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# Creating mosquito-free outdoor spaces using transfluthrin-treated chairs and ribbons

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**CREATING MOSQUITO-FREE OUTDOOR SPACES USING  
TRANSFLUTHRIN-TREATED CHAIRS AND RIBBONS**

**John Paliga**

**A Dissertation Submitted in Partial Fulfillment of the Requirement for the Degree of  
Master of Science in Public Health Research of the Nelson Mandela African Institution  
of Science and Technology**

**Arusha, Tanzania**

**March, 2020**

## ABSTRACT

Residents of malaria-endemic communities spend several hours outdoors performing different activities such as cooking, story-telling or eating; thereby exposing themselves to potentially-infectious mosquitoes. This compromises indoor interventions, notably long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS). This study characterized common peri-domestic spaces in rural south-eastern Tanzania, and assessed protective efficacies of transfluthrin-treated chairs and hessian ribbons against mosquitoes. Two hundred households were surveyed, and their most-used peri-domestic spaces physically characterized. Protective efficacies of these two prototyped interventions were tested outdoor in 28 households in dry and wet seasons, using volunteer-occupied exposure-free double net traps. Center for Diseases Control and Prevention miniature light traps (CDC-LT) were used to estimate host-seeking mosquito densities within outdoor kitchens. Field-collected *Anopheles arabiensis* and *Anopheles funestus* mosquitoes were exposed underneath the chairs to estimate 24h-mortality. Approximately half (52%) of houses had verandas. Aside from these verandas, most houses also had peri-domestic spaces where residents stayed most times (67% of houses with verandas and 94% of non-veranda houses). Transfluthrin-treated chairs reduced outdoor-biting *An. arabiensis* densities by 70-85% while transfluthrin-treated hessian ribbons caused 77-81% reduction in the general peri-domestic area. Field-collected *An. arabiensis* (99.4%) and *An. funestus* (100%) exposed under transfluthrin-treated chairs died. Most houses had actively-used peri-domestic spaces where exposure to mosquitoes occurred. The transfluthrin-treated chairs and ribbons reduced outdoor-biting malaria vectors in these peri-domestic spaces, and also elicited significant mortality among pyrethroid-resistant field-caught malaria vectors. These two new prototypes, if developed further, may constitute new options for complementing LLINs and IRS with outdoor protection against malaria and other mosquito-borne pathogens in areas where peri-domestic human activities are common.

**Keywords:** Peri-domestic spaces, transfluthrin-treated chairs, hessian ribbons, transfluthrin, spatial repellents, outdoor-biting, and malaria vectors.

## **DECLARATION**

I, John Paliga, I do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.



John Paliga

22.03.2020

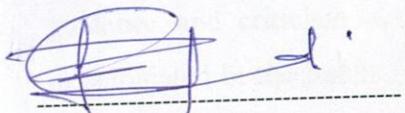
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## CERTIFICATION

I hereby confirm that the dissertation entitled “creating mosquito-free outdoor spaces using transfluthrin-treated chairs and ribbons” submitted by John Paliga to Nelson Mandela African Institution of Science and Technology, Tanzania in partial fulfillment of the requirements for the award of Master of Science in Public Health Research is an authentic work and has been done under my supervision.



Fredros Okumu, PhD  
**Supervisor**

23.03.2020

Date

## **ACKNOWLEDGEMENT**

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I would also like to thank Ms. Rukiyah Mohammad who helped me during the design of the experimental chairs evaluated in this study.

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Lastly and not least my deep appreciation goes to Ifakara Health Institute Training and Capacity Building Department for funding my project.

## **DEDICATION**

I dedicate this work to my father Mr. Masalu Mayenga Mchele and my mother Ng'wasi Maduhu Makalo and the rest of my brothers and sisters.

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## **LIST OF ABBREVIATION AND SYMBOLS**

CDC	Center for Diseases Control and Prevention
DDT	Dichlorodiphenyltrichloroethane
CI	Confidence interval
GLMM	Generalized Linear Mixed Effects model
IRB	Institutional Review Board
IRS	Indoor residual spraying
LLINs	Long-lasting insecticidal nets
NIMR	National Institute for Medical Research
PCR	Polymerase chain reaction
RR	Relative rate
&	And
<i>et al</i>	And others
°C	Degree centigrade
%	Percentage

# **CHAPTER ONE**

## **INTRODUCTION**

This chapter comprises of general introduction, objective, statement of the problem, rationale of the study, research questions, hypothesis, significant of the research and the delineation of the study. The overall objective was to create mosquito-free outdoor spaces using transfluthrin-treated chairs and transfluthrin-treated hessian ribbons and this was accomplished by creating three specific objectives: (a) to characterize peri-domestic spaces used by people for various early-evenings outdoor activities; (b) assess the efficacies of transfluthrin-treated chairs and ribbons in creating mosquito-free peri-domestic space and (c) test insecticide susceptibility of mosquitoes to pesticides commonly used in public health.

### **1.1 Background of the Problem**

Since 2000, malaria morbidity and mortality have tremendously declined in sub-Saharan Africa (Bhatt *et al.*, 2015; WHO, 2015b, 2017, 2018b), though the recent evidence suggests that such gains are starting to stagnate (WHO, 2017, 2018b, 2019). Most of the gains observed between 2000 and 2015 were estimated to have been contributed by the existing core indoor vector control interventions, i.e. Insecticide Treated Nets (ITNs) and indoor residual spraying (IRS) (Bhatt *et al.*, 2015; Noor *et al.*, 2014; O'Meara, Mangeni Steketee & Greenwood, 2010; Steketee & Campbell, 2010).

Long-lasting insecticide treated nets (LLINs) and IRS are effective against indoor-biting and indoor-resting mosquitoes, but are less effective against outdoor-biting mosquitoes, which are important vectors for residual malaria transmission (Durnez & Coosemans, 2013; Govella & Ferguson, 2012; Russell *et al.*, 2011; Sherrard-Smith *et al.*, 2019). It has been estimated that the *Anopheles* bites not preventable by LLINs could be causing up to 10 million additional malaria cases annually (Sherrard-Smith *et al.*, 2019). As a result, LLINs and IRS require complementary interventions to achieve the 2030 global targets of reducing malaria burden by at least 90% and elimination in 35 endemic countries (WHO, 2015a).

In many malaria-endemic communities, people spend several hours cooking, eating and socializing outdoors in the early evenings before they go to sleep (Monroe, Moore, Koenker, Lynch & Ricotta, 2019), and also in the early mornings after they wake up, when malaria vectors may be active and mediating transmission (Durnez & Coosemans, 2013). Some of

these outdoor activities, as well as sleeping outdoors (Monroe *et al.*, 2015), are partly attributable to warm climate (Moshi *et al.*, 2017), but they also have strong cultural determinants (Finda *et al.*, 2019; Moshi *et al.*, 2018). The importance of outdoor malaria transmission, and associated outdoor human activities, are now well-established (Finda *et al.*, 2019; Govella & Ferguson, 2012; Monroe *et al.*, 2019; Russell *et al.*, 2011). However, there are still gaps regarding appropriate interventions to address these gaps. The characteristics of the peri-domestic spaces where households conduct outdoor activities remain poorly documented, despite being essential for designing, creating and testing interventions to complement LLINs and IRS by protecting such outdoor spaces.

Several intervention options have been proposed as candidates for closing these malaria transmission gaps (Williams *et al.*, 2018). Examples include: (a) outdoor-baited traps (Homan *et al.*, 2016; Okumu, Govella, Moore, Chitnis & Killeen, 2010), (b) attractive targeted sugar baits (Müller *et al.*, 2010), (c) pyrethroid-treated clothing (Crawshaw *et al.*, 2017; Rowland *et al.*, 1999), zooprophylaxis (Rowland *et al.*, 2001) and (d) repellents (Gupta & Rutledge, 1994) among others. Topical repellents applied on human skin are widely available for personal protection in some areas. However, commercial formulations of government-sectioned scale-up campaigns of such topical repellents are limited because they protect only individual users (Moore, Davies, Hil & Cameron, 2007), have low user compliance rates and acceptance (Gryseels *et al.*, 2015; Maia, Kliner, Richardson, Lengeler & Moore, 2018; Makungu *et al.*, 2017), and have only short-term efficacy (Sangoro, Kelly, Mtali & Moore, 2014). They are also expensive for repeated use by the low-income populations at greatest risk.

On the other hand, spatial repellents are volatile insecticides that diffuse into the air as vapour, and may protect multiple people within the surrounding space against outdoor-biting malaria vectors (Achee *et al.*, 2012; Govella, Ogoma, Paliga, Chaki & Killeen, 2015; Masalu *et al.*, 2017; Ogoma *et al.*, 2017; Ogoma *et al.*, 2012). In recent years, several versions and delivery formats have been recently developed and have shown promising result to be able to provide both indoor and outdoor protection against diseases-transmitting mosquitoes without repeated application for several months (Govella *et al.*, 2015; Masalu *et al.*, 2017; Mmbando *et al.*, 2018; Mwanga *et al.*, 2019; Ogoma *et al.*, 2017; Ogoma *et al.*, 2012). In particular, a wide range of transfluthrin emanator prototypes based on treated hessian fabric products have been recently developed that protect indoor and outdoor spaces for several months without

repeated reapplication (Govella *et al.*, 2015; Masalu *et al.*, 2017; Mmbando *et al.*, 2018; Mwanga *et al.*, 2019; Ogoma *et al.*, 2017; Ogoma *et al.*, 2012). Transfluthrin also has additional properties beyond just spatial repellency that include toxicity to mosquitoes, and incapacitation that prevents blood-feeding, which could contribute to community-wide mass effects, even for non-users (Mwanga *et al.*, 2019; Ogoma *et al.*, 2014).

Improved understanding of the peri-domestic spaces coupled with new interventions that can be effective in such spaces, could potentially address current challenges related with exposure to outdoor-biting exposure and transmission risk. This study was therefore aimed at addressing two knowledge gaps by: (a) characterizing the common peri-domestic spaces used by communities in rural south-eastern Tanzania for various outdoor activities, and (b) assessing the protective efficacies of two hessian-based transfluthrin-emanator prototypes, specifically transfluthrin-treated chairs and transfluthrin-treated hessian ribbons wrapped around outdoor kitchen, against outdoor-biting malaria vectors and other pathogens-carrying mosquitoes in those peri-domestic spaces, and lastly (c) conducting WHO insecticide susceptibility test in malaria vectors.

## **1.2 Statement of the Problem**

The importance of outdoor malaria transmission and the outdoor human activities that facilitate it is now well-established (Finda *et al.*, 2019; Govella & Ferguson, 2012; Monroe *et al.*, 2019; Russell *et al.*, 2011). However, there are still gaps regarding appropriate interventions to address the challenges. The characteristics of the peri-domestic spaces where households conduct outdoor activities remain poorly documented, despite being essential for designing, creating and testing interventions to complement LLINs and IRS by protecting such outdoor spaces.

## **1.3 Rationale of the Study**

The fact that data on characteristics of the peri-domestic spaces used by household members for various outdoor activities remain poorly documented and these people are highly disproportionately exposed to potentially-infectious mosquitoes while are in these spaces; characterizing peri-domestic spaces, designing, creating and testing interventions that would be used specific in these spaces to complement LLINs and IRS worth investigation.

## **1.4 Objectives**

### **1.4.1 General objective**

Create mosquito-free outdoor spaces using transfluthrin-treated chairs and ribbons

### **1.4.2 Specific objectives**

- (i) To characterize peri-domestic spaces used by people for various early-evenings outdoor activities.
- (ii) To assess the efficacy of transfluthrin-treated chairs and transfluthrin-treated hessian ribbons in creating mosquito-free outdoor spaces, and
- (iii) To test insecticide susceptibility of mosquitoes to pesticides commonly used in public health.

## **1.5 Research questions**

- (i) Which are the peri-domestic spaces used by households members for various outdoor activities?
- (ii) By what magnitude do transfluthrin-treated chairs and transfluthrin-treated hessian ribbons create mosquito-free outdoor spaces?
- (iii) What is the current insecticide susceptibility status on malaria vectors in Lupiro village?

## **1.6 Hypothesis**

- (i) The study hypothesized that transfluthrin-treated chairs can create mosquito-free outdoor spaces, and
- (ii) The study hypothesized that transfluthrin-treated hessian ribbons can create mosquito-free outdoor spaces

## **1.7 Significance of the study**

As the data on the characteristics of common peri-domestic spaces used by household's members for various outdoor activities was lacking, this study filled this gap by clearly characterizing these spaces. This information were used as a benchmark for designing, creating and testing two interventions, notably transfluthrin-treated chairs and transfluthrin-treated hessian ribbons for specifically creating mosquito-free peri-domestic spaces in order to complement LLINs and IRS.

## **1.8 Delineanation of the Study**

Important of outdoor malaria transmission and the associated outdoor activities which coincide with peak hours of mosquitoes bites and this expose people to potentially-infectious mosquitoes is well documented. To date, data on the characterization of the peri-domestic spaces which are used by people for different activities before they go to sleep such as cooking, eating, watching television, story-telling among other is missing. This data is invaluable in the sense that it could be used as a basis for designing, creating and testing vector control intervention specific for these spaces to complement long lasting insecticidal nets and indoor residual spraying.

This study therefore focused on clearly characterizing peri-domestic spaces, which are used by people for various activities before they go to sleep and created and tested two vector control tools in creating mosquito-free outdoor spaces: (a) transfluthrin-treated chairs and (b) transfluthrin-treated hessian ribbons.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Introduction

To achieve global malaria elimination and eradication targets, protection of people against outdoor malaria transmission is well-advocated (Govella & Ferguson, 2012; Killeen, 2014; Russell *et al.*, 2011; Sherrard-Smith *et al.*, 2019). With the recent reiteration from WHO high-level panel on malaria eradication (WHO, 2019a) and the Lancet commission on malaria eradication (Feachem *et al.*, 2019), this call for the new innovative malaria vector control tools to complement LLINs and IRS. As people stay outdoors in early evenings before they go to sleep, they may be disproportionately exposed to potentially-infectious mosquito bites. Peri-domestic spaces are the common outdoor premises commonly used by community members for different activities, notably resting, cooking and eating, and this behavior coincides with peak mosquito bites (Matowo *et al.*, 2013). To date, data on characteristics of these peri-domestic spaces, which may be used as the basis for designing, creating and testing malaria vector control tools specific in these settings still poorly documented. The use of transfluthrin-treated hessian-based spatial repellent emanators is currently growing subject of interest in controlling outdoor-biting mosquitoes (Govella *et al.*, 2015; Masalu *et al.*, 2017; Mmbando *et al.*, 2018; Mwanga *et al.*, 2019; Ogoma *et al.*, 2012). This chapter, review on the global and national malaria burden, national adopted malaria control strategies, the threat of insecticides resistance malaria by vectors, early evenings peri-domestic spaces activities conducted by communities members before they go to sleep and the use of transfluthrin-treated chairs and transfluthrin-treated hessian ribbons as potential peri-domestic spaces malaria vector control tools.

#### 2.2 Global Burden of malaria transmission

In 2017, it was estimated that about 219 million cases and 435 000 deaths occurred globally and resulted to an increase of two million cases as compared to 2016 (WHO, 2018b). Even though 239 million cases occurred in 2010, the 20 million reduction in cases demonstrated that progress in malaria reduction has stalled (WHO, 2018b). The current evidence also show that 228 million malaria cases were recorded in 2018, which resulted to an increase of nine million cases as compared to 2017 (WHO, 2019). African region alone contributed to about

93% of the global malaria cases of which, 19 sub-Saharan countries and India contributed to an approximately 85% of global malaria burden (WHO, 2019) .

Generally, global malaria burden has substantially decreased since 2000, however, recent evidences show that these gains have stalled (WHO, 2017, 2018b, 2019) which may undermine the efforts toward malaria elimination and eradication.

### **2.3 Burden of malaria transmission in Tanzania**

In 2011-12, malaria prevalence in Tanzania was estimated to be 10% (NBS, 2012), whereas in 2015-16 the prevalence was estimated to be 14% (NBS, 2017), with the lake and southern region carrying relatively high burden of the disease. Besides, the goal of Tanzania was to reduce malaria prevalence from 10% in 2012 to 5% in 2016 and to the lesser than 1% in 2020 (MOHSW, 2014). Recent evidence show that malaria prevalence dropped from 14% in 2015-16 to 7.3% in 2017 (TMIS, 2018).

### **2.4 Malaria control strategies in Tanzania**

In Tanzania, vector control tools include LLINs most of which are distributed by the government (Renggli *et al.*, 2013), IRS and larvicide in selected areas as well as preventive and treatment strategies including the use intermittent preventive treatment in pregnancy (IPTp) in second and third trimesters, intermittent preventive treatment in infants (IPTi), the use of artemisinin combination therapy (ACTs) among others. Currently, Tanzania has developed a 2019-2024 vector control strategy, of which the use of repellent is one of the measures highlighted in the strategic objective one which entails to implement effective control measures against vectors to reduce transmission of vector borne diseases.

### **2.5 The challenges of malaria vectors resistance to insecticides commonly used in public health**

Long-lasting insecticidal nets and indoor residual spraying are key malaria vector control tools in sub-Saharan Africa. In Tanzania, LLINs are widely distributed and used as the main malaria vectors control tools (Renggli *et al.*, 2013). Due to the dermal toxicity safety of pyrethroid in mammals, this is the only class of insecticide used to impregnate these nets. Similarly, this class of insecticide is also widely used in control of crop pests in agriculture, as a result, insecticides contaminated water can permeates ground water which contain

mosquitoes larval, thereby exacerbating insecticides resistance in malaria vectors (Ranson *et al.*, 2009). The wide-scale spread of insecticide resistance in malaria vectors compromise the efforts toward malaria control and elimination (Cook *et al.*, 2018; Protopopoff *et al.*, 2018; Tiono *et al.*, 2018). To achieve malaria elimination and eradication targets, routine monitoring of this public health threat on the current mainstay malaria control tools such as LLINs and IRS is required in order to preserve and maintain the fragile gains accrued after the scale-up of these interventions (WHO, 2012).

## **2.6 Peri-domestic space activities conducted by household's members before bed time**

In many malaria-endemic communities, people spend several hours cooking, eating and socializing outdoors in the early evenings before they go to sleep, and also in the early mornings after they wake up (Monroe *et al.*, 2019), when malaria vectors may be active and mediate transmission (Durnez & Coosemans, 2013). Some of these outdoor activities, as well as sleeping outdoor (Monroe *et al.*, 2015), are partly attributable to warm climate (Moshi *et al.*, 2017) but also have strong cultural determinants (Finda *et al.*, 2019). The importance of outdoor malaria transmission and the outdoor human activities that facilitate it is now well-established (Finda *et al.*, 2019; Govella & Ferguson, 2012; Monroe *et al.*, 2019; Russell *et al.*, 2011). However, there are still gaps regarding appropriate interventions to address it. The characteristics of the peri-domestic spaces where households conduct outdoor activities remain poorly documented, despite being essential for designing, creating and testing interventions to complement LLINs and IRS by protecting such outdoor spaces.

## **2.7 The use of transfluthrin-treated chairs and hessian ribbon as outdoor-biting malaria vector control tools**

Following the scale up of LLINs across Africa to control indoor malaria transmission, approximately half of remaining residual transmission occurs outdoors, where it cannot be prevented with additional insecticide house spraying or screening methods (Killeen, 2014; Killeen *et al.*, 2013). It has been estimated that the *Anopheles* bites not preventable by LLINs could be causing up to 10 million additional malaria cases annually (Sherrard-Smith *et al.*, 2019). With this challenge, LLINs and IRS require complimentary interventions to achieve the 2030 global targets of malaria burden reduction of at least 90% and elimination in 35 endemic countries (WHO, 2015a).

Topical repellents applied on human skin offer one widely available option for personal protection, but commercially available formulations are limited because they protect only individual users (Moore *et al.*, 2007), have low user compliance rates and acceptance (Gryseels *et al.*, 2015; Maia *et*

*al.*, 2018; Makungu *et al.*, 2017), and have only short-term efficacy (Sangoro *et al.*, 2014), so they are too expensive for repeated use by the low-income populations at greatest risk. However, spatial repellents are volatile insecticides that diffuse into the air as vapour, and may protect multiple people within the surrounding space against outdoor-biting malaria vectors (Achee *et al.*, 2012) thereby, protecting multiple individuals occupying that space.

A recently developed long-lasting, low-tech formulation of the widely-used spatial repellent, transfluthrin, impregnated it into strips of locally available sacking fabric (Ogoma *et al.*, 2012) (Fig. 1a), can provides more than 90% protection against wild *An. gambiae* and *Culex* spp. for over four months (Govella *et al.*, 2015) (Fig. 1b). Besides, this experimental prototype provides up to a year of protection (Ogoma *et al.*, 2017), with no diversion to non-users (Ogoma *et al.*, 2017), and is equally efficacious for six months with a dosage of only 1 ml transfluthrin (Ogoma *et al.*, 2017). However, this experimental prototype is impractical for everyday use, as it requires the user to sit within the 1 m<sup>2</sup> perimeter of a strip, suspended on four poles (Fig. 1b).

This study specifically designed more practical prototypes, notably fitting a locally-popular chair (Fig. 1c) with transfluthrin-treated hessian mat underneath the seat (Fig. 1d), where there is no physical contact with the user. It is also worth noting that wooden chairs are popular across different demographic and socio-economic groups, making them an effective means to deploy and scale-up protective mosquito repellents, even in rural and marginalized communities. Similarly, as the first lead technology had hessian strip fitted about 1 m from the floor on the four poles making a 1 m<sup>2</sup> perimeter (Ogoma *et al.*, 2012), this way, made it impractical for programmatic scale up. With this limitation, this study changed the position of the strip from fitting it at 1 m above the floor (which restricted free entry of the user) to about 1.3 m (Figs. 1e and 1f) on the outdoor kitchen that is relatively convenient and allows free entrance of the user. This study therefore used low-tech and, scalable spatial repellents, notably transfluthrin-treated chairs and hessian ribbons for creating mosquito-free spaces.

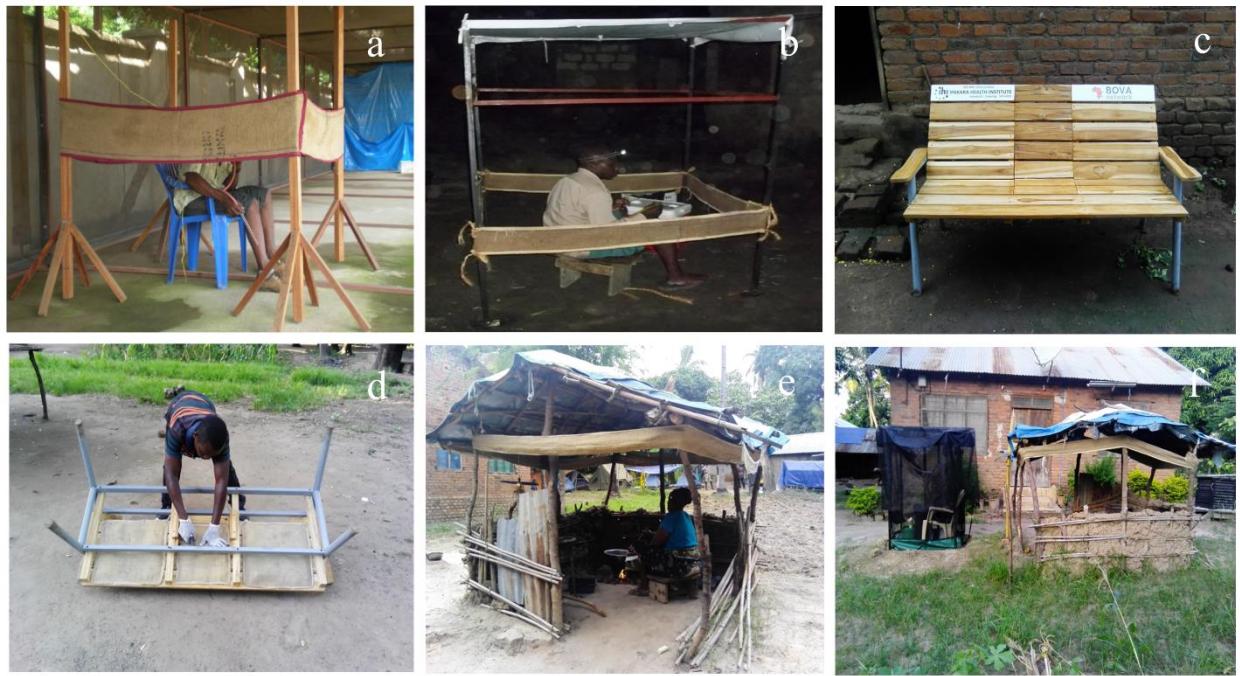


Figure 1: Stagewise development on the use of transfluthrin-treated chairs and transfluthrin-treated hessian ribbons

Key: a = Illustrating the original transfluthrin-treated hessian ribbon as previous described (Ogoma *et al.*, 2012), b = field evaluation of transfluthrin-treated hessian ribbon (Govella *et al.*, 2015), c = transfluthrin-treated chairs prototype evaluated in this study, d = fitting of transfluthrin-treated hessian mat underneath the chair, e = example of outdoor-kitchens installed with transfluthrin-treated hessian ribbon and f = example of outdoor-kitchens installed with transfluthrin-treated hessian ribbon with a miniaturized double net trap installed about 1.2 m from kitchen (Limwagu *et al.*, 2019)

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Hessian materials and the repellents

Used hessian sacks were purchased from kariakoo market, Dar es Salaam, Tanzania. A 99% technical grade of transfluthrin (Bayer CropScience AG, Monheim am Rhein, German), acetone from Sigma Aldrich and a domestic liquid detergent, tarmol (Tarmal soap and allied industries limited, Dar es Salaam, Tanzania). Timbers, nails and vanish were purchased from local hardware vendors in Lupiro village.

#### 3.2 Study area

The study was implemented in Lupiro village ( $8.385^{\circ}\text{S}$ ,  $36.670^{\circ}\text{E}$ ) (Fig. 2), in the Kilombero valley, south-eastern Tanzania. Households were selected from four sub-villages namely: (a) Ndoro; (b) Libaratula; (c) Mabatini and (d) Lupiro Kati. Most residents here were peasants, cultivating rice, maize and other crops. Houses have brick or mud walls, and metal (corrugated iron sheets) or grass-thatched roofs. Annual rainfall is 1200-1600 mm, and temperatures range between  $20.0^{\circ}\text{C}$  and  $32.6^{\circ}$  (TMA; World Weather Online). Principal malaria vectors in this area are *Anopheles funestus* and *Anopheles arabiensis* with the former contributing over 80% of transmission (Kaindoa *et al.*, 2017). Both *An. arabiensis* and *An. funestus* populations in the area have been shown to be resistant to multiple public health insecticides including pyrethroids, carbamates and organochlorides (Kaindoa *et al.*, 2017; Lwetoijera *et al.*, 2014; Matowo *et al.*, 2017). Long-lasting insecticidal nets are the main malaria prevention method, most of which are distributed by the government (Renggli *et al.*, 2013).

### **3.3 Characterization of peri-domestic spaces**

To achieve this objective, two hundred (200) households were surveyed, including 50 from each sub-village (Fig. 2), selected via stratified random sampling. Data were collected using electronic tablets using *KoboCollect™*, an open access software programmed using Open Data Kit (ODK) (KoBoToolbox, 2019). A trained research team was assigned to each sub-village. Written informed consent was obtained from each of the 200 households. For each household, the peri-domestic spaces were observed directly to characterize them physically based on use, physical setting (location) and whether they were built-up or not. Digital pictures were taken of the different peri-domestic environments. The research team also administered survey questions to the household heads on: (a) identification information such as age, (b) education level, (c) socio-economic data including source of income, possession of radio, television, cell phone among others, (d) information on peri-domestic spaces such as presence of other peridomestic spaces apart from veranda and their usage, presence of other peri-domestic spaces if the house had no veranda and their usage.

The peri-domestic spaces were classified as either: (a) built-up spaces attached to the main houses, i.e. veranda extensions; (b) built-up spaces not attached to the main houses, e.g. separate kitchens, and (c) non-built-up or other peri-domestic spaces commonly used for various outdoor activities.

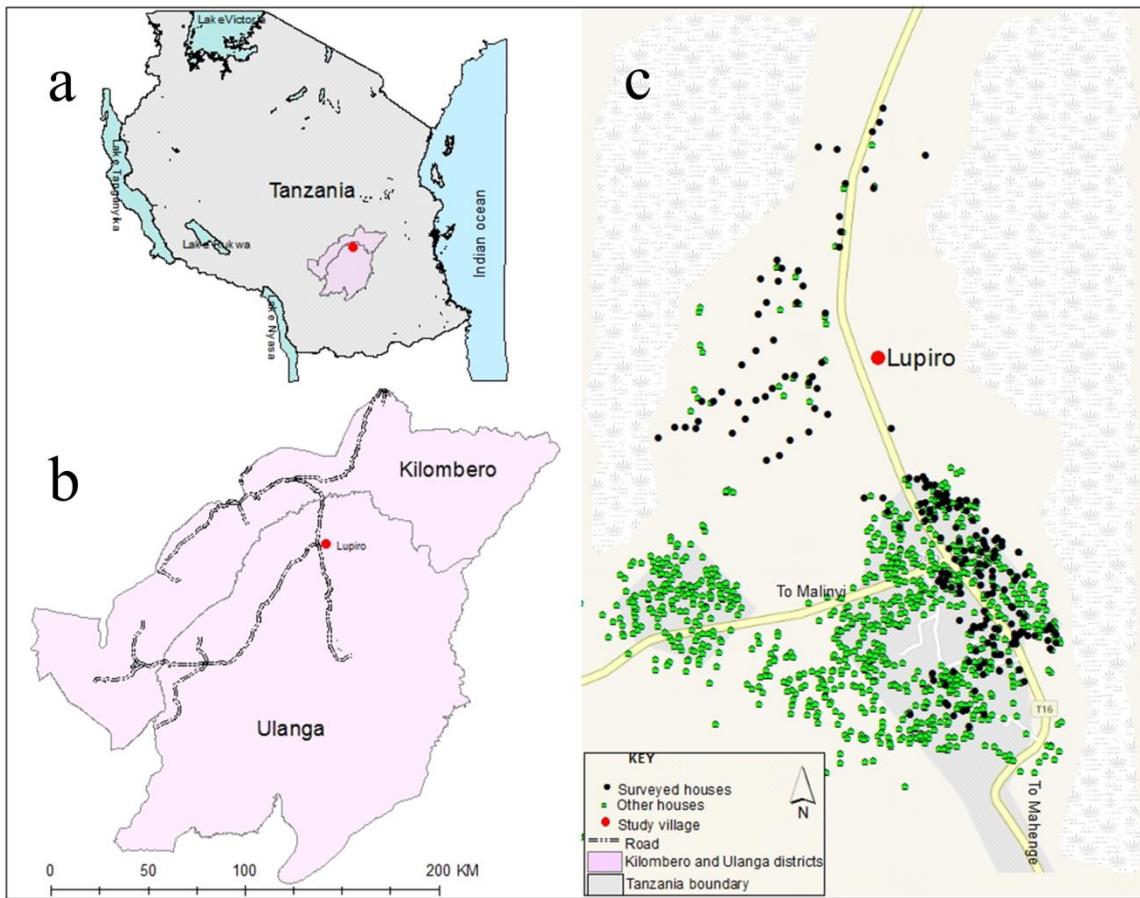


Figure 2: Map illustrating the study area

Key: a = Illustration of the location of Ulanga and kilombero district in the map of Tanzania, b = the location of Lupiro village in Ulanga district and c = household location in Lupiro village showing both surveyed and those did not

### 3.4 Making of transfluthrin-treated chairs and hessian ribbons

For the dry season experiment, six identical chairs made of wood and metal frame were constructed by a local carpenter, while for the wet season experiment 15 chairs were made (Fig. 3a). The chairs were fitted underneath with four standardized hessian fabric mats: two measuring 42 cm × 43 cm and fitted underneath the right and left sides of the chair and other two measuring 20 cm × 33 cm, which were fitted underneath the middle part of the chair (Fig. 3b). These mats were made by a local seamstress at the Ifakara Health Institute fabrication facility (the MozzieHouse). The hessian mats had been treated in emulsified solutions containing 2% transfluthrin (Bayer AG, Germany), prepared as previously described (Masalu *et al.*, 2017; Ogoma *et al.*, 2012).

Similarly, the hessian ribbons were prepared as previously described (Mmbando *et al.*, 2018). Each ribbon had 15 cm width and 10 m length, made locally at the MozzieHouse and treated

in a 2% emulsified solution of transfluthrin. Detailed descriptions of such hessian ribbons and treatment procedures have previously been published (Mmbando *et al.*, 2018; Ogoma *et al.*, 2012).

### **3.5 Determination of the efficacy of transfluthrin-treated chairs and ribbons**

The efficacy of transfluthrin-treated chairs and transfluthrin-treated hessian ribbons was evaluated as explained in section 3.5.1 and 3.5.2.

#### **3.5.1 Determine the efficacy of transfluthrin-treated chairs against outdoor-biting mosquitoes**

This assessment was conducted in two seasons: dry and wet seasons, between September to October, 2019 and between January to February, 2020 as dry and wet seasons respectively. Procedures: to address this objective, six houses with an outdoor kitchen were selected in the dry season. The houses were paired and assigned as follows: (a) a control arm, where no transfluthrin-treated chair was used, (b) a treatment arm where one transfluthrin-treated chair was used, and (c) a second treatment arm where two transfluthrin-treated chairs were used. One consenting male volunteer was assigned to each household and collected mosquitoes using the exposure-free miniaturized double nets trap (DN-Mini) (Limwagu *et al.*, 2019) from 1900 h to 2300 h, totaling 45 min of actively catching mosquitoes in each hour. For the households with transfluthrin-emanating chairs, the DN-Mini was installed 0.5 m from the chairs (Fig. 3c & 3d). After each experimental night, treatments were moving to the next houses using a 4×4 Latin square design for 32 experimental nights. As sections 3.5.1 and 3.5.2, were conducted at the same experimental night, the 4×4 Latin square design described here included other two households as a third treatment arm where transfluthrin-treated hessian ribbons were used around the outdoor kitchens as explained in section 3.5.2. As the experiments were conducted outdoor with enough airflow, there was no need to break for wash out. Similarly, volunteers were changing their position in each experimental night. Each morning the collected mosquitoes were sorted and identified using morphological keys (Gillies & Coetzee, 1987). The primary outcome was number of mosquitoes of different species caught in the DN-Mini light trap per house per night. In the wet season, 15 households were enrolled making five households in each arm for other 32 nights. The same procedure was adopted as described in dry season.

### **3.5.2 Determine the efficacy of transfluthrin-treated hessian ribbons against outdoor-biting mosquitoes**

Procedures: to address this activity, two addition houses with the outdoor kitchen were selected in the dry season. These kitchens were fitted with transfluthrin-treated hessian ribbon (Fig. 3e & 3f). The ribbon was fitted about 1.3 m above the floor of the kitchen. A CDC light trap was installed within the kitchen to collect host-seeking mosquitoes and the idea was to determine if the treated ribbon could offer protection to the active user within the kitchen. Additionally, D-Mini trap was installed about 1.2 m outside the kitchen enclosure (Fig. 3f), and the rationale for this was to determine if the fitted kitchen could offer protection to people sitting adjacent it, defined as general peri-domestic area. Furthermore, the houses without intervention (control) were the same as those used in section 3.5.1 above that means section 3.5.1 and 3.5.2 were conducted in the same experimental night. All experimental procedures were the same as those in section 3.5.1. The primary outcome was number of mosquitoes of different species caught in the DN-Mini or the CDC light traps per house per night. In the wet season, addition three households were enrolled making five households for this treatment arm. The same procedure was adopted as described in dry season.



Figure 3: Illustration for efficacy evaluation of transfluthrin-treated chairs and transfluthrin-treated hessian ribbon

Key: a = Design and prototyping of the wooden and metal flame chairs at the local carpentry, b = fitting tranfluthrin-treated hessian mat underneath the chair, c = one tranfluthrin-treated chair with the DN-Mini trap (Limwagu *et al.*, 2019) installed about 0.5 m from the chair, d = two tranfluthrin-treated chairs with DN-Mini trap installed about 0.5 m from each chair, e = outdoor kitchen fitted with transfluthrin-treated hessian ribbon with CDC light trap installed within it and f = outdoor kitchen fitted with transfluthrin-treated hessian ribbon with DN-Mini trap installed about 1.2 m from it

### 3.5.3 Assessing mortality effects of the transfluthrin-treated chairs on mosquitoes

Procedures: To address this activity, the assay was done using: (a) field-collected *An. arabiensis* and *An. funestus* of unknown age which are known to be pyrethroid resistant in this setting (Kaindoa *et al.*, 2017; Lwetoijera *et al.*, 2014; Matowo *et al.*, 2017), (b) laboratory-reared *An. arabiensis* from a pyrethroid-susceptible colony of local origin, and c) laboratory-reared *Aedes aegypti* from a pyrethroid-susceptible colony of local origin (Kahamba *et al.*, 2020).

The wild-caught *An. arabiensis* females were collected using a separate set of eight DN-Mini traps (Limwagu *et al.*, 2019) set outdoors at households without any transfluthrin treatments. Eight consenting adult male volunteers were involved in these collections each night from 1900 h to 0100 h. As population densities of *An. funestus* in this study area were very low, CDC light traps were used to collect adult females of this species from another village approximately 30 km away.

Each morning captured mosquitoes were sorted and *An. arabiensis* and *An. funestus* females separated in two cages containing 100 mosquitoes per species (four cages in total). Since the *Anopheles gambiae* complex in this area are known to consist exclusively of *An. arabiensis* (Masalu *et al.*, 2017), no molecular identification was required. Similarly, since indoor collections of *An. funestus* sensu lato have consistently been found to be >90% *An. funestus* sensu stricto (Masalu *et al.*, 2017), it was assumed that these were the dominant species in the collections. The separated mosquitoes were kept at a field insectary (average temperature:  $26.75 \pm 0.09^\circ\text{C}$ ; relative humidity:  $73.26 \pm 0.46\%$ ) for acclimatization before testing the next evening.

For the tests, two chairs were placed within open verandas of two separate houses. One of the chairs was fitted underneath with transfluthrin-treated hessian mats, while the other was fitted with an untreated hessian mat (control). The caged mosquitoes were placed underneath each chair overnight (1900 h to 0700 h). A simple water moat was used to prevent ants from eating the mosquitoes. Each morning, the cages were returned to the field insectary and monitored for further 12 h, totaling 24 h of observation since start of exposure. This procedure was repeated 10 times, totaling 1140 mosquitoes for field-collected *An. arabiensis* and five times, totaling 490 mosquitoes for field-collected *An. funestus* tested in control and treated arms.

Similar tests were conducted using cages containing 100 laboratory-reared *An. arabiensis* or 100 *Ae. aegypti*. Since *Ae. aegypti* mosquitoes are active during the day, they were exposed from 0800 h to 1900 h each day, as opposed to the *Anopheles* mosquitoes, which were exposed at night. Percentage mortality of mosquitoes was calculated for each species separately as a proportion of total exposed.

### **3.6 Determine susceptibility of malaria vectors in insecticides commonly used in public health and agriculture**

Procedures: In order to determine phenotypic resistance status of local mosquito populations to common pesticides, standard discriminatory tests were performed using standard WHO susceptibility bioassays (WHO, 2018a). Since transfluthrin is a pyrethroid, the tests also provided indication of how the transfluthrin-based interventions evaluated here (transfluthrin-treated chairs and transfluthrin-treated hessian ribbons) evaluated here would perform against wild mosquito populations. The susceptibility tests were done for: (a) 0.1% bendiocarb, a carbamate; (b) 4.0% dichlorodiphenyltrichloroethane (DDT), an organochloride; (c) 0.25%

pirimiphos methyl, an organophosphate, (d) 0.75% permethrin, a type I pyrethroid; and (e) 0.05% deltamethrin, a type II pyrethroid.

Female *An. arabiensis* mosquitoes were collected from the nearby rice fields as larvae, and reared to emergence at Ifakara Health Institute vector biology laboratory. The susceptibility tests were done using three days old adult females, using at least 100 mosquitoes per test (25 per replicate, with at least 4 replicates) as described in the most recent WHO guidelines (WHO, 2018a).

### **3.7 Data analysis**

The survey data was summarized in ODK analysis module (KoBoToolbox, 2019) to generate descriptive statistics of peri-domestic spaces and their usage. Data on efficacy of the transfluthrin-treated chairs and ribbons was analyzed using R open-source statistical software (Team, 2018), primarily using generalized linear mixed-effects models (Bates, Mächler, Bolker, & Walker, 2015), each time modeling the numbers of mosquitoes of a given species caught as a function of the treatments (fixed factors) assuming Poisson distributions. Volunteer, day and house codes were included as random factors in the models.

### **3.8 Ethics approval and consent to participate**

The study was approved by the Institute Review Board of Ifakara Health Institute IHI/IRB/No: 02-2019 and Medical Research Coordinated Committee of the National Institute for Medical Research of the United Republic of Tanzania (NIMR/HQ/R.8a/Vol.1X/3152). All study participants were recruited after signing informed consent forms.

## CHAPTER FOUR

### RESULTS AND DISCUSSION

#### **4.1 Introduction**

This study aimed at characterizing peri-domestic spaces used by households member for various activities and assessed the efficacies of transfluthrin-treated chairs and ribbons in creating mosquito-free outdoor spaces. The study specifically characterized these spaces, assessed the efficacies of these two interventions and finally, conducted WHO insecticide susceptibility test on malaria vectors to pesticide commonly used in public health. This chapter introduces the results from these three tasks and then discussed the meaning of each specific result in the general discussion section.

#### **4.2 Characteristics of households**

The demographic characteristics of household heads, and physical characteristics of all the 200 houses visited are summarized in Table 1. Most of the household heads were female (128/200). The main construction materials were bricks for the walls (153/200) and corrugated iron sheets for the roofs (140/200).

**Table 1:** Characteristics of the study participants and their houses in 200 surveyed households in Lupiro village, Ulanga District, south-eastern Tanzania

<b>Characteristics</b>	<b>Category</b>	<b>Total number surveyed (<i>n</i>)</b>	<b>Proportion (%)</b>
Gender	Male	72	36.0
	Female	128	64.0
Age	Average	38.5	NA
Wall type	Bricks	153	76.5
	Mud & stick	46	23.0
Roof type	Others	1	0.5
	Iron-sheets	140	70
Roof type	Thatched	56	28.0
	Others	4	2.0

### **4.3 Characteristics of the peri-domestic spaces**

Table 2 provides a summary of the physical characteristics of peri-domestic spaces where residents spend time outdoors before bedtime. Of the 200 households observed, 52% (103/200) had built-up veranda (Fig. 4), while 48% (97/200) did not have these verandas.

It was also observed that other than these verandas (Fig. 4), most houses had additional peri-domestic spaces where members congregated. Of the 103 that had verandas, 69 (67%) also had other active peri-domestic spaces, of which 23 were built-up structures and 46, were non-built up. These structures all had at least physical roofing, and 70% of them also had no wall. Two thirds of the built-up structures were used as outdoor kitchens (60% used for cooking) (Fig. 5). Many of the non-built structures (63%) were sited under trees (Fig. 6), while 35% were in open spaces. The peri-domestic spaces were used for multiple activities, e.g. cooking, eating, socializing among others.

Of 97 houses that did not have veranda extensions, 91 (93.8%) had active peri-domestic spaces, of which 32 had built up structures with roofs, and also walls in one third of the cases. Of the non-built structures, 42% were under trees. Common uses of these spaces were similar, i.e. resting, cooking, and eating etc.

**Table 2:** Peri-domestic space characteristic of the households surveyed in Lupiro village, Ulanga district, south-eastern Tanzania

House hold with veranda (N=103)			Household without veranda (N=97)		
Characterization	n	Percentage	Characterization	n	Percentage
Open veranda	69	67.0	N/A		
Closed veranda	34	33.0	N/A		
<b>Usage</b>					
Resting	92	42.2	N/A		
Cooking	67	30.7	N/A		
Eating	56	25.7	N/A		
Others	3	1.4	N/A		
<b>Other peri-domestic space</b>			<b>Other peri-domestic space</b>		
Yes	69	67	Yes	91	93.8
No	34	33	No	6	6.2
<b>Built structure</b>	<b>23</b>		<b>Built structure</b>	<b>32</b>	
Roof	23	100	Roof	31	96.9
No roof	0	0	No roof	1	3.1
Wall	7	30.4	Wall	10	31.3
No wall	16	69.6	No wall	22	68.7
Average Distance from the houses (m)	6.3		Average Distance from the houses (m)	6.8	
<b>Usage</b>			<b>Usage</b>		
Resting	9	24.3	Resting	19	29.2
Cooking	22	59.5	Cooking	30	46.2
Eating	6	16.2	Eating	16	24.6
<b>Non-built structure</b>	<b>46</b>		<b>Non-built structure</b>	<b>59</b>	
Under the tree	34	62.9	Under the tree	28	42.4
Open space	19	35.2	Open space	34	51.5
Others	1	1.9	Others	4	6.1
Average Distance from the houses (m)	6.8		Average Distance from the houses (m)	6.2	
<b>Usage</b>			<b>Usage</b>		
Resting	32	43.2	Resting	54	38.0
Cooking	20	27.0	Cooking	48	33.8
Eating	22	29.7	Eating	40	28.2

Key: n = total number of peridomestic space characterized, and N/A = as not required



Figure 4: Illustration of house with veranda extension physically characterized during survey



Figure 5: Illustration of houses with built-up peridomestic space away from the main house commonly used for cooking



Figure 6: Illustration of houses with non-built-up peridomestic space physically characterized as under the tree

#### 4.4 Overall collected mosquitoes

In the dry season, the total number of mosquitoes collected was 4960, including 2604 *Culex* spp; 2264 *An. gambiae* s.l.; 80 *An. coustani*; 6 *An. funestus*; 4 *Mansonia* spp; and 2 *Coquillettidia* mosquitoes. Polymerase chain reaction (PCR) was conducted on 81 samples of *An. gambiae* s.l to distinguish between sibling species. Of the 90.1% (73/81) successfully amplified in the PCR assays, all (100%) were identified as *An. arabiensis*. In the wet season the total number of mosquitoes collected was 14303, including 12224 *Culex* spp; 1978 *An. gambiae* s.l.; 42 *An. funestus*; 37 *Mansonia* spp; 15 *Ae. aegypti*; 6 *An. coustani*; and 1 *An. pharoensis*. No molecular assay was conducted to identify mosquitos' species in this particular season.

#### 4.5 Efficacy of transfluthrin-treated chairs and transfluthrin-treated hessian ribbons against outdoor-biting mosquitoes in the peri-domestic spaces

Findings on protective efficacy of the two interventions are summarized in Table 3 and Table 4. Using two transfluthrin-treated chairs significantly reduced outdoor-biting *An. arabiensis* mosquitoes by 76% (Relative rate (RR) = 0.24, 95% confidence interval, CI: 0.19-0.29,  $P<0.001$ ) and by 85% (RR= 0.15, 95% CI: 0.12-0.18,  $P<0.001$ ) in dry and wet seasons respectively. Using one transfluthrin-treated chair also significantly reduced *An. arabiensis* mosquitoes, in this case by 70% (RR = 0.30, 95% CI: 0.25-0.37,  $P<0.001$ ) and by 75% (RR = 0.25, 95% CI: 0.20-0.31,  $P<0.001$ ) in dry and wet seasons respectively. When the densities

of *Culex* mosquitoes were assessed, both the two-chair and one-chair interventions significantly reduced outdoor-biting, achieving 52% ( $RR = 0.48$ , CI: 0.37-0.63,  $P<0.001$ ) and 58% ( $RR = 0.42$ , 95% CI: 0.31-0.56,  $P<0.001$ ) protection in dry season respectively. In the wet season, both the two-chair and one-chair interventions significantly reduced outdoor-biting, achieving 51% ( $RR = 0.49$ , CI: 0.43-0.56,  $P<0.001$ ) and 40% ( $RR = 0.60$ , 95% CI: 0.53-0.68,  $P<0.001$ ) protection respectively.

Fitting the transfluthrin-treated hessian ribbons around the outdoor kitchens reduced outdoor-biting *An. arabiensis* by 81% in the area immediately outside this kitchen enclosure ( $RR = 0.19$ , 95% CI: 0.16-0.24,  $P<0.001$ ), and by 43% ( $RR = 0.57$ , CI: 0.32-1.03,  $P= 0.065$ ) inside the enclosures in the dry season. In the wet season, transfluthrin-treated hessian ribbons reduced outdoor-biting *An. arabiensis* by 77% in the area immediately outside this kitchen enclosure ( $RR = 0.23$ , 95% CI: 0.18-0.28,  $P<0.001$ ). The ribbons also reduced outdoor-biting *Culex* spp by 68% ( $RR = 0.32$ , CI: 0.24-0.43,  $P<0.001$ ) near the enclosures and by 77% ( $RR = 0.23$ , CI: 0.12-0.43,  $P<0.001$ ) within the enclosures in the dry season. In the wet season, the ribbons also reduced outdoor-biting *Culex* spp by 36% ( $RR = 0.64$ , CI: 0.56-0.72,  $P<0.001$ ) near the enclosures and by 48% ( $RR = 0.52$ , CI: 0.32-0.86,  $P<0.001$ ) within the enclosures.

**Table 3:** Comparison of nightly outdoor biting per person between houses with or without transfluthrin-treated chairs or ribbons (dry season)

Settings	Species	Treatment	Nights	n	Adjusted-Mean (95% CI)	RR (95% CI)	PP (95% CI)	P-value
Outdoor peri-domestic space	<i>Anopheles arabiensis</i>	Control	32	1056	15.05 (12.29-18.44)	1	0	
		Two TF-chairs	32	273	3.61 (2.87-4.55)	0.24 (0.19-0.29)	0.76 (0.71-0.80)	<0.001
		TF-treated ribbon	32	211	2.96 (2.33-3.75)	0.19 (0.16-0.24)	0.81 (0.75-0.84)	<0.001
	<i>Culex spp</i>	Control	28	910	14.86 (12.07-18.30)	1	0	
		One TF-treated chair	28	290	4.54 (3.60-5.73)	0.30 (0.25-0.37)	0.70 (0.62-0.75)	<0.001
		Control	32	889	10.52 (7.98-13.86)	1	0	
Inside outdoor kitchen enclosure	<i>Anopheles arabiensis</i>	Two TF-chairs	32	426	5.12 (3.84-6.83)	0.48 (0.37-0.63)	0.52 (0.36-0.63)	<0.001
		TF-treated ribbon	32	299	3.43 (2.55-4.61)	0.32 (0.24-0.43)	0.68 (0.57-0.75)	<0.001
		Control	28	744	9.99 (7.43-13.44)	1	0	
	<i>Culex spp</i>	One TF-treated chair	28	335	4.20 (3.07-5.75)	0.42 (0.31-0.56)	0.58 (0.43-0.68)	<0.001
		Control	25	152	1.17 (0.56-2.44)	1		
		TF-hessian ribbon	25	113	0.56 (0.26-1.22)	0.57 (0.32-1.03)	0.43 (-0.03-0.67)	0.065

Key: n = total number of mosquito collected, CI = confidence interval, PP = percentage protection, RR = relative rate, TF = transfluthrin 1 and 0 = references

**Table 4:** Comparison of nightly outdoor biting per person between houses with or without transfluthrin-treated chairs or ribbons (wet season)

Settings	Species	Treatment	Nights	n	Adjusted-Mean (95% CI)	RR (95% CI)	PP(95% CI)	P-value
Outdoor peri-domestic space	<i>Anopheles arabiensis</i>	Control	32	1116	5.71 (4.89-6.67)	1	0	
		One TF-chairs	32	308	1.42 (1.17-1.72)	0.25 (0.20-0.31)	0.75 (0.69-0.79)	<0.001
		Two TF-chairs	32	189	0.86 (0.69-1.07)	0.15 (0.12-0.18)	0.85 (0.81-0.88)	<0.001
		TF-treated ribbon	32	273	1.32 (1.08-1.60)	0.23 (0.18-0.28)	0.77 (0.71-0.81)	<0.001
	<i>Culex spp</i>	Control	32	4142	21.78 (18.11-26.18)	1	0	
		One TF-chairs	32	2598	13.17 (10.93-15.86)	0.60 (0.53-0.68)	0.40 (0.31-0.47)	<0.001
		Two TF-chairs	32	2216	10.68 (8.85-12.87)	0.49 (0.43-0.56)	0.51 (0.44-0.57)	<0.001
		TF-treated ribbon	32	2794	13.93 (11.56-16.78)	0.64 (0.56-0.72)	0.36 (0.27-0.44)	<0.001
Inside outdoor kitchen enclosure	<i>Anopheles arabiensis</i>	Control	32	68	Low catches			
		TF-hessian ribbon	32	24	Low catches			
	<i>Culex spp</i>	Control	32	302	0.49 (0.31-0.78)	1	0	
		TF-hessian ribbon	32	172	0.26 (0.15-0.43)	0.52 (0.32-0.86)	0.48 (0.13-0.67)	0.011

Key: n = total number of mosquito collected, CI = confidence interval, PP = percentage protection, RR = relative rate, TF = transfluthrin, 1 and 0 = references

#### 4.6 Mortality of field-collected and laboratory-reared mosquitoes exposed to transfluthrin-treated chairs

Findings on induced mortality of mosquitoes exposed to transfluthrin-treated chairs are summarized in Table 5. When field-collected *An. arabiensis* females and *An. funestus* females were exposed to the transfluthrin-treated chairs, 99.4% and 100% of them died within 24 h respectively. All (100%) of the laboratory-reared *An. arabiensis* or laboratory-reared *Ae. aegypti* mosquitoes exposed also died when exposed underneath the transfluthrin-treated chairs. Mortality of the mosquitoes exposed to untreated chairs however remained low (5.2% for field-collected *An. arabiensis*, 0.0% for field-collected *An. funestus*, 0.1% for laboratory-reared *An. arabiensis* and 1.1% for laboratory-reared *Ae. aegypti*).

**Table 5:** Comparison of induced mortality to mosquitoes exposed to house with or without transfluthrin-treated chairs

Settings	Species	Treatment	Days	Exposed	Dead 24hr	Mortality (%)
Wild mosquitoes	<i>Anopheles arabiensis</i>	Control	10	1142	60	5.2
		TF-treated chair	10	1140	1134	99.4
	<i>Anopheles funestus</i>	Control	5	490	0	0
		TF-treated chair	5	490	490	100
	<i>Anopheles arabiensis</i>	Control	9	860	10	1.1
		TF-treated chair	9	860	860	100
Lab-reared mosquitoes	<i>Aedes aegypti</i>	Control	9	900	3	0.3
		TF-treated chair	9	900	900	100

#### 4.7 Insecticide resistance status of mosquitoes in a study area

Results of the WHO resistance tests are summarized in Table 6. The field populations of *An. arabiensis* were fully susceptible to bendiocarb (100% mortality), pirimiphos methyl (100% mortality) and DDT (98.8% mortality). However, they were resistant to both permethrin (94.7% mortality) and deltamethrin (80.3% mortality).

**Table 6:** Show insecticide resistant status in *Anopheles arabiensis* mosquitoes to difference insecticides at Lupiro village

Insecticide tested	Mosquito species tested	Percentage mortality (%)	Resistance status
Bendiocarb	<i>Anopheles arabiensis</i>	100	Susceptible
Pirimiphos-methyl	<i>Anopheles arabiensis</i>	100	Susceptible
DDT	<i>Anopheles arabiensis</i>	98.8	Susceptible
Permethrin	<i>Anopheles arabiensis</i>	94.7	Resistant (After confirmation)
Deltamethrin	<i>Anopheles arabiensis</i>	80.3	Resistant

Key: DDT = dichlorodiphenyltrichloroethane

#### **4.8 General discussion of the results**

Several studies in tropical settings have documented that many people stay active outdoors in early evenings before they go indoors and then sleep under bed nets (Finda *et al.*, 2019; Monroe *et al.*, 2019; Moshi *et al.*, 2017). To date, this study is the first to characterize the peri-domestic spaces used by household members in a malaria-endemic setting for various outdoor activities.

The key finding was that most houses had active peri-domestic spaces (veranda extensions, open general areas and makeshift kitchens) where household members performed different activities, usually unprotected from potentially-infectious mosquitoes before they went indoors. In some of the peri-domestic spaces, residents constructed structures for cooking, eating and socializing, but these too were often open and not protective against mosquito bites (Fig. 5).

The study also demonstrated that the two simple interventions evaluated, i.e. transfluthrin-emitting chairs and ribbons both considerably reduced outdoor-biting by the important residual malaria vector, *An. arabiensis*. Furthermore, mosquitoes exposed to the chairs were killed rapidly, indicating that the interventions could offer not just personal or household protection, but also communal protection by reducing mosquito density, survival and malaria sporozoite infection prevalence (Mwanga *et al.*, 2019).

More than half the households surveyed had veranda extensions with roofed enclosing structures, mostly used for resting, cooking and eating. All these structures provide opportunities for mounting simple interventions in these spaces such as physical screening and complementary chemical measures like these transfluthrin emanator formats and turning them into mosquito-proof areas as they are predominantly used for early-evening human activities, notably resting, cooking and eating.

The findings that transfluthrin-emitting chairs provided useful levels of protection against *An. arabiensis* and *Culex spp.* corroborate previous observations with other prototypes in outdoor bars (Masalu *et al.*, 2017). Even though the prototype (chair) used in this study differs in design from previous studies (decoration) (Masalu *et al.*, 2017), it emphasizes the potential of these technologies for outdoor protection in such communities.

Outdoor kitchens were commonly used for cooking in early evening, and were among the commonest constructed spaces identified in households, regardless of whether they had verandas or not. Early-evening cooking within this space coincides with peak hours of mosquito bites (Finda *et al.*, 2019), amplifying the likelihood of malaria transmission in these spaces. In this study, the high levels of protection provided against *An. arabiensis* by the repellent-treated hessian ribbons around these outdoor kitchens is therefore encouraging and consistent with previous studies (Ogoma *et al.*, 2017) which demonstrated that transfluthrin-treated hessian ribbons protected non-users against *An. arabiensis* sitting within radius of 5 m. More recently, transfluthrin-treated hessian ribbons fitted to the eaves of houses prevented both indoor and outdoor-biting mosquitoes (Mmbando *et al.*, 2018; Mwanga *et al.*, 2019).

In addition to the substantial protection against *An. arabiensis* demonstrated in the areas immediately outside the ribbon-fitted kitchen, the catches by CDC light traps placed within the kitchens are reduced, albeit more modestly. This modest reduction may be due to the use of CDC light traps in these open spaces, may have resulted in exaggerated catches of mosquitoes attracted by the light alone and therefore not deterred by the repellent. It may also due to the smoke produced from these kitchens, which may have confounded the results observed on *An. arabiensis*. Interestingly, this emanator prototype provided much more satisfactory protection against nuisance-causing *Culex* spp. within the kitchens based on the same CDC light trap catches. It is not clear why such significant reductions observed for *Culex* spp were not observed for *An. arabiensis*, but it is nevertheless encouraging that reduced *Culex* spp. densities should motivate user acceptance. It is also encouraging that these observations are also broadly consistent with previous studies (Govella *et al.*, 2015; Ogoma *et al.*, 2012) demonstrating that outdoor use of transfluthrin-treated hessian provided more than 90% protection against both *An. gambiae* s.l. and *Culex* spp mosquitoes (Govella *et al.*, 2015; Ogoma *et al.*, 2012).

Pyrethroid-treated nets divert host-seeking mosquitoes from humans, or kill the mosquitoes attempting to feed on the protected persons (Lindsay, Adiamah, Miller & Armstrong, 1991; Miller, Lindsay & Armstrong, 1991). With these modes of action, pyrethroid-treated nets not only provide personal protection (to users), but also communal protection (to both users and non-users) by suppressing vectors population through the mass killing effect (Carnevale *et al.*, 1988; Magesa *et al.*, 1991). Transfluthrin, used to treat the hessian mats fitted underneath the chairs induced high mortality on caged mosquitoes exposed underneath the experimental chairs (100% in most cases). This implies that the chairs may not only provide personal

protection, but also community benefit through killing of mosquitoes. This effect was particularly important since the field-collected mosquitoes were from villages where *Anopheles* populations were pyrethroid-resistant (Table 6).

Even though excito-repellency effects maximize person protection by chasing mosquitoes away, it may attenuate more important mass killing effects by deterring mosquitoes from making fatal contact with lethal doses of the repellent insecticide itself or with complementary solid-phase insecticides applied as LLINs or IRS (Killeen, Chitnis, Moore & Okumu, 2011; Killeen & Moore, 2012; Killeen *et al.*, 2014). However, these observations of mortality amongst wild malaria vectors exposed to transfluthrin suggest that mass population suppression could be achieved even without mosquitoes necessarily touching treated surfaces. It is also encouraging that (Ogoma *et al.*, 2017) demonstrated that transfluthrin-treated emanator provided more than 90% biting reduction against *An. arabiensis* without any obvious diversion to non-users (Ogoma *et al.*, 2017). Furthermore another study (Ogoma *et al.*, 2014) also observed that transfluthrin-treated coils could protect non-users within 20 m radius. More recently, Mwanga and colleague demonstrated that transfluthrin-treated ribbons fitted to the eave gaps of houses protected volunteers both inside and outside the houses (Mwanga *et al.*, 2019).

The spread of pyrethroid resistance in malaria vectors clearly compromises ongoing control and elimination efforts (Cook *et al.*, 2018; Protopopoff *et al.*, 2018; Tiono *et al.*, 2018), so it is obviously a concern that transfluthrin is also a pyrethroid. It is therefore encouraging that transfluthrin killed almost all wild-caught *An. arabiensis* and *An. funestus* exposed to emanated vapour from the chairs, even though local populations of both species are clearly resistant to the conventional solid-phase pyrethroids used for LLINs and IRS (Kaindoa *et al.*, 2017).

However, one important limitation of this study was that caged mosquitoes were placed underneath the transfluthrin-treated chairs for 12 h. This long-time exposure may well greatly exceed true exposure levels in the field, where mosquitoes can freely fly around and way upon encountering airborne insecticide. Nonetheless, since transfluthrin effects are vapor-mediated, this initial attempt to quantify possible lethal modes of action is encouraging and offers a basis for future improvements in study designs for developing and evaluating these technologies.

## **CHAPTER FIVE**

### **CONCLUSION AND RECOMMENDATIONS**

#### **5.1 Conclusion**

Most houses in this rural African context had well-used peri-domestic spaces (veranda extensions, makeshift kitchens and completely open spaces) where members performed different activities before bed time, usually unprotected from potentially-infectious mosquitoes before they went indoors. Both the transfluthrin-emanating chairs and ribbons reduced outdoor exposure to biting malaria vectors in these peri-domestic spaces and also caused significant mortality of caged, field collected malaria vector mosquitoes. The two emanator prototypes, may require additional improvements, optimizations and assessments in future studies, and could constitute new options for outdoor malaria prevention to complement LLINs and IRS in areas where peri-domestic human activities are common.

#### **5.2 Recommendations**

Based on the findings above, this study provided the following opportunities for the next step work

- (i) Conduct additional improvements and optimizations of the two candidate interventions.
- (ii) Conduct a small-scale randomized field trial to evaluate epidemiological endpoints of these two candidate interventions in term of reducing malaria transmission.
- (iii) Evaluate the new intervention in other settings to validate the findings and explore options for scaling up the technologies as a complementary malaria control interventions.
- (iv) Assess cost-effectiveness of the interventions.

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