Efficacy of *Ocimum suave* volatile oil formulation against man-biting mosquitoes in Muheza, north-east Tanzania

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Abstract: The effectiveness of *Ocimum suave* volatile oil in repelling mosquitoes in the laboratory and natural field conditions was evaluated in Muheza, north-eastern Tanzania. *O. suave* plant materials were steam-distilled and the oil formulation tested for repellency against laboratory and field mosquitoes. A Latin Square Design was applied for randomly assigning the treatment and control to the tests over different nights. The white petroleum formulation containing 10% of *O. suave* volatile oil provided protection against mosquito bites from *Anopheles funestus* (66.67%), *An. gambiae* (45.40%), *An. coustani* (50.00%), *Culex quinquefasciatus* (73.31%), *Cx cinereus* (62.36%) and *Aedes aegypti* (100%). The 20% *O. suave* volatile oil formulation was more effective with better protection against mosquito bites from *An. gambiae* (83.3%), *Cx quinquefasciatus* (75.34%), and *Cx cinereus* (100%) ($P<0.0001$). The formulation containing 10% DEET showed stronger protection against mosquito bites from *An. funestus* (100%), *An. gambiae* (77.3%), *Cx quinquefasciatus* (99.02%), *Cx cinereus* (100%) and *Ae. aegypti* (100%) ($P < 0.0001$). The 20% DEET formulation showed 100% ($P<0.0001$) repellency to all mosquito vector species. In conclusion, our findings indicate *O. suave* volatile oil can offer cost-effective alternative as additional means of personal protection, and a useful complement to other mosquito control measures, particularly for the early part of the evening before retiring to bed.

Key words: *Ocimum suave*, mosquitoes, repellents, Tanzania

Introduction

Among the mosquito-borne diseases occurring in Tanzania, malaria by far is the commonest and of greatest public health importance. In over 80% of the regions, malaria endemicity ranges from hyperendemic to holoendemic. Furthermore, malaria is the largest cause of hospital attendance, hospital admissions and one of the leading causes of hospital deaths in the country (Kitua, 2003). Lymphatic filariasis is also of great public health importance as one of the major causes of disabilities in the more humid parts of the country.

Because of drug and insecticide resistance and social and environmental changes, malaria situation has deteriorated in many parts of the Sub-Saharan Africa. The failing malaria chemotherapy strategy in Tanzania is a strong indication of a medical emergency from multi-drug resistant malaria in the near future. The fact that new, effective and affordable antimalarial drugs by the poor majority are unlikely to be available in the near future, vector control approaches need to be strengthened.

Mosquito control and personal protection from mosquito bites are currently the most important measures for the control of malaria and lymphatic filariasis. Hopes for controlling malaria have recently been revitalized by the demonstration in Tanzania that insecticide treated nets (ITNs) can reduce morbidity and mortality (Abdulla *et al.*, 2001). Although ITNs have been demonstrated to protect people against mosquito bites and reduce malaria morbidity and mortality, in many parts of the world, people may still contract the disease in early evening hours before they go to bed. In such situations, there is need to find protective measures to complement ITNs. Insect repellents have been shown to play an important role in preventing humans from mosquito bites and can therefore be used as supplemental protective measures to ITNs that can easily be adopted in rural communities of Africa (Curtis *et al.*, 1987, 1991; Seyoum *et al.*, 2003).

The use of mosquito repellents is an obvious practical and economical means of preventing mosquito-borne diseases. The most common mosquito repellent formulations available on the market contain DEET (*N,N*-diethyl-3-methylbenzamide). DEET has shown excellent repellence against mosquitoes and other biting insects. However, human toxicity and reactions after the applications of DEET vary from mild to severe (Qiu and McCall, 1998). To avoid these adverse effects, research on repellents that are derived from plant extracts are needed to replace DEET. Already research has demonstrated that traditional mosquito repellent plants are effective against vector mosquito species under laboratory and field conditions (Tawatsin *et al.*, 2001). Despite the widespread use of mosquito repellent plants in Muheza district in

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Tanzania (White, 1973), only few studies have been carried out to demonstrate the effectiveness of the oil from these plants in protecting people from mosquito bites under field natural conditions. On this background, field trials of oils from these plants are required to prove their mosquito repellency effectiveness under natural conditions. We therefore compared the repellence effectiveness of *Ocimum suave* volatile oil with DEET against man-biting mosquitoes in Muheza, north-eastern Tanzania.

Materials and methods

Study area

The study was carried out at Mbaramo in Muheza district (4°52'S; 38°48'E) in north-east Tanzania. Mbaramo is a sub-urban village characterised by ridges and valleys lying at an altitude of 200m. The climate is characterised by two rainy seasons, the main one from March to May and a short rainy season from October to December. The area receives an average rainfall of 1000mm annually with temperature of 26°C. Both malaria and lymphatic filariasis are endemic in the district.

Test plant and extraction of essential oil

O. suave was selected for extraction of oil for the field trial based on its wider use by people in the area. The plant is usually burnt or its branches placed in the house to drive away mosquitoes (Curtis *et al.*, 1991). The *O. suave* plant materials were extracted for volatile oils by steam distillation. Three kilograms of chopped fresh leaves, seeds and flowers were placed in a distillation flask with three litres of water and ceramic anti-bump chips added. The distillation flasks were heated and allowed to boil until the distillation was completed. The distillate was collected in a Buckner flask and cooled in ice. The volatile oil was separated from water by a separating funnel and dried of traces of water by passing through a chromatographic column packed with 2g of anhydrous sodium sulphate. A total of 150ml of *O. suave* volatile oil was collected and kept in a refrigerator at 4°C in a stoppered reagent bottle.

O. suave volatile oil and DEET was formulated in absolute ethanol (v/v) to obtain serial concentrations containing 0.625%, 1.25%, 2.5%, 5% or 10% of *O. suave* volatile oil and DEET, for the laboratory repellence test. The concentrations for the field efficacy test, 10% and 20% (w/w) mixtures of *O. suave* volatile oil and DEET formulations were made up with a mixture of white Vaseline, respectively.

Test mosquitoes

The mosquitoes used in the laboratory based tests were obtained from an *An. gambiae*, R70 strain colony reared at the Tropical Pesticides Research Institute, Arusha. *An. gambiae* females, 3-5 day old were used for the laboratory tests.

Laboratory tests

Mosquito repellency activity of *O. suave* volatile oil against laboratory reared *An. gambiae* was assessed by following a standard method. The mosquito colony was kept in an insectary maintained at 25°C and 50-70% relative humidity. The right arm was used as the test arm and the left one was used as the control. The forearms were washed with water and soap and allowed to dry. The test person cleaned the hands with 70% ethanol (w/w). After air-drying the hands of the test person were covered with gloves to cover the hands and flexor region of the forearms. The exposed part of the right arm 42 cm^2 was treated for each test with 10ml containing 0.625%, 1.25%, 2.5%, 5% or 10% of serial concentrations of *O. suave* volatile oil in absolute ethanol. The left arm was treated with 10ml of absolute ethanol only. Test cages were positioned securely on the arms of the volunteer with rubber bands to ensure that only the test areas were exposed to mosquito bites. Fifty non-blood fed female mosquitoes starved for three days were introduced into each cage and the numbers of probing mosquitoes were counted at the end of 30 seconds. The test procedure was replicated twice. DEET, applied in a similar manner was used as the standard mosquito repellent.

Field trials

The field trial was carried out in April 2002 when high densities of *Cx quinquefasciatus, An. gambiae* and *An. funestus* were expected (Mboera *et al.*, 1997). Prospective study participants were screened for malaria one week before the field trial. Those tested blood slide positive were treated with sulphadoxine/pyrimethamine and excluded from the study. Study participants who were blood slide negative (male adults more than 45 kg bodyweight each) were given daily two proguanil (Paludrine®) tablets as malaria chemoprophylaxis one week before and after the start of the field trial.

Six adult human volunteers applied the mosquito repellent formulations and white Vaseline alone all over the ankles and feet at 18:15 hour each day for 18 days. The first two pairs of mosquito collectors applied either *O. suave* volatile oil formulation (treatment 1) or DEET formulation (treatment 2), whereas the third pair applied only the white Vaseline (treatment 3) and acted as controls.

A Latin-square design (3 x 3 two blocks) was used whereby the individuals sat 10m apart. The collectors were allowed 15 minutes to apply the treatments and take up their positions ready to mosquito landing catches, which lasted between 18:30 and 21:30 hours. The sitting positions were randomly assigned for either treatment 1, treatment 2 or treatment 3 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 and temporal variations of mosquito density in individual sitting positions selected for experimentation. The whole procedure was repeated in a second round with 20% treatment 1, treatment 2 and treatment 3. After each night's collection, the collectors washed their ankles and feet thoroughly with water and bar soap to make sure that the treatment effect would not be carried over to the next day.

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

Where: NC = Number of mosquito landings on control
NT = Number of mosquito landings on treated feet

Table 1: Percent repellency of *O. suave* essential oil and DEET product against caged *An. gambiae*

Serial dose	Concentration (%)	% repellency *O. suave*	% repellency DEET
1	0.625	68	100
2	1.25	100	100
3	2.50	100	100
4	5.00	100	100
5	10.0	100	100
Mean	3.89	93.6	100
SD	3.8	14.3	0.0
SE	1.7	6.4	0.0

Data analysis

For the 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:

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

Where: T = Number of probing mosquitoes on treated arm
C = Number of probing mosquitoes on control arm

For the field tests, hourly collections and night totals for each species were summed and biting rates calculated for treated and untreated (control) collectors. The percent protection provided by *O. suave* volatile oil and DEET formulations were expressed as:

An analysis of variance and contrast analysis was performed to determine the significant difference among the treatments and the significant variation of mean mosquito density between the first, second, third and fourth hours of treatments tested.

Results

Results of the laboratory tests are summarised in Table 1. In the laboratory reared mosquitoes, the mean repellency of *O. suave* volatile oil and DEET in absolute ethanol formulations at mean concentration of 3.9% was calculated as 93.6% and 100% respectively. There were significant overall differences between the repellency of the treatments, including the controls ($P < 0.001$).

A total of 1567 culicine and 117 anopheles mosquitoes were caught from all the treatments and control for both study rounds, of which 93.0% were *Cx quinquefasciatus* and 5.0% were *An. gambiae* (Table 2).

Table 2: Landing catches and species composition of the mosquitoes

Species	Number landing	% composition
Cx quinquefasciatus	1540	93.0
Cx cinereus	23	1.3
An.gambiae	85	5.0
An. funestus	4	0.2
An.coustani	5	0.3
An. aegypti	4	0.2
Total	1661	100

O. suave oil formulation showed significant repellency effect against field mosquitoes landing on the treatments. The overall repellency effectiveness of *O. suave* oil and DEET formulations for the duration of four hours against the total number of field mosquitoes landing on the treatments is shown in Figure 1.

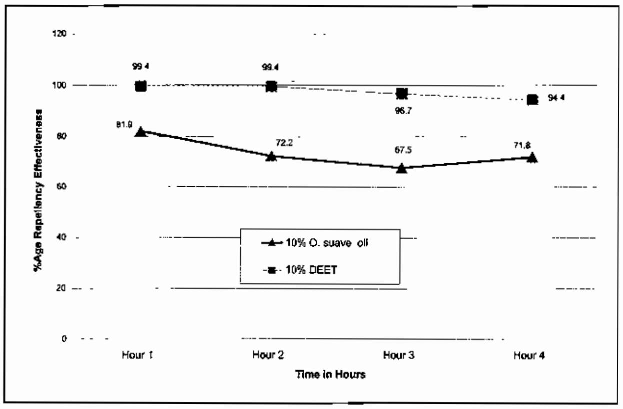

Figure 1: Estimated reduction in mosquito biting activity (% repellency) of 10% *O. suave* oil formulation as compared to DEET

Significant overall differences were found between the protective efficacies of all treatments, including the controls ($P<0.001$). Increasing concentration of the treatments to 20% resulted into a significant increase in mosquito repellency ($P<0.001$).

The 20% *O. suave* oil formulation showed a substantial improvement in protective effect from an overall of 72.9% to 76.0%, where as that of 20% DEET formulation reached 100% protective effectiveness. Decline in repellency effectiveness of *O. suave* oil formulation attributable to the evaporation of the oil was observed with time.

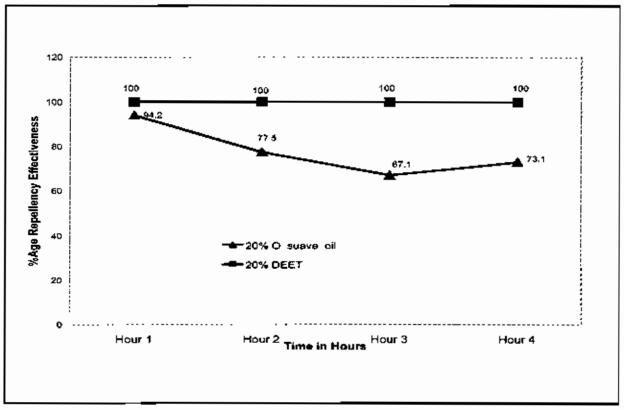

Figure 2: Estimated reduction in mosquito biting activity (% repellency) of 20% *O. suave* oil formulation as compared to DEET

Significant differences were observed between the repellency effect of *O. suave* oil and DEET formulations for the duration of four hours against *Cx quinquefasciatus, Cx cinereus, An. gambiae, An. funestus, An. coustani* and *An. aegypti* ($P<0.001$). White Vaseline was used as blank control, and is used as reference treatment (Table 3).

The overall protective effect for the duration of four hours of a Vaseline jelly containing 10% and of the *O. suave* oil against mosquito bites is shown in Table 4. By doubling the concentration to 20%, *O. suave* oil formulation showed improved protection against bites by *An. gambiae* (83.58%), *Cx quinquefasciatus* (75.34%), and *Cx cinereus* (100%) ($P<0.001$). DEET which was used as a standard showed stronger protection against mosquito bites from all species ($P<0.001$) in a 10% formulation whereas the 20% DEET formulation showed 100% ($P<0.001$) repellency effectiveness to all mosquito vector species.

Table 3: Number of mosquitoes collected with each treatment of DEET or *O. suave* oil

Species	No. mosquitoes intensity in the 1st round			No. mosquitoes intensity in the 2nd round		
	Control	10% DEET	10% *O. suave*	Control	20% DEET	20% *O. suave*
Cx quinquefasciatus	715	7	191	503	0	124
Cx cinereus	16	0	6	1	0	0
An. gambiae	44	10	24	6	0	1
An. funestus	3	0	1	0	0	0
An. coustani	2	2	1	0	0	0
An. aegypti	4	0	0	0	0	0
Total	784	19	223	510	0	125

Table 4: Comparison of repellent effect of DEET and *O. suave* at 10 and 20% concentrations

Species	Percent repellency				
	Control (100%Vaseline)	10% DEET	10% *O. suave*	20% DEET	20% *O. suave*
Cx quinquefasciatus	0.00	99.02	73.31	100	75.34
Cx cinereus	0.00	100	62.36	100	100
An. gambiae	0.00	77.30	45.40	100	83.58
An. funestus	0.00	100	66.67	100	100
An. coustani	0.00	100	50.00	100	100
An. aegypti	0.00	100	100	100	100

Discussion

Our findings have shown that *O. suave* oil formulation under natural field conditions is effective in repelling *An. gambiae* and *Cx quinquefasciatus* the principal malaria and/ lymphatic filariasis vectors in Tanzania. The method used in this study was simple and highly adaptable under varied local situations in rural communities of Tanzania, whereby traditional methods such as direct burning of plant materials and rubbing the leaves of the plants and hanging them in the vicinity of the bed are being practiced to repel mosquitoes away from houses (Seyoum *et al.*, 2002).

In recent years, extracts of several traditional mosquito repellent plants including *Azadirachta indica* A. Jusss, *Ocimum basilicum* L, *O. canum, O. gratissimum* L, *O. americanum* L, *O. tenuiflorum* L, *O. kilimandscharicum, O. suave, Cymbopogon nardus* Rengle, *Limonia acidissima* L, *Alpinia galanga* L, *Hyptis suaveolens, Piper guineense, Syzygium aromaticum* L, *Curcuma longa* L, *Cymbopogon winterianus* Jowitt, *Corymbia citriodora, Citrus hystrix* DC, *Lantana camara, Lippia ukambensis, L. javanica* and *Thymus vulgaris* L, have been studied as potential mosquito repellents (Lindsay *et al.*, 1998; Tawatsin *et al.*, 2001; Seyoum *et al.*, 2002). Studies in western Kenya have shown that thermal expulsion of *Corymbia citriodora* (formerly *Eucalyptus maculates citriodora*), *Ocimum suave* and *O. kilimandscharicum* repelled up to 74% of host-seeking *An. gambiae* s.s. in semi-field experimental huts, employing a simple modification of classical African traditional stoves (Seyoum *et al.*, 2002). Furthermore, evaluation of intact potted plants of *O. americanum, L. camara* and *L. ukambensis* also indicated reduction in mosquito biting by *An. gambiae* in semi-field experimental huts by 30–40% (Seyoum *et al.*, 2002).

Although the level of repellency of *O. suave* oil formulation in this study was lower than that of the standard repellent DEET, it was however, within the required repellency effect to substantially reduce mosquito bites. The plant may therefore be able to offer an added vector mosquito control tool in an integrated vector control programme. *O. suave* oil formulation is likely to offer a cheaper and effective tool that can be used to prevent as well as drive away vector mosquitoes from human dwellings.

The mosquito repellency of *O. suave* oil obtained in this study is significantly higher than that previously obtained from thermal expulsion and directs burning of its leaves and seeds (Seyoum *et al.*, 2002). Recent studies by Seyoum *et al.* (2002) have shown that when thermally expelled, *O. suave* leaves have 53.1% repellent effect against *An. gambiae* mosquitoes. In this study, *O. suave* oils showed overall repellent effectiveness of 72.9% and 76.0% against field mosquito species for the 10% formulation and 20% formulations. This indicates that oil formulations have more repellent effects than that of burnt *O. suave* leaves.

In addition to their effects on adult mosquitoes, plant extracts have also been shown to have ovicidal/ larvicidal effect against immature stages of *Cx quinquefasciatus* in experiments carried out in Tanzania. In Muheza, Tanzania, Mboera *et al.* (2003) observed a failure of eggs to hatch in water treated with neem seed cake extract. The authors also found that a combination of synthetic oviposition pheromone and neem seed cake extract can offer excellent potential tools that could supplement currently available chemical and biological strategies developed for control of *Cx quinquefasciatus*. In another study in Morogoro, Tanzania, Kabula and Kilonzo (2005) showed that *Tephrosia vogelii* leaf extract has insecticidal effects against *Cx quinquefasciatus* larvae.

In conclusion, *O. suave* oil formulation can significantly repel anopheline and culicine mosquitoes under natural field conditions, and offers great scientific promise for incorporation into integrated vector management in the control of malaria and lymphatic filariasis.

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