Journal of Cell and Animal Biology Vol. 3 (10), pp. 171-174, October, 2009
Available online at http://www.academicjournals.org/JCAB
ISSN 1996-0867 © 2009 Academic Journals

Full Length Research Paper

Efficacy of *Ocimum kilimandscharicum* plant extracts after four years of storage against *Anopheles gambiae* ss

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Accepted 19 August, 2009

Reducing vector-human contact is of priority in breaking the transmission chain of malaria parasites. The use of plant extracts as repellents against malaria vectors have been advocated in different studies. The feeding inhibition of four years old *Ocimum kilimandscharicum* in liquid paraffin or glycerin was compared with N, N-diethyl-3-methylbenzamide (DEET) using cage evaluation method. The four years old extracts of *O. kilimandscharicum* performed similarly when mixed either in glycerine or liquid paraffin. Blood feeding succession was highest in negative control (glycerine/liquid paraffin alone) while low in OK and DEET. Therefore, promotion of plant extracts for commercialization is of priority in rural Tanzania where whole plants are currently used as repellents against malaria vectors.

Key words: *Ocimum kilimandscharicum,* DEET, *Anopheles gambiae s.s*, feeding inhibition.

INTRODUCTION

The most efficient malaria vector *Anopheles gambiae ss* (Giles) is responsible for the transmission of *Plasmodium falciparum* in more than 70% of malaria cases which occur in Africa (Greenwood, 2002; Hay et al., 2009). The efficiency of this vector has been attributed to its anthropophilic behaviour and its breeding habitats which are mostly found within human domiciles (Coetzee et al., 2000; Mutuku et al., 2006).

The control of *A. gambiae* ss is currently achieved by using synthetic insecticides for indoor residual spray, larvicides and insecticide treated materials (Lengeler, 2004; Magesa et al., 2005; Malima et al., 2008). Due to low social economic status in rural areas, the affordability and maintenance of ITN and IRS efficacy for protection is limited (Gallup and Sachs, 2001; Rugemalila et al., 2006; Goesch et al., 2008). The emerging of resistance against commonly used insecticides for ITNs and IRS as worsen the malaria vector control efforts (Greenwood and Mutabingwa, 2002).

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Mosquitoes have bee found tracing the host by using the cues emanated by the host (Takken and Knols, 1999). Repellents have been deployed to interrupt the mosquitoes which are attracted by the cues emanated by the host hence desisting the blood feeding process. *Ocimum kilimandscharicum* blocks electrophysiological responses to olfactory sensory neurons to attractive odors in *Anopheles gambiae ss (*Syed and Leal, 2008). The use of plant based repellents for personal protection against *A. gambiae* ss has been previously reported (Hassanali et al., 1990; Omolo et al., 2004; Odalo et al., 2005, Kweka et al., 2008a, b). The studies which have been reported the findings of plant based repellents have shown significant improvement and insights of developing cheap and available resource for community use against malaria vectors (Kweka et al., 2008b). Based on mosquitoes biting behaviour and human activities in malaria endemic areas, cheap personal protection seemed necessary in complementing bed nets and indoor residual spray when community members stay outdoor for long before retiring to bed net /indoor residual spray protection. Most of the plant extracts are degradable upon exposure to direct light which is more

Table 1. Chemical composition of the essential oil of *Ocimum kilimandscharicum.*

Component	Percentage composition
Camphor	70.4
1,8-Cineole	7.2
Limonene	6.2
Trans-Caryophyllene	2.8
Camphene	5.1
4-Terpineol	1.4
Myrtenol	1.3
∝-Terpineol	0.6
Endo-borneol	0.6
Linalool	0.5

difficult to store in rural setting and they are most volatile once applied on skin (Jembere et al., 1995). Mixing up with oily material such as liquid paraffin and glycerine can assist in making the repellent to last longer on the human skin surface hence more protection.

This paper presents results of essential oil of *O. kilimandscharicum* feeding inhibition after four years of storage and their active ingredients.

MATERIALS AND METHODS

Plant material collection

The leaves of *O. kilimandscharicum* (OK) were collected from Naivasha district Kenya in December, 2004. The identification of the plants was confirmed by botanist at International Centre of Insect Physiology and Ecology, Kenya before distillation.

Extraction of essential oil

Essential oil was extracted from the leaves of *O. kilimandscharicum* by steam distillation at ICIPE following protocol by Peter and Amala, (1998). Distillation was done on December, 2004. The material (plant leaves) filled in the chamber at once was 11.5 Kg. The yield was 2.3 g (0.02%) for 11.5 Kg of plant leaves. From that time, the essential oil was kept in room temperature (20 - 25°C) in darkness.

Mosquito rearing

All laboratory tests were conducted using female 2 - 3 days old *Anopheles gambiae* ss mosquitoes from Tropical Pesticides Research Institute insectary. A colony of *An.gambiae* ss (from Kisumu, Kenya), was maintained in the laboratory since 1972. They were reared and maintained at 27 ± 2°C, 75 - 85% relative humidity with 12:12 light / dark photoperiod. Adult mosquitoes, 2-3 days old, mated females that were only provided a sugar meal prior were used in the assays. Sugar solution was taken off six (6) hours before experiment so as to make them active in seeking for blood meal.

Repellents preparation

The repellents were prepared in the laboratory in the same composition as standard repellent composition. DEET (positive control), OK and negative control (oil only) were prepared in both glycerine and liquid paraffin. Liquid paraffin and Glycerine were used to make up the repellent mixture with essential oils to be applied on human skin. The mixture was made up in 2 parts of essential oil and 8 parts of liquid paraffin or glycerine by volume, the mixture was miscible. The OK essential oil since 2004 when distilled was stored in a dark store with a temperature ranging 20 - 25°C. Experiments were performed in laboratory with temperature 27 ± 2°C and relative humidity range from 75 - 85%.

Essential oil analysis

Gas-chromatography- mass spectrometer was carried out using a low resolution mass spectrometer VG Quattro (Fisson, Manchester, UK). The chemical contents of the oil were identified**.**

Feeding inhibition bioassay

Evaluations were done in Insectary using *An.gambiae* ss (strongly anthropophilic) mosquitoes. The arm treated with *O. kilimandscharicum* in glycerine (OKGLY) or *O. kilimandscharicum* in liquid paraffin (OKLP), DEET and Control (only glycerine or liquid paraffin) were inserted in the cage with 50 unfed female mosquitoes of two to three days old for one hour as described elsewhere (Yuwadee et al., 2005). The repellent was applied on arm as the common application of glycerine is done. Each evaluated repellent or control combination had twenty five replicates. After one hour, any full or partially blood fed mosquito was considered fed.

Statistical analysis

Data were entered twice for validation in MS-excel spreadsheet and analyzed using the SPSS version 15 for windows (SPSS, Inc., Chicago, IL, U.S.A.). Feeding inhibition percentage (FIP) was calculated using the FIP = [(mosquitoes fed in control – Mosquitoes fed in test)/ (mosquitoes fed in control)] (Mehr et al., 1985). Data were analyzed with analysis of variance (ANOVA) for comparison between the feeding responses in DEET (positive control), OK and negative control (glycerine or paraffin oil alone) were made. The comparison between the feeding inhibition between DEET, OK and negative control was done using samples paired t-tests, and the significance level was determined at $P < 0.05$.

Ethical consideration

The TPRI ethics committee reviewed and approved the study. Experiments were conducted by EJK and AMM, where EJK did mosquitoes feeding inhibition experiments and AMM took care of mosquitoes before and after experiments.

RESULTS

The chemical composition of the essential oils of *O. kilimandscharicum* was analysed. The major component was found to be camphor and minor content was Linalool (Table 1). The feeding inhibition calculated using the formula by Mehr et al. (1985) was 95.0, 87.9 and 84.7%

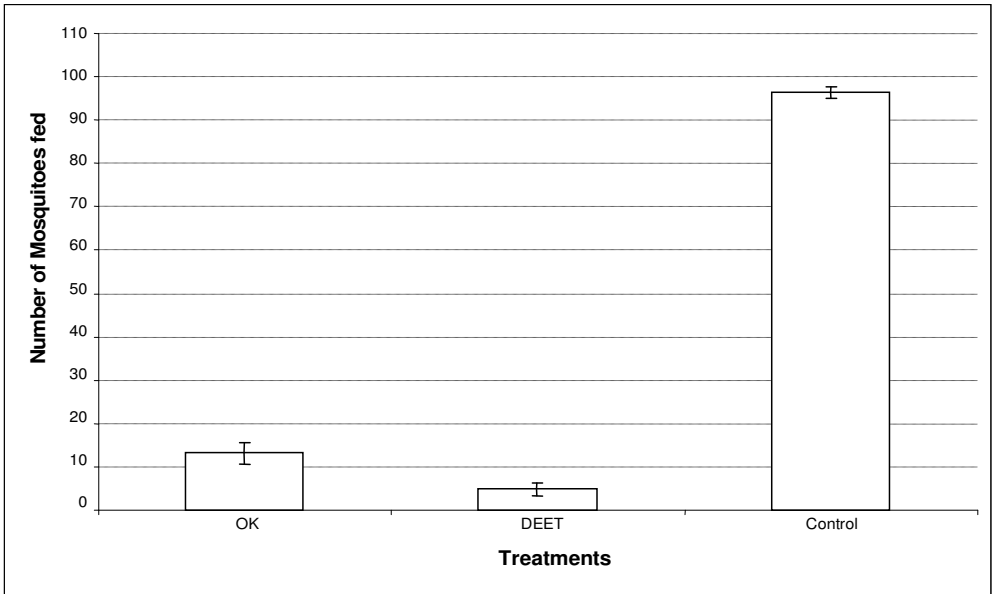

Figure 1. Comparative feeding inhibition of DEET, OK and Control against *Anopheles gambiae* ss insectary colony.

for DEET, OKLP and OKGLY respectively. Statistically, the feeding inhibition between Control, OK and DEET was significant (Figure 1). There was no significant difference between the four years stored extract when mixed with glycerine or liquid paraffin ($t = 1.48$, $df = 24$, $p = 0.97$). Comparison of the mean between *O. kilimandscharicum* in glycerine and in paraffin had no difference ($t = 1.84$, $df = 24$, $p = 0.621$). The comparison of feeding inhibition between OK and control was statistically significant ($t = 81.81$, $df = 24$, $p \leq 0.0001$).

DISCUSSION

The findings of this study are supported by the previous studies (Omolo et al., 2004; Odalo et al., 2005; Kweka et al., 2008b) which showed similar results from botanical products. Despite of been stored for four years, the effectiveness of OK has been competitively similar to that of the approved synthetic repellent, DEET. These results have granted important information for possibilities of producing and using this plant product for long time once formulated and preserved in good conditions. In these experiments, the overall DEET feeding inhibition was 95% while that of OK was 87.4%, which statistically had not different. Therefore, this feeding inhibition guarantees the use of these plant based products in community to supplement already existing malaria control tools to reduce man-vector contacts. *O. kilimandscharicum* when was freshly prepared, showed significant protection efficiency against *An.*gambiae ss (Kweka et al., 2008b). After four years of the storage, the difference in protection against anthropophilic malaria vector in Africa was only 3.1%. This repellency efficient of OK essential oil is attributed by the chemical contents available at different percentages which did not change for all four years of storage (Table 1).These chemical contents of *O. kilimandscharicum* have not been reported to have effect on volunteers in any of the previous studies (Chogo et al., 1981; Seyoum et al., 2003; Omolo et al., 2004; Kweka, 2008b). Linalool which seemed to be of least amount in chemical composition has been associated with the repellency efficacy of the *O. kilimandscharicum* essential oils (Jembere et al., 1995). The longevity of these plant extracts which are found abundantly in majority of rural areas of Tanzania (Chogo et al., 1981) and other part of Africa (Omolo et al., 2004) can be guaranteed to be produced and formulated from these local resources for coupling malaria control existing tools. It's use in community can increase the protection of vulnerable groups (children and pregnant women) as one way of attaining Abuja declaration for reducing malaria burden (Rugemalila et al., 2006); therefore, these plant extracts as to be commercialized in areas where the plants are available.

In this study OK extracts has shown that, the proper formulation and storage of natural productions can give a strong tool to supplement existing malaria vectors control

programmes in rural areas were people are predisposed to disease burdens. This will improve the livelihood of the rural community which bears the burden of the disease.

ACKNOWLEDGEMENTS

We would like to thank Ester Lyatuu, Edward Stephen, Michael A. Mboya, Asteria Maggebo and Sebastian Massawe (Baba Mrema) for preparing mosquitoes during experiments. This study had no funds; it used available resources at the Tropical Pesticides Research Institute, Arusha, Tanzania for the completion of her objective.

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