

## The Bio-Efficacy of Flower Liquid Mosquito Repellent in Laboratory and Field Conditions

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### Abstract

Large-scale use of long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) have contributed largely to the decline of malaria in many countries. However, these may be less effective in endemic areas with residual malaria transmission where people are being bitten before they go to bed. In such situations, repellents may offer protection from such bites hence reducing morbidity from malaria. In the present study, synthetic Flower liquid Mosquito Repellent was studied for its efficacy against laboratory strains of *Anopheles gambiae* s.s. and wild population of *Anopheles arabiensis* in the field. The Flower Liquid Mosquito Repellent was evaluated in comparison to Risasi (prallethrin as its active ingredient) slow release, a registered product in Tanzania as a positive control. Protection efficiency was higher in flower liquid mosquito repellent (97.3%) than in Risasi Slow Release Mat (90.9%). There was no significant difference in per cent protection against mosquito bites between the Flower Liquid Mosquito Repellent and Risasi slow release. The worn sock alone showed the normal mosquito attraction trend for all trapping hours which could be indicator of mosquito biting for unprotected group. The Flower liquid Mosquito Repellent and Risasi slow release provided more than 90% protection from *Anopheles* mosquitoes for upto 8 hrs. In conclusion, the Flower liquid Mosquito Repellent was comparable to the known repellent cream Risasi slow release for prolonged protection against malaria vectors in laboratory and field conditions. The use of these formulations as a control tool in reducing man-vector contact is important in controlling residual malaria transmission in endemic areas.

**Keywords:** Malaria; Mosquito; Liquid mosquito repellent; Bio-efficacy

### Introduction

Mosquitoes are major disease vectors found in tropic and sub-tropic regions including Sub-Saharan Region. Mosquitoes cause human illness such as malaria, dengue fever, yellow fever, and lymphatic filariasis [1]. Repellent applications constitute one of the most reliable means of personal protection measures to reduce human contact with vector and nuisance mosquitoes [2]. Use of repellents seems to be a simple, practical and economical approach to prevent mosquito-borne diseases not only for local people, but also for travellers in disease risk areas, particularly in tropical countries.

Malaria vector control efforts especially through LLINs and IRS programs have resulted to decline of mosquito vector population and transmission rates [3,4]. Although malaria decline is reported throughout endemic areas, residual malaria transmission is still a problem [5,6]. Residual malaria control needs more tools than the use of Insecticide treated nets and Indoor residual spraying. The use of mosquito repellent can be considered for the protection of residual malaria control [7,8]. Historically, reduction of vector – human contact has been used to control disease vector either by burning whole plants [9] or by hanging the plants indoors [10,11]. In advancement of science and technology, extraction and isolation of active repellent compounds of plant materials has been achieved and evaluated against disease vectors [12-15]. Different plants based repellents have shown protection efficiency of six to eight hours [15,16]. The protection efficiency shown by these plants based repellents is similar to that of the chemical compound N,N-diethyl-3-methylbenzamide (formerly known as N,N-diethyl-meta-toluamide) and commonly referred to as DEET. DEET is the gold standard known repellent which has offered a protection of up to 8 hours since its inception in 1954 [17]. The short comings of topical repellents are skin irritations, allergic reactions

[18] and inconsistent application by people [19]. Therefore, the more sensitive and reliable tools should be in place for better protection. The use of mosquito coils for repelling vectors has been found to be efficient for 6-8 hours [20,21], especially during the first biting cycle of the principal malaria vectors (18:00-22:00 hours).

Different slow releasing mats and vaporizing tools with different active ingredients such as allethrin and bioallethrin have been evaluated and proved to have protective efficacy against different species of human biting mosquitoes including strains of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* [22-24]. The Slow release chemical compounds such as prallethrin and transfluthrin have shown to have impact on *An. gambiae* s.l behaviour [25-27]. The current study evaluated the bioefficacy of flower liquid mosquito repellent in laboratory and field conditions against *Anopheles gambiae* s.l. The flower contains transfluthrin as active ingredient.

### Materials and Methods

#### Study area

Laboratory evaluation was conducted at Tropical Pesticides

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Research Institute (TPRI) (Latitude -3.37053 and Longitude 36.695913) where laboratory is maintained at standard temperature ( $\pm 27^{\circ}\text{C}$ ) and relative humidity of 85%. The field trials were carried out at Mabogini village in Lower Moshi, (Latitude -3.432546 and Longitude 37.341843). The village is situated in the Lower Moshi rice irrigation scheme with rice paddies providing conducive environment for mosquito breeding. *Anopheles arabiensis* is the principal malaria vectors in the area [28].

### Test products

**Flower liquid mosquito repellent:** This is a mosquito repellent with transfluthrin 1.6% w/w as active ingredient manufactured by KAPI limited, Kenya.

**Risasi slow release mosquito mat:** This is a mosquito repellent with Prallethrin active ingredient 18 mg/mat which is 12% of mat net content (150 mg/mat) .. Each mosquito mat is approximately 1 gram and can last up to 10 hours. The mosquito mat was used as a positive control.

### Study design and sampling procedure

This study was experimental randomized control design. In the laboratory, mosquitoes were selected randomly while in the field; cluster sampling technique was used for selection of houses.

In each experimental set up a total of 100 female unfed mosquito 3 days old were released in the room. Mosquitoes were released at 18:00 hrs and recaptured mosquito counted at 07:30 hrs.

### Laboratory tests

A nylon sock was worn for 10 hours by a volunteer to absorb the human odour emanated by foot which has been considered be most attractive to mosquitoes [29]. In laboratory three treatments were prepared, the worn sock alone (negative control), a worn sock placed thirty (30) centimetres close to Risasi slow release mosquito mat (Positive control) and a worn sock placed 30 centimetres to Flower Liquid Mosquito Repellent (Evaluated Product).

The worn sock (Negative Control), Risasi (prallethrin) slow release mosquito mat (Positive control) and Flower Liquid Mosquito Repellent (Evaluated Product) were hanged 30 centimetres from CDC – miniature light trap for attracting released 50 unfed female *An.gambiae* s.s. mosquitoes that were repelled by the tested products. Each CDC – miniature light trap was hanged 2 feet above the surface in different rooms with dimension of 5 by 4 by 3 m. The power source was connected to automated switch which cut-off the power after 8 hours stopping the slow release of mosquito repellent Risasi and Flower Liquid.

### Field trials

Twelve houses were selected in Lower Moshi rice irrigation scheme community during rice transplantation. The houses were grouped into three clusters, of four houses each. The three clusters included a cluster without any treatment, a cluster installed with Risasi (prallethrin) slow release mosquito mat and a cluster installed with slow releasing flower liquid mosquito repellent. A field trial was conducted for 60 days to validate the protection duration given by manufacturer (KAPI industries limited).

### Percent protection

The percentage protection was calculated by comparison of the mean collections between the control and treatments arms for both the

Liquid Mosquito Repellent and Risasi slow release using the formula below;

$$\text{Percentage Protection} = 100 \times (1 - (\text{mean treatment} / \text{mean control})).$$

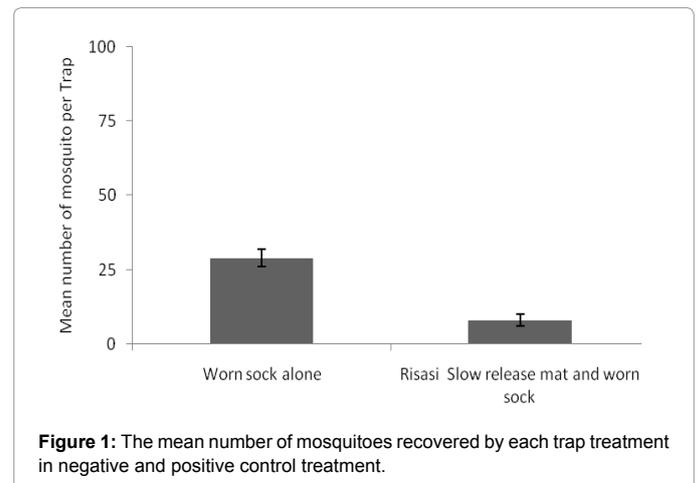
### Statistical analysis

Data were recorded in sheets and entered in excel spreadsheet. The data were transferred to statistical package for social scientists (SPSS) version 25 (SPSS Inc., Chicago, IL). The paired T-test two tailed-distribution with equal variance (homoscedastic) was performed for each comparison of two comparative samples.

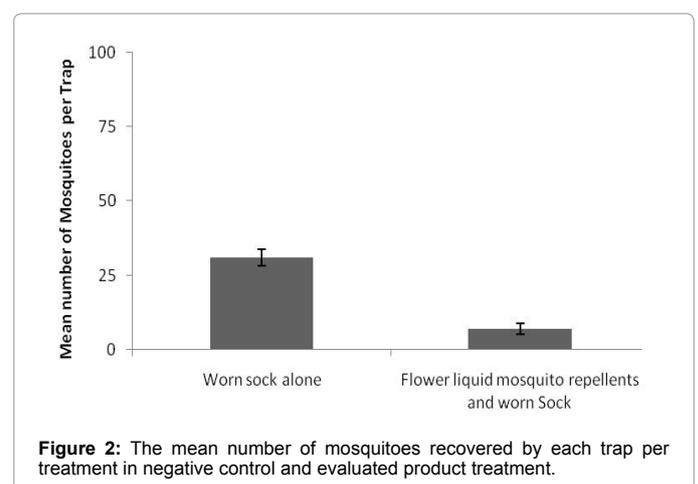
### Results

#### Laboratory results

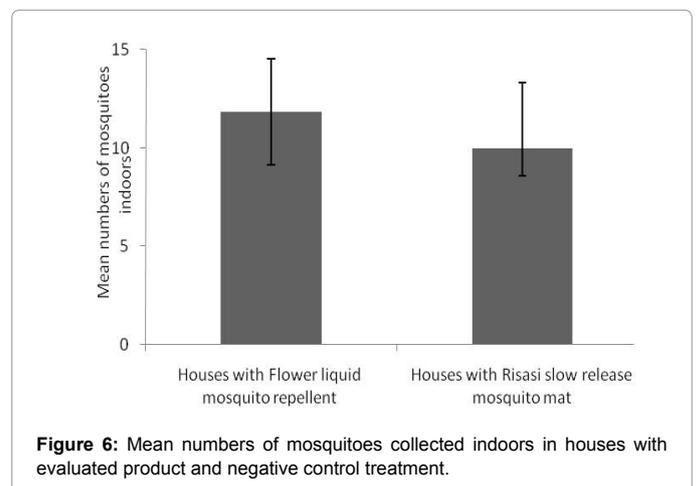
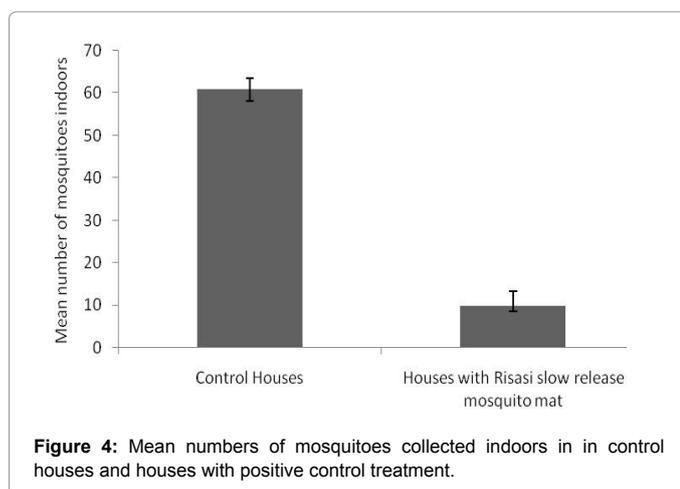
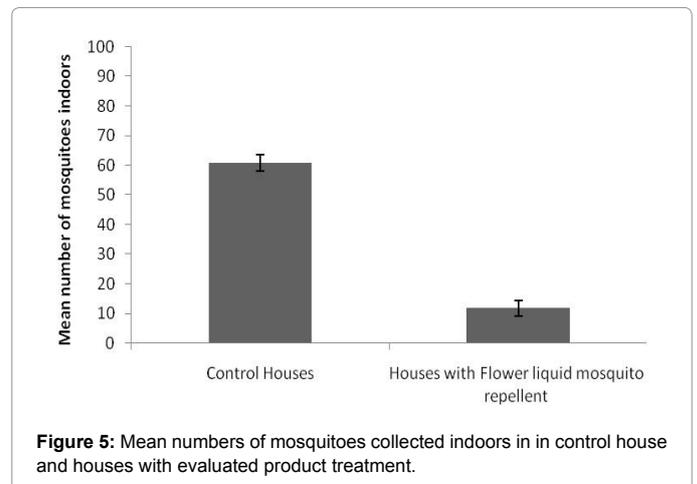
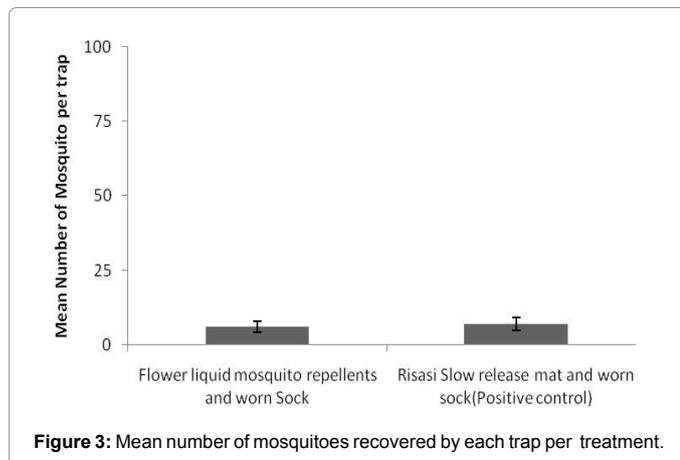
The mean number of mosquitoes sampled by trap with untreated worn sock was statistically significant higher than those recaptured in a trap with a worn sock treated with Risasi slow release mosquito mat ( $t=16.28, P<0.001$ ) (Figure 1). The number of mosquitoes recaptured in a trap with worn sock alone was higher and significantly different with a worn sock in a trap close to flower liquid mosquito repellent ( $t=18.8, P<0.001$ ) (Figure 2). The mean numbers of recaptured mosquitoes in both traps close to Risasi slow release mat and Flower liquid mosquito repellent were low and had no statistical difference between them ( $t=1.08, P=0.721$ ) (Figure 3).



**Figure 1:** The mean number of mosquitoes recovered by each trap treatment in negative and positive control treatment.



**Figure 2:** The mean number of mosquitoes recovered by each trap per treatment in negative control and evaluated product treatment.



## Field results

The total of 796 female mosquitoes was collected from the three arms for sixty days. The indoor resting mosquitoes in houses treated with Risasi slow release mosquito mat was reduced by 90.9% compared to control house which was statistically significant with  $P < 0.001$  (Figure 4).

The mean number of mosquitoes collected in houses with liquid flower repellents decreased by 97.3% compared to control which was statistically significant ( $P < 0.001$ ) as shown in Figure 5. There was no significant difference in the mean number of mosquitoes ( $P < 0.621$ ) between the two treatments in terms of mosquito sampled in those houses (Figure 6).

## Discussion

The present study assessed bioefficacy of Flower liquid Mosquito Repellent against laboratory strain and wild population of *Anopheles gambiae* s.l. In laboratory tests and field trials both Liquid Mosquito Repellent and Risasi slow release provided high protection against *Anopheles* mosquitoes. However, the Flower liquid mosquito repellent showed higher protection efficiency than that of the standard registered product Risasi slow release mosquito mat. The Flower liquid mosquito product can therefore be used for reducing indoor resting vectors and subsequently reduces indoor residual malaria transmission. Repellents have shown to have impact on indoor vector reduction both traditional

plants and synthetic repellents [30]. They reduce vector populations in houses, adding value to the attained impact of IRS and LLINs (WHO, 2016).

Repellents may form an attractive option in areas where mosquitoes bite during daytime or in the early evening, as has been reported in Asia [31]. Repellents may also be quite useful in some areas for example Dar-es-salaam, Tanzania, where outdoor feeding among malaria vectors have been reported to be increasing due to wide ITNs coverage, hence increasing biting pressure on unprotected individuals outdoors [32]. The vectors have also changed feeding behaviour and tend to feed outdoors (exophagic) due to massive IRS, house modifications and ITN coverage. As expected, there were no adverse events like local irritations or nausea experience in laboratory and the house occupants.

## Conclusion

Although the Flower liquid mosquito repellent did not provide complete protection (100% protection from *Anopheles* mosquitoes), its protective efficacy is almost comparable to the known repellent cream DEET for prolonged protection against malaria vectors

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## Author's Contribution

EJK: Conceived and designed the study. LL, VT, SM, JM did data analysis and interpretation. All authors read and agreed for the submission of this manuscript.

## Ethics Approval and Consent to Participate

The study was approved by TPRI ethics committee and given experimental permit No. 1833 by registrar of pesticides in Tanzania.

## Competing Interest

The author's declare to have no competing interests.

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