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Repellency of essential oils of some plants from the Kenyan coast against *Anopheles gambiae*

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Abstract

Volatile oils extracted by hydrodistillation from six plant species growing in the Kenyan coast, *Croton pseudopulchellus* Pax, *Mkilua fragrans* Verdc. (Annonaceae), *Endostemon tereticaulis* (poir.) Ashby, *Ocimum forskolei* Benth., *Ocimum fischeri* Guerke and *Plectranthus longipes* Baker (Labiateae), were evaluated for repellency on forearms of human volunteers against *Anopheles gambiae sensu stricto*. All oils were found to be more repellent (RC_{50} range = 0.67–9.21 × 10^{-5} mg cm^{-2}) than DEET (RC_{50} = 33 × 10^{-5} mg cm^{-2}). The individual components of the oils were identified by GC–MS and GC co-injections with authentic standards. The repellency of 15 of the main constituents of the different oils (which had not been previously assayed) was evaluated. Although some of these showed relatively high individual repellencies, none was comparable to the parent essential oils. Partial synthetic blends of selected constituents with moderate or relatively high individual repellency against the vector were also assayed. Four of these exhibited activities comparable to or higher than those of the corresponding parent oils, indicating interesting blend effects in the repellent action of the oils against the mosquito. The implication of these results in the utilization of the plants is discussed.

Keywords: Repellent plants; Eurphorbiaceae; Annonaceae; Labiateae; *Anopheles gambiae*; Essential oils

1. Introduction

Mosquito-borne diseases, like malaria, yellow and dengue fevers, are a major threat to over 2 billion people in the tropics (Service, 1993). Integration of disease treatment with vector control (the latter, comprising of insect population control and personal protection from mosquito bites) is considered the most effective means for disease control (Tawatsin et al., 2001). Currently, repellents and insecticide-treated bed nets (ITNs) represent the most practical and economic methods of controlling vectors (Gupta and Rutledge, 1994; Copeland et al., 1995; WHO, 1995).

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The most common mosquito repellent products available in the market contain DEET (*N*,*N*-diethyl-3-toluamide) (Schreck, 1985; McCabe et al., 1954). It is a broad-spectrum repellent that is effective against mosquitoes and other biting insects (Yap, 1986; Coleman et al., 1993). However, its allergic reactions and toxicity to man (Robbins and Cherniack, 1986; Edwards and Johnson, 1987; Qui et al., 1998), as well as its ability to act as a good solvent for plastics and other synthetic materials, has led to the search for alternative synthetic and natural repellents (Trigg, 1996; Trigg and Hill, 1996; Walker et al., 1996; Fradin and Day, 2002; Debboun et al., 2000; Peterson and Coats, 2001; Badolo et al., 2004).

The potential of plants as sources of essential oils or fumigants that are repellent to mosquitoes and other insect pests and vectors is well known (Granett, 1940; Roark, 1947; Snow et al., 1987; Thorsell et al., 1998). Essential oils from a large number of plants, including *Ocimum* spp. (Chokechaijaroenporn et al., 1994; Tawatsin et al., 2001), *Cymbopogon* spp. (Rutledge et al., 1983; Ansari and Razdan, 1995), *Eucalyptus maculata citriodon* (Collins and Brady, 1993), *Pelargonium citrosum* (Matsuda et al., 1996), *Artemisia vulgaris* (Hwang et al., 1985), *Lantana camara* (Dua et al., 1996; Seyoum et al., 2002a,b), *Mentha piperita* (Ansari et al., 2000), *Vitex rotundifolia* (Grayson, 2000), *Curcuma* spp. (Pitasawat et al., 2003), *Conyza newii*, *Plectranthus marrubioides*, *Tetradenia riparia*, *Tarchonanthus camphoratus*, *Lippia javanica* and *L. ukambensis* (Omolo et al., 2004), have been demonstrated to exhibit good repellent activities against mosquitoes.

In our bio-prospecting initiative for useful repellent plants, our overall objective is to identify a pool of candidates with potential for use in African traditional methods of reducing human–vector contacts, such as fumigation of households by direct burning, thermal expulsion from hot surfaces or use of intact potted plants (Pålsson and Jaenson, 1999a,b; Seyoum et al., 2002a,b, 2003), and as sources of essential oils or specific components that can be incorporated in personal protection products (Omolo et al., 2004, 2005). As part of these studies, we report here the repellent activities of essential oils of six plants collected from the coastal region of Kenya and those of some of their constituents and partial synthetic blends against *An. gambiae s.s.*

2. Experimental

2.1. Plant materials

The leaves, flowers, or whole aerial parts of the plants were collected from the coastal region of Kenya at altitudes of 0–1829 m in June and December 2001. Selection of plants was based on chemotaxonomic (phytochemical) consideration and ethnobotanical information. The collected plants were identified at the Department of Botany, University of Nairobi (UoN), Kenya. Voucher specimens of the plant materials were deposited at the UoN Herbarium: *Croton pseudopulchellus* (SGM-2001/2), *Mkilua fragrans* (SGM-2001/1), *Endostemon tereticaulis* (SGM-2001/17), *Ocimum forskolei* (SGM-2001/16), *Ocimum fischeri* (SGM-2001/18) and *Plectranthus longipes* (SGM-2001/15). The plant materials were dried under shade for 1 week before hydrodistillation.

2.2. Extraction and analysis of essential oils

The essential oils were isolated by steam–distillation using Clavenger apparatus, dried over anhydrous sodium sulphate, and stored in amber-coloured vials at 0 °C until required for further work.

Characterization, identification and determination of relative amounts of the components of the essential oils from the selected plants were performed through gas chromatography (GC), gas chromatography–linked mass spectrometry (GC–MS), and GC co-injection of the essential oils with standards. GC analyses were carried out on a Hewlett Packard (HP) 5890 Series II fitted with a split-less capillary injector system, 50 m × 0.20 mm (i.d.) × 0.33 μm (film thickness) cross-linked methylsilicone capillary column, and FID coupled to Hewlett Packard HP 3393A Series II integrator. Analytical conditions were: split ratio, 1:60; injector and detector temperature 250 °C; oven temperature programme, 50 °C (5 min) to 100 °C at 10 °C/min, to 180 °C at 2 °C/min, to 250 °C at 5 °C/min. The carrier gas was nitrogen at a flow rate of 0.84 ml/min. The flow rates of air and hydrogen were 400 and 30.5 ml/min, respectively.

Qualitative and quantitative analyses were done by combined gas chromatography–mass spectrometry (GC–MS). HP 8060 Series II gas chromatograph coupled to VG Platform II mass spectrometer (manu-

factured by Micromass, UK, formerly known as VG Biotech) was used for identification of the essential oil constituents. The column used was similar to the one for GC analysis except for the film thickness (0.5 μm). The GC operating conditions were also the same as described above but using helium as the carrier gas. MS conditions were as follows: ionization potential, 70 eV; ion source temperature, 180 °C; resolution, 1000; scan time, 1 s; interscan delay, 0.5 s. and ionization current, 1 A. The instrument was calibrated using heptacosafluorotributyl amine, $[CF_3(CF_2)_3]_3N$ (Apollo Scientific Ltd., UK). The preliminary identification of the constituents was based on the computer matching of mass spectral data of the essential oil components against standard Wiley and National Institute of Science & Technology (NIST) library spectra. These were confirmed by comparison of GC retention time as well as co-injection/co-elution with standards where possible. The relative amounts (%) of the individual components of the essential oil were computed from GC peak areas without using correction factors.

3. Mosquito repellency assays of essential oils

The mosquitoes used in this study were laboratory reared female *Anopheles gambiae s.s.* (cultured in 1998 from specimens originally obtained from Njage, 70 km from Ifakara, south-east Tanzania). The insects were reared according to the WHO (1996) protocol at ICIPE, Nairobi, Kenya. The larvae were reared at 32–36 °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 temperatures and relative humidity in the adult insectaria were 26–28 °C and 70–80%, respectively.

The repellency of the volatile oils was evaluated using the human-bait technique to simulate the condition of human skin to which repellents will be eventually applied (Schreck and McGovern, 1989; WHO, 1996). Six human volunteers were selected from those who showed mild or no allergic reaction to mosquito bites or candidate oils. They had no contact with lotions, perfumes, oils or perfumed soaps on the day of the assay. Evaluation was carried out in a 7 m × 5 m × 3 m room, at 30–32 °C and relative humidity of 65–80% using 5–7 days old female *An. gambiae* that had been starved for 18 h, but previously fed on 6% glucose solution. Bioassay of the essential oils was carried out in aluminium-frame cages (50 cm × 50 cm × 50 cm), with aluminium sheet bottom, window screen (mesh size 256) 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^{-1} concentration levels (WHO, 1996). Briefly, test solutions (0.5 ml), in 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 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.

3.1. Repellency assays of individual constituents and selected blends

Of the 65 compounds identified from the essential oils of the 6 plants, 36 were also constituents of another set of plants, which had been previously assayed and reported (Omolo et al., 2004). Of the remaining 29, 15 that were commercially available in sufficient amounts were tested individually in the concentration range 10^{-5} to 10^{-2} g ml^{-1} as detailed above. Synthetic blends of components that demonstrated relatively potent individual repellencies in this and previous (Omolo et al., 2004) studies were also assayed in the same concentration range. The blends were constituted in approximate ratio in which they occur in the essential oils as follows:

1. *M. fragrans* – linalool, camphor, 4-isopropylbenzenemethanol, carvone, caryophyllene oxide (2:1:12:8:77).

2. *C. pseudopulchellus* – linalool, caryophyllene oxide, γ-terpinene, 1-methylpyrrole (45:39:8:8).
3. *E. tereticaulis* – terpene-4-ol, fenchone, γ-terpinene, terpinolene (40:26:21:13).
4. *O. fischeri* – eugenol, terpinolene, β-myrcene (89:6:5).
5. *O. forskolei* – fenchone, camphor, α-pinene, β-myrcene (83:10:2:5).
6. *P. longipes* – carvacrol, caryophyllene oxide, terpene-4-ol, β-myrcene, γ-terpinene, α-terpinene (75:4:2:2:15:2).

3.2. Data analysis

The repellency, expressed as protective efficacy (PE) at each concentration was calculated from six replicates using the formula, PE = ((% control mean − % test mean)/% control mean) (Mehr et al., 1985; Sharma and Ansari, 1994; Matsuda et al., 1996; Yap et al., 1998). The data was transformed and subjected to analysis of variance (ANOVA) (SAS® Institute, 2002). Means were ranked using the Student–Newman–Kuels (SNK) test (SAS® Institute, 2002). Dose-response relationships were determined using probit analyses; and RC_{50} and RC_{75} values obtained from the regression equations (SAS® Institute, 2002).

4. Results

4.1. Repellency assays of the volatile oils

Table 1 summarises the results of the repellent activity of the essential oils of the six plant species. Interestingly, all the six oils were found to be more repellent (RC_{50} 0.67–9.62 × 10^{-5} mg cm^{-2}) than DEET (RC_{50} 3.3 × 10^{-4} mg cm^{-2}) under the same experimental conditions.

4.2. Essential oil composition

The analysis of the essential oils revealed complex mixtures of constituents. A total of 65 compounds were identified in the essential oils of the 6 plant species by GC–MS and GC co-injections with authentic standards (Table 2).

4.3. Repellency assays of individual constituents and partial blends

Table 3 gives RC_{50} values of the 15 constituents assayed. The more potent repellents included carvacrol, 4-isopropylbenzenemethanol, phytol, thymol, 3-carene, myrcene, and 1-methylpyrrole. However, none of these showed repellency comparable to those of the parent essential oil blends.

The repellency data of synthetic partial blends of the more potent repellent constituents of the oils of the six plant species are given in Table 4. The blends corresponding to *C. pseudopulchellus* and *P. longipes* essential oils exhibited higher activities (95% CI) than the respective parent oils. Those of *M. fragrans* and *E. tereticaulis* were comparable to the parent oils. However, those of *O. fischeri* and *O. forskolei* were less repellent than the respective parent oils.

5. Discussion

In the present study, essential oils of six plants that were screened have been found to be between 49- and 3.4-fold more repellent to *An. gambiae s.s.* than DEET. Interestingly, none of the more prominent individual constituents of these plants, which were assayed in this or previous (Omolo et al., 2004) phase of the study, demonstrated repellency comparable to those of the parent essential oils. On the other hand, the

Table 1
Repellent activities (RC values) of plant essential oils

Plant	RC_{50} (×10^{-5} mg cm^{-2})	RC_{75} (×10^{-5} mg cm^{-2})
M. fragrans	9.21 (2.45, 5.34)	481 (11, 145)
C. pseudopulchellus	3.74 (1.85, 4.98)	2503 (458, 1154)
E. tereticaulis	1.52 (1.12, 3.56)	3421.7 (578, 1256)
O. fischeri	0.67 (0.35, 2.51)	791.2 (149, 275)
O. forskolei	9.62 (6.83, 8.95)	3370.7 (546, 1175)
P. longipes	1.93 (1.54, 3.56)	1436.8 (558, 985)

Values in parentheses represent lower and upper confidence limits at 95%.

Table 2
Chemical composition of essential oils of the repellent plants

Compound	% Peak area					
	Mf	Cp	Et	Ofi	Ofo	Pl
α-Amorphene[a]			0.33			
Alloaromandedrene			0.57			t
(+)-Aromadendrene		0.55				
δ-Amorphene[a]		0.64	3.41	1.08		0.69
Benzaldehyde				0.11		
Borneol					0.15	
Bornyl acetate			0.16			
Camphene	3.01	0.15	1.22		0.75	t
m-Cymene	0.24					
2-Carene	0.14		1.61	t	0.20	
3-Carene			0.30	0.38		
o-Cymene	t					
p-Cymene	0.27	6.79	0.52	0.17		9.83
Camphor	0.15		0.34		5.93	t
Carvacrol						47.17
Carvone	0.93					
α-Cubebene		t	0.57	0.99		
p-Cymen-8-ol[a]	0.92					
Caryophyllene oxide	8.63	5.47	0.52	0.38		2.73
β-Caryophyllene	0.25	14.95	4.69	1.13		
α-Caryophyllene[a]				3.08	4.46	3.04
β-Cubebene[a]		0.96	0.14			
(−)-Dehydroaromadendrene	7.50					
Eugenol				19.35		
Fenchone			3.96	0.52	49.86	0.76
α-Fenchol					0.31	
E-β-Farnesene			0.58		0.66	
Geranial					t	
Hexanal	0.59					
Cis-3-hexenol				t		
α-Humulene[a]		3.45	2.69			
Cis-3-hexenylacetate				0.20		
4-Isopropylbenzaldehyde		t				
4-Isopropylbenzenemethanol	1.29					
(−)-Isoledene			t		0.20	
Isocaryophyllene					0.90	8.33
Limonene	2.23	12.48	7.54	1.69	14.08	1.08
Linalool	0.19	6.33		3.50		t
Cis-linalool oxide		t				
Trans-linalool oxide		0.13				
Cis-limonene oxide	0.27					
2-Methylbutanol		0.25				
β-Myrcene		8.22		1.08	2.91	1.71
Myrtenol					0.34	
Methyleugenol[a]	0.34		0.34	0.26		t
1-MethylPyrrole	t	1.11				
Nerolidol			0.26			
Octane		t				
1-Octen-3-ol			0.15	t		
β-Ocimene		1.44	1.89	11.97	2.42	0.13

Table 2 (*Continued*)

Compound	% Peak area					
	Mf	Cp	Et	Ofi	Ofo	Pl
α-Phellandrene		6.70	0.80		0.25	0.41
Phytol	t		t			
α-Pinene	0.75	3.98	5.51	0.56	1.47	0.41
β-Pinene		2.70	5.07	0.85	t	t
Sabinene		0.45			t	t
Cis-sabinene hydrate			0.10	0.10		
Trans-sabinene hydrate		0.10	0.17	t	t	0.13
Terpinolene		0.89	1.99	1.26		t
α-Terpinene						1.57
γ-Terpinene	t	1.13	3.22	0.29	0.37	9.16
Terpine-4-ol		0.24	6.23	0.91	0.68	1.22
α-Terpineol		0.65	0.60		0.70	
Thymol			t			0.28
Thymyl acetate						0.73
α-Ylangene	2.65	0.55	3.00	1.65	0.21	0.82

Isomer not established; 't': trace amount (<0.1%); Mf, Cp, Et, Ofi, Ofo and Pl refer to *M. fragrans*, *C. pseudopulchellus*, *E. tereticaulis*, *O. fischeri*, *O. forskolei* and *P. longipes*, respectively.

[a] Except this all components confirmed by co-injection with standard.

repellency of four of the synthetic blends of selected constituents of the oils were either higher (*C. pseudopulchellus*, *P. longipes*) or comparable (*M. fragrans*, *E. teriticaulis*) to the corresponding parent essential oils (Table 4) indicating additive or synergistic effects of such constituents in conferring the repellent activity of the essential oils. Somewhat lower activities of two of the synthetic blends (*O. fisheri* and *O. forskolei*) suggest that other components that were not included in these blends also contribute to the activities of the parent oils. A similar pattern of results was obtained with synthetic blends of the main components (present in ≥1.5% in respective oils) of the essential oils of six other plants reported previously (Omolo et al., 2004), indicating the generality of blend effects in conferring mosquito repellent properties to this group of phytochemicals.

Blend effects in the bioactivities of plant products now represent a recurring theme in phytochemical

Table 3
Repellent activity (RC values) of selected individual constituents of the essential oils

Compound	RC_{50} ($\times 10^{-5}$ mg cm^{-2})	RC_{75} ($\times 10^{-5}$ mg cm^{-2})
1-Methylpyrrole	367 (143, 785)	8746 (4672, 5635)
2-Carene	6980 (1286, 5690)	18041 (9848, 11451)
3-Carene	85.5 (45, 87)	15802 (5784, 9473)
3-Hexenyl acetate	4416 (1273, 1695)	13417 (765, 1156)
4-Isopropylbenzenemethanol	274 (84, 1236)	738.5 (245, 378)
Benzaldehyde	4583.7 (2315, 1237)	10631.7 (875, 1514)
Carvacrol	24 (17, 27)	410.3 (134, 165)
γ-Gurjunene	2290 (478, 1125)	12447 (956, 1045)
Hexanal	5728 (3658, 4275)	14173 (1127, 2984)
Linalool oxide	65 (39, 57)	15727 (4514, 8473)
Myrcene	84.3 (35, 58)	9400 (569, 892)
1-Octen-3-ol	2521 (789, 1025)	11836 (957, 1284)
Phytol	64 (45, 56)	3826 (674, 986)
Thymol	187.2 (115, 152)	731.1 (95, 124)
Thymyl acetate	4585.2 (1474, 2373)	10632.1 (6862, 9764)
DEET	33 (26, 42)	

Values in parentheses represent lower and upper confidence limits at 95%.

Table 4
Repellent activity (RC_{75} values) of plant essential oil mixtures and synthetic blends of potent repellent constituents

Plant	Essential oil RC_{75} ($\times 10^{-5}$ mg cm^{-2})	Synthetic blend[a] RC_{75} ($\times 10^{-5}$ mg cm^{-2})
M. fragrans	481 (11, 145)	472.2 (18, 195)
C. pseudopulchellus	2503 (458, 1154)	835 (10, 275)
E. tereticaulis	3421.7 (578, 1256)	3866 (115, 1048)
O. fischeri	791.2 (149, 275)	1965.5 (158, 256)
O. forskolei	3370.7 (546, 1175)	7278 (954, 1415)
P. longipes	1436.8 (675, 1086)	641 (235, 354)

Values in parentheses represent lower and upper confidence limits at 95%.
[a] See experimental.

research (Berenbaum and Zangerl, 1996; Cates, 1996; Bekele and Hassanali, 2001; Omolo et al., 2004, 2005), and have important implications for the way bioprospecting for useful plant natural products is carried out and how these products are exploited for practical use. Until the advent of single-component based synthetic repellents, essential oils and their mixtures formed the basis of most commercial repellent formulations for personal protection (Curtis, 1990). Their major handicap has been the relatively high volatility of many of their monoterpenoid constituents, and the resulting rapid loss of activity in personal protection uses, which saw their gradual displacement in favour of synthetic repellents (Fradin, 1998; Goodyer and Behrens, 1998). On the other hand, at household level, it may be worthwhile to re-examine the role of potent essential oils derived from repellent plants in substantially reducing entry of blood-seeking mosquitoes and vector–human contacts. The use of repellent plants as sources of repellent blends has been widespread among different communities in Africa (Pålsson and Jaenson, 1999a,b; Seyoum et al., 2002a,b). Two principal traditional uses have been documented: production of fumes from plant materials placed on burning charcoal stove (Pålsson and Jaenson, 1999a; Seyoum et al., 2002a), and hanging leafy branches near mosquito entry points (Kokwaro, 1993). A recent study has shown that fumes generated by burning foliage materials of different plants give varying levels of protection and that the traditional method can be substantially 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 from such arrangements have indicated a richer compositional profile of the volatiles than from direct burning (Seyoum, 2003). Further studies on the effects of varying periods of post-harvest drying of foliage and different thermal expulsion temperatures and conditions are needed to exploit the full potential of the volatile phytochemical blends available in promising repellent plants. An alternative approach would be to develop a simple device for controlled vaporization of appropriately diluted essential oils, such as that described for control vaporization of transfluthrin, a pyrethroid insecticide, in vegetable oil placed in a tin just above traditional kerosene lamps (Pates et al., 2002).

No study designed to evaluate the efficacy of leafy branches has been reported. However, a variant of this involving intact potted plants has been described. Three species (*Ocimum americanum*, *L. camara* and *L. ukambensis*) from a pool of nine plants evaluated were shown to emit sufficient quantities of volatiles to provide significant repellency against *An. gambiae* under semi-field conditions (Seyoum et al., 2002b, 2003). In view of its convenience and simplicity, screening of a broader profile of potted plants, including those with potent essential oils described in this and previous (Omolo et al., 2004, 2005) reports, is warranted.

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References

Ansari, M.A., Razdan, R.K., 1995. Relative efficacy of various oils in repelling mosquitoes. Ind. J. Malariol. 32, 104–111.

Ansari, M.A., Vasudevan, P., Tandon, M., Razdan, R.K., 2000. Larvicidal and mosquito repellent action of peppermint (*Mentha piperita*) oil. Biores. Technol. 71, 267–271.

Badolo, A., IIboudo-Sanogo, E., Ouédraogo, P.A., Costantini, C., 2004. Evaluation of the sensitivity of *Aedes aegypti* and *Anopheles gambiae* complex mosquitoes to two insect repellents: DEET and KBR 3023. Trop. Med. Int. Health 9, 330.

Bekele, J., Hassanali, A., 2001. Blend effects in the toxicity of the essential oil constituents of *Ocimum kilimandscharicum* and *Ocimum kenyense* (Labiateae) on two post-harvest insect pests. Phytochemistry 57, 385–391.

Berenbaum, M.R., Zangerl, A.R., 1996. Phytochemical diversity: adaptation or random variation? In: Romeo, J.T., Saunders, J.A., Barbosa, P. (Eds.), Phytochemical Diversity and Redundancy in Ecological Interactions. Plenum Press, New York, pp. 1–24.

Cates, R.G., 1996. The Role of Mixtures and Variation in the Products of Terpenoids in Conifer–Insect–Pathogen Interactions. Plenum Press, New York, pp. 179–216.

Chokechaijaroenporn, O., Bunyapraphatsara, N., Kongchuensin, S., 1994. Mosquito repellent activities of Ocimum volatile oils. Phytomedicine 1, 135–139.

Coleman, R.E., Robert, L.L., Roberts, L.W., Glass, J.A., Seeley, D.C., Laughinghouse, A., Perkins, P.V., Wirtz, R.A., 1993. Laboratory evaluation of repellents against four anopheline mosquitoes (Diptera: Culicidae) and two phlebotomine sand flies (Diptera: psychodidae). J. Med. Entomol. 30, 499–502.

Collins, D.A., Brady, J.N., 1993. Assesment of the efficacy of quwenling as a mosquito repellent. Phytother. Res. 7, 17–20.

Copeland, R.A., Walker, T.W., Robert, L.L., Githure, J.I., Wirtz, R.A., Klein, T.A., 1995. Response of wild *Anopheles funestus* to repellent-protected volunteers is unaffected by malaria infection of the vector. J. Am. Mosquito Contr. Assoc. 15, 342–347.

Curtis, C.F., 1990. Appropriate Technology in Vector Control. CRC Press Inc., Boca Raton, FL, pp. 125–128.

Debboun, M., Strickman, D., Solberg, V.B., Wilkerson, R.C., McPherson, K.R., Golenda, C., Keep, L., Wirtz, R.A., Burge, R., Klein, T.A., 2000. Field evaluation of DEET and piperidine repellent against *Aedes communis* (Diptera: Culicidae) and *Simulium venustrum* (Diptera: Simulidae) in the Adirondack mountains of New York. J. Med. Entomol. 37, 919–923.

Dua, V.K., Gupta, N.C., Pandey, A.C., Sharma, V.P., 1996. Repellency of *Lantana camara* (Verbenaceae) flowers against *Aedes* mosquitoes. J. Am. Mosquito Contr. Assoc. 12, 406–408.

Edwards, D.L., Johnson, C.E., 1987. Insect–repellent-induced toxic encephalopathy in a child. Clin. Pharm. 6, 496–498.

Finney, D.J., 1971. Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve, 3rd ed. Cambridge University Press, Cambridge, pp. 9–158, 318.

Fradin, M.S., 1998. Mosquitoes and mosquito repellents: a clinician's guide. Ann. Int. Med. 128, 931–940.

Fradin, J.L., Day, G.B., 2002. Efficacy of DEET and its analogues in mosquito control. Am. Rev. Entomol. 16, 207–209.

Goodyer, L., Behrens, R.H., 1998. Safety and toxicity of insect repellents. Am. J. Trop. Med. Hyg. 59, 323–324.

Granett, P., 1940. Studies of mosquito repellents. II Relative performance of certain chemicals and commercially available mixtures as mosquito repellent. J. Econ. Entomol. 36, 625–629.

Grayson, H.D., 2000. Monoterpenoids. Nat. Prod. Rep. 17, 385–419.

Gupta, R.K., Rutledge, C., 1994. Role of repellents in vector control and disease prevention. Am. J. Trop. Med. Hyg. 50, 82–86.

Hwang, Y.S.M., Wu, K.H., Kumamato, J., Akelord, J., Mulla, M.S., 1985. Isolation and identification of mosquito repellent in *Artemisia vulgaris*. J. Chem. Ecol. 11, 297–298.

Kokwaro, J.O., 1993. Medicinal Plants of East Africa, 2nd ed. Kenya Literature Bureau, Nairobi, pp. 73–245.

Matsuda, B.M., Surgeoner, G.A., Heal, J.D., Tucker, A.O., Maciarello, M.J., 1996. Essential oil analysis and field evaluation of the citrosa plant, *Pelargonium citrosum*, as a repellent against populations of *Aedes* mosquitoes. J. Am. Mosquito Contr. Assoc. 12, 69–74.

McCabe, E.T., Barthel, W.F., Gertler, S.I., Halls, S.A., 1954. Insect repellents. III. *N,N*-diethylamides. J. Org. Chem. 19, 493–498.

Mehr, Z.R., Rutledge, L.C., Morales, E.L., Meixsall, V.E., Korte, D.W., 1985. Laboratory evaluation of controlled release insect repellent formulations. J. Am. Mosquito Contr. Assoc. 1, 143–147.

Omolo, M.O., Okinyo, D., Ndiege, I.O., Lwande, W., Hassanali, A., 2004. Repellency of essential oils of some Kenyan plants against *Anopheles gambiae*. Phytochemisrty 65, 2797–2802.

Omolo, M.O., Okinyo, D., Ndiege, I.O., Lwande, W., Hassanali, A., 2005. Fumigant toxicity of the essential oils of some African plants and constituents of *Conyza newii* (Compositeae) against *Anopheles gambiae sensu stricto*. Phytomed. Res. 12, 241–246.

Pålsson, K., Jaenson, T.G.T., 1999a. Comparison of plant products and pyrethroid-treated bednets for protection against mosquitoes (Diptera: Culicidae) in Guinea-Bissau, West Africa. J. Med. Ent. 36, 144–148.

Pålsson, K., Jaenson, T.G.T., 1999b. Plant products used as mosquito repellents in Guinea-Bissau, West Africa. Acta Tropica 72, 39–52.

Pates, H.V., Lines, J.D., Keto, A.J., Miller, J.E., 2002. Personal protection against osquitoes in Dar es Salaam, Tanzania, by using kerosene oil lamp to vapourize transfluthrin. Med. Vet. Entomol. 16, 277–284.

Peterson, C., Coats, J., 2001. Insect repellents – past, present and future. Pesticide Outlook, 154–158.

Pitasawat, B., Choochote, W., Tuetun, B., Tippawangkosol, P., Kanjanapothi, D., Jitpakdi, A., Riyong, D., 2003. Repellency of aromatic turmeric *Curcuma aromatica* under laboratory and field conditions. J. Vect. Ecol. 28, 234–240.

Qui, H., Jun, H.W., McCall, J.W., 1998. Pharmacokinetics, formulation, and safety of insect repellent *N,N*-diethyl-3-

methylbenzamide (DEET): A review. J. Am. Mosquito Contr. Assoc. 14, 12–27.

Roark, R.C., 1947. Some promising insecticidal plants. Econ. Bot. 1, 437–438.

Robbins, P.J., Cherniack, M.G., 1986. Review of biodistribution and toxicology of the insect repellent *N*,*N*-diethyl-*m*-toluamide (DEET). J. Toxicol. Environ. Health 18, 503–525.

Rutledge, L.C., Collister, D.M., Meixsell, V.E., Eisenberg, G.H.C., 1983. Comparative sensitivity of representative mosquitoes (Diptera: Culicidae) to repellents. J. Med. Entomol. 20, 506–510.

SAS Institute, 2002. Proprietary Software Release 8.1 (TS1M0). SAS Institute Inc., Cary, NC, USA.

Schreck, C.E., 1985. The status of DEET (*N*,*N*-diethyl-*m*-toluamide) as a repellent for Anopheles albimanus. J. Am. Mosquito Contr. Assoc. 1, 98–100.

Schreck, C.E., McGovern, T.P., 1989. Repellents and other personal protection strategies against *Aedes albopictus*. J. Am. Mosquito Contr. Assoc. 5, 247–252.

Service, M.W., 1993. Mosquitoes (Culicidae). In: Lane, R.P., Crosskey, R.W. (Eds.), Medical Insects and Arachnids. Chapman & Hall, London, pp. 723–725.

Seyoum, A.A., 2003. Studies on the use of repellent plants and plant products against the main malaria vectors in Eastern Africa. PhD dissertation. Kenyatta University, Kenya.

Seyoum, A., Palsson, K., Kung'a, S., Kabiru, E.W., Lwande, W., Killeen, G.F., Hassanali, A., Knols, B.G.J., 2002a. Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against *Anopheles gambiae*: Ethnobotanical studies and application by thermal expulsion and direct burning. Trans. R. Soc. Trop. Med. Hyg. 96, 225–231.

Seyoum, A., Kabiru, E.W., Lwande, W., Killeen, G.F., Hassanali, A., Knols, B.G.J., 2002b. Repellency of live potted plants against *Anopheles gambiae* from human baits in semi-field experimental huts. Am. Soc. Trop. Med. Hyg. 67, 191–195.

Seyoum, A., Killeen, G.F., Kabiru, E.W., Knols, B.G.J., Hassanali, A., 2003. Field efficacy of thermally expelled or live potted repellent plants against African malaria vectors in western Kenya. Trop. Med. Int. Health 8, 1005–1011.

Sharma, V.P., Ansari, M.A., 1994. Personal protection from mosquitoes (Diptera: Culicidae) by burning neem oil in kerosene lamps. J. Med. Entomol. 31, 505–507.

Snow, R.W., Bradley, A.K., Hayes, R., Byass, P., Green-wood, B.M., 1987. Does woodsmoke protect against malaria? Ann. Trop. Med. Parasitol. 81, 449–451.

Tawatsin, A., Wratten, S.D., Scott, R.R., Thavara, U., Techadamrongsin, Y., 2001. Repellency of volatile oils from plants against three mosquito vectors. J. Vect. Ecol. 26, 76–82.

Thorsell, W., Mikiver, A., Malander, I., Tunón, H., 1998. Efficacy of plant extracts and oils as mosquito repellents. Phytomedicine 5, 311–323.

Trigg, J.K., 1996. Evaluation of *Eucalyptus*-based repellent against *Anopheles* spp. in Tanzania. J. Am. Mosquito Contr. Assoc. 12, 243–246.

Trigg, J.K., Hill, N., 1996. Laboratory evaluation of *Eucalyptus*-based repellent against four biting arthropods. Phytother. Res. 10, 43–46.

Walker, T.W., Robbert, L.L., Copeland, R.A., Githeko, A.K., Wirtz, R.A., Githure, J.I., Klein, T.A., 1996. Field evaluation of arthropod repellents, DEET and a piperidine compound, A13-37220, against *Anopheles funestus* and *Anopheles arabiensis* in West Kenya. J. Am. Mosquito Contr. Assoc. 12, 172–176.

WHO. International travel and health vaccination requirement and health advice. World Health Organization, Geneva, 1995.

WHO. Report of the WHO Informal Consultation on the Evaluation and Testing of Insecticides. WHO, Geneva, 1996, pp. 32–36, 50–52.

Yap, H.H., 1986. Effectiveness of soap formulations containing deet and permethrin as personal protection against outdoor mosquitoes in Malaysia. J. Am. Mosquito Contr. Assoc. 2, 63–67.

Yap, H.H., Jahangir, K., Chong, A.S.C., Adanam, C.R., Chong, N.L., Malik, Y.A., Rohaizat, B., 1998. Field efficacy of a new repellent, KBR 3023, against *Aedes albopictus* (Skuse) and *Culex quinquefasciatus* (Say) in a tropical environment. J. Vect. Ecol. 23, 62–68.