

REPELLENCY OF LIVE POTTED PLANTS AGAINST *ANOPHELES GAMBIAE* FROM HUMAN BAITES IN SEMI-FIELD EXPERIMENTAL HUTS

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Abstract. The repellency of potted plants against the malaria vector *Anopheles gambiae sensu stricto* Giles was quantified in experimental huts under semi-field conditions inside a screen-walled greenhouse. *Ocimum americanum* Linnaeus (Labiatae), *Lantana camara* L. (Verbenaceae), and *Lippia uckambensis* Spreng (Verbenaceae) repelled at an average of 39.7% (95% confidence interval [CI] = 29.6–48.4%), 32.4% (95% CI = 19.7–43.1%), and 33.3% (95% CI = 21.5–43.3%) of the mosquitoes, respectively ($P < 0.0001$ for all treatments). This was determined by logistic regression, allowing for variations associated with different bait hosts, sampling huts, and replicate test nights. In contrast, *Ocimum kilimandscharicum* Guerke (Labiatae), *Ocimum suave* Willd. (Labiatae), *Corymbia citriodora* Hook (Myrtaceae), *Azadirachta indica* A. Juss (Meliaceae), *Tagetes minuta* L. (Asteraceae), and *Hyptis suaveolens* Poit. (Lamiaceae) did not significantly repel mosquitoes. The combination of *O. americanum* with either *L. camara* or *L. uckambensis* repelled 31.6% (95% CI = 19.7–41.7%) and 45.2% (95% CI = 34.7–54.0%) of the mosquitoes, respectively ($P < 0.0001$ for both treatments). This study is the first to show that live intact plants can reduce domestic exposure to malaria vector mosquitoes. As such, they may represent a new, sustainable and readily applicable malaria vector control tool for incorporation into integrated vector management programs.

INTRODUCTION

Vector-borne diseases are among the most important public health problems and obstacles to socioeconomic development of developing countries, particularly in the tropics, with malaria alone causing an estimated 1.5–2.7 million deaths and 300–500 million cases per year.¹ To date, no method of malaria control has proven effective enough to control the high transmission intensities found in sub-Saharan Africa.^{2–5} Even the most efficacious of these, such as pyrethroid-treated bed nets, have proven difficult to implement on a sustainable basis for reasons of availability, acceptability, and cost.^{6–8} However, with increasing problems of toxicity to non-target organisms and the resistance of mosquitoes to synthetic insecticides,⁹ interest in natural or botanical insecticides has been revived. Natural and synthetic insect repellents are well established as means of personal protection against biting arthropods and the various pathogens they may carry.^{10,11}

We have examined the potential use of mosquito repellent plants and their modes of application, with an emphasis on materials and methods that can be locally used by communities with minimal external input. Ethnobotanical studies of traditionally used mosquito repellent plants in western Kenya showed that many of the most commonly used plants are indeed effective against malaria vectors when burned or thermally expelled with domestic charcoal stoves.¹² Moreover, these surveys also revealed that some of the plants were commonly applied by cutting the branches and placing them inside houses, particularly around beds. We therefore hypothesized that live, intact, potted plants may work in a similar way and represent a sustainable and cost-effective way to protect humans against host-seeking malaria vectors. Here we report the repellency of selected potted plants against the malaria vector *Anopheles gambiae sensu stricto* Giles by using a novel semi-field experimental system.

MATERIALS AND METHODS

Mosquito and plant species. Semi-field tests of the repellency of nine individual candidate plants and some combinations thereof were carried out with a laboratory colony of *An. gambiae s.s.* maintained at the Mbita Point Research and Training Center at the International Centre of Insect Physiology and Ecology, as previously described.¹²

The plant species tested were *Ocimum americanum* Linnaeus (Labiatae), *Lantana camara* L. (Verbenaceae), *Azadirachta indica* A. Juss (Meliaceae), *Tagetes minuta* L. (Asteraceae), *Hyptis suaveolens* Poit. (Lamiaceae), *Ocimum suave* Willd. (Labiatae), *Ocimum kilimandscharicum* Guerke (Labiatae), *Corymbia citriodora* Hook (Myrtaceae), previously referred to as *Eucalyptus maculata citriodon* (Myrtaceae), and *Lippia uckambensis* Spreng (Verbenaceae). The latter four species were included in the test as candidate repellent plants based on empirical information from our own unpublished work and from the literature.^{13–15} The other five species were selected for experimentation based on ethnobotanical information obtained locally.¹² Furthermore, the impact of the combined use of two repellent plants (*O. americanum* with either *L. camara* or *L. uckambensis*) in reducing house entry and human feeding by mosquitoes was also evaluated.

Semi-field quantification of the repellency of selected plants. The effect of potted plants on human-vector contact of *An. gambiae* was determined under semi-field conditions inside a screen-walled (shade netting = 90%) greenhouse (7.1 × 11.5 meters) with two experimental huts (Figure 1). These were rectangular (3.2 × 2.7 meters) and made of hard board (plywood) and internally painted with white paint. The roofs were covered with black cloth inside and reed mats outside. Each night, 10 potted test plants (fresh branches of the tree for *C. citriodora*) were suspended under the eaves of one of the huts (five on each side) while 10 pots with *Hyparrhenia rufa* Stapf (Poaceae), a local wild grass, were placed in a similar position on the other hut to serve as a control one hour before the start of the experiment. Some leaves of the

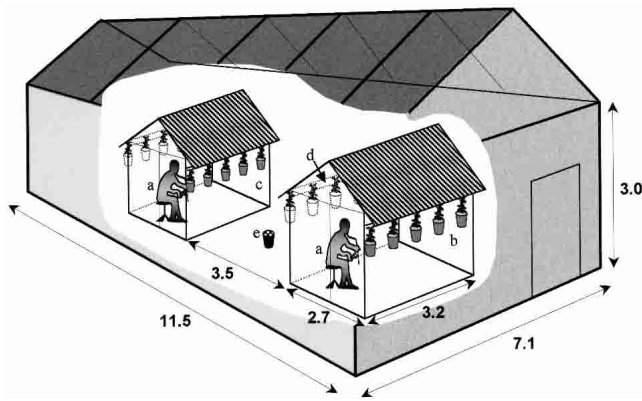


FIGURE 1. Set up for testing the repellency of potted plants. Experimental huts were constructed inside a screen-walled greenhouse, within which human baits sat (a) and the test plants (b) as well as control plants (c) were placed under the eaves (d). Mosquitoes were released from a paper cup (e) placed half way between the huts. Numbers are dimensions in meters.

plants were bruised by hand five minutes before the onset of the experiment to enhance release of repellent volatiles. Five potted plants of each species were used for testing the combined use of two repellent plants. A cross-over design was used in which the treatment and control were exchanged between the two huts on different nights. Each night 200, 5–7-day-old, unfed female *An. gambiae* s.s. from a colony that was originally initiated from adults collected in 1996 at Njage village in southeastern Tanzania were used in the experiments. They were starved for six hours, released from a paper cup at the center of the screen house at 8:00 PM local time, and recaptured in the two huts by the human landing catch until midnight. Human collectors (two African men 35 and 20 years old) exchanged positions between the huts every 30 minutes to minimize bias caused by differential attractiveness and collection skills. Experiments were replicated four times on four different nights by changing the arrangement of treatment and control plants to the huts. Ethical clearance was obtained from the Kenya Medical Research Institute (KEMRI) National Ethical Review Committee (KEMRI/RES/7/3/1) for the involvement of human subjects involved in this experiment.

Data analysis. Logistic regression analysis was used to determine the significance of the effect due to plant treatment and other sources of variations using SAS 8.1 for Windows.¹⁶ The proportion of recaptured mosquitoes recovered in the treatment hut for each candidate plant was analyzed by logistic regression. By considering only those mosquitoes that actually chose to bite in a given 30-minute period, the probability (P) of biting in the treated huts was estimated from the data on the proportion biting in the treatment huts. A forward stepwise selection procedure was used to fit the model, including only parameters reflecting other potential sources of variation that were found to be significant ($P < 0.05$). The list of parameters from which these were chosen includes arrangement of huts, arrangement of collectors, replicates, and time. Standard errors of the means and 95% confidence intervals (CIs) of the fitted parameters were estimated by maximum likelihood analysis. The percentage repellency (R) was calculated using the proportions of the total catch in the control P(C) and treatment huts P(T):

$$R (\%) = 100 \times (P(C) - P(T))/P(C)$$

Given that all the mosquitoes recaptured either chose the treatment or control hut:

$$P(C) = 1 - P(T)$$

Substituting into equation 1:

$$R = 100 \times (1 - 2P(T))/(1 - P(T))$$

Repellency was estimated using the parameter estimate of the intercept (β_0). By definition,¹⁷ β_0 is the natural log of the treatment over the control:

$$\beta_0 = \ln (P(T)/1 - P(T))$$

Which by rearrangement,¹⁷ yields:

$$T = \exp(\beta_0)/(1 + \exp(\beta_0))$$

Mean repellency and confidence intervals for that estimate were therefore calculated by substituting equation 5 into equation 3, using the estimates for β_0 and its confidence intervals respectively, as derived from logistic regression analysis.

RESULTS

When the possible effects due to other factors such as the arrangement of huts, collectors, time of collections and replicates were taken into account, *O. americanum*, *L. camara*, and *L. uckambensis* were all found to be effective repellents, reducing domestic exposure by 30–40% (Table 1). Combining *O. americanum* with either *L. camara* or *L. uckambensis* was similarly effective, but no obvious synergism was observed. *Ocimum kilimandscharicum*, *O. suave*, *C. citriodora*, *A. indica*, *T. minuta*, or *H. suaveolens* were not even mildly repellent to *An. gambiae*, even though some of these plants were found to be quite potent when directly burned or thermally expelled.¹²

The effects of other sources of variation in our experimental setup are shown in Table 2. Logistic regression using forward stepwise selection indicated that the arrangement of huts was a significant source of variation during the test periods for five candidate plants. Large differences were also observed between the catches of mosquitoes by the two persons involved in human landing catches for all but one of the trials, but replicate was found to be a significant source of variation on only two occasions. No significant effect due to time of collection was observed in any of the trials, indicating that live plants do not appreciably vary in their repellent properties at different times of the night.

DISCUSSION

This study is the first to our knowledge to demonstrate that live intact plants can repel mosquitoes to reduce domestic exposure. Although the mosquito plant *Pelargonium citrosum* Voigt (Geraniaceae) is reported to repel host-seeking mosquitoes from areas near the plant because of its citronella-like scent, it appears ineffective when evaluated under field conditions.¹⁸ Intact plants may represent a new and readily applicable vector control tool to be integrated into vector management programs and indeed one of these plants, *L. camara*,

TABLE 1
Repellency of potted plants against *Anopheles gambiae* s.s. in semi-field experimental hut trials*

Plant species	English name	Local name (Dholuo)	No. recaptured†		Intercept parameter estimate ($\beta_0 \pm$ SEM)	% Repellency (95% CI)	P
			Treatment	Control			
<i>Azadirachta indica</i>	Neem	Arubaini	379	339	0.0479 \pm 0.082	-4.9 (-2.3-10.9)	0.5595
<i>Corymbia citriodora</i>	Lemon eucalyptus	Bap-karadali	319	302	0.0656 \pm 0.0824	-6.8 (-25.9-9.4)	0.4263
<i>Hyptis suaveolens</i>	Wild spikenard	Abuba	179	202	-0.1209 \pm 0.1027	11.4 (-8.8-27.8)	0.2390
<i>Lantana camara</i>	Lantana	Nyabend winy	264	432	-0.3915 \pm 0.0863	32.4 (19.7-43.1)	<0.0001
<i>Lippia uckambensis</i>	Fever tea	Ang'we-rao	273	408	-0.4055 \pm 0.0814	33.3 (21.5-43.3)	<0.0001
<i>Ocimum americanum</i>	Lime basil	Mweny	283	456	-0.5063 \pm 0.0779	39.7 (29.6-48.4)	<0.0001
<i>Ocimum kilimandscharicum</i>	African blue basil		335	325	-0.0353 \pm 0.0826	3.5 (-13.9-18.2)	0.6693
<i>Ocimum suave</i>	Tree basil	Mieny madongo	343	372	-0.1170 \pm 0.0793	11.0 (-4.2-24.1)	0.1403
<i>Tagetes minuta</i>	Khaki weed	Nyanjaga	388	351	0.0817 \pm 0.0772	-8.5 (-26.6-7.0)	0.2901
<i>L. camara</i> + <i>O. americanum</i>			290	414	-0.3800 \pm 0.0800	31.6 (19.7-41.7)	<0.0001
<i>L. uckambensis</i> + <i>O. americanum</i>			221	408	-0.6008 \pm 0.0875	45.2 (34.7-54.0)	<0.0001

* CI = confidence interval.
† Total caught for four test nights.

is already commonly used for hedges around the huts in many villages around Lake Victoria.

We caution, however, that field studies are required to fully evaluate the effectiveness of these plants in community dwellings. Although this semi-field system has many clear advantages,¹² full field trials are essential to rule out possible artifacts resulting from overlap of the repellent range of the treatment hut with the control hut and possible differences in the behavior of mosquitoes where they are not constrained. In this semi-field set up, where mosquitoes are released at a mid-way point between control and treatment huts at a distance of 1.75 meters, mosquitoes can readily choose to fly towards the control hut and avoid the treatment hut when the candidate plant is indeed repellent. However, under real field conditions, where distances between dwellings are usually much greater, the use of the same plant-derived repellent may not have the same effect without an alternative, unprotected host nearby, so mosquitoes may be less easily diverted from humans by repellent volatiles. Field evaluations of potted plants that resulted in significant reductions in exposure under semi-field conditions are now ongoing in western Kenya.

Of the three plants found to be repellent, *O. americanum* is the most commonly used repellent plant by the community on Rusinga Island in western Kenya.¹² Although there are no

previous reports on the repellency of intact plants considered in this study, topical skin treatment of juices from the green leaves and inflorescences of *O. americanum* and *O. suave* have reduced the feeding rates of *An. gambiae* mosquitoes from Tanzania by 56% and 45%, respectively.¹⁹ However, direct comparisons cannot be made with the results of this study due to differences in the methods of application of the plants and the experimental setup. Recent studies have shown that volatile oils extracted by steam distillation of *O. americanum*, especially with addition of 5% vanillin, repelled *Aedes aegypti* L., *An. dirus* Peyton & Harrison, and *Culex quinquefasciatus* Say under cage conditions for up to eight hours.²⁰ Furthermore, extracts from the closely related species *O. canum* Sims (Labiatae) showed strong larvicidal properties against *An. gambiae* s.s. in Tanzania.²¹ Similarly, topical application of *L. camara* flower extract in coconut oil resulted in 94.5% protection against *Ae. albopictus* Skuse and *Ae. aegypti* with a mean protection time of 1.9 hours in India.²² Essential oil from leaves of *L. uckambensis* also has mosquito repellent properties (Lwande W, unpublished data).

Tagetes minuta, *A. indica*, *O. kilimandscharicum*, *O. suave*, *H. suaveolens*, and *C. citriodora* have not shown significant repellency in this study, even though they all have documented repellent or insecticidal properties. Whole plant

TABLE 2

Sources of variation affecting host choice, other than plant treatment, as determined by logistic regression analysis (time was not found to be significant)*

Plant species	Hut location		Person		Replicate	
	P	OR†	P	OR‡	P	OR§
<i>Azadirachta indica</i>	NS	NA	<0.0001	0.195	0.0361	1.96
<i>Corymbia citriodora</i>	0.0128	0.664	<0.0001	0.470	NS	NA
<i>Hyptis suaveolens</i>	NS	NA	NS	NA	NS	NA
<i>Lantana camara</i>	NS	NA	<0.0001	0.157	NS	NA
<i>Lippia uckambensis</i>	NS	NA	<0.0001	0.322	NS	NA
<i>Ocimum americanum</i>	0.0021	0.622	<0.0001	0.494	NS	NA
<i>Ocimum kilimandscharicum</i>	NS	NA	<0.0001	0.266	NS	NA
<i>Ocimum suave</i>	NS	NA	<0.0001	0.282	0.0193	0.586
<i>Tagetes minuta</i>	0.0001	0.554	<0.0001	0.324	NS	NA
<i>L. camara</i> + <i>O. americanum</i>	0.0029	0.620	<0.0001	0.341	NS	NA
<i>L. uckambensis</i> + <i>O. americanum</i>	0.0183	0.663	<0.0001	0.304	NS	NA

* OR = odds ratio; NS = not significant; NA = not applicable.
† Hut 1 versus hut 2.
‡ Person 1 versus person 2.
§ Greatest difference between replicates.

Soxhlet and steam distillation extracts of *T. minuta* are larvicidal to *Ae. aegypti* and *An. stephensi* Liston.²³ The repellent action of neem (*A. indica*) cream (5% neem oil in vanishing cream) was also reported to afford more than 90% protection against both anopheline and culicine mosquitoes in the field.²⁴ Kerosene lamps containing neem oil also reduce the biting and indoor resting density of mosquitoes, with better protection against *Anopheles* than *Culex*.²⁵ *Ocimum suave* and *O. kilimandscharicum* repel insect pests of stored products^{13,14} and cloth treated with quwenling (the waste distillate after extraction of oil from the lemon eucalyptus *C. citriodora*) was effective against *Ae. albopictus* and *An. quadrimaculatus* Say.¹⁵

The four species *O. americanum*, *O. kilimandscharicum*, *O. suave*, and *H. suaveolens* belongs to the Basil family (Lamiaceae/Labiatae) and are morphologically easy to distinguish. *Ocimum americanum* is 14–18 inches tall with small (1½'' × ½'') shiny, dark green leaves and approximate 100 seeds per pack. *Ocimum kilimandscharicum* has pink flowers instead of the usual white ones found on most Basils, and has long flower stems, up to 18 inches long. *Ocimum suave* is a dense woody shrub, reaching heights of two meters. *Hyptis suaveolens* has quadrate hairy stems and ovate to obovate leaves 3–5 cm long and 2–4 cm wide, the margins of which are serrulate, and the lower surface is densely hairy and has petioles up to 3 cm long. It has flowers in small cymes along branch ends with reduced leaves.

The precise effect of potted plants in the reduction of human vector contact is difficult to compare with effects obtained with existing vector control tools, given the inherent differences in the methods of evaluation of their impact. However, the repellent plants observed in this study can be considered as effective (a reduction of approximately 40% in human vector contact) and may be used in combination with other vector control tools in integrated vector management programs. Although such reductions by repellent plants are unlikely to yield significant reductions of disease burden in malaria-endemic settings^{2,4,5} integrated control with a few modestly effective tools can meaningfully reduce malaria transmission.³ Intact repellent plants may therefore contribute to control programs that combine several tools and may be the most easily implemented method of domestic protection reported to date.

The results obtained in the present study can also help to develop push-pull or stimulo-deterrent diversionary strategies,²⁶ whereby certain species of vector mosquitoes (zoophilic anophelines) could be repelled from human hosts and simultaneously attracted to alternative hosts such as cattle. Recent research on agricultural pests has demonstrated that such approaches can be very successful: intercropping with the non-host molasses grass *Melinis minutiflora* Beauv. (Poaceae) significantly repelled female *Chilo partellus* Swinhoe stem borers from maize crops and simultaneously attracted foraging female *Cotesia sesamiae* Cameron, a larval parasitoid of this important pest.²⁷ There is also some evidence that domestic use of insecticides with excito-repellent properties can lengthen the feeding cycle of anthropophilic mosquitoes and discourage them from feeding on humans.^{3,28–30} Thus, bed nets and insecticides seem to have the potential to enhance or enable zooprophylaxis and similar approaches could be explored for the use of intact repellent plants. Although this approach is unlikely to work for highly

anthropophilic species such as *An. gambiae* and *An. funestus*, malaria transmission across much of tropical Africa is dominated by *An. arabiensis*³¹ and appears to be very sensitive to changes in the relative availability of cattle and humans.³²

In conclusion, our study showed that potted plants of *O. americanum*, *L. camara*, and *L. uckambensis* significantly reduce domestic human exposure to the malaria vector *An. gambiae* in semi-field trials. Potted plants may represent a new, sustainable, and readily applicable malaria control tool for incorporation into integrated programs.

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