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ORIGINAL PAPER

Laboratory evaluation of traditional insect/mosquito repellent plants against *Anopheles arabiensis*, the predominant malaria vector in Ethiopia

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Abstract Laboratory study was carried out to evaluate the repellent efficiency of most commonly known four traditional insect/mosquito repellent plants Wogert [vernacular name (local native language, Amharic); *Silene macroserene*], Kebercho [vernacular name (local native language, Amharic); *Echinops* sp.], Tinjut [vernacular name (local native language, Amharic); *Ostostegia integrifolia*], and Woira[vernacular name (local native language, Amharic); *Olea europaea*] against *Anopheles arabiensis* under the laboratory conditions. One hundred (4–5 days old) female *A. arabiensis* were introduced into the both 'control' and 'test' repellent chamber through the hole on top. Traditional charcoal stoves were used for direct burning. The experiment was conducted by applying the smoke into the repellent "test" mosquito cage by direct burning of 25 gm of dried plant materials (leaves and roots) until plant materials completely burned. The number of mosquitoes driving away from the "test" and "control" cage was recorded for every 5 min. In the present investigation, the results clearly revealed that the roots of *S. macroserene* has potent repellent efficiency (93.61%) and was the most effective. The leaves of *Echinops* sp. (92.47%), leaves of *O. integrifolia* (90.10%) and *O. europaea* (79.78%) were also effective. Roots of *S. macroserene* exhibited the highest repellent efficiency by direct burning. The present study identified these four traditional indigenous insect/mosquito repellent plant materials are very promising and can be used as safer alternative to modern synthetic chemical repellents against mosquito vectors of disease. Since people have been using these plants for some medicinal purposes, no side effects have been found.

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Introduction

Malaria continues to be a major public health problem in the tropical world. Of the total world population of about 5.4 billion people, 2,200 million are exposed to malarial infections in some 90 countries or areas. The most recent estimates indicate that there may be 300–500 million clinical cases each year, with countries in tropical Africa accounting more than 90% of these. Malaria is also the cause of an estimated 1–4 to 2–6 million deaths worldwide every year, with more than 90% in Africa alone (WHO 1995; Snow et al. 1999; Breman 2001). About 75% of the people in Africa live in areas of highly endemic stable transmission. Another 18% live in epidemic-prone areas where malaria transmission is seasonal and unstable and where all age groups are vulnerable to infection and disease (WHO 1995).

Malaria is one of the leading causes of morbidity and mortality in Ethiopia. In Ethiopia, about three quarter of the land is malarious and 65% of the populations live in this area. About 64% (36 million) of the total population resides in areas where malaria could occur in epidemic form. Only 1.5% (about 1 million) of the total population is living in highly endemic western low lands (MOH 2000).

Repellents have an important place in protecting man from the bites of insect pests. An effective repellent will be useful in reducing man-vector contact and in the interruption of disease transmission. Repellent compounds should be non-toxic, non-irritating and long lasting (Kalyanasundaram 1991).

Majority of commercial repellents are prepared by using chemicals like allethrin, *N*-*N*-diehyl-*m*-toluamide (DEET), dimethyl phthalate (DMP) and *N*,*N*-diethyl mendelic acid amide (DEM). It has been reported that these chemical repellents are not safe for public use (Zadikoff 1979; Ronald et al. 1985). Repellents of plant origin do not pose hazards of toxicity to human and domestic animals and are easily biodegradable. Natural products are safe for human when compared to that of synthetic compounds (Sharma et al. 1993; Sharma and Ansari 1994).

The repellent properties of plants to mosquitoes and other pest insects were well known before the advent of synthetic chemicals. In southern India, leaves of *Vitex negundo* are burned to repel mosquitoes from houses (Curtis et al. 1989). The plant products have been used traditionally to repel or kill the mosquitoes in many parts of the world (Novak 1985).

The burning of some herbs such as *Artemisia* (Astraceae) and *Calmus* species in rural areas in China is used to keep away mosquitoes and protect cattle from blood sucking insects (Hwang et al 1985). Smoke is still the most widely used means of repelling mosquitoes utilized throughout the rural tropics. Several field evaluations, where plants were burned to repel mosquitoes, have shown good reduction in mosquito landings (Palsson and Jaenson 1990a, b).

Various plants have been reported to possess repellent activity against mosquitoes, *Azadirachta indica*, *Eucalyptus* sp. (Myrtaceae), *Lantana camara* (Verbanaceae), Cymbopogon spp. (Gramineae), *Mentha piperita* (Labiatae), *Tagetes minuta* (Compositae), and some other plant products have been studied more extensively during the past one decade. Smoke produced by burning of dried leaves of *Azadirachta indica* has been used for the protection against mosquitoes since ancient times (Sukumar et al. 1991).

The first methods man used to repel insects were with smoke, covering the skin with mud, or by applying a variety of animal fats and greases (Novak and Gerberg 2005). Smoke is a common method of repelling biting insects that is used throughout the world. Fresh or dried plants are frequently added to fires to enhance the repellent properties of the smoke (Sangat-Roemantyo 1990). The use of traditional repellents is widespread among the different cultures and communities of Africa and beyond (Seyoum et al. 2002).

People are living in the remote rural areas and poorer section of the society endlessly suffers from many vector-borne diseases, particularly malaria due to lack of simple and cheap methods of personal protection. In Ethiopia, rural population have been using some plant materials as a source to drive away mosquitoes/other blood-sucking insects to prevent from their bites. However, the repellent efficiency of these indigenous traditional plant materials has to be evaluated. Therefore, in the present investigation, an attempt has been made to evaluate the most commonly known four traditional insect/mosquito repellent plants against *A. arabiensis* under laboratory conditions.

Objectives

The aim of this study was to evaluate the most commonly known four traditional insect/mosquito repellent plants (*S. Macroserene, Echinops* sp., *O. integrifolia* and *O. europaea*) against *A. arabiensis*, under laboratory conditions.

Materials and methods

Collection of repellent plant material

The four indigenous traditional insect/mosquito repellent plant materials are most commonly known and have been widely used by the local community since ancient times to drive away insects/mosquitoes from the households in Addis Zemen town, South Gonder, Ethiopia. Therefore, repellent plants materials roots of Wogert [vernacular name (local native language, Amharic); *S. macroserene*], leaves of Kebercho [vernacular name (local native language, Amharic); *Echinops* sp.], Tinjut [vernacular name (local native language, Amharic); *O. integrifolia*] and Woira [vernacular name (local native language, Amharic); *O. europaea*] were collected from in and around of Addis Zemen town, South Gonder, North Western Ethiopia.

Adult mosquitoes

The larvae of *A. arabiensis* were collected from the Boye River, Jimma town, Ethiopia and maintained at 70–85% relative humidity, 27±2°C temperature, and 14:10 light and dark photoperiod cycle. The larvae were fed on a powdered mixture of dog biscuits and yeast tablets in the ratio of 3:1. Female mosquitoes (4–5 days old), which had never received a blood meal, were starved for 6 h before experiment start.

Repellent test chamber

Both “test” and “control” cages were made with nylon meshes excluding bottom of the cage to make easy access

of traditional insect/mosquito repellent plant smokes into the "test" cage. The cages measure about (30×30× 62.5 cm). In order to stay away from the effect of smokes "control" and "test" cages were placed on the stand in the adjacent rooms.

Laboratory evaluation of repellent plant materials

The repellent efficiency of four traditional repellent plants Wogert (*S.macroserene*), Kebercho (*Echinops* sp.), Tinjut (*O. integrifolia*) and Woira (*O. europaea*) were conducted by using repellent test cage, under laboratory conditions. The common method/process was adopted to drive away the mosquitoes by direct burning of repellent plants products (leaves and roots). Customarily, the local people are used to apply smoke in the early evening to drive away mosquitoes and other blood-sucking insects by direct burning of traditional insect/mosquito repellent plant materials. Therefore, all the experiments were carried out during early evening.

Repellent efficiency testing method

One hundred (4–5 days old) female *A. arabiensis* were introduced into the both 'control' and 'test' repellent cages through the holes on top. Traditional charcoal stoves were utilized for direct burning. Experiments were carried out by applying smoke into the repellent "test" cage by direct burning of 25 gm of dried plant materials (leaves and roots) until total plant materials completely burned. Simultaneously, exit holes of "control" and "test" cages were opened.

Mosquitoes driving away from 'control' as well as 'test' repellent cage through the exit hole were collected with the help of a suction tube and a flash light by insect collectors. Mosquitoes collected every 5 min were kept separately and recorded up to 35 min. Insect collectors were rotated at an interval of 10 min to avoid bias collections. Experiments were replicated four times for each of the traditional repellent plant. Percentage of repellent efficiency of plant was calculated by using the following formula:

$$\%\ \text{of repellent efficiency} = \frac{C - T}{C} X\ 100$$

where

C = no. of mosquitoes drive away from the control chamber.
T = no. of mosquitoes drive away from the test chamber.

Results

Results of laboratory evaluation of four most commonly known traditional insect/mosquito repellent plants Wogert (*Silene macroserene*), Kebercho (*Echinops* sp.), Tinjut

Table 1 Repellent efficiency of root of Wogert (*Silene macroserene)* against *Anopheles arabiensis*

Minutes	Percent of mosquitoes drive away (control)	Cumulative percent of mosq. drive away (control)	Percent of mosquitoes drive away (test)	Cumulative percent of mosquitoes drive away (test)
00–05	1	1	33	33
05–10	0	1	22	55
10–15	1	2	12	67
15–20	1	3	4	71
20–25	0	3	2	73
25–30	1	4	0	73
30–35	2	6	6	79

Table 2 Repellent efficiency of leaves of Kebercho (*Echinops sp.)* against *Anopheles arabiensis*

Minutes	Percent of mosquitoes drive away (control)	Cumulative percent of mosq. drive away (control)	Percent of mosquitoes drive away (test)	Cumulative percent of mosquitoes drive away (test)
00–05	0	0	79	79
05–10	0	0	8	87
10–15	2	2	3	90
15–20	2	4	0	90
20–25	1	5	1	91
25–30	2	7	0	91
30–35	0	7	2	93

Table 3 Repellent efficiency of leaves of Tinjut (*Ostostegia integrifolia)* against *Anopheles arabiensis*

Minutes	Percent of mosquitoes drive away (control)	Cumulative percent of mosq. drive away (control)	Percent of mosquitoes drive away (test)	Cumulative percent of mosquitoes drive away (test)
00–05	0	0	46	46
05–10	4	4	15	61
10–15	0	4	6	67
15–20	0	4	4	71
20–25	2	6	5	76
25–30	3	9	7	83
30–35	0	9	6	89

Table 4 Repellent efficiency of leaves of Woira (*Olea europea)* against *Anopheles arabiensis*

Minutes	Percent of mosquitoes drive away (control)	Cumulative percent of mosq. drive away (control)	Percent of mosquitoes drive away (test)	Cumulative percent of mosquitoes drive away (test)
00–05	1	1	24	24
05–10	0	1	15	39
10–15	0	1	13	52
15–20	1	2	13	65
20–25	0	2	07	72
25–30	0	2	03	75
30–35	4	6	06	81

(*Ostostegia integrifolia*) and Woira (*Olea europaea*) against *A. arabiensis* are presented in Tables 1, 2, 3 and 4 and Figs. 1, 2, 3 and 4. The results showed that all four traditional insect/mosquito repellents were significantly more effective against *A. arabiensis*. The percentage of repellent efficiency of *S. macroserene* (93.61%) against *A. arabiensis* was higher than *Echinops* sp., *O. integrifolia* and *O. europaea* (92.47%, 90.10% and 79.78%) were, respectively (Tables 1, 2, 3 and 4 and Figs. 1, 2, 3 and 4). Remarkably, the roots of *S. macroserene*, has demonstrated highest repellency (93.61%) and was the most effective. Furthermore, the leaves of *Echinops* sp. (92.47%), *O. integrifolia* (90.10%) and *O. europaea* (79.78%) were also effective and efficient to drive away mosquitoes. Results of laboratory evaluation of four most commonly known traditional insect/mosquito repellent plants against *A. arabiensis* comparing all the four plants are summarized in Table 5 and Fig. 5.

Conclusion and discussion

The present study clearly revealed that the direct burning of four most commonly known traditional insect/mosquito repellent plants Wogert (*S. macroserene*), Kebercho (*Echinops* sp.), Tinjut (*O. integrifolia*) and Woira (*O. europaea*) and application of smokes drive away of *A. arabiensis* mosquitoes from the test cage ranging from 79.78–93.61%, under the laboratory conditions(Tables 1, 2, 3, 4 and 5 and Figs. 1, 2, 3, 4 and 5). A study indicated that the effect of fresh leaves and shoots of *O. forskolei* hanging on walls at the head and foot of beds was tested in Eritrea against *A. arabiensis* and 53% reduction in mean number of mosquitoes per house was achieved (Waka et al. 2004).

Another study conducted to assess the repellent efficiency of various plant products against *A. arabiensis*, *s*tudy conducted to assess the repellency effect of three local plants; fever tea (*Lippia javanica*), rose geranium (*Pelargonium reniforme*), and lemon grass (*Cymbopogon excavatus*) against laboratory reared *Anopheles arabiensis* mosquitoes. All three alcohol plant extracts provided significantly more protection (p=0.012) than alcohol control. The alcohol plant extract of L. javanica provided 76.7% protection against *A. arabiensis* after a 4-h period, and *C. excavatus* and *P. reniforme* provided 66.7% and 63.3% protection for 3 h, respectively. At 5 h post-application, only L. javanica alcohol extract provided appreciable protection (59.3%) against *A. arabiensis* (Govere et al. 2001).

In addition, few more study showed that when people sit outside during the evening, the number of bites by the

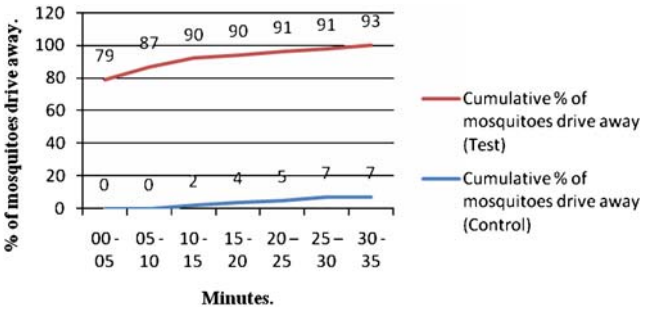

Fig. 2 Repellent efficiency of leaves of Kebercho

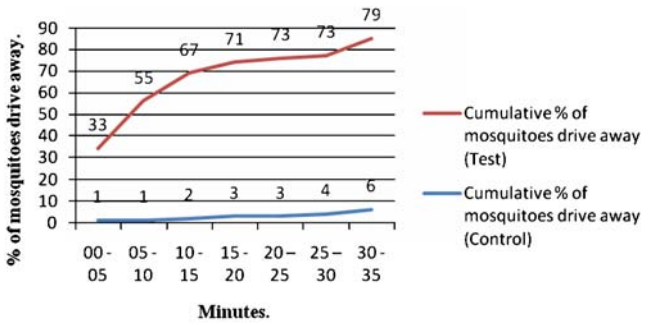

Fig. 1 Repellent efficiency of root of Wogert

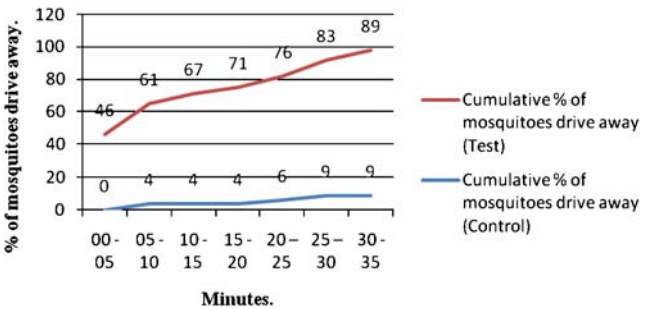

Fig. 3 Repellent efficiency of leaves of Tinjut

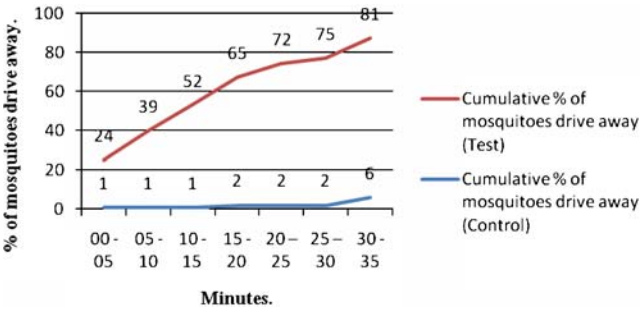

Fig. 4 Repellent efficiency of leaves of Woira

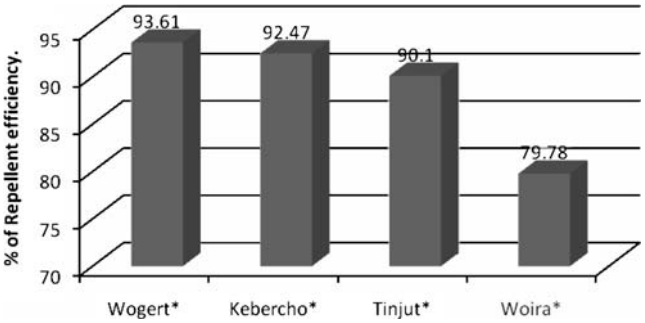

Fig. 5 Percent of repellent efficiency of four traditional insect/mosquito repellent plants

malaria vector *A. arabiensis* was reduced more than threefold (from 26 to 8/person/evening), simply by treating ankles and feet with a consumer brand of DEET repellent (Waka et al. 2006). About 70 plant extracts were tested for their ability to repel the attacks of blood-sucking arthropods. It was found that a CO_2 extract of the seeds of the Mediterranean plant *Vitex agnus castus* (monks pepper) can be used as a spray to keep away especially *Ixodes ricinus* and *Rhipicephalus sanguineus* ticks from animals and humans for at least 6 h. In addition, mosquitoes, biting flies, and fleas are also repelled for about 6 h (Mehlhorn et al. 2005).

There are several studies particularly in Africa found that burned plant materials effective to drive away mosquitoes. Thirteen percent of rural Zimbabweans using plants and 15% using coils (Lukwa et al. 1999). Thirty-nine percent of Malawians burn wood dung or leaves (Ziba et al. 1994). Up to 100% of Kenyans burned plants to repel mosquitoes (Seyoum et al. 2002), and in Guinea Bissau 55% of people burned plants or hung them in the home to repel mosquitoes (Palsson and Jaenson 1999). These studies are indicated that natural fumigants are extensively used, to drive away mosquitoes and other blood-sucking insects across the Africa. In addition, one more study found that traditional insect/mosquito repellent plants provide significant protection from mosquito bites. *Attalea princeps* (name not verified) husks burned on charcoal in the traditional way provided 35% and 51% protection against *An. darlingi* and *Mansonia* spp., respectively (Moore et al. 2007).

Table 5 Percent of repellent efficiency of four traditional insect/mosquito repellent plants

S. no.	Name of the plants	No. of *A. arabiensis* mosquitoes drive away		No. of mosquitoes remain	Percent of repellent efficiency
1	Olea europaea (Woira)	Control	06	94	79.78
		Test	81	19	
2	*Ostostegia integrifolia* (Tinjut)	Control	09	91	90.10
		Test	89	11	
3	*Silene macroserene* (Wogert)	Control	06	94	93.61
		Test	79	21	
4	*Echinops sp.* (Kebercho)	Control	07	93	92.47
		Test	93	07	

The present investigation, established that all four traditional insect/mosquito repellent plants were shown significant repellent efficiency. Remarkably, the roots of *S. macroserene*, has demonstrated highest repellency (93.61%) and was the most effective. Furthermore, the leaves of *Echinops* sp. (92.47%), *O. integrifolia* (90.10%) and *O. europaea* (79.78%) were also effective and efficient to drive away mosquitoes (Tables 1, 2, 3 and 4 and Figs. 1, 2, 3 and 4). In general, the roots of S. *macroserene* (93.61%), leaves of *Echinops* sp. (92.47%), *O. integrifolia* (90.10%) and *O. europaea* (79.78%) were exhibited the significant repellency by direct burning (Table 5 and Fig. 5). Virtually, all four traditional plants showed significant repellency effect more than 90% against *A. arabiensis*, except *O. europaea* (79.78%) and none of them shown negative control.

The existing modern synthetic chemical repellents are generally more expensive. Besides their toxicity, adverse side effect and few of them require electricity for their usage. Traditional repellent plant products are extremely useful essentially in the inaccessible rural areas, where there is lack of electricity. Furthermore, plant-based repellent products are inexpensive, easily available, locally known, and culturally acceptable. Especially in Africa, particularly in a country like Ethiopia, the use of traditional fumigants is well-known. Therefore, we recommend that these indigenous traditional insect/mosquito repellent plant products can be used as potential device to reduce man-vector contact.

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References

Breman J (2001) The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. Am J Trop Med Hyg 64:1–11

Curtis CF, Lines JD, Baolin L, Renz A (1989) Natural and synthetic repellents. In: Curtis CF (ed) Appropriate technology in vector control. CRC Press, Boca Raton, FL, USA, pp 75–92

Govere J, Braack LEO, Durrheim DN, Hunt RH, Coetzee M (2001) Repellent effects on *Anopheles arabiensis* biting humans in Kruger Park, South Africa. Med Vet Entomol 15(3):287–292

Hwang YSM, Wu KH, Kumamato J, Akelord J, Mulla MS (1985) Isolation and identification of mosquito repellent in *Artemisia vulgaris*. J Chem Ecol 11:1297

Kalyanasundaram M (1991) Chemical control of mosquito vectors. ICMR Bulletin 21(8):77–83

Lukwa N, Nyazema NZ, Curtis CF, Mwaiko GL, Chandiwana SK (1999) People's perceptions about malaria transmission and control using mosquito repellent plants in a locality in Zimbabwe. Cent Afr J Med 45:64

Mehlhorn H, Schmahl G, Schmidt J (2005) Extract of the seeds of the plant *Vitex agnus castus* proven to be highly efficacious as a repellent against ticks, fleas, mosquitoes and biting flies. Parasitolo Res 95:363–365

MOH, Ministry of Health, Ethiopia (2000) Malaria and other vector-borne diseases control unit, epidemic forecasting, prevention, detection and control. P-3–15

Moore SJ, Hill N, Ruiz C, Cameron MM (2007a) Field evaluation of traditionally used plant-based insect repellents and fumigants against the malaria vector *Anopheles darlingi* in Riberalta, Bolivian Amazon. J Med Entomol 44(4):624–630

Novak D (1985) Nonchemical approaches to mosquito control in Czechoslovakia. In: Control methodologies, v 2. Academic, San Diego, CA, USA pp. 185–96

Novak RJ, Gerberg EJ (2005) Natural based repellent products: efficacy for military and general public uses. J Am Mosq Cont Assoc 21(4):7–11

Palsson K, Jaenson TG (1999a) Plant products used as mosquito repellents in Guinea Bissau, West Africa. Acta Trop 72:39–52

Palsson K, Jaenson TG (1999b) Comparison of plant products and pyrethroid-treated bed nets for protection against mosquitoes (*Diptera: Culicidae*) in Guinea Bissau, West Africa. J Med Entomol 36:144

Ronald EH, Jan JE, Rigg JM (1985) Toxic encephalopathy in child after brief exposure to insect repellent. Canadian Med Assoc J 132:155–156

Sangat-Roemantyo H (1990) Ethnobotany of Javanese incense. Econ Bot 44:413–416

Seyoum A, Palsson K, Kunga S, Kabiru EW, Lwande W, Killeen GF, Hassanali A, Knols BGJ (2002) Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against Anopheles gambiae: ethnobotonical studies and application by thermal expulsion and direct burning. Trans R Soc Trop Med Hyg 96:225–231

Sharma VP, Ansari MA (1994) Personal protection from mosquitoes (Diptera: Culicidae) by burning neem oil in kerosene. Indian J Med Entomol 31(3):505–507

Sharma VP, Nagpal BN, Srivastava A (1993) Effectiveness of neem oil mats in repelling mosquitoes. Trans R Soc Trop Med Hyg 87:627–628

Snow RW et al (1999) Estimating mortality, morbidity, and disability due to malaria among Africa's non-pregnant population. Bull WHO 77:624–640

Sukumar K, Perich MJ, Boobar LR (1991) Botanical derivatives in mosquito control: A Review. J Am Mosq Cont Assoc 7:210

Waka M, Hopkins RJ, Curtis C (2004) Ethnobotanical survey and testing of plants traditionally used against hematophagous insects in Eritrea. J Ethnopharmacology 95(1):95–101

Waka M, Hopkins RJ, Glinwood R, Curtis C (2006) The effect of repellents *Ocimum forskolei* and deet on the response of *Anopheles stephensi* to host odours. Med Vet Entomol 20(4):373–376

WHO (1995) Vector control for malaria and other mosquito-borne diseases, in WHO Technical Report Series 857, Vol. 857, World Health Organization, Geneva

Zadikoff CM (1979) Toxic encephalopathy associated with use of insect repellent. J Paediatr 95(I):140–142

Ziba C, Slutsker L, Chitsulo L, Steketee RW (1994) Use of malaria prevention measures in Malawian households. Trop Med Parasito 45:70