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Field efficacy of thermally expelled or live potted repellent plants against African malaria vectors in western Kenya

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Summary

OBJECTIVE To estimate the effectiveness of live potted plants and thermal expulsion of plant materials in repelling African malaria vectors in traditional houses in western Kenya.

METHODS *Ocimum americanum*, *Lantana camara* and *Lippia ukambensis* were tested in live, intact potted form whereas leaves of *Corymbia citriodora*, leaves and seeds of *O. kilimandscharicum* and *O. suave* were tested by thermal expulsion from modified traditional stoves. A latin square design was applied for randomly assigning the treatment and control plants to experimental houses over different nights.

RESULTS All plant species showed significant repellency against *Anopheles gambiae sensu lato* Giles (Diptera: Culicidae) (81.5% *An. arabiensis* Patton and 18.5% *An. gambiae sensu stricto* Giles), the main vectors of malaria in Africa, with the highest repellency by *C. citriodora* (48.71%, $P < 0.0001$) followed by an equal level of repellency of *O. kilimandscharicum* and *O. suave* (44.54%, $P = 0.001$) during application of plant material by thermal expulsion. All three plant species also showed a residual effect against *An. gambiae s.l.* with 36–44% repellency post-application period (22.30–06.30 hours) after a period of thermal expulsion. Similarly, intact potted plants of *O. americanum* and *L. camara* repelled *An. gambiae s.l.* significantly (37.91%, $P = 0.004$; and 27.22%, $P = 0.05$, respectively). Thermal expulsion of leaves and seeds of *O. kilimandscharicum* significantly repelled *An. funestus* Giles, although none of the potted plants repelled this species.

CONCLUSION Both methods of application may offer cost-effective alternatives as additional means of household protection, and a useful complement to bed nets, particularly for the early part of the evening before bedtime.

keywords repellent plants, mosquito, malaria, thermal expulsion, potted plants, Kenya

Introduction

Malaria remains an important public health problem, and is endemic in more than 100 countries in the world (Remme *et al.* 2001). The major impact of malaria is in sub-Saharan Africa where at least 90% of deaths from malaria occur (Greenwood & Mutabingwa 2002). Because of the poor performance of health service delivery systems for malaria control and other diseases, vector control has had limited success in highly endemic countries (Breman 2001). There is, however, growing interest in the control of mosquitoes both with classical vector control technologies to prevent and control epidemics and personal protection methods such as insecticide-treated bed nets (Lengeler & Snow 1996; Nevill *et al.* 1996; Schellenberg *et al.* 2001; Guyatt *et al.* 2002; Maxwell *et al.* 2002) and insect repellents (Curtis *et al.* 1987, 1991).

Although insecticide-treated bed nets protect against mosquitoes and malaria in many parts of the world, people may contract disease in the early evening before they retire to the confines of the net, since exposure to malaria vectors and nuisance mosquitoes starts in the early evening (Maxwell *et al.* 1998). Thus, there is the need to find supplemental protective measures to insecticide-treated nets that can easily be adopted in rural communities of Africa.

In an effort to develop low cost plant-based household protection methods that can be used by communities with minimal external input, several plant species were recently evaluated in terms of their repellent properties under semi-field experimental huts in western Kenya (Seyoum *et al.* 2002a,b). Thermal expulsion of *Corymbia citriodora* (previously referred to as *Eucalyptus maculatus citriodora*), *Ocimum suave* and *O. kilimandscharicum* repelled up to 74% of host-seeking *Anopheles gambiae sensu stricto* in semi-field experimental huts, using a simple modification of typical African traditional stoves (Seyoum *et al.* 2002a). Furthermore, intact potted plants of *O. americanum, Lantana camara* and *Lippia ukambensis* also reduced biting by *An. gambiae* in semi-field experimental huts by 30–40% (Seyoum *et al.* 2002b).

However, field trials are required to unambiguously demonstrate the impact of promising repellents in community dwellings under fully natural conditions. Furthermore, different mosquito species may display different behavioural responses towards a given vector control tool under natural field conditions where transmission is often mediated by two or more mosquito species. We therefore evaluated the impact of selected potted repellent plants and thermal expulsion of plant materials against the malaria vectors in Lwanda village, western Kenya where transmission is mediated by *An. gambiae* Giles, *An. arabiensis* Patton and *An. funestus* Giles, the three major vectors of malaria in Africa.

Materials and methods

Study area

The studies were carried out in Lwanda village (00°28.621′S and 034°17.331′E), western Kenya, about 420 km west of Nairobi in the basin region of Lake Victoria. The study village has an altitude of 1169 m above sea level, with the main rainy season from March to May, and a short rainy season from October to November. It is a rural village with many small artificial ponds, swamps, hoof prints and a variety of other mosquito breeding habitats (Minakawa *et al.* 1999). Mosquito density is high in the main and small rainy seasons but lower in the dry season. Although the bulk of anopheline populations comprises of members of the *An. gambiae* complex, *An. funestus* can also found at quite high densities. No previous vector control programme had been in operation in the village, nor were there any on-going control activities.

Five homesteads were selected for the field tests of the candidate plants by thermal expulsion and four for potted plants. All experimental houses were mud-walled with grass-thatched roofs in which the open eaves and the unscreened windows allowed ready access to mosquitoes (Lindsay & Snow 1988). The distances between huts vary from approximately 100–300 m.

Test plants

Candidate plants were selected for the field trial based on the screening of plants for their repellency in semi-field experimental huts trials: potted plants of *O. americanum, L. camara* and *L. ukambensis* (Seyoum *et al.* 2002b) and, for thermal expulsion, leaves of *C. citriodora* (Lemon eucalyptus); and leaves and seeds (as traditionally used) of *O. kilimandscharicum* and *O. suave* (Seyoum *et al.* 2002a) were tested for their ability to reduce mosquito density in individual huts under natural field conditions. Mosquito coils (Baygon®, product of BAYER East Africa Ltd) with 0.20% (w/w) pyrethrins as the active ingredient was also tested to serve as a positive control.

Repellency tests

Ten potted plants of each species were placed under the eaves of houses (one species per house) from 18.30 to 06.30 hours during each experimental night. The leaves of the plants were bruised by hand at 18.30 and 21.00 hours to enhance the release of repellent volatiles. For thermal expulsion the plant materials were placed on the top of thin metal plates placed directly above the charcoal in a traditional stove or 'Jiko' (Seyoum *et al.* 2002a) and the houses were fumigated by applying fresh plant material every hour from 18.30 to 21.30 hours local time. Each hour 10 g of pre-weighed plant materials were placed on the thin metal plate of the traditional stove with burning charcoal for thermal fumigation of individual huts. Three hundred grams of charcoal was used to light the traditional stoves followed by additional 150 g of charcoal every hour.

A 4 × 4 by four latin square design was used for the assignment of the treatments (plants) and the control plant, a local wild grass, *Hyparrhenia rufa*, to the experimental units (huts) for the potted plants. Similarly, a 5 × 5 latin square design was used for thermal expulsion of plant material in three treatment huts, one hut with the mosquito coil (positive control) and one with only burning charcoal stove without plant material (negative control). The huts were randomly assigned for the candidate plants and the control(s) on the first sampling night. The treatments and the control(s) were then assigned by rotation in consecutive sampling nights in different huts to compensate potential spatial variations of mosquito density in individual huts selected for experimentation.

Communicable Diseases Control (CDC) miniature light traps (model 512, John W. Hock company, USA) set close to occupied untreated bed nets inside bedrooms were used for sampling mosquitoes. Each light trap was operated on a 6 V 10 Ah battery and fitted with a 150 mA bulb (6.3 V) and a lid. The traps were positioned at the foot end of the bed near the top of the bed net (Mboera *et al.* 1998). Traps were operated between 18.30 and 06.30 hours. The collection bags were collected and replaced at 22.30 hours for experiments by thermal expulsion, to evaluate the impact on mosquito density during and after fumigation of individual huts, and only at 06.30 hours for experiments with potted plants. The mosquitoes were identified to species level using morphological characteristics (Gillies & De Meillon 1968). Samples (5%) of *An. gambiae s.l.* were preserved in 95% ethanol for further identification to sibling species by polymerase chain reaction (Scott *et al.* 1993). The tests were carried out with minimum intervening periods of three nights to avoid potential residual effect of the plants by periodic thermal expulsion. Tests with potted plants were carried out over consecutive nights. The experiments were replicated 24 nights for potted plants (six completed blocks) and 30 nights (six completed blocks) for experiments by thermal expulsion. Blocks of either four or five nights were run during different seasons (June 2001 to May 2002). Thus, each treatment or control was assigned six times to all experimental huts for both methods.

Data analysis

A generalized linear model (GLM) procedure was used to determine the significance of differences of the total catch of mosquitoes in each treatment and control huts using SPSS 10 for Windows. The data were analysed separately for the two most common anopheline species, *An. gambiae s.l.* and *An. funestus*, and for the two application methods. For experiments with thermal expulsion, data for the catches during and after application of the treatments were analysed separately. All-night catches were used in the analysis of data from experiments with potted plants.

We analysed the effects of repellent plants and allowed for differences between experimental units by generalized linear modelling of the relationship between mosquito catches in control (C) and treatment huts (T). The effects of treatment repellency (R) and the effects of different household experimental units (E) were modelled as:

$$T = (1 - R)E \cdot C. \tag{1}$$

Because mosquito catches are usually highly aggregated and vary over wide ranges (Smith 1995) they are best expressed in logarithmic form to minimize heterogeneity of variances for generalized linear modelling. We therefore add one to all counts to enable the inclusion of nights with zero catches, assume $T + 1 \approx T$ and $C + 1 \approx C$ and log transform both sides of equation 1.

$$\text{Log } T = \text{Log}(1 - R)E \cdot C = \text{Log}(1 - R) + \text{Log } E + \text{Log } C \tag{2}$$

which can be fitted to a generalized linear model of the form:

$$y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 \tag{3}$$

where, β_1 represents the effects of different repellent plants, β_2 represents the effects of different experimental units (huts and residents), and β_3 represents the equivalence of catches where treatment and experimental unit effects are negligible. We therefore treated replicate or sampling night as a randomly varying factor determining overall catch size and estimated values for β_1 and β_2 as fixed factors that influence this outcome. Substituting β_1 into the first term of equation 2 and rearranging allows calculation of proportional repellency as follows:

$$R = 1 - 10^{\beta_1} \tag{4}$$

Results

Trap catches and species composition

Anopheles gambiae sensu lato and *An. funestus* were the main species present during the study period, with a higher proportion of the former than the latter. A total of 16 347 anopheline mosquitoes were caught from all the treatment and control huts for both studies, of which 79.5% were *An. gambiae s.l.* and 20.5% *An. funestus*. Of 649 *An. gambiae s.l.* samples identified using PCR, 18.5% were *An. gambiae s.s.* and 81.5% *An. arabiensis*.

Repellency by thermal expulsion and potted plants

All the three candidate plants, *C. citriodora*, *O. suave* and *O. kilimandscharicum* showed significant repellency against *An. gambiae s.l.* both during application of plant treatments and residual effect in post-application periods (Tables 1 and 2, respectively). The highest repellency against *An. gambiae s.l.* both during and after application of the plant treatments was by *C. citriodora* followed by *O. kilimandscharicum* and *O. suave*. None of the candidate plants except thermal expulsion of *O. kilimandscharicum* showed significant protection against *An. funestus*.

Intact potted plants of *O. americanum* and *L. camara* also showed significant repellency against *An. gambiae s.l.* under field conditions (Table 3). However, none of the candidate potted plants showed significant protection against *An. funestus* under natural field conditions.

Table 1 The repellency of plants against *Anopheles gambiae sensu lato* and *An. funestus* by thermal expulsion during application of plants (18.30–22.30 hours sampling period) in Lwanda, western Kenya

Plant species	*Anopheles* species	Parameter estimate of treatments (β_1 ± SEM)	Repellency percentage (95% CI)	*P*
Corymbia citriodora	*An. gambiae s.l.*	−0.290 ± 0.072	48.71 (28.88, 63.02)	<0.0001
	An. funestus	−0.07054 ± 0.071	15.0 (−17.58, 38.48)	0.323
Ocimum kilimandscharicum	*An. gambiae s.l.*	−0.256 ± 0.072	44.54 (23.09, 60.01)	0.001
	An. funestus	−0.205 ± 0.071	37.63 (13.69, 54.92)	0.005
Ocimum suave	*An. gambiae s.l.*	−0.256 ± 0.072	44.54 (23.09, 60.01)	0.001
	An. funestus	−0.07472 ± 0.071	15.81 (−16.45, 39.19)	0.295
Mosquito coil (control)	*An. gambiae s.l.*	−0.349 ± 0.072	55.23 (37.91, 67.72)	<0.0001
	An. funestus	−0.186 ± 0.071	34.84 (9.89, 52.90)	0.010

Table 2 The residual repellency of plant volatiles against *Anopheles gambiae sensu lato* and *An. funestus* between 22.30 and 06.30 hours, following a period of thermal expulsion (18.30–22.30 hours) in Lwanda, western Kenya

Plant species	*Anopheles* species	Parameter estimate of treatments (β_1 ± SEM)	Repellency percentage (95% CI)	*P*
Corymbia citriodora	*An. gambiae*	−0.252 ± 0.057	44.02 (27.56, 56.75)	<0.0001
	An. funestus	−0.07459 ± 0.067	15.78 (−12.45, 37.91)	0.267
Ocimum kilimandscharicum	*An. gambiae s.l.*	−0.219 ± 0.057	39.61 (21.84, 53.44)	<0.0001
	An. funestus	−0.164 ± 0.067	31.45 (6.96, 49.42)	0.016
Ocimum suave	*An. gambiae s.l.*	−0.194 ± 0.057	36.03 (17.12, 50.57)	0.001
	An. funestus	0.01682 ± 0.067	−3.95 (−40.93, 23.44)	0.802
Mosquito coil (control)	*An. gambiae s.l.*	−0.351 ± 0.057	55.43 (42.19, 65.57)	<0.0001
	An. funestus	−0.232 ± 0.067	41.39 (20.51, 56.75)	0.001

Table 3 The repellency of potted plants against *Anopheles gambiae sensu lato* and *An. funestus* in Lwanda, western Kenya

Plant species	*Anopheles* species	Parameter estimate of treatments (β_1 ± SEM)	Repellency percentage (95% CI)	*P*
Ocimum americanum	*An. gambiae s.l.*	−0.207 ± 0.069	37.91 (14.51, 54.81)	0.004
	An. funestus	0.02651 ± 0.103	−5.92 (−71.00, 33.93)	0.798
Lantana camara	*An. gambiae s.l.*	−0.138 ± 0.069	27.22 (0.04, 47.16)	0.050
	An. funestus	0.06801 ± 0.103	−16.95 (−87.93, 27.22)	0.513
Lippia ukambensis	*An. gambiae s.l.*	−0.125 ± 0.069	25.01 (−3.08, 45.55)	0.075
	An. funestus	−0.129 ± 0.103	25.70 (−19.46, 53.87)	0.216

Discussion

Here, we evaluated the level of protection conferred by thermal expulsion of plants and intact potted plants under natural field conditions in western Kenya, which indeed are effective in repelling *An. gambiae s.l.*, the principal malaria vector in tropical Africa. These methods are simple and highly adaptable under varied local situations in rural communities of Africa, where traditional methods such as direct burning of plant materials and bruising the leaves of the plants and hanging them around the bed are already being practised to repel house-entry mosquitoes (Seyoum *et al.* 2002a).

The repellency of all candidate plants against *An. gambiae s.l.* by thermal expulsion was comparable with that of a mosquito coil that was used as a positive control in this field trial. The level of repellency of mosquito coil in this study is in agreement to reports of

24–88% reduction from Tanzania and Papua New Guinea (Hudson & Esozed 1971; Charlwood & Jolly 1984). The continuing repellency of plants after application by thermal expulsion showed that thermal fumigation in the early hours of the night may be sufficient to effectively repel mosquitoes for the remaining duration of the night. This finding may have potential for integration with untreated bed nets (Takken 2002) and (partially) replace the excito-repellent effect exerted by pyrethroid-impregnated nets.

A similar approach to thermal expulsion of plant materials has been recently developed using kerosene-burning lamps (*korobois*) modified to heat and vaporize a volatile pyrethroid insecticide to repel host-seeking mosquitoes in Tanzania (Pates *et al.* 2002). In this study, a modified lamp by mixing the insecticide (0.1% transfluthrin) with vegetable oil and heating it to 120 °C in a tin held just above the flame gave 50–75% reduction in the biting rate of *Culex quinquefasciatus* Say. However, direct comparisons cannot be made with our results because of differences in mosquito species.

Live potted plants of *O. americanum* best repelled *An. gambiae s.l.* compared with slightly lower effects by the other plant species tested in this manner. This confirms results obtained in semi-field tests (Seyoum *et al.* 2002b), and suggests that such readily applicable use of local plant products merits further investigation. It also validates the importance of semi-field systems (Knols *et al.* 2002; Seyoum *et al.* 2002a,b) to screen large numbers of plants in a short period of time.

Although the level of repellency of intact potted plants and thermal expulsion of plant materials in this study is lower than required to substantially reduce the incidence of malaria in highly endemic areas, it may usefully contribute to integrated programmes. Integrated vector management with a number of modestly effective control tools can significantly lower entomological inoculation rates (Killeen *et al.* 2000).

Most plant species have no significant ($P > 0.05$) reduction against *An. funestus* by both methods of applications. However, thermal expulsion of the leaves and seeds of *O. kilimandscharicum* significantly repelled *An. funestus* both during and after application periods (37.63% and 31.45%, respectively). The principal constituent of volatiles of thermally expelled *O. kilimandscharicum* is camphor (A. Seyoum and A. Hassanali, unpublished data) and may be effective alone or in combination with other constituents against this species. Differences in the sensitivity of mosquito species to synthetic repellents such as diethyl-methyl toluamide (DEET) are also widely documented (Curtis *et al.* 1987; Walker *et al.* 1996; Tawatsin *et al.* 2001). Walker *et al.* (1996) reported that *An. funestus* was significantly less sensitive ($P < 0.001$) than *An. arabiensis* to DEET and a piperidine compound, AI3-37220, in western Kenya. Similarly DEET provided protection for at least 8 h against *Ae. aegypti* L. and *Cx. quinquefasciatus*, but for only 6 h against *An. dirus* Peyton & Harrison in Thailand (Tawatsin *et al.* 2001). Laboratory tests of six insect repellents (DEET, di-methyl phthalate, ethyl-hexanediol, permethrin, citronella and cedarwood oil) by different methods showed that *An. stephensi* Liston was consistently more susceptible than *An. gambiae* Giles, *An. albimanus* Wiedemann or *An. pulcherrimus* Theobold (Curtis *et al.* 1987).

Similar studies in rural Papua New Guinea (Paru *et al.* 1995) also demonstrated the repellent activity of various plant products by burning the wood outdoors, and bruised (rubbed) leaves of the plants on to the legs of human baits against anopheline and culicine mosquitoes. Wood smoke and topical applications reduced biting of human volunteers by 79% and 51%, respectively. More recently, Pålsson and Jaenson (1999a,b), showed that direct burning of several plant species in Guinea-Bissau reduced biting rates by up to 80% in the field. Overall, different results obtained in this and other reports can be accounted for by differences in the type of mosquito species, plant species, methods of applications and evaluation procedures.

We used CDC light traps to evaluate the impact of mosquito repellent plants in the reduction of mosquito density. Studies by E. M. Mathenge *et al.* (personal communication) in the same village revealed that catches by CDC light traps are proportional to human landing catches of *An. gambiae s.l.* and *An. funestus* in the area and sample equivalent host-seeking cohorts of the vector population. Similarly, studies in Tanzania and Sierra Leone showed that catches of *An. gambiae s.l.* in light traps set beside occupied untreated nets were proportional to the human biting catches, and the age distribution of the mosquitoes caught by the two methods was similar (Lines *et al.* 1991; Magbity *et al.* 2002), and recommended the use of light traps as a surrogate for human bait catches in estimating biting rates of *An. gambiae* mosquitoes. Therefore, light traps set beside occupied bed nets present an alternative to human biting catches and avoid the possible ethical problem that arises when mosquito collectors deliberately expose themselves to disease vectors.

In conclusion, thermal expulsion of *C. citriodora*, *O. kilimandscharicum* and *O. suave*, and intact potted plants of *O. americanum* and *L. camara* significantly repelled *An. gambiae* under natural field conditions in western Kenya, and can be incorporated into integrated vector management in areas where the plant species are available and where the dominant vector of malaria is *An. gambiae s.l.* Similarly, thermal expulsion of *O. kilimandscharicum* can also be considered in areas where *An. funestus* is an important vector of malaria. Both methods of application may offer cost-effective alternatives as additional means of household protection, and a useful complement to bed nets, particularly for the early part of the evening before bedtime.

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