamethrin	10	32.11 (12.3 – 92.11)	103.3 91.37 – 2342)	99 ^b

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

DISCUSSION

In this study, peppermint oil had the lowest mean value of mosquitoes aligned to the left side of volunteers' body and aligned/Bit with 2.5, followed by ginger oil 6.0 and the highest was 21.0 for turmeric oil. This is similar to with the findings of a research by the Liverpool School of tropical Hygiene in 2019, which states that mosquitoes bite mostly bite on the left side of the body. Not because of the characteristics of the person, but due to wind and air flow, sleeping and seating position, type and positioning of your clothing and nearby objects. A similar study reported reasons such as skin chemicals, temperature, humidity and exhaled Carbon dioxide. This implicates that mosquito bites can be prevented by wearing clothes that cover the whole body and that mosquito's disease are likely to increase and dominate populations that do not wear proper and complete body clothing.

This study reports that the Daily Bio-efficacy test of repellent cream on day 1 indicates high indoor activity against *Anopheles gambiae*. The efficacy is comparable but lower than DEET. A conducted comparative study on the persistence of 30% DEET and 10% *H. suaveolens* essential oil for personal protection against mosquitoes in field conditions, discovered that the mean number of mosquitoes that landed on treated volunteers 6 hours post-application was 0.50 and 0.45 for 10%. *H. suaveolens* essential oil and DEET respectively, against 6 mosquitoes for the control people. Statistical analysis revealed that there is no significant difference between 10% *H. suaveolens* essential oil and DEET

indicating that both products are similarly effective. Similar findings were reported by Sritabutra *et al.* (2011), who reported high indoor activity of repellents against *Anopheles gambiae*.

In this study, the dose response of concentration of essential oils on *Anopheles gambiae* showed that pepper mint had the highest repellence of 95% by pepper mint oil. This is higher than the findings of Ansari *et al.* (2000) who reported that Pepper mint oil 1 ml without dilution has a repellency of 92.3% and protection time of 96 hours on *An. culicifacies*; and repellency of 100% and protection time of 11 hours on *An. Annularis*, which is higher than that of the current study. Similarly, Sritabutra *et al.* (2011) found that Peppermint (*M. piperita*) essential oil (0.1 ml) protected for 1.08 hour against *An. dirus*. The repellency effect of Peppermint oil against *Anopheles* is because it contains biologically active constituents and have high menthone, menthol and methyl esters in addition to limonene, α -pinene, myrcene and linalool.

Furthermore, Ansari *et al.* in a field trial revealed that 1 ml peppermint oil without dilution completely repels *Anopheles annularis*, *An. culicifacies* and *Anopheles subpictus* for 11, 9.6 and 7.3 h, respectively and the corresponding percentage repellency were 100%, 92.3% and 83.1%. The study by Sritabutra *et al.* also found that 0.1 ml of peppermint essential oil protect against *An. dirus* for 1.08 h. In addition, a 100% peppermint oil exhibited the highest efficacy against *A. dirus* (180 min), longer than the 64 min recorded for 10% peppermint oil in soybean oil. A previous study reported 100% protection by

peppermint oil against *A. aegypti* for 150 min. These results are higher than the findings of this current study. However, in another study, 20% oil solutions of peppermint had 57% repellency and complete protection time for 6.5 h against *An. stephensi*, which is lower than the findings of the current study.

Additionally, Govindarajan *et al.* (2015) reported that *Zingiber nimmonii* essential oil 1 mg/cm² used on *An. stephensi*, led to a 100% repellency and 3 hours protection. Ginger contains some chemicals that are responsible for their pharmacological properties. And it contains many volatile oils (sesquiterpenes) and aromatic ketones (gingerols) as the most active pharmacological active constituents. The authors further reported that the gingerols could be the most active pharmacological constituents found in ginger. The mortality observed upon the utilisation of ginger on *Anopheles* species during this current study could be as a result of the presence of bioactive ingredients that are associated with ability to kill pests such as Borneol, β -Bisabolene, Cineole, α -Cedrene, α -Curcumene, β -Farnesene (E), β -Sesquihelladiene, β -Thujene and Zingiberene, and lemon grass contain Citral, Geranic Acid, Geranyl Acetate, Linalool, Nerolic acid, (Z) Citral, β -myrcene and β -Thujene in their essential oil, in addition to other phytochemical constituents of ginger such as gingerols, zingerone, shogaols, and paradols. Furthermore, other authors have reported the presence of flavonoids, tannins, alkaloids, saponins, triterpenes and glycoside in ginger as the most pharmacological active constituent (Bhargava *et al.*, 2012; González-Guevara *et al.*, 2017). The presence of alkaloids could account for the ability of ginger to ward off pests (Kigigha *et al.*, 2015; Eidi *et al.*, 2016; Izah, 2019).

In this study, comparison of Knockdown times (KdT50 and KdT90), and percentage mortality of *Anopheles gambiae* exposed to various concentrations of essential oils, deltamethrin and solvent control showed that mint oil inflicted 99% mortality, equivalent to deltamethrin. Ginger oil also imposed a high mortality (80%). These findings are lower than those of Auysawasdi *et al.* (2015) who found that 25% turmeric (*Curcuma longa*) essential oil provided 100% protection for 8 hours against *An. dirus*. However, Tawatsin *et al.* (2000) reported that 3 ml turmeric volatile oil provided 100% repellency and 6 hours protection against *An. dirus*. Other studies also found that turmeric oil repels *An. dirus* for 6 h (Tawatsin *et al.* (2001) and *An. minimus* (Phasomkusolsil and Soonwera, 2010) for 1 h. The insecticidal effects of turmeric observed in this study could be because of its major constituents of its essential oil such as β -

bisabolene (13.9%), trans ocimene (9.8%), myrcene (7.6%), 1,8-cineole (6.9%), α -thujene (6.7%) and thymol (6.4%) (Ameen *et al.*, 2009).

The repellent activity of essential oils depends on their chemical constituents. Contents of repellent oils like camphor, cineol, linalool and α -pinene have been identified as insect repellents (Campos *et al.*, 2018; Huff *et al.*, 2019; Saad *et al.*, 2019; Tian *et al.*, 2020). Additionally, limonene and linalool act as contact poisons or nerve toxins (Dubey, 2011). Furthermore, essential oils are composed mainly of monoterpenes, sesquiterpenes, alcohols and phenols that can be neurotoxic to mosquitoes (Dubey, 2011). However, the use of essential oils as insect repellents is limited by their volatility and oxidation. Stabilizing the oils may involve additives such as vanillin, coconut oil and olive oil (Auysawasdi *et al.*, 2016) to extend protection times and substitute for synthetic repellents (Soonwera, 2015; Rehman *et al.*, 2014).

In this study, the Skin Sensitivity factor test for cream recorded slight but negligible itching, rash, redness and skin discolouration but there were no records of development of skin hardening, bumps, vesicles, swelling and pain after 24 hours. This is similar to the findings of other authors (Tawatsin *et al.*, 2001; Phasomkusolsil and Soonwera, 2010).

This study has determined through effectivity test, that the higher the concentration of biooil in the repellent, the higher the protection. In general, repellents will manipulate the smell and taste from skin by resisting the lactate acid receptor in mosquito, so it will prevent the mosquito from coming near the skin, usually by adding some dominant smell on mosquito repellent such as orange skin or lavender. This principle also applied to pepper mint and ginger repellents utilised in this study, where beside their potential as pesticides, the biooils also had a dominant smell so they can manipulate mosquito by covering the Carbon dioxide (CO₂) smell that was produced by the human skin.

CONCLUSION

Peppermint oil had the lowest mean value of mosquitoes aligned to the left side of volunteers and aligned/Bit with 2.5, followed by ginger oil 6.0 and the highest was 21.0 for turmeric oil. The Daily Bio-efficacy test of repellent cream showed that the cream had the highest indoor activity. The higher the concentration of the biooil used, the stronger the odor. The Dose response of concentration of essential oils on *Anopheles gambiae* showed that pepper mint had the highest repellence of 95% and the lowest was 21% for turmeric. Comparison of Knockdown times

(KdT50 and KdT90), percentage mortality of *Anopheles gambiae* exposed to various concentrations of essential oils, deltamethrin and solvent control showed that mint oil inflicted 99% mortality, equivalent to deltamethrin. Ginger oil also imposed a high mortality (80%).

Mosquito Repellents need to be integrated into mosquito control programmes because this study has shown that pepper mint is very effective against *Anopheles gambiae*. Further field trials needs to be conducted to evaluate the efficacies of pepper mint and ginger so as to establish their performance in the field. Special consideration should be accorded to the patenting of Bioinsecticides by the CAC so as to protect current and future research results on mosquito repellents. There is the need to conduct GC-MS analysis of the oils of mosquito repellents to confirm the active components. Further research is needed to conduct phytochemical screening of plant based repellent oils of ginger and pepper mint. Oil repellents need to be stabilized by using additives such as vanillin, coconut oil and olive oil to extend protection times and substitute for synthetic repellents.

Ethical statement

The study was conducted after obtaining certified ethical approval from the Faculty of Life Sciences, Federal University Dutsin-Ma Ethical Review Committee.

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REFERENCES

Abdelkarim, A. & Heinz, M. (2006). Repellency effect of forty-one essential oils against *Aedes*, *Anopheles* and *Culex* mosquitoes. *Journal of Medical Parasitology*, 9:1 - 23

Adelaja, O.J., Oduola1, A.O., Omotayo, A.I., Adeogun, A.O., Ande, A.T. & Obembe, A. (2025). Repellency and Toxicity Effects of Selected Plant Essential Oils against *Anopheles gambiae*. *Ceylon Journal of Science*, 54(4):1095-1104 doi: <http://doi.org/10.4038/cjs.v54i4.8397> RESEARCH

Adeniran, O.I. & Fabiyi, E. (2012). Natural products from plants as insecticides. *Journal of Natural Produce Plant Resources*, 2:322-327.

Adeogun, A.O., Adewuyi, G.O., Etatuvie, S.O., Fawehinmi, A.B. & Lawal, H.O. (2012). Bioassay of

Herbal Mosquito Repellent Formulated from the Essential Oil of Plants. *Journal of Natural Products*, 5: 109-115

Afify, A. & Potter, C.J. (2020). Insect repellents mediate species-specific olfactory behaviours in mosquitoes. *Malaria Journal*, 19:127.

Agu, G.C., & Thomas, B.T. (2012). Antibacterial Activities of Ethanol and Aqueous Extracts of Five Nigerian Medicinal Plants on Some Wound Pathogens. *Nature and Science*, 10(2): 78-84

Ameen, O.M., & George, O.C., Hamid, A.A., Muhammad, N.O., Lawal, A., (2009). Chemical Composition of Rhizome Essential Oil of *Curcuma longa* L. Growing in North Central Nigeria. *World Journal of Chem*, 4: 178-181.

Amer, A. & Mehlhorn, H. (2006). Repellency effect of forty-one essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes. *Parasitol. Res.*, 99: 478-490.

Ansari, M., Mittal, P., Razdan, R., & Sreehari, U. (2005). Larvicidal and mosquito repellent activities of pine (*Pinus longifolia*, Family: Pinaceae) oil. *Journal of Vector Borne Dis.*, 42:95.

Aremu, O.I., Femi-Oyewo, M.N. & Popoola, K.O.K. (2009). Towards an understanding of interdisciplinarity. *Inter. Multi-Disciplinary journal*, 3: 12-22.

Ausawasdi, N., Chuntranuluck, S., Phasomkusolsil, S., & Keeratinijakal, V. (2016). Improving the effectiveness of three essential oils against *Aedes aegypti* (Linn.) and *Anopheles dirus* (Peyton and Harrison). *Parasitology Research*, 115:99-106

Bhargava, S., Dhabhai, K., Batra, A., Sharma, A., & Malhotra, B. (2012) *Zingiber Officinale*: Chemical and phytochemical screening and evaluation of its antimicrobial activities. *Journal of Chemical and Pharmaceutical Research*, 4(1): 360-364

Campos, E.V.R., Proenca, P.L.F., Oliveira, J.L., Pereira, A.E.S., de Moraes Ribeiro, L.N. Fernandes, F.O., Goncalves, K.C., Polanczyk, R.A., Pasquoto-Stigliani, T. & Lima, R. (2018). Carvacrol and linalool co-loaded in beta-cyclodextrin-grafted chitosan nanoparticles as sustainable biopesticide aiming pest control. *Sci. Rep.* 8: 7623.

Damalas, C.A. (2011). Efficacy of neem oil and turmeric powder against *Sitobion avenae* and *Rhopalosiphum padi*. *Plant Omics Journal*, 4: 136-140.

Dubey, N.K. (2011). *Natural Products in Plant Pest Management*; CABI: Wallingford, UK, 2011

Durrani, F., Ismail, M., Sultan, A., Suhail, S., Chand, N., & Durrani, Z. (2006). Effect of different levels of feed added turmeric (*Curcuma longa*) on the performance of broiler chicks. *Journal of Agric Biol Sci.* 1:9-11.

- Epidi, J.O., Izah, S.C., Ohimain, E.I., & Eidi, T.T. (2016). Antibacterial and synergistic potency of tissues of *Vitex grandifolia*. *Biotechnological Research*, **2**(2): 69-76.
- Ghalandari, R., Heidari, S., Kashani, H.H., & Shooshtari, M.B. (2013). Field evaluation of naturally occurring mosquito repellents in Mt. Kenya Region, Kenya. *African Journal of Pharm Pharmacol*, **7**: 310-314.
- González-Guevara, J.C., Madrigal-Redondo, G.L., Vargas-Zúñiga, L., & Rodríguez-Sibaja, C. (2017). Physicochemical characterization of medicinal essential oil from the rhizome of *Zingiber officinale* (ginger), grown in San Carlos, Costa Rica. *Journal of Applied Pharmaceutical Sciences*, **4**(1): 9-18.
- Govindarajan, M., & Sivakumar, R. (2015). Laboratory evaluation of Indian medicinal plants as repellents against malaria, dengue, and filariasis vector mosquitoes. *Parasitol Res.*, **114**:601–12.
- Hardin, J.A. & Jackson, F.L.C. (2009). Applications of natural products in the control of mosquito-transmitted diseases. *African Journal of Biotechnology*.
- Hazarika, H., Boruah, S., Islam, J., Paul, A. & Zaman, M.K. (2020). Antimicrobial, Mosquito Larvicidal And Repellent Activity Of Essential Oils Isolated From Three Local Species Of Ginger Grown In Upper Assam Region. *India Current Trends In Pharmaceutical Research*, **17**:(1).
- Huff, R.M., & Pitts, R.J. (2019). An odorant receptor from *Anopheles gambiae* that demonstrates enantioselectivity to the plant volatile, linalool. *PLoS ONE*, **14**: e0225637.
- Innocent, E., Joseph, C.C., Gokonyo, N.K., Nkunya, M.H.H., & Hassanali, A. (2010). Constituents of the Essential oil of *Suregada zanzibariensis* leaves are repellent to the mosquito *Anopheles gambiae*. *Journal of Insect Science*, 10:57
- Izah, S.C. (2019). Activities of Crude, Acetone and Ethanolic Extracts of *Capsicum frutescens* var. minima Fruit Against Larva of *Anopheles gambiae*. *Journal of Environmental Treatment Techniques*, **7**(2): 196-200.
- Jufri, M., Irmayanti, E., & Gozan, M. (2016). Formulation of Tobacco Based Mosquito Repellent to Avoid Dengue Fever. *International Journal of PharmTech Research*, **9**(7): 140-145
- Kain, P., Boyle, S.M., Tharadra, S.K., Guda, T., Pham, C., Dahanukar, A. & Ray, A. (2013). Odour receptors and neurons for DEET and new insect repellents. *Nature*, 502::507 – 12. Doi:10.1038/nature12594.
- Kigigha, L.T., Izah, S.C., & Ehizibue, M. (2015). Activities of *Aframomum melegueta* Seed Against *Escherichia coli*, *S. aureus* and *Bacillus* species. *Point Journal of Botany and Microbiology Research*, **1**(2): 23-29.
- Mahour, K., Prakash, S., Singh, S. (2009). Ethanopharmacological and Phytochemical Aspects of *Ocimum sanctum* Linn-The Elixir of Life. *Journal of Herbal Med Toxicol*, **3**: 87-90.
- Makhaik, M., Naik, S.N., & Tewary, D.K. (2005). Evaluation of anti-mosquito properties of essential oils. *Journal of Sci Ind Res*, **64**: 129-133.
- Miot, H.A., Batistella, R.F., Batista, K.D.A., Volpato, D.E.C., Augusto, L.S.T., Madeira, N.G. ... & Miot, L.D.B. (2004). Comparative study of the tropical effectiveness of the andiroba oil (*Carapa guianensis*) and DEET 50% as repellent for *Aedes* sp. *Revista do instituto de Medicina Tropical de Sao Paulo*, 46(5):253 - 256
- Musau, J.K. (2024). Effectiveness of a plant-based mosquito repellent formulation. *International Journal of Novel Research and Development (IJNRD)*.
- Pandey, D.M., Rani, N., Vidyarthi, A.S., & Wany, A. (2013). Study of Citronella leaf based herbal mosquito repellents using natural binders. *Curr Res Microb Biotechnol*, **1**: 98-103.
- Patil, N., Chandel, V., Rana, A., Jain, M., & Kaushik, P. (2023). Investigation of cannabis sativa Phytochemicals as anti- Alzheimer's Agents: An In Silico Study. *Plants*, 12:510
- Phasomkusolsil, S., & Soonwera, M. (2010). Insect repellent activity of medicinal plant oils against *Aedes aegypti* (Linn.), *Anopheles minimus* (Theobald) and *Culex quinquefasciatus* Say based on protection time and biting rate. *Southeast Asian Journal of Trop Med Public Health*, **41**:831.
- Poungjai, S., Soonwera, M., Sritabutra, D., & Waltanachanobon, S. (2011). Repellent activity of herbal essential oils against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say.). *Asian Pacific Journal of Tropical Biomed*, **3**: 124 -128.
- Pradhan, S.S., & Patel, R.K. (2014). Preparation of Low-Cost Herbal Mosquito Repellent from Begunia Leaf. *Rourkela National Institute of Technology*, **4**: 1-25.
- Ranasinghe, M.S.N., Arambewela, L., & Samarasinghe, S. (2016). Development of Herbal Mosquito Repellent Formulations. *International Journal of Collaborative Research on Internal Medicine & Public Health*, **8**(6): 241 - 380
- Rehman, J.U., Ali, A., & Khan, I.A. (2014). Plant based products: Use and development as repellents against mosquitoes: A review. *Fitoterapia*, **95**: 65–74.
- Saad, M.M.G., El-Deeb, D.A. & Abdelgaleil, S.A.M. (2019). Insecticidal potential and repellent and

biochemical effects of phenylpropenes and monoterpenes on the red flour beetle, *Tribolium castaneum* Herbst. *Environ. Sci. Pollut. Res. Int.* **26**: 6801–6810.

Scott, R.R., Tawatsin, A., Techadamrongsin, Y., Thavara, U., & Wratten, S.D. (2001). Behavioral responses of *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, and *Anopheles minimus* against various synthetic and natural repellent compounds. *Journal of Vector Ecology*, **26**: 76-82.

Soonwera, M. (2015). Efficacy of essential oils from Citrus plants against mosquito vectors *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say). *IJAT*, **11**: 669–681.

Sritabutra, D., Soonwera, M., Waltanachanobon, S. & Pongjai, S. (2011). Evaluation of herbal essential oil as repellents against *Aedes aegypti* (L.) and *Anopheles dirus* Peyton & Harrion, **1**:S124–8.

Sukumar, K., Perich, M.J., & Boobar, L.R. (1991). Botanical Derivatives in Mosquito Control. A Review. *Journal of the American Control Association*, **7**:210 - 237

Sutthanont, N., Choochote, W., Tuetun, B., Junkum, A., Jitpakdi, A., Chaitong, U., Riyong, D., & Pitasawat, B. (2010). Chemical Composition and Larvicidal Activity of Edible Plant-derived Essential Oils against the pyrethroid-susceptible and resistant strains of *Aedes aegypti* (Diptera: Culicidae). *Journal of Vector*

Ecology, **35**:106 – 115. Doi:10.1111/j.1948-7134.2010.00066.x

Tawatsin, A. Wratten, S.D., Scott, R.R., Thavara, U. & Techadamrongsin, Y. (2001). Repellency of volatile oils from plants against three mosquito vectors. *Journal of Vector Ecology*, **26**:76–82.

Tian, Y., Dong, F., Zhou, X. & Yang, X. (2020). Repellent, Insecticidal and Antimicrobial Activities of Leaf Essential Oils from Three Eucalyptus Species. *Chem. Biodivers*, **17**: e1900580.

Tyagi, A.K. & Malik, A. (2011). Antimicrobial potential and chemical composition of *Mentha piperita* oil in liquid and vapour phase against food spoiling microorganisms. *Food Control*, **22**: 1707–1714.

WHO (1996). The world health report 1996: Fighting disease, fostering development. <http://iris.who.int/handle.10665/178481>

WHO (1998). Standard operating procedure for testing insecticide susceptibility of adult mosquitoes in WHO tube tests. <http://iris.who.int/handle.10665/178481>

WHO (2009). The world health report 2009 – health system: Improving Performance. World Health Organisation, Geneva.

WHO (2013). The World Health Report. Research for universal health coverage. http://.app.who.int/iris/bitstream/10665/85761/2/9789240690837_eng.pdf