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Full Length Research Paper

Repellence effectiveness of essential oils from some Tanzanian *Ocimum* and *Hyptis* plant species against afro-tropical vectors of malaria and lymphatic filariasis

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Essential oils from three plant species growing in Dar es Salaam, Tanzania, *Ocimum gratissimum* (OG), *Ocimum tenuiflorum* (OT) and *Hyptis suaveolens* (HS) all from family Labiateae, were extracted by hydrodistillation and evaluated for laboratory and field based repellency against afro-tropical vectors of malaria and lymphatic filariasis. All oils were found to exhibit high mosquito repellency activity against *Anopheles gambiae* sensu stricto in the laboratory tests with RC_{50} values ranging from 2.0- to 15-$\times10^{-5}$ mg cm^{-2}, whereas standard *N*,*N*-diethyl- 3-toluamide (DEET) was more repelling with RC_{50} value of 0.1×10^{-5} mg cm^{-2}. The formulation containing 10% of *O. gratissimum* provided protection against mosquito bites from *Anopheles funestus* of 70.5 to 95.7%, *A. gambiae* of 63.2 to 91.5% and *Culex quinquefasciatus* of 83.8 to 89.5%. The 30% *O. gratissimum* formulation was more effective with better protection against mosquito bites from *A. funestus* of 84.2 to 96.6%, *A. gambiae* of 73.7 to 91.5% and *C. quinquefasciatus* of 89.5 to 91.5%. The formulation containing 15% DEET showed slightly lower protection against mosquito bites from *A. funestus* of 70.5 to 78.9%, *A. gambiae* of 68.4 to 94.0%, *C. quinquefasciatus* of 85.5 to 89.5% (P<0.05). The individual components of the oils were identified by GC-MS. The implication of these results in the development and promotion of repellent plant extracts for commercialization is of priority in rural Tanzania where whole plants are currently used as repellents against mosquito vectors.

Key words: Repellent plants, Labiatae, *Anopheles gambiae*, *Culex quinquefasciatus*, essential oils.

INTRODUCTION

Mosquitoes are among the most disturbing blood sucking insects afflicting human beings. The behavior of female mosquitoes involving feeding on human blood is responsible for the transmission of a number of diseases. The most prevalent ones being malaria, bancroftian filariasis, yellow fever, dengue fever and several arbovirus infections (White, 1973; Curtis et al., 1991; White, 1996). It is a matter of fact that the transmission of these infections to humans totally depends upon the availability of competent mosquito vectors; hence, mosquito control initiatives remain the most successful mechanism for disease prevention and control.

Among the efforts that have been made in recent decades in seeking to reduce mosquito bite and malaria transmission is the introduction and use of insecticides treated nets (ITNs), especially with pyrethroids, to enhance the protective utility of the nets (Curtis, 1991; Chavasse et al., 1999). Hopes for controlling malaria was revitalized by the demonstration in Tanzania that ITNs can reduce morbidity and mortality (Abdulla et al., 2001). Freshly treated pyrethroid based insecticides on nets are well known for their excito-repellent activity against indoor host seeking mosquitoes (Malima et al., 2008).

Since, unlike ITNs, untreated nets do not repel or kill mosquitoes, they allow mosquitoes to bite through them, or if the net is torn, to enter through a hole. Mosquitoes can bite people before retiring to bed or wait and bite when people get out of the net at night. Hence, the need of an additional mosquito control tool to completely curtail human-mosquito contacts when not under ITNs is urgently needed. Mosquito control and personal protection from mosquito bites are currently the most important measures for the control of malaria. The mosquito repellent products commonly available on the market contain *N*,*N*-diethyl- 3-toluamide (DEET) as the active ingredient (Schreck, 1985). DEET is a wide-spectrum repellent that is effective against mosquitoes and other biting insects (Coleman et al., 1993). However, DEET has operational disadvantages due to its allergic reactions and toxicity to man (Qui et al., 1998). Moreover, it is well known for its ability to act as a good solvent for synthetic materials and plastics, which has led to the search for more safer alternative synthetic and natural repellents (Fradin and Day, 2002; Debboun et al., 2000; Peterson and Coats, 2001; Badolo et al., 2004).

Earlier hopes of malaria eradication by getting rid of mosquito vectors had only limited success due to the development of insecticide resistance by mosquitoes. However, the major jolt to human fight against malaria has come from the emergence of drug resistant strains of *Plasmodia*. Chloroquine and sulfadoxine/pyrimethamine (SP), the most widely used cheap and effective drugs for clinical treatment of malaria have lost efficacy in many malaria endemic countries. In sub Saharan Africa, the increasing prevalence of mosquito-transmitted diseases such as malaria and lymphatic filariasis, together with the appearance of mosquitoes which are resistant to pyrethroid insecticides in South and West Africa, stresses the necessity for the search for new additional tools to combat the infection. A viable alternative strategy for controlling mosquito-borne diseases would involve the assessment of plant compounds or extracts as natural repellents or insecticides which will counteract the effect of rapid development of insecticide resistance to the available synthetic insecticides including pyrethroids. Scientific investigations has for long appreciated that, co-evolution has equipped plants with a variety of chemical defenses against insect predators. While aware of this effect, mankind has used plant parts or extracts to control insects since ancient times. Plant derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticidal properties (Balandrin, 1985; Rawls, 1986; Sukamar et al., 1991; Tawatsin et al., 2001; Malebo et al., 2005; Odalo, et al., 2005; Shaalan et al., 2005; Isman, 2006; Ogendo et al., 2008).

Natural insecticides such as pyrethrum, rotenone and nicotine, among others, have been extensively used for insect control (Balandrin, 1985). Limonoids such as azadirachtin and gedunin, present in species from the Meliaceae and Rutaceae are recognized for their toxic effects against insects and are used in several insecticide formulations in many parts of the world (Dua et al., 1995; Nagpal et al., 1996; Nkunya, 2002). About two decades ago, the discovery of insecticide activity of phototoxins present in Asteraceae species has stimulated the interest in botanicals as part of the search for new plant derived insecticides (Rawls, 1986).

The application of repellent plants for mosquito control has been widespread among different communities in Africa (Lindsay et al., 1998; Tawatsin et al., 2001; Seyoum et al., 2002a; Seyoum et al., 2002b; Malebo et al., 2005; Odalo et al., 2005). Two main traditional uses of repellent plants have been recognized: production of fumes from plant materials placed on burning charcoal stove (Pålsson and Jaenson, 1999; Seyoum et al., 2002a) and suspending branches of plant materials near mosquito entry points (Kokwaro, 1993). Recently, it has been shown that fumes produced by burning foliage materials of different plants provide varying levels of protection and that the traditional method can be considerably improved if direct contact of the plant material with burning charcoal is avoided by placing it on a hot plate above a stove (Seyoum et al., 2002a, 2003). Collection and analyses of fumes resulting from thermal expulsion have indicated a richer compositional profile of the volatiles higher than from direct burning (Seyoum et al., 2003).

Natural anti-mosquito agents are likely to offer a cheaper and effective tool that can be used to prevent as well as drive away vector mosquitoes from human dwellings (Seyoum et al., 2002a, b; Omolo et al., 2004; Malebo et al., 2005; Odalo et al., 2005; Shaalan et al., 2005). The potential of plants as sources of essential oils with mosquito repellency effect offers great scientific promise and deplorable opportunity for incorporation into integrated vector management in the control of malaria (Odalo et al., 2005). In our bio-prospecting initiative for safe and effective anti-mosquito agents, we investigated

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essential oils from anti-mosquito plants: *Ocimum gratissimum*, *Ocimum tenuiflorum* and *Hyptis suaveolens* as possible candidates with potential for incorporation in the available strategies as additional supplementary means of malaria prevention and control, and to provide a useful complement to ITNs, particularly for the early part of the evening before bedtime and for people who get out of the net during night time.

MATERIALS AND METHODS

Study area

The laboratory study was carried out at Ubwari field station of the National Institute for Medical Research (NIMR) situated 1 km near Muheza town, whereas field trials were carried out in Zeneti village (5°13'S and 38°39'E, altitude 193 m) situated 24 km near Muheza town. Muheza district is located in Northeastern Tanzania at 4° 52' S; 38° 48' E. Zeneti is a peri-urban village built in ridges and with valleys characterized by swampy ground preferred by the small-scale rice growers. Muheza district located at an altitude of 200 m above sea level, with the main rainy season from March to June, and a short rainy season from October to December with an average rainfall of 1000 mm annually and it is about 40 km to the west coastal town of Tanga. The area experiences a mean temperature of 26°C. Most people in the peri-urban village do practice small-scale farming and some are engaged in small-scale businesses. Mosquito density is high in the main and small rainy seasons in the period between April and August (Clyde, 1967; Ellman et al., 1998). Malaria is holo-endemic with perennial transmission and transmission is the highest during and following the long rainy season, which usually extends from April through August (Clyde, 1967; Ellman et al., 1991). *Anopheles gambiae* sensu stricto Giles and *Anopheles funestus* Giles are the main vectors of malaria in Muheza district where the study village is located (Malima et al., 2008). Entomological inoculation rate (EIR) computations show an average of 405 infective bites per person per year in unprotected individual (Ellman et al., 1998) and up to 7 infective bites per person during peak transmission season (Magesa et al., 1991). *Plasmodium falciparum* has been shown to be an exclusive infecting species accounting for more than 90% of all malaria cases with low levels of *Plasmodium malariae* and *Plasmodium ovale* (Lyimo et al., 1991; Ellman et al., 1998).

Test plants

Two kilograms of leaves and aerial parts from three plants species, namely *O. gratissimum*, *O. tenuiflorum* and *H. suaveolens* (formerly *Ocimum basilicum*) were separately collected for essential oils extraction. The plants were collected from Dar es Salaam at the altitude of 0 to 56 m between January and March, 2007. Selection of plants was based on the ethno-botanical information and taxonomic consideration, the plants are widely used by people in Muheza and other parts of Tanzania to drive away mosquitoes with the smoke created by burning them or simply by laying the branches in the house (Curtis et al., 1991). The identification of plants was done by Mr. L. B. Mwasumbi, an experienced botanist from the Botany Department of the University of Dar es salaam Tanzania.

Extraction and analysis of essential oils

The essential oils were isolated by hydro-distillation using Clevenger apparatus, dried over anhydrous sodium sulphate and the yields were immediately determined. Oils were stored in vials at 4°C until when required for further work. Yield of essential oils were determined in percentage (w/w) using the formula:

$$\text{Yield (\%)} = \frac{\text{Weight of oils extracted}}{\text{Weight of leaves}} \times 100$$

Determination of physical parameters

Physical parameters determined included; viscosity, optical rotation, density, refractive index and solubility in ethanol. Standard methods for determination of the parameters were used. Specific gravity (SG) was determined using a density bottle. Viscosity (v) was measured by Ostwald viscometer at 27°C. Optical rotation (OR) was measured with 343-model polarimeter. Refractive index (RI) was measured with ABBE '60' Refractometer, at 30°C. Solubility in ethanol (SE) was done by complete solubilization of 1 ml of essential oil in 5 ml of 85% ethanol, at 30°C.

Determination of chemical composition of essential oils

Analysis of the chemical composition of the oils was conducted at the Government Chemist Laboratory Agency (GCLA). Gas chromatography-mass spectrometry (GC-MS) analyses were performed with a Varian Q 1200 LMS equipped with a capillary column VF-IMS (30 m × 0.25 mm; coating thickness, 0.25 µm). The analytical conditions included: oven temperature from 50 (1 min) to 230°C at 6°C min^{-1}; injector temperature, 240°C; carrier gas was helium at a flow rate of 1.0 ml/min; the ionization energy employed was 70 eV and the source temperature was 25°C. The oil components were identified by MS library searches (Wiley Nist MS database) using retention indices as pre-selection routine and visual inspection of the mass spectra from literature for confirmation. The relative amounts (%) of the individual components of the essential oil were computed from GC peak areas.

Evaluation of mosquito repellency effectiveness of essential oils

Laboratory based mosquito repellency assay

The mosquitoes used in this study were laboratory reared female *A. gambiae* s.s. (Originally obtained from Njage, 70 km from Ifakara, Southeast Tanzania). The insects were reared according to the WHO (1996) protocol at Ubwari, Muheza, Tanzania. The larvae were reared at 32 to 36°C and fed on TetraMin® (manufactured by Tetra GmbH, Germany). The adults were maintained on 6% glucose solution and the females fed on human blood thrice a week. Rearing conditions in the adult insectaria were maintained at temperatures ranging between 26 and 28°C and relative humidity ranging between 70 and 80%. The repellency of the volatile oils was evaluated using the human-bait technique to simulate the condition of human skin to which repellents were eventually to be applied. Six human volunteers were selected from those who showed mild or no allergic reaction to mosquito bites or candidate oils. The volunteers were asked not to apply any lotions, perfumes, oils or perfumed soaps on the day of the assay. Evaluation were carried out in a 3 × 3.5 × 3.5 m bioassay room, at 26 to 27°C and relative humidity of 65 to 80% using 5 to 7 days old female *A. gambiae* that had been starved for 1 h, but previously fed on 10% glucose solution. Bioassay of the essential oils were carried out in aluminium-frame cages (30 × 30 × 30 cm), with aluminium sheet bottom, window screen (mesh size 156) on top and back, clear acrylic (for viewing) on the right and left sides, and a cotton stockinet sleeve for access on the front, at 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} and 10^{-1} g/ml concentration levels (WHO, 1996). Test solutions (0.5 ml), in high performance liquid chromatography (HPLC) grade acetone, were dispensed on one of the forearms of a volunteer from the wrist to the elbow. The rest of the hand was covered with a glove. HPLC grade acetone (0.5 ml) was dispensed on the other forearm to serve as control. The control and test arms were interchanged regularly to eliminate any bias. Experimental 5-day-old female mosquitoes (100) were released into the bioassay cage in paper cups and left for 3 min to settle. By gently tapping the sides of the experimental cages, the mosquitoes were activated; the control arm were introduced into the cage first and kept there for 3 min. The mosquitoes that landed on the hand were recorded and then shaken off before imbibing any blood. Subsequently, the test arm was introduced into the cage for the same duration and the number of landing insects recorded. The different sample concentrations were tested sequentially starting with the lowest one.

Repellency field trials of essential oils formulation

The field trial was carried out at Zeneti village in Muheza in April at a season whereby high density of *Culex quinquefasciatus* and *A. gambiae* is expected. Ten adult human volunteers applied mosquito repellent formulations and white vaseline alone at 18.15 h each day for 10 days to all over the ankles and feet. The first three pairs of mosquito collectors applied either the essential oil formulations (treatments 1, 2, and 3) or DEET formulation (treatment 4), whereas the fifth pair applied only the white vaseline (treatment 5) and act as controls. A Latin-square design (5 × 5 two blocks) was used whereby each individual sat on a stool 10 m away from the next person. The collectors were allowed for 15 min to apply the treatments and take up their positions ready to start collecting landing mosquitoes from 18.30 to 23.30 h. The sitting positions were randomly assigned for either treatment 1 to 3 or treatment 4 or treatment 5 on the first sampling night. The treatments and the control(s) were then assigned by rotation in consecutive sampling nights in different sitting positions to counteract potential spatial variations of mosquito density in individual sitting positions selected for experimentation. As a result of rotating the treatments nightly between the pairs of collectors over ten days, each pair tested treatments 1 to 3, treatment 4, and treatment 5 on five nights for 6 h. The whole procedure was repeated in a second round with the second set of formulations. After each night's collection, the collectors washed their ankles and feet thoroughly with water and soap to make sure that the treatment effect would not be carried over to the next day. Participants were provided with the antimalarial prophylaxis Malarone® (atovaquone proguanil) and were screened weekly by a specially trained laboratory assistant for malaria parasites with both rapid diagnostic tests and microscopy. None was diagnosed as positive for a blood-stage malaria infection, but if they had, these participants would have been provided free of charge the standard front-line malaria treatment in Tanzania, namely Co-Artem® (Artemether-Lumefantrin). In the case of withdrawal of any participant prior to completion of the study, replacement was made as soon as possible with a new recruit continuing with the field experiment as described earlier.

Data analysis

Laboratory tests

The numbers of probing mosquitoes on treated arm at different concentrations relative to the number probing on the control arm were recorded. Percentage repellency was calculated as:

$$\text{Mosquito repellency (\%)} = \frac{100\ (1 - T)}{C}$$

where T = Number of probing mosquitoes on treated arm; C = Number of probing mosquitoes on control arm.

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Field tests

Hourly collections and night totals for each species were summed and biting rates calculations for treated and untreated (control) collectors were done. The percent protection provided by volatile oiland DEET formulations was expressed as:

$$\text{Percent protection} = \frac{NC - NT}{NC} \times 100$$

where NC = Number of mosquito landings on control; NT = Number of mosquito landings on treated feet

The repellence data were submitted to a Probit analysis and a Chi-square test was used to test as to whether there were significant differences between the formulations (P = 0.05) with POLO PLUS software (Version 1.0, LeOra Software) (Finney, 1971). The repellence concentrations (RC) for 50 and 90% values were obtained from the regression equations calculated with a confidence intervals of 95% (CI 95%).

RESULTS AND DISCUSSION

Table 1 summarizes the yields of essential oils, among the three species of plants; *O. gratissimum* appeared to have the highest amount of essential oils. Table 2 provides physical parameters of the oils. Viscosity appeared to be optimal for effective spreading on the skin surface.

Considering optical rotation, the L-form appeared to be more active than the D-forms. The analysis of the essential oils revealed complex mixtures of constituents. A total of 27 compounds were identified in the essential oils of the 3 plant species by GC-MS (Table 3). Table 4 summarizes the results of the repellent activity of the essential oils of the three plant species. All the three essential oils were found to exhibit high mosquito repellent activity with RC_{50} values ranging from 2.0 to 15×10^{-2} mg cm^{-2} in the laboratory test. However, the observed repellency was lower than that of the standard repellent, DEET that exhibited an RC_{50} value of 0.1×10^{-5} mg cm^{-2} under the same experimental conditions. In the field experiments, *C. quinquefasciatus* was significantly the most abundant human biting mosquito vector species found outside houses (63.9%), whereas *A. funestus*

Table 1. Percentage yields of essential oils from the three plant species.

Species name	Yield (%)
*H. suaveolens (*formerly *Ocimum basilicum)*	0.6-0.8
O. tenuiflorum L.	0.25-0.38
O. gratissimum	0.8-1.2

(45.8%) and *A. gambiae* (39.8%) were together found to be the two most abundant human biting mosquito species inside houses. The number of mosquitoes landing and the overall repellency effectiveness of essential oil formulations and DEET formulation for the duration of 6 h against the total number of field mosquitoes landing on the treated individuals are shown in Tables 5, 6 and 7.

A total of 159 culicine and 83 anopheles mosquitoes were caught from all the treatments and controls. Essential oil formulations showed repellency effect against field mosquitoes landing on the treatments. The formulation containing 10% of *O. gratissimum* provided protection against mosquito bites from *A. funestus* of 70.5 to 95.7%, *A. gambiae* of 63.2 to 91.5% and *C. quinquefasciatus* of 83.8 to 89.5%. The 30% *O. gratissimum* formulation was more effective with better protection against mosquito bites from *A. funestus* of 84.2 to 96.6%, *A. gambiae* of 73.7 to 91.5% and *C. quinquefasciatus* of 89.5 to 91.5%. The formulation containing 15% DEET showed slightly lower protection against mosquito bites from *A. funestus* of 70.5 to 78.9%, *A. gambiae* of 68.4 to 94.0%, *C. quinquefasciatus* of 85.5 to 89.5% ($P<0.05$). Interestingly, *O. gratissimum* formulations were more repellent than DEET against *Anopheles* and *Culex* mosquitoes.

There were slight differences found between the protective efficacies of all treatments, including the controls. Increasing concentration of the *O. gratissimum* essential oil formulation from 10 to 30% resulted into an increase in mosquito repellency activities both indoors and outdoors. The 30% *O. gratissimum* essential oil formulation was found to exhibit promising protective effect which was higher than that of standard DEET ($P<0.05$).

Our findings are supported by previous studies (Omolo et al., 2004; Odalo et al., 2005; Malebo et al., 2005; Kweka et al., 2008, 2009) which revealed similar results from traditional mosquito repellent plants. Interestingly, *O. gratissimum* essential oil formulations were found to be more repellent against *Anopheles* and *Culex* mosquitoes than the approved synthetic repellent, DEET. These results have provided important information for possibilities of developing this plant product for use as a mosquito repellent product to supplement the existing malaria control tools used to reduce man-vector contacts. The observed repellency efficiency of *O. gratissimum* essential oil is attributed to its volatile chemical contents (Table 3). The repellency efficiency can be associated among others to the presence of camphor, eugenol and linalool which are well known for their mosquito repellency efficacy in essential oils (Jembere et al., 1995).

Previous trials carried out in Muheza district, found the level of mosquito repellency of *Ocimum suave* oil formulation to be slightly lower than the standard repellent DEET, but it was within the required repellency effect to substantially reduce mosquito bite incidence (Malebo et al., 2005).

The currently reported repellency effectiveness of *O. suave* oil was found to be higher than that previously reported by Seyoum et al. (2002a), where *O. suave* leaves showed 53.1% repellency effect against *A. gambiae*, whereas direct burning showed 28% which is a lower repellency effectiveness as compared to that observed with thermal expulsion. In the study by Malebo et al. (2005), *O. suave* oils showed overall repellency effectiveness of 72.9% against field mosquito species for the 10% formulation, whereas the 20% formulation exhibited 76.0% which is more superior to previously reported repellency effectiveness from thermal expulsion and burning of *O. suave* leaves.

In this study, *O. gratissimum* essential oil formulation has shown promising efficacy such that it can reduce the incidence of malaria and filariasis if it could be used as a complementary mosquito vector control tool. This product has a potential to improve the livelihood of the rural community by reducing the burden due to malaria and lymphatic filariasis infection.

Conclusion

It is evident that 30% *O. gratissimum* essential oil formulation repelled anopheline and culicine mosquitoes (principal vectors of malaria and lymphatic filariasis) under natural field conditions in Muheza district, Northestern Tanzania. The product offers great scientific promise for incorporation into integrated vector management in the control of malaria and lymphatic filariasis. *O. gratissimum* can be formulated in lotions or ointments for topical application. An alternative approach would be to devise a simple device for controlled slow heating to elicit vaporization of appropriately formulated essential oils.

On this regard, 30% *O. gratissimum* essential oil formulation can offer cost-effective alternative as additional means of household protection, and a useful complement to bed nets, particularly for the early part of the evening before bedtime. On the other hand, it may be worthwhile to re-examine the role of potent essential oils derived from repellent plants for possible production of mosquito coils which could substantially be utilized in repelling of blood-seeking mosquitoes and break the vector-human contacts.

Table 2. Physical parameters of essential oils from *Ocimum gratissimum*, *O. tenuiflorum* and *H. suaveolens*.

Parameter	Plant species		
	O. gratissimum	***O. tenuiflorum***	***H. suaveolens***
Density (g/cm^3)	0.87	0.82	0.83
Optical rotation (OR)	-110	12.9	9.9
Viscosity (mpas)	1.874	1.82	Not determined
Refractive index (RI)	1.516	1.466	1.466
Solubility in ethanol	Readily soluble	Readily soluble	Readily soluble

Table 3. Chemical compositions of essential oils from the plant species.

Compound	Plant species		
	O. tenuiflorum	***O. gratissimum***	***H. suaveolens***
	Peak area (%)	**Peak area (%)**	**Peak area (%)**
Camphene	4.8	6.0	9.5
Sabinene	0.6	0.6	4.6
Myrcene	0.5	0.6	5.8
Cineole (1,8)	6.8	7.9	-
Ocimene (trans)	7.6	9.2	-
Linalool	0.9	1.9	-
Terpinene (alpha)	1.7	2.1	-
Camphor	2.8	7.2	1.9
Eugenol	3.3	19.5	-
Copaene (alpha)	5.6	3.9	-
Caryophyllene	6.9	6.3	6.8
Germacrene	2.1	1.1	6.4
Dipentene	4.1	2.6	-
Alpha-pinene	-	-	1.8
P-cymol	-	-	2.1
Terpinene gamma	-	-	1.8
Terpinole	-	-	1.8
Fenchone	-	-	3.0
Borneol	-	-	7.6
Terpineol (-4-)	-	-	7.0
Bergamotene	-	-	1.8
Humulene	-	-	3.3
Aromadendrene	-	-	1.0
Selinene	-	-	1.7
Spathulenol	-	-	3.7
Bergamotol trans	-	-	5.1

Table 4. Repellent activities (RC values) of plant essential oils.

Plant species	RC_{50} (×10^{-5} mg cm^{-2})	RC_{90} (×10^{-5} mg cm^{-2})
H. suaveolens	15.0 (7.0, 27.0)	560.0 (310.0, 1230.0)
O. Tenuiflorum	8.0 (1.0, 28.0)	1700.0 (550.0, 9600.0)
O. gratissimum	2.0 (0.1, 7.0)	1200.0 (520.0, 4100.0)
DEET	0.1 (0.1)	89 (26.0, 270.0)

Values in parentheses represent lower and upper confidence limits at 95%, P<0.05.

Table 5. Species percent composition of landing mosquitoes collected in 6 hours duration during the five days rounds outside and inside houses.

Mosquito species	Composition (%)	
	Outside houses	Inside houses
C. quinquefasciatus	63.9	14.4
A. gambiae	19.1	39.8
A.s funestus	17.0	45.8
Total	100	100

Table 6. Total number of landing catches of mosquitoes collected per treatment in 6 h duration during the five days rounds outside and inside houses.

Mosquito species	Density outside houses					Density inside houses				
	10% OG	30% OG	40% OG + OT	15% DEET	Vaseline	10% OG	30% OG	40% OG + OT	15% DEET	Vaseline
C. quinquefasciatus	19	10	28	17	73	2	2	2	2	4
A. gambiae	10	0	7	7	20	7	5	4	6	11
A. funestus	5	4	4	2	24	17	3	10	4	4

OG: *Ocimum gratissimum*; OT: *Ocimum tenuiflorum*.

Table 7. Comparison of percent protection from mosquito bites of the tested products in 6 hours duration during the five days rounds outside and inside houses.

Mosquito species	Protection outside houses (%)				Protection inside houses (%)			
	10% OG	30% OG	40% OG + OT	15% DEET	10% OG	30% OG	40% OG + OT	15% DEET
C. quinquefasciatus	83.8	91.5	76.1	85.5	89.5	89.5	89.5	89.5
A. gambiae	91.5	100	94.0	94.0	63.2	73.7	78.9	68.4
A. funestus	95.7	96.6	96.6	98.3	70.5	84.2	47.4	78.9

OG: *Ocimum gratissimum*; OT: *Ocimum tenuiflorum*.

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