

## Research Article

## Preliminary Study of the Mosquito Repellent and Adulticidal Effects of Volatile Oils of Lemon grass (*Cymbopogon winterianus*) in Imo State, Southeast Nigeria

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### Abstract

Lemon grass (*Cymbopogon winterianus*) has been widely acclaimed to possess therapeutic, cosmetic and insecticidal activities. This study was conducted using a field controlled trial by human volunteers to confirm the effectiveness and applicability of locally-produced Lemon grass oil as a mosquito repellent and adulticidal agent for the prevention of mosquito-borne diseases in Nigeria. Volatile oil was extracted using petroleum ether as solvent while olive oil was used as oil base. The study was conducted between September and October 2017 and made use of 12 volunteers across three locations. The lotions were applied on exposed body parts of the volunteers and they worked three shifts daily. The biting rate, percentage repellence complete protection time and whole night protection were measured and recorded. Topical application of each lotion reduced the biting rate of mosquitoes across the three locations, with the 60% (v/v) exhibiting higher impact of 341 vs 32; 438 vs 44 and 388 vs 33. The 60% (v/v) formulation also had the highest percentage repellence of 91.92% at Location I, offered a whole night protection against mosquito in location I and O and offered a Complete Protection Time (CPT) of 21.6, 18.93 and 20.8 respectively across all three locations. There was a significant statistical association  $r=0.94$  ( $p<0.05$ ) between concentrations of the Lemon grass oil formulations and mean duration protection time against mosquitoes. All formulation exhibited adulticidal activities, with the 60% (v/v) formulation having higher impact, causing fast immobilization or paralyzing effect on some mosquitoes that were at close range to treated volunteer's body. The study concludes that volatile oils of Lemon grass possess mosquito (*A. gambiae* and *An. funestus*) repellent and adulticidal effects, especially at higher concentrations and can be used to reduce human-mosquito contacts and hence mosquito-borne diseases and irritation caused by their bites.

**Keywords:** Adulticidal; *Cymbopogon winterianus*; Formulation; Insecticidal; Lemon grass; Mosquito; Repellent; Volatile oil

### Introduction

Control of mosquitoes is something of utmost importance in the present day, with rising number of mosquito borne illnesses. Mosquito-borne diseases affect millions of people worldwide each year. The bite of a mosquito can result in anything from a skin irritation to contracting malaria. Malaria transmitted by *Anopheles* (Diptera: Culicidae) mosquitoes is the leading cause of morbidity and mortality in Nigeria, and in Africa as a whole, it causes about 1–2 million deaths annually (Annual Report in Nigeria, 2005; Breman, 2001). In southeastern Nigeria, *Anopheles gambiae* is the major vector transmitting malaria

followed by *An. funestus* (Uneke and Ibeh, 2009). Diseases of mosquitoes are transmitted to human beings through bites only.

Mosquito control and personal protection from mosquito bites is one of the main strategies to control or minimize incidence of these diseases. The common approach for the control of mosquito vectors and reducing the transmission of human pathogens is based on the chemical insecticide-based intervention measures (Donald, 2005). Synthetic pesticides have been extensively used for mosquito control by either killing, preventing adult mosquitoes to bite human beings or by killing

mosquito larvae at the breeding sites of the vectors (Brown and Hebert, 1997.) However, its deleterious impact on non-target population and the development of resistance prompted for the search of alternative, simple and sustainable methods of mosquito control (Miro *et al.*, 2010).

Fradin (1998), noted that protection against mosquito bites can be best achieved by avoiding infested habitats, wearing protective clothing and applying insect repellent. The use of repellent has been advocated. Insect repellents date back to ancient times, with the use of tars, smokes, plant oils and other modalities (Peterson and Coats, 2001). Insect repellents work by masking human scent, making it impossible for the mosquito to sense the carbon dioxide and lactic acid present in sweat in humans and act as attractive substances for mosquitoes (Peterson and Coats, 2001).

Plants products are emerging as a potential source of mosquito control and among them essential oils have special interest due to their insecticidal properties (Benner, 1993). Essential oils are volatile naturally occurring, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites (Madhumathy *et al.*, 2007).

Lemon grass (*Cymbopogon winterianus*) is native to India and tropical Asia. It is widely used as herb in Asian cuisine. It is a perennial, shallowly rooted rhizome. Culms are tufted, robust, up to 2 m tall. Leaf sheaths are glabrous, reddish inside; leaf blades relatively thin, drooping 2/3 of their length. Lemon grass is known as *achara ehi* or *achara tea* in Igbo. Its essential oils are natural products that exhibit a variety of biological properties, such as analgesic anticonvulsant and anxiolytic (Almeida *et al.* 2001, 2003 and 2004). The steam volatile essential oils extracted from its leaves are used in perfumery, cosmetics, pharmaceuticals and flavoring industries. In traditional medicine, the oil has been used as an aromatic tea, vermifuge, diuretic, and antispasmodic (Nandini *et al.*, 2013; Aakanksha *et al.*, 2013).

This study was conducted to investigate the effectiveness and applicability of Lemon grass oil as a natural mosquito repellent and adulticide on human subjects in outdoor conditions.

## Material and Methods

### Study Design

This is a field study that examined a locally acclaimed plant that grows in Imo State, South East Nigeria, for its mosquito repellence and adulticidal effects. Volatile oil was extracted from the leaves of the test plants formulated into two different concentrations (two formulations) and tested on volunteered human subjects. The study was conducted between September through October, 2017, using the extracted essential oils as test material and olive oil both as base material and as negative control.

### Description of Study Area

This study was conducted in Owerri. Owerri is made up of three local government areas, which are Owerri West, Owerri North and Owerri Municipal. All these places lie within the rainforest zone of the south-eastern Nigeria with typical tropical climate. The temperature range during the study period was 35–40°C. Owerri has a tropical wet climate according to the Köppen-Geiger system. Rain falls for most months of the year with a brief dry season. The study was carried out in three different locations Ihiagwa, Royce Road and Obinze.

### Study Population

The study population for this study comprise individuals living in the three locations. Selection of houses to be included in the investigation was done by the researcher. Selection criteria included the presence of stagnant water collections in gutters and potholes as well as refuse dumps littering the vicinity of the houses. Such practices promote proliferation of mosquitoes. Houses where mosquitoes were found inside bathroom floors and around water closets and septic tanks were enlisted.

### Sample Size

In each of the three locations, four (4) human baits who voluntarily consented were selected and were used. Since it has been established that only about 20% in a population receive intensive bites from mosquitoes due to chemical makeup and other factors (Du, 2005), the investigator selected only those who claim to experience much mosquito bites naturally. With the help of the University Director of Health Services, the subjects were given chemo prophylactics before the investigation and they continued it throughout the period of investigation and two weeks after the exercise. Thus, the sampling size for this study was twelve (12).

### Sample Preparation

Fresh leaves of *C. winterianus* were collected from a garden along World Bank Housing Estate, Owerri and were confirmed by a Botanist. The leaves were air dried until the moisture content was reduced to barest minimum. The collected leaves of each of the plant materials were pulverized into powder using a Binatone Mx10 blender and sieved to obtain a fine powder of the plant part. 150g of each pulverised plant part was placed in a plain sheet of white paper, then placed in the timbel of the soxhlet apparatus compartment using petroleum ether extraction solvent (Okigbo *et al*, 2010). The oils extracted were stored in an appropriate sample bottles at a temperature of 4<sup>0</sup>C till the period of formulation and repellency testing.

### Formulation of Oil

Olive oil was used as the natural oil base for the formulations. Two different concentrations (30% <sup>v/v</sup>, and 60% <sup>v/v</sup>,) were made for the essential oil. The concentrations were made following the method employed by earlier researchers (Oyedele, *et al.*, 2000; Oparaocha, *et al.*, 2010). The olive oil was bought from priceless supermarket in Owerri. The different formulations of Citronella oil were labeled B<sub>1</sub> and B<sub>2</sub>. The ordinary olive oil which served as negative control was labeled CONTROL.

### Mosquito Repellent Test Procedure

The method adopted for mosquito repellency test is that developed by Oparaocha *et al*, (2010) but was adapted using WHO (2005) procedures for field trial. The volunteers worked in pairs and for each of the six in each location, a volunteer used the negative Control. All the six pairs worked for three sessions (morning, evening and night). The pairs for the three sessions worked for three consecutive days, twice a month and rotated lotion type and session every day. All the formulations were tested the same day so as to allow equal exposure to the same environmental conditions.

For the testing, 2ml of the test lotion was given to the volunteers to rub on the exposed parts of the body (the arms, legs and on the face). The pairs that worked in the morning and evening sessions stayed outside the house at about 20 m distance from each other from 5:30am to 7:30am and from 6:00pm to 8:00pm, while the pair for the night stayed in different rooms in the same house. The volunteers were informed to note the number of flies resting on

the exposed body parts by catching them with inverted cylinder tube.

For those working at night, since they were not expected to get up from bed to catch mosquitoes, a grading system was introduced to them to record palpable bites and/or number of times they were woken up by the buzzing sound of mosquitoes as below; 0 bites/buzzing sound (no bite), 1–2 bites/buzzing sound at the same time and woken up to three times (mild bite), 3 and above bites at the same time and woken up more than three times (heavy bites). Total bites for each month (24 h) of morning and evening as well as the two-month totals were calculated for each lotion type for the treated and control. For whole night bites a summary of their reports was made. A randomized species identification of the mosquitoes was done by an entomologist.

### Mosquitocidal Test Procedure

The method utilized was developed by Oparaocha *et al.*, (2010). It is a very simple method, in which the volunteers were told to note any dead mosquito or any immobilizing effect of the lotion on the fly. The investigator confirmed the action before recording.

### Ethical clearance

Ethical clearance was obtained from the Department of Public Health before commencing the investigation. Informed and free consents were obtained from the volunteers before they were recruited for the study.

### Data Analysis

The repellence for the treated and the control in each test location was recorded and the relationship between concentrations of repellence of formulations and mean protection time was measured using Pearson's correlation coefficient. Complete Protection Time (CPT) and Percentage Repellency were calculated for each of the formulations. The results were presented in tables and charts.

### Terminologies:

**Biting Rate:** Biting rate important parameter for assessing malaria transmission and evaluating vector control interventions, is commonly estimated by Human Landing Collections (HLC) (Kwon *et al.*, 2011).

**Percentage Repellency:** This is the number of mosquitoes landing or attempting to bite at given

time intervals without re-treatment is recorded and the repellence of the substance is calculated with the formula:

$$\% \text{ Protection} = \frac{C-T}{C} \times 100\%$$

Where C corresponds to the number of bites by exposing the control limb (untreated or with a solvent such as Olive oil as a control substance) and T by exposing the repellent treated limb.

**Complete Protection Time (CPT):** This is the time period between repellent application and the first two mosquito landing/probing/bites during the same observation period or one bite each in two consecutive intervals.

## Results

### Repellence Result: Biting Rate

The monthly biting rates of mosquitoes on both treated and control test volunteers at different concentrations and at different locations is shown in Table 1. The result showed that there was a significant reduction in the biting rate for the two different formulations across the three locations (Ihiagwa, Obinze and Royce Road), with 60% ( $\frac{v}{v}$ ) formulation exhibiting highest impact (341 vs 32; 438 vs 44 and 388 vs 33) at all the centres respectively, for the two months' totals. The highest biting rate (432 vs 55) was observed in Royce and the least (293 vs 34) was recorded in Obinze.

### Percentage Repellence

The results of the percentage repellence showed values ranging from 85.71 to 91.92% for the two-month averages, with the 60% ( $\frac{v}{v}$ ) formulation having the highest values (90.62%, 90.00% and 91.50%), while the 30% ( $\frac{v}{v}$ ) formulation had the least (87.66, 87.77 and 88.40%) in Ihiagwa, Obinze and Royce road respectively (Table 2).

### Complete Protection Time (CPT)

The result of the complete protection time for the 24 hours/2-months biting time is given in table 3 below. It can be seen that across the three test locations, the 60% ( $\frac{v}{v}$ ) formulation offered a CPT of 21.6, 18.93 and 20.8 respectively across the three locations. Similarly, the 30% ( $\frac{v}{v}$ ) formulation offered a CPT of 19.2, 20.3 and 19.5 respectively across the three locations

### Whole Night Biting Rate

The result of the whole night protection offered by the different formulations of repellents is shown in table 4 below. The table indicated that the 60% ( $\frac{v}{v}$ ) formulations afforded whole night protection to a greater extent, dawn and dusk in Ihiagwa and Obinze, while 30% ( $\frac{v}{v}$ ) formulation offered whole night protection in Obinze. Mild bite was reported Royce for the 60% ( $\frac{v}{v}$ ) formulation.

### Correlation between Concentration of Lemon Grass Oil and Mean Protection Time

$r = \frac{[\sum(x-x)(y-y)]}{\sqrt{\sum(x-x)^2 \sum(y-y)^2}}$ , Where 'r' is correlation coefficient

$$\sum x=19.04, \sum y=13.53, \sum xy=102.12, \sum x\sum y=71.4$$

$$\text{Therefore, } r=30.72/32.57=0.94$$

Value of correlation coefficient 'r' indicates that there is close association between concentrations of the Lemon grass oil formulations and mean duration protection time against mosquitoes. To test whether the observed correlation is due to chance or not, a student t- test is used to determine the significance at 5% level.

$$t = \{r\sqrt{[(n-2)/(1-r^2)]}, df = n-2$$

$$t = \{0.95\sqrt{[3/(1-0.885)]}\} = 4.79, df = 3$$

This is significant at 5% level, confirming the significance of the apparent association between concentrations of the formulations cream and mean duration protection time.

### Adulticidal Effects

Observations for effect of the 2 formulations showed varying levels of paralyzing and knock down effects. Observation showed that the 60% ( $\frac{v}{v}$ ) formulations had the higher knock down action across the three test locations, by causing fast immobilization or paralyzing effect on some mosquitoes that were at close range to treated volunteer's body. The affected mosquitoes were immobilized and can easily fall off the volunteers' hand to the ground. In all, only 14 mosquitoes demonstrated this fast knockdown effect of the volatile oil during the two-month study and the observation was made only in Royce. A random specie identification shows that 81.7% of the collected mosquitoes were *A. gambiae* and 18.3% were *An. funestus*.

### Adverse Reaction Enquiry Result

The volunteers were also asked on the occurrence of any skin reaction as a result of the topical application of the repellents. None of the volunteers reported any adverse reaction on the body parts to which the repellents were applied (arm, leg and face) across the three test locations. This was also confirmed by a preliminary physical observatory test carried out on the volunteers by a dermatologist. However, volunteers reported high sensation of warmth (heat) as a result of application of the test lotions.

### Discussion

Repellents are substances that act locally or at a distance, deterring an arthropod (insect/mosquito) from flying to, landing on or biting human or animal skin (Sah *et al.*, 2010). Volatile oils of Citronella have shown from this study, to possess great mosquito repelling abilities, as well as adulticidal effects. The result showed that all the two types of repellent formulations offered a considerable amount of protection from mosquito species. Findings from previous research gave similar results. For instance, Ansari & Razdan (1994) in their study on Repellent action of essential oils of *Cymbopogon martinii*, reported 100% repellency against Anopheles sp. mosquitoes in field tests for 12 h.

The highest biting rate reported for Royce can be attributed to the fact that Royce, being an urban area has many economic activities taking place. These activities would also lead to high volume of waste generation. With increased population, poor housing design, poor sanitation practice, poor sewage management and poor waste disposal methods in the capital City, it is expected that there would be large numbers of mosquito because the immediate environment offers conducive breeding place for mosquito.

The high percentage repellence and complete protection time offered by the 60% (v/v) formulations of volatile oil of *Cymbopogon winterianus*, can be attributed to the presence of citronellal, geraniol, citronellool, geranyl acetate (Nakahara *et al.*, 2003), which have been shown to have repellent effects (Salkulku, 2009). Similar results from studies on mosquito repellent of Lemon grass were obtained by Oyedele *et al.*, 2002 & Lawal *et al.*, 2012). Syed and Leal (2008) carried

out a field study in Bolivia with *C. winterianus*. The result showed that topical application of the essential oil offers 74% protection against *An. darlingi* for 2.5h 95% protection against *Mansonia spp.* for 2.5 hours. In a laboratory study conducted by Salkulku and colleagues in 2009, methanol leaf extract of *C. winterianus* was formulated and applied topically (2.5mg/m<sup>2</sup>). It offered 78.8 % protection against *An. arabiensis* for 12 hours (Salkulku *et al.* 2009).

This study shows that at higher concentrations, *C. winterianus* oil offer higher protection from mosquito species. This was evident from the correlation coefficient 'r' that indicated that there is significant statistical association  $r=0.94$  ( $p<0.05$ ) between concentrations of the Lemon grass oil formulations and mean duration protection time against mosquitoes. Thus, it is not by chance. in a study to demonstrate the repellent activity of *C. winterianus* oil against *Aedes aegypti* mosquitoes, Murugan *et al.*, (2012) demonstrated the microencapsulation method based on coacervation method, in which 15%, 30% and 50% repellency effect was studied, 50% concentrated repellents gave the best mosquito repellency rather than the other two.

The results also indicated that the test oil afforded whole night and to a greater extent, dawn and dusk protection at 30% (v/v) concentration in the olive oil bases. Malaria mosquitoes bite mainly during night, dusk and dawn. Volatile oil of Lemon grass vaporizes easily, reducing the protection it offers, because the oils rapidly evaporate causing loss of efficacy and leaving the user unprotected (Das *et al.*, 2003). However, by mixing the essential oil of *C. winterianus* with a large molecule like vanillin (5%) protection time can be considerable prolonged by reducing the release rate of the volatile oil (Tawatsin *et al.*, 2001). Similarly, a randomised, double-blind control trial of *C. winterianus* repellent against mosquito in Bolivia by Hill *et al.*, (2007), proved that 100% essential oil of *C. winterianus* combined with vanillin 5% applied topically offered 100% protection against *Ae. aegypti* for 6.5 hours, 100% protection against *C. quinquefasciatus* for 8 hours and 100% protection against *An. dirus* for 8 hours.

Observation on the adulticidal activity formulations indicated that the 60% (v/v)

Table 1: Monthly and average mosquito biting rate (24hrs/month) on treated and control volunteers

Formulation/Month	Ihiagwa		Royce Road			Obinze	
	Treated	Control	Treated	Control	Treated	Control	
<b>30% (v/v) of <i>C. winterianus</i> in olive oil (B<sub>1</sub>)</b>							
September	15	147	22	187	15	136	
October	23	161	31	245	19	157	
Total	38	308	53	432	34	293	
<b>60% (v/v) of <i>C. winterianus</i> in olive oil (B<sub>2</sub>)</b>							
September	11	143	19	197	13	161	
October	21	198	25	241	20	227	
Total	32	341	44	438	33	388	

Table 2: Monthly percentage repellents of the formulations in the three locations

Formulation/Month	Percentage repellent in the three locations		
	Ihiagwa	Royce Road	Obinze
<b>30% (v/v) of <i>C. winterianus</i> in olive oil (B<sub>1</sub>)</b>			
September	89.80	88.23	88.97
October	85.71	87.35	87.90
Total	87.66	87.77	88.40
<b>60% (v/v) of <i>C. winterianus</i> in olive oil (B<sub>2</sub>)</b>			
September	92.31	90.34	91.92
October	89.39	89.63	91.19
Total	90.62	90.00	91.50

Table 3: Complete Protection Time for 24hrs/month testing for September

Lotion type	Ihiagwa	Royce Road	Obinze
	CPT	CPT	CPT
Formulation B <sub>1</sub>	19.2	20.3	19.5
Formulation B <sub>2</sub>	21.6	18.9	20.8

Table 4: Whole night biting rates for 24hrs/month test duration

Lotion type	Ihiagwa		Royce Road		Obinze	
	T	C	T	C	T	C
Formulation B <sub>1</sub>	+	++	+	++	0	++
Formulation B <sub>2</sub>	0	++	+	++	0	++

**Key:** T=Treated, C=control, 0=No bite, +=Mild bite, ++=Heavy bite

formulation had some adulticidal action by causing fast immobilization or paralyzing effect on some mosquitoes that were at close range to treated volunteer's body. The affected mosquitoes were immobilized and can easily fall off the volunteers' hand to the ground. Boonyuan *et al.*, (2014) in their study "Excito-Repellency of Essential Oils against an *Aedes aegypti* (L.) Field Population in Thailand", they reported that the insecticidal activities of the essential oils lemongrass and citronella oil are comparable and more potent than some other essential oils such as basil, cloves, and eucalyptus. In another laboratory study on the adulticidal efficacy of Lemon grass, Phasomkusolsil and Soonwera, (2011), reported that Lemongrass essential oil at 10% concentration killed 100% of all three species of mosquitoes within 24 hours of exposure.

The testing was conducted by the standard WHO protocol using diagnostic kits that exposed mosquitoes in tubes to paper impregnated with the essential oils. Additionally, citronella oil at 10% also killed 100% of *Culex quinquefasciatus* and *Anopheles dirus* and 97.6% of *Aedes aegypti* adults in 24 hours post exposure.

A preliminary dermatological enquiry on the effects from the topical application of all the repellent formulations and oral interview of the volunteers for the study showed that there was no adverse impact in any form on their skins during the two-month study. Documented evidence has shown that repellents of plant origin do not pose hazards of toxicity to humans and domestic animals and are easily biodegradable. Several studies have reported the safety natural products when compared to that of synthetic compounds (Mittal and Subbaro, 2003). Citronella oil has

been used as a natural insect repellent for more than 50 years and is unlikely to cause harmful effects on humans, pets, the environment, and the ecosystem, as compared to synthetic insecticides (EPA, 1997; N'Guessan *et al.*, 2010).

### Conclusion

Volatile oils of *C. winterianus* possess mosquito (*A. gambiae* and *An. funestus*) repellent efficacies. This repellent efficacy is improved when the concentration is increased and when, especially at higher concentrations and when mixed with a large molecule. Another finding from this study is that volatile oils of *C. winterianus* possess adulticidal effects, in which mosquitoes are immobilized and paralyzed on contact with a skin treated with the formulation. Malaria mosquitoes bite mainly during night, dusk and dawn. Consequently, the oil of *C. winterianus* could be used as an alternative, relatively safe, natural, insect-repellent to protect people from mosquito bites at night when they are on bed, and at dawn and dusk when often due to poor housing and harsh climatic conditions as well as social and religious responsibilities stay outside their houses. Since the extraction technique is simple, the colour, fragrance and consistency of the lotions remained unchanged during the two months' study, and the oil base (olive oil) is readily available with a religious acclaimed benefit, the local people could be taught how to prepare the lotions. The formulation could equally help to supplement the protection afforded by window and door nets for those who because of cost and/or odour of permethrin do not like to sleep under insecticide-treated bed nets. This study thus concludes that the topical application of *C. winterianus* oil to human skin can be used as a

potential alternative for outdoor activities on the basis of its high repellency effect against mosquito bites and on the participants' high satisfaction with its relative little to no toxicity and irritation to the skin

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