UNIVERSITY OF THE WITWATERSRAND

FACULTY OF HEALTH SCIENCES

MASTERS DISSERTATION

THE USE OF INSECTICIDE TREATED EAVE RIBBONS AS A PROTECTION TOOL AGAINST PYRETHROID-RESISTANT POPULATIONS OF MOSQUITOES THAT TRANSMIT MALARIA AND DENGUE FEVER

Student:
Ruth Severin Shirima
Student No: 2504959

Supervisors:
Dr Fredros O. Okumu, PhD
Prof. Lizette L. Koekemoer

A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science in Medicine (Virology)

Johannesburg, June 2023

DECLARATION

I Ruth Severin Shirima declare that this Dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science in Medicine (Virology) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signature of candidate

Date: 12/06/2023

DEDICATION

I dedicate this work to my parents, Mr. Severin Shirima and Mrs. Lucia Shirima.

PRESENTATIONS ARISING FROM THIS RESEARCH PROJECT

i. Ruth S. Shirima, Emmanuel E. Hape, Emmanuel W. Kaindoa, Arnold S. Mmbando, Lizette L. Koekemoer, and Fredros O. Okumu. '*The use of insecticide treated eave ribbons as a protection tool against populations of mosquitoes that transmit malaria and dengue fever*'. Wits Research Institute for Malaria Research Day presentation on 20th April 2022

Role: Preparation of the abstract and submission, preparation of the slides and presentation.

ABSTRACT

Vector control methods such as insecticide treated nets (ITNs) and indoor residual spraying (IRS) have been successful in preventing mosquito-borne diseases like malaria and dengue. However, these methods face challenges including insecticide resistance, high costs, logistical difficulties, low adoption rates, and limited durability. Therefore, there is a need for simpler and more affordable interventions that can be used on a large scale in disease-endemic communities to supplement current approaches. This study evaluated the effectiveness of using insecticide-treated eave ribbons as a potential tool for complementing the current vector control methods. Eave ribbons are pieces of hessian fabric that can be placed around the eave spaces of poorly constructed houses to kill or repel mosquitoes. Laboratory cone bioassays were conducted to assess the efficacy of eave ribbons treated with the organophosphate, pirimiphos-methyl, for killing the malaria vectors, *Anopheles funestus* and *Anopheles arabiensis*, and the dengue vector, *Aedes aegypti*, under varying exposure durations and insecticide doses. In addition, a semi-field experiment was done to assess the efficacy of eave ribbons treated with pirimiphos-methyl against the malaria vectors. Indoor and outdoor biting was assessed by the number of mosquitoes captured indoors in window exit traps and outdoors by human landing catches, respectively. Mortality of recaptured mosquitoes was recorded after 24, 48, and 72 hours. The findings revealed that treated eave ribbons resulted in higher mosquito mortality than the untreated ribbons, but the impact increased with increased exposure duration or dose. The semi-field study indicated moderate levels of bite prevention and mortality of the mosquitoes. At the doses of 1 g a.i./m^2 and 2 g a.i./m^2 pirimiphos-methyl, there was no significant protection against *An. arabiensis*, but at the dose of 4 g a.i./m^2 pirimiphos-methyl, there was only significant protection against outdoor biting *An. arabiensis*, but not *An. funestus*. In conclusion, while insecticide-treated eave ribbons may have potential for controlling malaria and dengue vectors, further research is needed to validate their efficacy in field settings and to identify suitable insecticides or insecticide combinations that are safe and effective.

ACKNOWLEDGEMENT

First and foremost, I am deeply grateful to God for bestowing upon me an abundance of blessings although I haven't deserved them. I am thankful for his constant presence and guidance. He has been my rock and my strength throughout my journey and I am forever grateful for all that he has done for me. I don't have good words to express my gratitude but all I can is that, this far you have brought me, you are Ebenezer.

I would like to offer my heartfelt thanks to my supervisor, Dr. Fredros Okumu for his constant support to me. Thank you for being there to offer your support whenever I needed it. I remember clearly your words telling me that I have 100% of your support and for sure that's what you have shown me. I also remember the moments of singing with you and colleagues at Ifakara. I remember you tuning the key of the song "*Tarumbeta ya mwana itakapolia mara*" and we were all happy. I hope we have the opportunity to sing more in the future as we continue the journey of fighting against vector-borne diseases.

I am truly grateful for the constant guidance and support provided by my supervisor Prof. Lizette Koekemoer. Her patience, encouragement and willingness to assist at all times have been invaluable to me. Thank you so much for your quick responses to my inquiries and for your dedication in reviewing my thesis even during the busy holiday season. Thank you so much for invaluable contribution to my academic journey.

To the volunteers who assisted in mosquito collection in the semi-field systems and during cone bioassays, this work would have not been possible without you. Thank you for devoting your time and efforts to work with me.

To the colleagues at Ifakara Health Institute team, I cannot express enough gratitude for the support, guidance and knowledge you have imparted on me. When I first arrived at the institute, I had little experience on research but through your support I have grown and developed a range of skills such as writing skills, statistical analysis and experimental designs. I am truly grateful for the opportunity to learn and grow with you and thank you for the positive impact you have had on my professional development.

To my parents and siblings, thank you for your prayers, encouragement and love that you have shown me. Your support gives me strength to keep going and face any challenges that come my way. Thank you for being there for me always.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

SPECIMEN NOMECLATURE

°C	Degrees Celsius
a.i.	Active Ingredient
AChE	Acetyl Cholinesterase
ATSB	Attractive Targeted Sugar Baits
CI	Confidence Interval
DDT	Dichlorodiphenyltrichloroethane
DEET	N, N-diethyl-3-methylbenzamide
DMP	Di-methyl phthalate
GLMM's	Generalized Linear Mixed Models
GTS	Glutathione S-transferase
HLC	Human Landing Catches
IRS	Indoor Residual Spraying
ITNs	Insecticide Treated Nets
LC_{99}	Time taken for 99% of mosquitoes to die
MD	Median Difference
MOH	Ministry of Health
NMCP	National Malaria Control Program
OP's	Organophosphates
OR	Odds Ratio
RH	Relative humidity
SAP	Sensory Appendage Proteins
VBD's	Vector-borne Diseases
VGSC	Voltage Gated Sodium Channel
WHO	World Health Organization

CHAPTER ONE

1 INTRODUCTION

1.1 The burden of malaria

Vector-borne diseases (VBD's) are a major public health concern, with nearly 80% of the global human population at risk. These diseases account for about 17% of all infectious diseases (WHO Regional Office for South-East Asia, 2014). Mosquitoes are major vectors responsible for transmitting various pathogens, including malaria parasites and dengue fever virus, which mostly affect the poor and low-income communities in tropical and subtropical regions (WHO Regional Office for South-East Asia, 2014). The World Health Organization (WHO) has reported that malaria caused around 247 million cases and 619,000 deaths in 2021, a slight decrease from 627,000 deaths in 2020 (WHO, 2022c). Africa bore the greatest share of the burden, accounting for approximately 95% of cases and 96% of deaths that occurred in 2021 (WHO, 2022c). Children under the age of five were the most affected group, accounting for about 78.9% of deaths reported in 2021 in the WHO African region (WHO, 2022c). More than half of the malaria deaths globally were in just four African countries, one being Tanzania accounting for 4% of malaria deaths globally (WHO, 2022c). One study predicted the average parasite prevalence in 2019 to be 10.6% (95% Bayesian Credible Interval 3.4– 39.2) in Tanzania (Alegana *et al.*, 2021). The burden of malaria in Tanzania is not uniform. It varies both, within and between regions which makes a sub-national stratification of malaria risk a crucial component for a targeted deployment of interventions as recommended by WHO (WHO, 2018) (Figure 1). About 37% of the population live in areas with a high malaria burden (> 30% parasite prevalence), 23% in moderate prevalence areas (5-30% parasite prevalence), 28% in low prevalence areas (1-5% parasite prevalence) and 12% in very low prevalence areas (< 1% parasite prevalence) (Thawer *et al.*, 2020).

The national malaria strategic plan 2021-2025 has a target to reduce the prevalence of malaria in children under the age of five from 7% in 2017 to less than 3.5% by 2025 (NMCP-Tanzania, 2021). The stratified targets aim at reducing malaria prevalence in high and moderate transmission areas from 15% in 2017 to less than 7.5% in 2025 while maintaining and further reducing the prevalence to less than 0.5% in low and very low transmission areas (NMCP-Tanzania, 2021). It has been predicted that combining all available interventions could reduce the malaria burden in the high

prevalence stratum by 76%, with ITNs and high case management accounting for 70% of that reduction (Runge *et al.*, 2022)

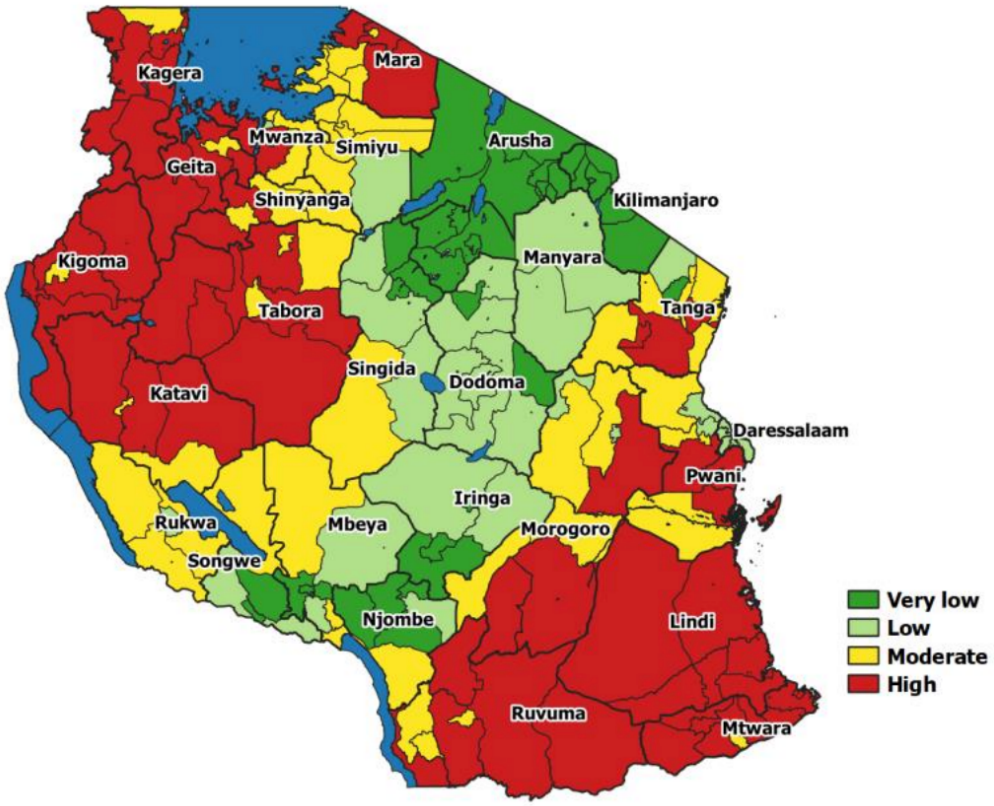

Figure 1: Map showing the stratification of malaria risk at district council in Tanzania. Source (NMCP-Tanzania, 2021)

1.2 The burden of dengue

Dengue, on the other hand, is the world's fastest-growing vector-borne disease, with a continuous increase in the number of cases and the number of countries reporting dengue for the first time. Before the 1970s, only nine countries reported dengue cases but recently, more than 100 countries in various regions have reported dengue cases (WHO, 2022a). There were an estimated 96 million apparent and 294 million inapparent cases of dengue worldwide in 2010, Asia being the highest contributor (~70%), while Africa and the Americas were estimated to bear nearly the same burden (Bhatt *et al.*, 2013). Apparent cases are those in which an individual exhibits visible signs or symptoms of the disease, whereas inapparent cases are those in which individuals have the condition but are asymptomatic. Between 1990 and 2019, dengue incident cases have increased by 85.5%, with a higher proportion of incident cases and deaths occurring in adults (Yang *et al.*, 2021) rather than the young population as previously documented (Stanaway *et al.*, 2016).

The WHO estimated an eight-fold increase in dengue cases over the last two decades with a total of 5.2 million cases reported in 2019 and the spread in new areas such as Afghanistan where dengue transmission was reported for the first time (WHO, 2022a). It was reported that Africa bears a large burden, but dengue is underreported, misdiagnosed with other febrile diseases such as malaria, and receives little attention and resources which results in insufficient data available to accurately estimate the burden (Bhatt *et al.*, 2013; Gainor, Harris and Labeaud, 2022).

In Tanzania, dengue fever was detected on Zanzibar Island in 1823 and 1870. The disease was referred to as "kidinga pepo" in Swahili, which means cramp-like pain caused by an evil spirit (Christie, 1872; Amarasinghe *et al.*, 2011). Later in 2010, travelers returning from Tanzania to Japan and Europe were diagnosed with dengue fever shortly after arriving in their respective countries. This indicated that they became infected in Tanzania, implying that the virus was circulating there (Gautret *et al.*, 2010; Moi *et al.*, 2010). In 2014, dengue outbreaks in Tanzania resulted in 1,969 and 961 suspected and confirmed dengue cases respectively (SACIDS, 2019). In 2019, Tanzania experienced its worst outbreak ever, which resulted in 6,873 cases and 13 deaths (SACIDS, 2019). All four dengue serotypes have been reported to circulate in the country (Vairo *et al.*, 2016; SACIDS, 2019; Chipwaza *et al.*, 2021). Although there is no overall country prevalence of dengue reported, the burden is high and the prevalence was reported to be 38.2% in Kilosa (Chipwaza *et al.*, 2014), 20% in Dar es Salaam (Vairo *et al.*, 2016) and 9.9% in Kilombero (Chipwaza *et al.*, 2021).

1.3 Reasons for high burden of vector-borne diseases Africa

The burden of VBD's in sub-Sahara Africa is very high compared to other parts of the world and their control is very challenging due to various reasons. Malaria, for example, has been linked to poverty and it mostly affects low-income communities most of which are found in sub–Saharan Africa (Gallup and Sachs, 2001). These areas are presented with weak health systems, poor housing, nutrition and education which contribute to a high disease burden (Kigadye *et al.*, 2012; Lowassa *et al.*, 2012; Kienberger and Hagenlocher, 2014; Hagenlocher and Castro, 2015; Snyman *et al.*, 2015; Oleribe *et al.*, 2019). Weak health systems compromise the quality and on-time provision of care. The availability of resources and competent personnel to deliver services is critical to the positive impact of health services provided to the population (Okumu *et al.*, 2022).

The climatic conditions in Africa are also crucial for the growth and survival of parasites and vectors. Suitable temperature and rainfall provide ideal conditions for the development of parasites within the mosquito as well as the development of vector aquatic stages and adult survival (Rossati *et al.*, 2016). The climate in sub-Saharan Africa favors the growth and survival of both parasites and vectors, leading to a significant disease burden in the area (Craig, Le Sueur and Snow, 1999).

Thirdly, the distribution and efficiency of the vectors that transmit the disease also play a vital role in the disease's severity. Africa is home to four major efficient vectors for malaria transmission namely, *Anopheles gambiae sensu stricto (s.s)*, *Anopheles arabiensis*, *Anopheles funestus* and *Anopheles coluzzii* (Sinka *et al.*, 2012; Coetzee *et al.*, 2013). While *An. funestus*, *An. gambiae s.s* and *An. coluzzii* are efficient vectors because of their primarily anthropophilic behavior (prefer to feed on humans) which makes them more likely to come into contact with humans (Githeko *et al.*, 1994; Takken and Verhulst, 2013; Akogbéto *et al.*, 2018), *An. arabiensis* is an opportunistic vector that can feed on humans and other hosts (Githeko *et al.*, 1994; Takken and Verhulst, 2013). Its ability to feed and rest outdoors when people are not protected by nets makes it a suitable vector for residual outdoor transmission (Kibret and Wilson, 2016; Killeen *et al.*, 2016). The two main vectors responsible for dengue transmission can also be found in Africa. *Aedes aegypti* is anthropophilic and the primary vector for dengue (CDC, 2017). It is considered to have originated in Africa (Mattingly, 1957). *Aedes albopictus* on the other hand was introduced into Africa from Asia in 1991 (Savage *et al.*, 1992). *Aedes albopictus* is more opportunistic but a potential vector for dengue (Valerio *et al.*, 2010; CDC, 2017). In Africa, it has been involved in the transmission of dengue and chikungunya viruses in central Africa (Leroy *et al.*, 2009; Paupy *et al.*, 2010).

Other factors impeding control efforts include biological threats such as insecticide resistance in mosquitos, behavioral adaptations (Durnez and Coosemans, 2013) and the invasion of *Anopheles stephensi* to the horn of Africa (Faulde, Rueda and Khaireh, 2014; Carter *et al.*, 2018; Ahmed *et al.*, 2021), and recently in East Africa (Ochomo *et al.*, 2023) which is capable of transmitting malaria in urban areas. Sub-optimal use of the available interventions by people (Ahorlu *et al.*, 2019), civil wars, conflicts and outbreaks of other infectious diseases also affect the control efforts of VBD’s in Africa (Okumu *et al.*, 2022).

To combat VBD's in Africa, strong cross-sectoral cooperation is required. Different sectors should consider health issues when developing policies (Danasekaran *et al.*, 2014). The environmental sector, housing and infrastructure sectors should be integrated to control mosquito vectors. Environmental management and sanitation as well as improved housing are among the long-term measures against mosquito control and may significantly reduce mosquito-borne diseases (Okumu *et al.*, 2022). The government should support through funding and increase investment in public health. The healthcare systems should be strengthened to ensure quality and proper provision of health services (Okumu *et al.*, 2022). It is important to promote development strategies that will help African countries overcome adversity and eventually combat VBD's (Badmos *et al.*, 2021). Supplementary vector control interventions are necessary to allow targeting the vectors at different stages of their development (Govella and Ferguson, 2012; Russell *et al.*, 2013)

1.4 Transmission and control

1.4.1 The vectors

Both malaria parasites and the dengue fever virus are transmitted by a bite of an infected female mosquito. While malaria is transmitted by an infective bite from female *Anopheles* mosquitoes (Ross, 1923; Fantini, 1999; Cox, 2010), dengue is transmitted by a bite from an infected female of the genus *Aedes*. *Anopheles funestus, An. arabiensis, An. gambiae s.s.* and *An. coluzzi* are the four primary species responsible for malaria transmission in Africa (Figure 2) (Sinka *et al.*, 2012; Coetzee *et al.*, 2013; Kyalo *et al.*, 2017). In Tanzania, the major malaria vectors are *An. funestus*, *An. arabiensis* and *An. gambiae s.s* the majority being *An. funestus* (56.3%) followed by *An. arabiensis* (24.9%) and lastly *An. gambiae s.s*. (18.8%) (NMCP-Tanzania, 2021). In rural southern Tanzania *An. funestus* has been found to mediate more than 80% of malaria transmission (Kaindoa et al., 2017a; Mapua et al., 2022).

Dengue is spread by *Ae. aegypti* and *Ae. albopictus* the former being the principal vector (WHO, 2009). *Aedes aegypti* is widespread in tropical and subtropical climates and is an efficient vector for dengue because of its anthropophilic behavior, capacity to feed multiple times in different hosts to complete one gonotrophic cycle, and ability to nest close to human settlement (WHO, 2009). It feeds mostly during the day, both indoors and outdoors. *Aedes albopictus* is largely involved in the transmission of dengue in central Africa where it is also more abundant than *Ae. aegypti* (Leroy *et al.*, 2009; Paupy *et al.*, 2010).

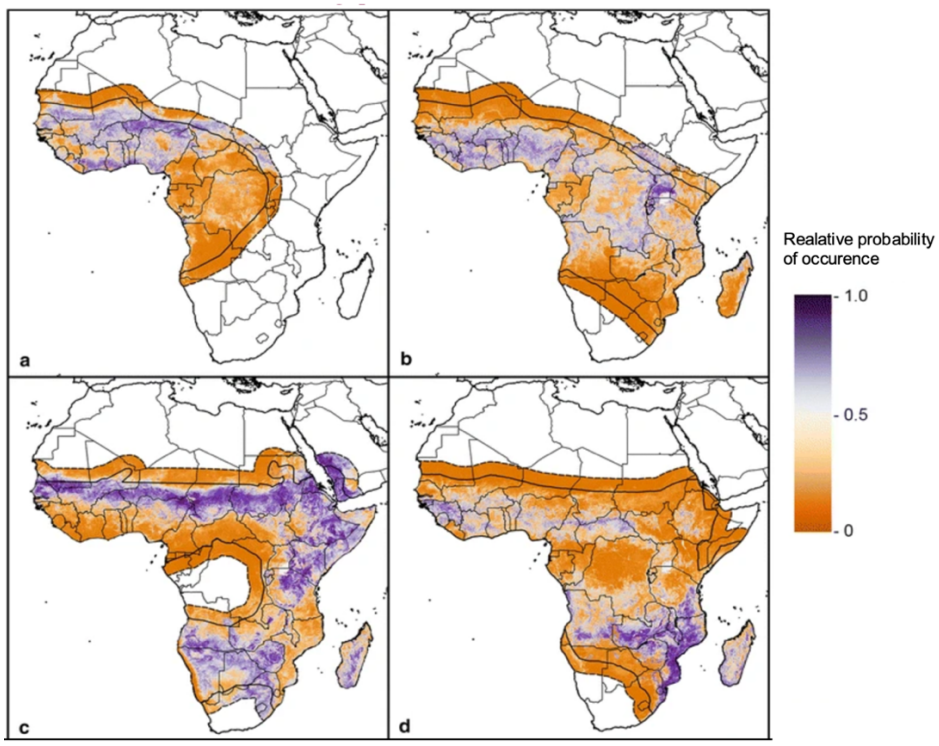

Figure 2: Predictive maps for occurrence of sibling species of Africa malaria vectors. "**a"** *An. coluzzii*, "**b"** *An. gambiae* "**c**", *An. arabiensis*, "**d"** *An. funestus*. Source (Wiebe *et al.*, 2017)

1.4.2 The control of the vectors

Efforts to control VBD's began in the 1900s, with various initiatives such as environmental management, biological control and larviciding (WHO, 2009; Wilson *et al.*, 2020). The discovery of DDT (dichlorodiphenyltrichloroethane) in the 1930s sparked a global drive to eradicate malaria by indoor residual spraying (IRS) with DDT. The program was successful in some areas but was later abandoned because it was realized that it was not feasible to eradicate malaria with the available resources within a short period, nor with a single tool (Nájera, González-Silva and Alonso, 2011). Synthetic pyrethroids were introduced in the 1980s for use in malaria vector control in the form of IRS and ITNs. To date, six classes of insecticides are pre-qualified by WHO for adult vector control. These classes are organophosphates (OP), pyrethroids, carbamates, organochlorines, pyrroles, and neonicotinoids. Examples of active ingredients and products used in vector control for each class are shown in Table 1.

Since 2000, the increased use of insecticide treated nets (ITNs) and IRS as primary vector control interventions has resulted in a considerable decrease in malaria incidence and deaths across Africa (Bhatt *et al.*, 2015). ITNs alone contributed to about 68% of the malaria cases averted from 2000 to 2015 (Bhatt *et al.*, 2015). Despite these

achievements, it has been noted that the progress has begun to level off (WHO, 2022c). As discussed below, several factors limit the effectiveness of vector control interventions.

Table 1: Examples of WHO prequalified insecticides for adult malaria vector control. The full list is available online on https://extranet.who.int/pqweb/vector-control-products/prequalified-product-list.

Insecticide class	Active ingredient (Representative compounds)	Use	Product name
Pyrethroids	Bifenthrin, Lamda-cyalothrin, Alpha-cypermethrin, Deltamethrin	IRS	Bistar 10WP, Icon WP, REVIVAL 100 WP, CS, RUBI 100 SC, WP, PALI 250 WG, etc.
	Alpha-cypermethrin, Deltamethrin, Permethrin, Piperonyl Butoxide	ITN	MiraNet, DuraNet LN, DuraNet Plus, Panda Net 2.0, PermaNet 2.0, Olyset plus
Organophosphates	Pirimiphos-methyl EC, CS	IRS	Actellic CS 300
Carbamates	Bendiocarb	IRS	Ficam, FastM
Neonicotinoids	Clothianidin	IRS	SumiShield 50WG, Fludora fusion
Organochlorines	DDT	IRS	NA
Pyrrole	Chlorfenapyr	ITN	Interceptor G2

1.5 Vector control challenges

1.5.1 Inadequate use of ITNs among targeted populations

ITNs have contributed greatly to the massive reduction of malaria in sub-Saharan Africa since 2000 (Bhatt *et al.*, 2015). Achieving and maintaining universal access to and use of ITNs as recommended by WHO is necessary for sustaining the gains. In 2021, only 38% of the households in sub-Saharan Africa were reported to own at least one ITN for every two people; and 54% had access to an ITN within their households, a considerable increase from 3% in 2000 (WHO, 2022c). The percentage of people

sleeping under ITNs has increased from 2% in 2000 to 47% in 2021, however, since 2017 the access to and use of ITNs started to decline in sub–Saharan Africa (WHO, 2022c). High temperatures when sleeping under the bed net, skin irritations, difficulty in hanging the nets, and low mosquito densities are some of the reasons given by people for not sleeping under bed nets (Iwashita *et al.*, 2010; Ahorlu *et al.*, 2019). ITN use also varies by season, with the highest usage often reported during the rainy seasons when mosquito densities are high and the climate is cooler than in the dry season (Thwing *et al.*, 2008).

In Tanzania, free ITN distribution to the communities resulted in increased ownership and access to ITNs for the populace (Renggli *et al.*, 2013; Koenker *et al.*, 2023). However, there is a gap between access to and use of ITNs. According to the 2017 malaria indicator survey, 63% of the population had access to ITNs, but only 52% slept under bed nets the night before the survey (Figure 3). The gap was greater in rural areas, where 60% of people had access to ITNs but only 40% used them (MOH-Tanzania *et al.*, 2017).

Non-compliance to bed net use may compromise malaria control efforts. According to a study conducted in Uganda, failure to use ITNs has been linked to a 15-fold increase in the risk of contracting malaria, thereby underlying the importance of continuing to use ITNs even if malaria has considerably decreased (Rek *et al.*, 2020). Although the majority of the community members seem to understand the importance of using nets (Kanyangarara *et al.*, 2018), more community involvement, education, and communication are required to understand their perceptions and attitudes regarding vector control initiatives. Understanding their preferences such as design and color could boost acceptance and compliance.

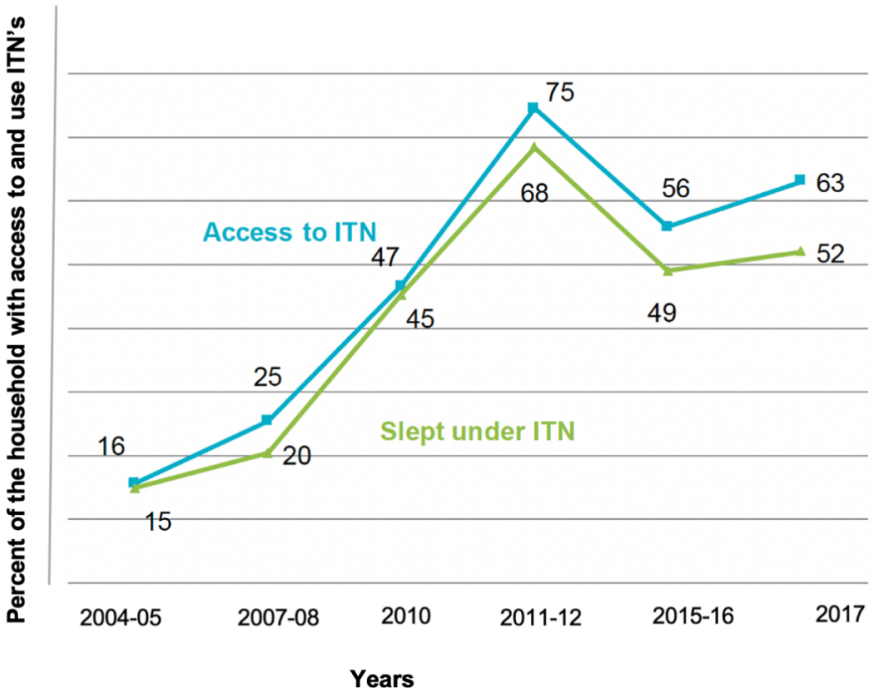

Figure 3: Percentage of the households with access to and percentage of the population that slept under an ITN the night before survey. Source (MOH-Tanzania *et al.*, 2017)

1.5.2 Mosquito resistance to insecticides

Insecticide resistance is defined as "a development of an ability in a strain of an organism to tolerate doses of a toxicant which would prove lethal to the majority of individuals in a normal (susceptible) population of the species" (WHO, 1957). This occurs as a result of repeated exposure of the mosquito to insecticides. This results in the selection of resistant mosquitoes which pass resistant genes to their offspring (IRAC, 2011). There are various mechanisms used by vectors to prevent the lethal impacts of the insecticide as discussed below. Figure 4 shows a summary of these mechanisms.

1.5.2.1 Target site resistance

When mosquitoes are exposed to insecticides, the insecticide acts at a specific site within the mosquito known as the active site. Target site resistance occurs when mutations alter the active site in such a way that the insecticide can no longer bind to the active site and act (Figure 4).

The active site for pyrethroids and DDT is a voltage-gated sodium channel (VGSC) in mosquito neurons. The insecticides act on VGSC, causing a prolonged influx of sodium

ions and prolonged membrane depolarization, resulting in mosquito paralysis and death (Davies *et al.*, 2007). Resistance develops when an insecticide fails to bind to the VGSC due to mutations that alter the active site. This type of resistance is commonly referred to as knockdown resistance because the mosquitos can withstand exposure without being knocked down. Knockdown is defined as when the mosquito is unable to stand, or fly in a coordinated manner, or the female can stand and take off briefly but rapidly falls (WHO, 2016b).

Organophosphates and carbamates are cholinesterase inhibitors. They inhibit the acetylcholinesterase (AChE) enzyme, resulting in nerve impulse disruption and mosquito death. Mutations in the *AChE* genes have been shown to modify the AChE active site, resulting in reduced sensitivity to OP or carbamate insecticides (Weill *et al.*, 2004). Both, malaria and dengue vectors have been found to have mutations in *VGSC* and *AChE* genes (Verhaeghen *et al.*, 2006; Sombié *et al.*, 2019; Diouf *et al.*, 2020) which confer target site resistance to these vectors.

1.5.2.2 Metabolic resistance

This resistance mechanism occurs as a result of increased enzymatic activity that metabolizes insecticides before they reach the active site (Figure 4). Increased enzymatic activity can result from increased gene expression or gene duplication, which leads to increased enzyme production. Three enzyme families are involved in mosquito insecticide detoxification namely, cytochrome P450s, esterases, and glutathione S-transferases (GTS). The cytochrome P450s confer resistance to pyrethroids and carbamates, as well as organochlorines and OP's to a lesser extent. Esterases confer resistance to OP's, carbamates, and, to a lesser extent, pyrethroids (Vaughan, Rodriguez and Hemingway, 1995), whereas elevated GSTs aid in the detoxification and excretion of OP's and DDT in insects (Hayes and Wolf, 1988). There is reported evidence of overexpression of metabolic resistance genes in the major malaria vectors (Stevenson *et al.*, 2011; Riveron *et al.*, 2014) and in *Ae. Aegypti*, a vector responsible for the transmission of dengue (Kasai *et al.*, 2014).

1.5.2.3 Cuticular resistance

The cuticles of mosquitos are the first point of contact with insecticides and allow insecticides to penetrate the mosquito body. Cuticular resistance refers to changes in insect cuticles, such as increased thickness or altered cuticle structure, that render or reduce insecticide uptake (Balabanidou, Grigoraki and Vontas, 2018) (Figure 4). One

of the first studies showed that, resistant *An. funestus* had thicker cuticles than susceptible *An. funestus* (Wood *et al.*, 2010). Cuticular resistance has been shown to work together with metabolic resistance by reducing the amount of insecticides that penetrate the body which is then easily metabolized by metabolic enzymes or by increasing the amount of time available for metabolic enzymes to detoxify the insecticide before it reaches the target site (Balabanidou *et al.*, 2016; Yahouédo *et al.*, 2017). More research is required to understand the contribution of cuticular resistance to insecticide resistance. This is critical because cuticular resistance can have an impact on a wide range of insecticides that require absorption through the cuticular membrane.

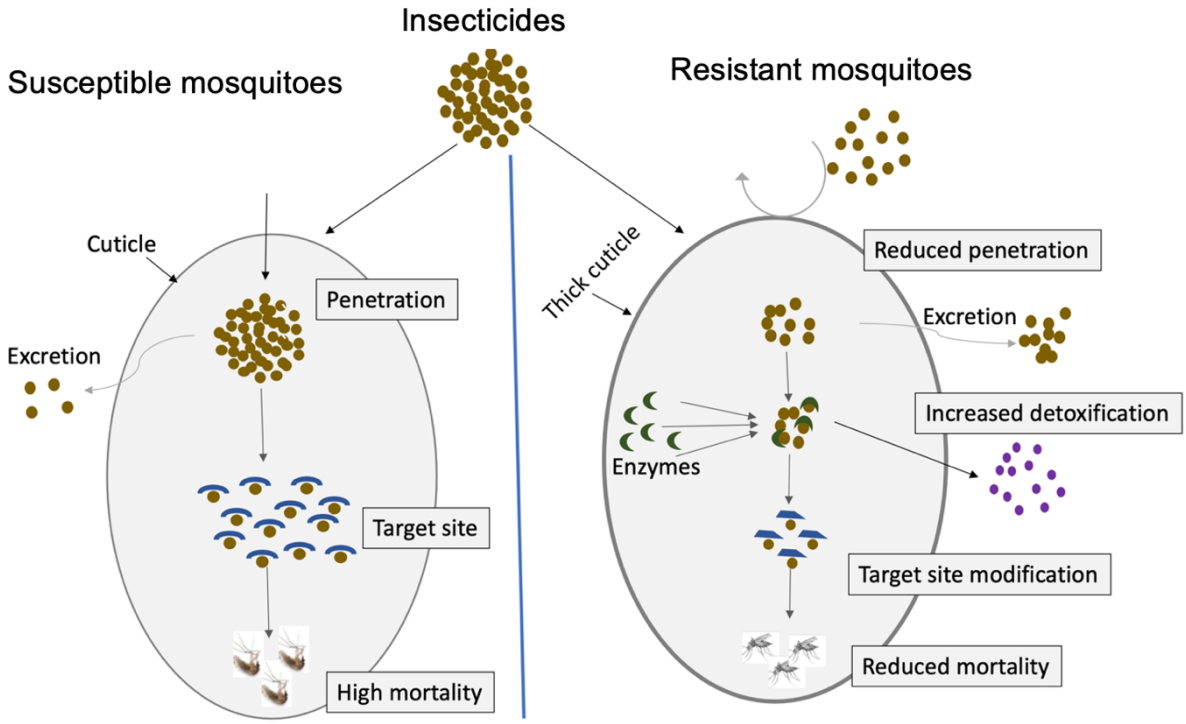

Figure 4: Mechanisms of insecticides resistance in insects. Modified from (Lapied *et al.*, 2009)

1.5.2.4 Sensory appendage protein

Sensory appendage protein (SAP2) is one of the chemosensory proteins which is enriched in the legs of *Anopheles* mosquitoes (Ingham *et al.*, 2020). This protein was demonstrated to confer resistance against pyrethroids in malaria vectors (Ingham *et al.*, 2020). This protein was found to be overexpressed in resistant species and was further elevated following exposure to pyrethroids. Silencing the *SAP* gene resulted in the restoration of the susceptibility of the mosquitoes (Ingham *et al.*, 2020).

1.5.2.5 Impact of mosquito resistance to malaria vector control

Mosquito resistance to public health insecticides has been a matter of great concern for the future of vector control (Ranson and Lissenden, 2016). It can result in reduced effectiveness of vector control interventions or control failure which occurs when the interventions are no longer preventing transmission (WHO, 2012a). Insecticide resistance is caused by the repeated use of insecticides in both the public health and agriculture sector, inducing selective pressure for the selection of resistance (Nkya *et al.*, 2014; Reid and McKenzie, 2016; Implications of Insecticide Resistance Consortium, 2018; Urio *et al.*, 2022).

The history of resistance dates back to the 1950s when DDT was widely used for malaria control through IRS. This led to the development of resistance which was among the reasons that contributed to the failure of the campaign to eradicate malaria globally (Livadas and Georgopoulos, 1953; Nájera, González-Silva and Alonso, 2011; Sharma, 2012). As of now, evidence of resistance to four classes of insecticides has been found in several countries (WHO, 2016a, 2021, 2022c) (Figure 5). Pyrethroid resistance is the most prevalent and widespread in the principal malaria vectors (WHO, 2016a; Hancock *et al.*, 2020; Moyes *et al.*, 2020). Since most of the ITNs contain pyrethroids, resistance to this class is of great concern particularly, in sub-Saharan Africa where the burden of malaria is still very high and ITNs are the main control tool (Strode *et al.*, 2014; Toé *et al.*, 2014).

In Zanzibar for example, apart from the opportunistic feeding behavior of *An. arabiensis,* resistance to pyrethroids used in ITNs and IRS also added to the challenge of eliminating malaria (Haji *et al.*, 2013). In Mozambique, strong resistance of *An. funestus* to pyrethroid was reported which reduced the efficacy of ITNs and even piperonyl butoxide nets in a laboratory assay (Riveron *et al.*, 2019). In South Africa, DDT spraying led to the disappearance of *An. funestus* in 1950s. In 1996, IRS was switched from DDT to pyrethroids, resulting in the reappearance of *An. funestus*, which was resistant to pyrethroids. This resulted in an increase in malaria cases from 4,117 cases in 1995 to 27,238 cases in 2000 (Hargreaves *et al.*, 2000, 2003). The reintroduction of DDT resulted in a decrease in malaria cases to 9,473 cases in 2001 (Hargreaves *et al.*, 2003).

Apart from pyrethroid resistance which is the most widespread, resistance and possible resistance to other classes of insecticides, such as carbamates,

organochlorines, and OP, has been reported. This is of critical concern as it will have an impact on other types of insecticide available for IRS (Hargreaves et al., 2003; Ranson et al., 2009; Wanjala et al., 2015; Fang et al., 2019; WHO, 2022c).

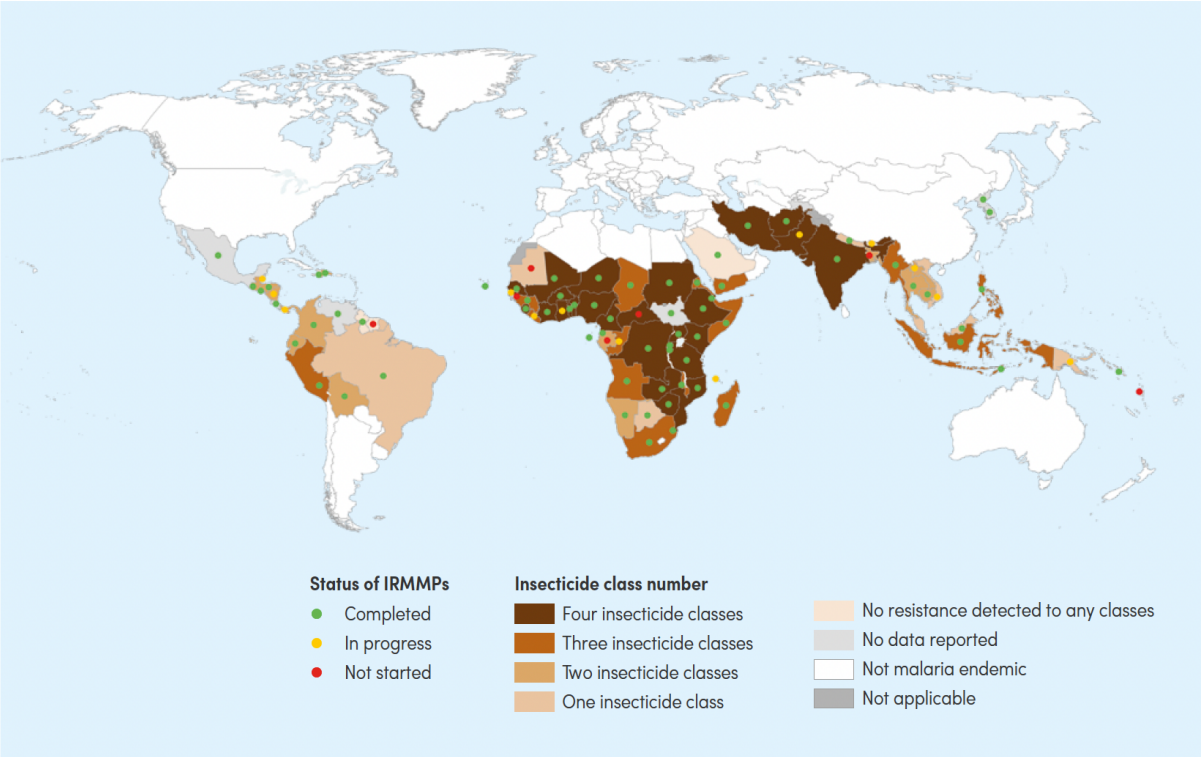

Figure 5: Insecticide classes for which resistance in at least one malaria vector has been confirmed between 2010 and 2020. Source (WHO, 2022c)

1.5.2.6 Insecticide Resistance Management

The WHO has created a global plan for managing insecticide resistance, which contains several suggestions for managing resistance (WHO, 2012a). Rotations entail switching insecticides with different modes of action from year to year. Combination of interventions involves using two or more insecticide interventions with different modes of action in one house. Mosaic spraying involves using one insecticide in one geographic area and a different insecticide in the neighboring areas, the two insecticides being from different insecticide classes. Mixtures involve the use of several insecticides with different modes of action to create a single formulation that exposes the vector to multiple classes of insecticides at once. All these methods can help to manage resistance by removing the selection pressure that causes resistance or by killing the resistant vectors through exposure to more than one insecticide at once (WHO, 2012a).

Since the use of insecticides in agricultural pest control also contributes to the development of resistance, it is necessary to integrate the two sectors in managing resistance (Hawkes and Ruel, 2006). Additionally, community involvement through education on the proper use and disposal of insecticides, and the impact improper use of insecticides in agriculture may have on public health is necessary (Matowo *et al.*, 2020, 2022; Urio *et al.*, 2022)

1.5.3 Behavioral avoidance

The use of vector control interventions to target indoor vectors has resulted in changes in mosquito behavior to avoid the impact of the interventions (Pates and Curtis, 2005; Durnez and Coosemans, 2013; Carnevale and Manguin, 2021). Vectors adapt by switching from indoor to outdoor feeding, indoor to outdoor resting, changing biting time, or changing blood meal hosts.

Changes in mosquito feeding behavior were observed in the British Solomon Islands Protectorate in the 1970s where the main vector control tool was IRS using DDT. The ratio of numbers of *An. farauti* biting outdoors and indoors was 657: 330 after DDT spraying compared to 1767: 2060 before DDT spraying (Taylor, 1975). In southern Tanzania, the scale up of ITNs in 2004, resulted in a decrease of more than 50% in indoor feeding by *An. funestus* compared to 1997 where ITNs were not used (Russell *et al.*, 2011). In Bioko Island where the dominant malaria vector was *An gambiae s.s*., similar observations were made in 2009 where an increased proportion of outdoor host-seeking mosquitoes was observed following the implementation of the Bioko Island malaria control project which involved the applications of insecticide indoors (Reddy *et al.*, 2011). In Benin, the application of bendiocarb IRS resulted in a decrease in indoor feeding *An. gambiae s.l.* mosquitoes from 67% before intervention to 42% after intervention (Padonou *et al.*, 2012).

Apart from the changes in feeding behavior, resting behavior has also been influenced by ITNs and IRS. In Kenya, a study by Mbogo *et al* (1996) indicated a decline in indoor mosquito collections and increased outdoor collections following the introduction of permethrin-treated nets and pyrethrum sprays. The use of permethrin impregnated nets resulted in a reduction in the number of *An. gambiae* and *An. funestus* mosquitoes resting indoors by more than 90% (Bøgh *et al.*, 1998). In Benin, one study indicated a reduction of indoor resting mosquitoes from 67% before the implementation of IRS to 4.5% after the application of IRS (Padonou *et al.*, 2012).

Shifts in blood meal hosts from humans to animals have been reported following the use of vector control interventions. The use of permethrin-treated nets in Kenya has resulted in the shift of the anthropophilic *An. gambiae* and *An. funestus* vectors to feed on alternative hosts instead of humans. Prior to the intervention, the human blood index (the proportion of blood meal taken from humans by mosquitoes) of *An. gambiae* and *An. funestus* was 99% in both control and intervention villages. However, after the distribution of treated nets, the proportion of human blood meals was significantly reduced to 87.5% and 52.2% in *An. gambiae* and *An. funestus*, respectively, with 14.3% of *An. gambiae* blood meal and 47.8% of *An. funestus* blood meal originating from other hosts (Bøgh *et al.*, 1998). Similar observations were made in Western Kenya, with *An. gambiae s.s* (Ndenga *et al.*, 2016) and in Burkina Faso (Lefèvre *et al.*, 2009).

The changes in the biting time of the major malaria vectors have also been observed following the use of indoor interventions. Different studies indicated shifts in the biting time of malaria vectors from midnight biting to early evening and even daylight biting when people are not protected by ITNs or IRS (Taylor, 1975; Moiroux *et al.*, 2012; Sougoufara *et al.*, 2014; Thomsen *et al.*, 2017; Sangbakembi-Ngounou *et al.*, 2022).

Although indoor interventions have been beneficial in protecting individuals from malaria vectors (Kigozi *et al.*, 2012; Musiime *et al.*, 2019), the shift in mosquito biting behavior to avoid these interventions may interfere with malaria control efforts. There is a need for new tools that can control indoor and outdoor transmission to eliminate malaria (Govella and Ferguson, 2012; Russell *et al.*, 2013). Therefore, understanding the role of human behavior at night and how it contributes to residual malaria transmission is crucial for deploying appropriate supplementary interventions (Monroe *et al.*, 2019).

1.6 Supplementary interventions for malaria vector control

Following the challenges of the primary vector control interventions such as resistance to insecticides and changes in mosquito behavior as discussed above, there is a need for supplementary interventions that target changing mosquito behavior (Govella and Ferguson, 2012; Russell *et al.*, 2013). Some of the supplementary malaria control interventions are discussed below.

1.6.1 Interventions that target aquatic stages

Larval control has been shown to be successful in reducing vector abundance and malaria transmission in various settings (Fillinger and Lindsay, 2006; Fillinger *et al.*, 2008, 2009; Geissbühler *et al.*, 2009). When combined with ITNs, larval control provides up to two fold reduction in malaria infections compared to when ITNs are used alone (Fillinger *et al.*, 2009). However, larval control remains ineffective in settings where breeding sites are widespread, flooded, and difficult to access on foot (Majambere *et al.*, 2010), particularly the rural areas. As a result, WHO currently recommends only a restricted deployment strategy in areas where mosquito aquatic habitats are fixed, few and easy to find (WHO, 2012b).

1.6.2 Interventions that target mating behavior

Male mosquitoes typically congregate in swarms, competing for the opportunity to mate with female mosquitoes looking for a mate. The swarms form at consistent locations and times of day, usually at sunset throughout the year. Studies have indicated that mosquito swarms can be located and sprayed with insecticides to reduce vector density and malaria transmission (Sawadogo *et al.*, 2017). In Burkina Faso, spraying of mosquito swarms resulted in a reduction in mosquito densities by 73%, while the female insemination rate was also significantly reduced (Sawadogo *et al.*, 2017). Trapping swarming mosquitos resulted in a significant reduction in indoor resting mosquitos as well as malaria prevalence in The Gambia, but there was no significant reduction in biting rate, sporozoite rate, or entomological inoculation rate (Assogba *et al.*, 2022). In Tanzania, swarms of both *An. arabiensis* and *An. funestus* have been located in the Kilombero valley (Kaindoa et al., 2017b; Kaindoa et al., 2019) implicating that the swarms could be targeted for malaria control. Targeting male mosquitoes in swarms can potentially be an effective vector control intervention where the vectors are resistant to insecticides, or they evolved behavioral change to escape the core interventions (Sawadogo *et al.*, 2017). Since most of the interventions target females which have led to the emergence of resistance and behavioral avoidance, targeting the males in swarms can be a potential way to address those challenges.

1.6.3 Interventions that target sugar feeding behavior

Both male and female mosquitoes obtain sugar on plant nectars as a source of energy. Attractive targeted sugar baits (ATSBs) target mosquitoes when feeding on sugar sources by attracting them to a bait station and killing them upon feeding (Qualls *et al.*, 2015; Traore *et al.*, 2020). Indoor application of ATSBs resulted in a four-fold and

thirteen-fold decrease in the indoor female and male populations of *An. gambiae s.l* respectively. The older females (females with greater than four gonotrophic cycles) which are potentially more likely to carry and transmit malaria were also significantly reduced (Qualls *et al.*, 2015). A large-scale field trial of ATSB in Mali indicated a significant reduction in vector densities and the number of females infected with sporozoites. Females with greater than three gonotrophic circles were significantly reduced by 95% in June and 100% in December after the introduction of ATSBs (Traore *et al.*, 2020). Apart from malaria vector control, ATSB has been shown to be effective in controlling *Ae. aegypti* a major vector for dengue transmission (Qualls *et al.*, 2014; Sissoko *et al.*, 2019). More studies to assess the epidemiological impact in different settings (including settings with high vegetation cover), as well as community acceptance are also needed (Maia *et al.*, 2018).

1.6.4 Topical and spatial repellents

Topical repellents can be used to safeguard people when not protected by ITNs or IRS (Critchley, 1971; Goodyer *et al.*, 2010). The most commonly used topical repellent is N, N-diethyl-3-methylbenzamide (DEET), though there are several other products including di-methyl phthalate (DMP), Picaridin, Insect repellent 3535 and plant based repellents such as citronella and cedarwood oil (Curtis *et al.*, 1987; Fradin, 1998; Fathy Khater *et al.*, 2019). Topical repellents are effective at providing personal protection against mosquito bites and can reduce mosquito bites by more than 80% within five hours from the time of application (LeGoff, Robert and Pierre, 1994; Coetzee, 2000; Rowland *et al.*, 2004; Dadzie *et al.*, 2013). However, some of the studies indicated that topical repellents could not offer protection against clinical malaria (Chen-Hussey *et al.*, 2013; Sangoro *et al.*, 2014; Wilson *et al.*, 2014), inconvenience on daily applications of repellents such as skin irritations, headache, flu, dizziness (Dadzie *et al.*, 2013; Gryseels *et al.*, 2015) and risks of diversion of mosquitoes to non-users (Moore *et al.*, 2007; Maia *et al.*, 2013).

Spatial repellents on the other hand work by emitting repelling vapors in the air thereby protecting multiple people within a specified area by preventing human-vector contact (Achee *et al.*, 2012). Different delivering formats of spatial repellents have been investigated such as mosquito coils (Ogoma, Moore and Maia, 2012), hessian strips (Ogoma *et al.*, 2012, 2017; Tambwe *et al.*, 2020) and controlled release devices (Stevenson *et al.*, 2018) all shown to be effective in delivering a repellent thereby, preventing mosquito bites. In recent years, a different use case, i.e., the deployment

of repellent-treated materials around eave spaces of dwellings, has become increasingly common. This technology, commonly referred to as the “eave ribbons”, involves the hessian material treated with a candidate spatial repellent, and suspended around dwellings at the level of the eave spaces, through which malaria vectors typically enter dwellings (Mmbando *et al.*, 2018). Its development, potential, and reason for employing the same technology in this study are further discussed below.

1.7 The development of eave ribbon technology

Ogoma *et al* (2012) described the use of hessian strips treated with a spatial repellent, transfluthrin for the prevention of mosquito bites in the semi-field tunnel. Transfluthrin is a fast-acting pyrethroid insecticide, which strongly repels and kills mosquitoes. Transfluthrin treated hessian strips provided more than 90% protection against mosquito bites for six months (Ogoma *et al.*, 2012). In 2015 a field study in Dar es Salaam demonstrated that the transfluthrin treated hessian strips could protect against malaria and lymphatic filariasis by providing more than 90% protection against *An. gambiae* and *Culex* mosquitoes (Govella *et al.*, 2015). Another field study in 2017 found that hessian strips can provide long-term protection against the vectors that transmit malaria, arboviruses, and filariasis. People who were not using the treated strips within 5 m away from treated strips were protected and there was no evidence of mosquito diversion to nearby unprotected persons within an 80 m radius (Ogoma *et al.*, 2017).

The eave ribbon technology was developed by adapting the use of the same hessian material used in previous studies. It is 0.15 m wide, but the length varies depending on the size of the house where it is installed (Mmbando *et al.*, 2018). The ribbon is placed around the eave spaces of houses, based on observations that mosquitoes use these gaps to enter human houses, (Lindsay and Snow, 1988; Spitzen *et al.*, 2016; Mburu *et al.*, 2018), thus allowing repellent or mosquitocidal effect to be delivered at the point of entry. The first study with transfluthrin treated eave ribbons published by Mmbando *et al* (2018) indicated that transfluthrin treated eave ribbons could prevent people from mosquito bites by more than 90%, both indoors and outdoors

When transfluthrin treated eave ribbons were tested on a push-pull system (with a push system consisting of treated eave ribbons and a pull component consisting of traps), the treated eave ribbons could still offer high protection on their own and the addition of pull (traps) component resulted in a slight increase in protective efficacy (Mmbando

et al., 2019; Tambwe *et al.*, 2020). When evaluating the possibility of diverting the mosquitoes repelled by treated eave ribbons to non-users, one semi-field study demonstrated that the treated eave ribbons could offer about 57% and 48% indoor and outdoor protection to non-users respectively with the protection to non-users increasing with increase in coverage or the number of huts with treated ribbons (Mwanga *et al.*, 2019). In rural locations where agricultural communities relocate from their houses to the farms for the entire length of the farming season and sleep in makeshift huts, transfluthrin-treated eave ribbons have been demonstrated to protect these populations as well (Swai *et al.*, 2019). Other research used the same hessian material to make innovative transfluthrin-treated hessian decorative, chairs, and sandals which were found to provide significant protection against malaria vectors (Masalu *et al.*, 2018, 2020a; Sangoro *et al.*, 2020)

Although most of the research has focused on eave ribbons treated with transfluthrin, there are still opportunities to explore more on the potential of using eave ribbons treated with other insecticides. In this study, eave ribbons treated with the residual pesticide pirimiphos-methyl (Actellic® 300CS) were tested as a tool of protection against malaria and dengue vectors. Pirimiphos-methyl was chosen since it is currently an effective insecticide used in IRS and mosquitoes in Kilombero valley are susceptible to this insecticide (Kahamba *et al.*, 2020; Pinda *et al.*, 2020) . If the use of eave ribbons treated with pirimiphos-methyl proves to be successful, it could offer a simpler alternative intervention that can be used in large scale compared to IRS which is costly in terms of logistics and operations.

1.8 Rationale of the study

Mosquito resistance to insecticides limits the effectiveness of ITNs and IRS (Hemingway *et al.*, 2016; Ranson and Lissenden, 2016). Most of the ITNs are pyrethroid based on which strong resistance has been reported against this class of insecticide. Although ITNs containing a pyrrole, Chlorfenapyr have been approved by WHO (WHO, 2022b), pyrethroid based ITNs are still abundant and mostly used in vector control.

IRS on the other hand uses different insecticide classes apart from pyrethroids such as carbamates, OP's and neonicotinoids which are still effective (WHO, 2022b). However, the percentage of the population in endemic countries protected by IRS has decreased from 5.5% in 2010 to 2.4% in 2021. Globally, the number of people

protected by IRS has decreased from 153 million in 2010 to 80 million in 2021 (WHO, 2022c). The implementation of IRS is limited by high costs and logistical challenges (Conteh *et al.*, 2021). For example, it requires the removal of household belongings before spraying, significantly slowing operations. It also requires well-trained teams, further limiting coverage and effectiveness (Kaindoa *et al.*, 2021). Moreover, large quantities of insecticides are needed to spray all indoor surfaces which add up to the cost of IRS (Conteh *et al.*, 2021; Kaindoa *et al.*, 2021)

Another limitation is that not all mosquitoes rest on surfaces that have been sprayed (Smith, 1962; Msugupakulya *et al.*, 2020) and therefore current IRS monitoring approaches are sub-optimal. Communities refuse IRS due to reasons such as bad smell and stains left by insecticides, difficulties in removing household assets and not willing to allow strangers inside their houses (Magaço *et al.*, 2019). Modification of the walls such as plastering, washing, painting, or adding new rooms after IRS reduces the actual coverage as well as the effectiveness of IRS (Opiyo *et al.*, 2022). The risk of accidental physical contact with insecticide treated walls poses a safety threat to the users. Children may unknowingly touch the wall and ingest the insecticide sprayed on the walls (Kaindoa *et al.*, 2021). Temporary shelters such as farm huts (temporary shelters used by farmers while staying on the farms during the farming season), mining camps and refugee camps are in most cases not included in IRS operations due to logistical and feasibility challenges further lowering the coverage of IRS.

To circumvent these challenges, there is a need to simplify house spraying, without compromising its effectiveness. One option is to use insecticide treated eave ribbons, such as those previously used to deliver spatial repellents for protecting people indoors and outdoors against mosquito bites (Mmbando *et al.*, 2018, 2019; Mwanga *et al.*, 2019; Swai *et al.*, 2019; Masalu *et al.*, 2020b; Kaindoa *et al.*, 2021). Such a technique targets mosquitoes at the point of house entry but would be far more scalable and cheaper. This could potentially address the challenges associated with standard IRS as it utilizes lower quantities of insecticides, as only the ribbons to be used in the eaves will be sprayed. Operations and implementation might be simplified because there will be no need of removing household belongings before spraying or large spray teams which may simplify the operation resulting in high coverage.

Mosquitoes which rest on other surfaces not sprayed during IRS may be targeted as they enter indoors potentially increasing the impact. It is safer because it is less likely

that the users may accidentally touch the treated eave ribbon on the eaves. Temporary shelters can also be targeted, a good example is demonstrated in rural south-eastern Tanzania by (Swai *et al.*, 2019) where people living temporarily in farm huts were protected against malaria and non-malaria vectors both, indoors and outdoors when using transfluthrin treated eave ribbons. In addition, the intervention is accepted by community members and more than 90% were willing to use and pay for the intervention (Swai *et al.*, 2019). It may also help to manage resistance when used in combination with ITNs which are mostly treated with pyrethroids or when treated with a combination of two or more insecticide classes with different modes of action (Kaindoa *et al.*, 2021)

However, despite the evidence of the effectiveness of transfluthrin treated eave ribbons in protecting people against malaria and non-malaria vectors, no study has tested eave ribbons treated with alternative insecticides. This study was therefore aimed at assessing the potential of using eave ribbon treated with a residual insecticide pirimiphos-methyl, as a protection tool against malaria and dengue vectors and its potential to be used as an alternative for IRS by addressing the challenges posed by standard IRS.

1.9 Objectives

1.9.1 Overall objective

To evaluate the potential of eave-ribbons treated with a residual insecticide pirimiphos-methyl for controlling malaria and dengue vectors in rural Tanzania.

1.9.2 Specific objectives

1. To assess the efficacy of eave-ribbons treated with pirimiphos-methyl at WHO standard dose (1 g a.i./m^2) and exposure time (30 minutes) against malaria and dengue fever vectors.

2. To assess the efficacy of eave-ribbons treated with different doses of pirimiphos-methyl, against malaria and dengue fever vectors at different exposure durations.

3. To assess the efficacy of the eave-ribbons treated with different doses of pirimiphos-methyl for providing household-level protection against malaria and dengue fever vectors in the semi-field systems.

CHAPTER TWO

2 MATERIALS AND METHODS

2.1 Description of the study area & facility

The study was conducted in south-eastern Tanzania in Ifakara town, using specialized facilities at the Ifakara Health Institute for the bioassay work as well as for the semi-field studies. The laboratory bioassays were carried out at the vector biology laboratory, also known as, the VectorSphere. Semi-field studies were carried out inside a large, tunnel-shaped semi-field cage at the Mosquito City facility (Figure 6), located in Kining'ina Village, ~6km north of Ifakara town. The tunnel (also known as the Mosquito Marathon Tunnel) is 110 m long, 3 m wide and 2.5 m high, and has previously been described in multiple reports (Lorenz *et al.*, 2013; Mmbando *et al.*, 2015; Mwanga *et al.*, 2019).

Four portable experimental huts (3.0m length × 2.7m width × 2.0m height) were placed in the four chambers inside the tunnel, 25 meters apart from each other. Chambers are compartments separated by fiberglass mesh in which experimental huts with either treated or untreated eave ribbons were placed. This enables mosquitoes to remain in a specific compartment with a specific treated or untreated eave ribbon without crossing to the other huts when released during the experiment. In this experiment, we had four chambers, and one experimental hut was placed in each chamber. The treated eave ribbons were fitted to the eave spaces of experimental huts while control huts had no eave ribbon or untreated ribbon.

Each hut was fitted with exit traps on windows. Exit traps are rectangular box-shaped structures made up of metal rods joined together by wooden blocks covered with fiberglass netting (Okumu *et al.*, 2012). They were attached to the windows on the experimental huts using Velcro adhesive facing inside of the huts to catch mosquitoes trying to exit the huts. They have a sealable sleeve on the outside to allow retrieving mosquitoes in the trap by using a mouth aspirator (Govella *et al.*, 2011; Okumu *et al.*, 2012).

Figure 6: The large tunnel-shaped semi-field cage, within which the study was conducted, and the experimental huts used (**A:** Inside view; **B:** outside view; **C:** experimental hut with open eaves represented by the orange arrow, without exit trap or eave ribbon; **D:** hut with fitted ribbon and window exit trap). Source (Mmbando *et al.*, 2015)

2.2 Design and Treatment of the eave ribbons.

The eave ribbons were made of hessian fabric purchased locally in Tanzania. Two pieces of hessian were joined together to make a double layered ribbon. The lengths usually vary with the size of the house where it is fixed (Mmbando *et al.*, 2018), and the weight of a single layer ribbon was 360 g/m^2 and was capable of adsorbing ~300 mL of liquid when freshly treated. The ribbons used in this study had two different dimensions as follows:

i. The ribbons used in cone bioassays had final dimensions of 0.15 m wide and 11.6 m long, treatable surface area of 2 x 1.74 m^2 = 3.48 m^2
ii. The ribbons used in semi-field studies had final dimensions of 0.15 m wide and 7.7 m long, consisting of a treatable area of 2 x 1.155 m^2 = 2.31 m^2.

The eave ribbons were treated by spraying using a Hudson Micronair CS10 backpack sprayer with an 8002E flat fan nozzle equipped with a control valve as recommended by WHO for IRS (WHO, 2015). The control valve ensures uniform discharge as the

pressure in the spray tank decreases. Separate sprayers were used for the control arm (using water only) and treatment arm (using the organophosphate, pirimiphos-methyl). For each specific test or experiment, freshly treated ribbons were used, and the tests were done one day after treating the eave ribbons. The sprayer was pressurized by manual pumping to 55 pounds per square inch. The nozzle was kept ~10 cm away from the eave ribbon during treatment to ensure that the spray swath covered the entire surface of the eave ribbon. The ribbons were sprayed on both sides, each time allowing the ribbon to dry under a shade before spraying the other side. Once dry they were rolled in aluminium foil and placed in buckets ready to be taken to the study site.

2.3 Test insecticide and doses

This study assessed the eave ribbons treated with Actellic® 300CS, a long lasting formulation of the organophosphate, pirimiphos-methyl in a micro-encapsulated format (co-developed by Syngenta and Innovative Vector Control Consortium (IVCC, 2013)). The doses of pirimiphos-methyl tested were 0.5 g a.i./m^2, 1 g a.i./m^2, 2 g a.i./m^2, 4 g a.i./m^2, and 8 g a.i./m^2 for cone bioassay, and 1 g a.i./m^2, 2 g a.i./m^2 and 4 g a.i./m^2 for semi-field studies. A detailed description of the tank mix preparation for each dose is attached in Appendix 1 of this document.

2.4 Biological material

Laboratory reared 3-5 days old nulliparous (female mosquitoes that have never laid eggs), non-blood fed females of *An. arabiensis, An. funestus and Ae. aegypti* were used. The mosquitoes were maintained in the VectorSphere insectary. The larvae were fed on Tetramin® fish food and maintained at 26 - 28^{0}C. Adults were fed with a 10% glucose solution and the insectary was maintained on a 12h:12h light and dark cycle (Siria *et al.*, 2018). Previous studies had shown that the malaria mosquito colonies were susceptible to common public health insecticides (Urio *et al.*, 2022)

2.5 Efficacy of eave-ribbons treated with pirimiphos-methyl at WHO standard dose (1 g a.i./m^2) and exposure time (30 minutes) against malaria and dengue fever vectors.

To assess the efficacy of treated eave ribbons, cone bioassays tests were done following the standard WHO procedure for IRS cone bioassays (WHO, 2006) (Figure 8). One eave ribbon, treated with 1 g a.i./m^2 pirimiphos-methyl was cut into four pieces, each measuring ~60 cm long and 15 cm wide. An untreated piece of similar dimensions was used as a control.

On each eave ribbon piece, four WHO cones were affixed using adhesive tape (Figure 7). Five female mosquitos of *An. arabiensis*, *An. funestus* and *Ae. aegypti* were then transferred by mouth aspirator into each cone and exposed for 30 minutes as per WHO guidelines (WHO, 2006). Each of the three mosquito species was tested separately. A total of 80 female mosquitoes for each species were tested on the treated eave ribbons (four treated pieces, each tested with four cones, and each cone containing five mosquitoes). Additionally, 20 mosquitoes were exposed to the untreated eave ribbons (one untreated piece of ribbon with four cones each with five mosquitoes exposed). After 30 minutes of exposure, mosquitoes were removed using a mouth aspirator and placed into labelled paper cups, then provided with glucose and monitored for mortality after 24, 48 and 72 hours post-exposure.

Figure 7: Picture showing WHO cone attached to eave ribbon with a cotton wool plugged on the aspiration hole to prevent mosquitoes from escaping "**A**" and "**B**" showing aspiration of mosquitoes in the cone using mouth aspirator.

2.6 Efficacy of eave-ribbons treated with different doses of pirimiphos-methyl, against malaria and dengue fever vectors at different exposure durations.

This objective involved two different activities (Figure 8) as follows:

- Cone bioassays on eave ribbons treated with pirimiphos-methyl at the WHO-recommended IRS dose of 1 g a.i./m^2 for reduced exposure durations (one, three and 10 minutes, and 30 minutes as reference).
- Cone bioassays on eave ribbons treated with varying doses of pirimiphos-methyl at the reduced exposure durations (one and three minutes)

The first activity was done using the three mosquito species (*An. arabiensis* and *An. funestus* and *Ae. aegypti*) while the second activity was done using only *An. arabiensis* and *Ae. aegypti*

2.6.1 Cone bioassay on eave ribbons treated with pirimiphos-methyl at the WHO-recommended IRS dose of 1 g a.i./m^2 for reduced exposure durations

It was hypothesized that since the eave ribbon technology targets mosquitoes at the eave spaces as they attempt to enter human dwellings, the mosquitoes may spend less than 30 minutes near the eaves before entering houses. Therefore, this experiment was conducted to assess the mortality of mosquitoes exposed to treated eave ribbons at reduced exposure durations of one, three and 10 minutes, as well as the 30 minutes exposure as a reference. Similar procedures were followed as detailed above in section 2.5.

Four eave ribbon pieces (~60 cm long and 15 cm wide) treated with 1 g a.i./m^2 pirimiphos-methyl and one untreated piece were tested. On each eave ribbon piece, four WHO cones were affixed using adhesive tape and five female mosquitos were exposed to each cone. Separate cohorts of female mosquitoes of each species were exposed for one, three, 10 or 30 minutes exposures. Mortality of the mosquitoes was recorded after 24, 48 and 72 hours post-exposure.

2.6.2 Cone bioassay on eave ribbons treated with varying doses of pirimiphos-methyl at the reduced exposure durations

The experiments described under section 2.6.1 yielded very low mortality of the mosquitoes exposed at one and three minutes (< 25% mortality). Therefore, additional investigations were conducted to assess if higher mortality could be achieved using a higher concentration of the active ingredient at these exposure times. This experiment involved treating eave ribbons with different concentrations of pirimiphos-methyl (0.5 g a.i./m^2, 1 g a.i./m^2, 2 g a.i./m^2, 4 g a.i./m^2, and 8 g a.i./m^2). The ribbons were prepared and treated as described in section 2.6.1 above. Similarly, additional untreated pieces were included as controls.

Four plastic cones were attached to each piece of treated and untreated ribbon and five female mosquitoes were transferred into each cone using a mouth aspirator. Female mosquitoes of *An. arabiensis* and *Ae. aegypti* were exposed to different doses of treated eave ribbons for one and three minutes. This activity did not assess *An. funestus* due to the limited availability of this species. After exposure, mosquitoes were removed using a mouth aspirator and placed in labelled paper cups, provided with 10% glucose. Mortality was recorded after 24, 48 and 72 hours post-exposure.

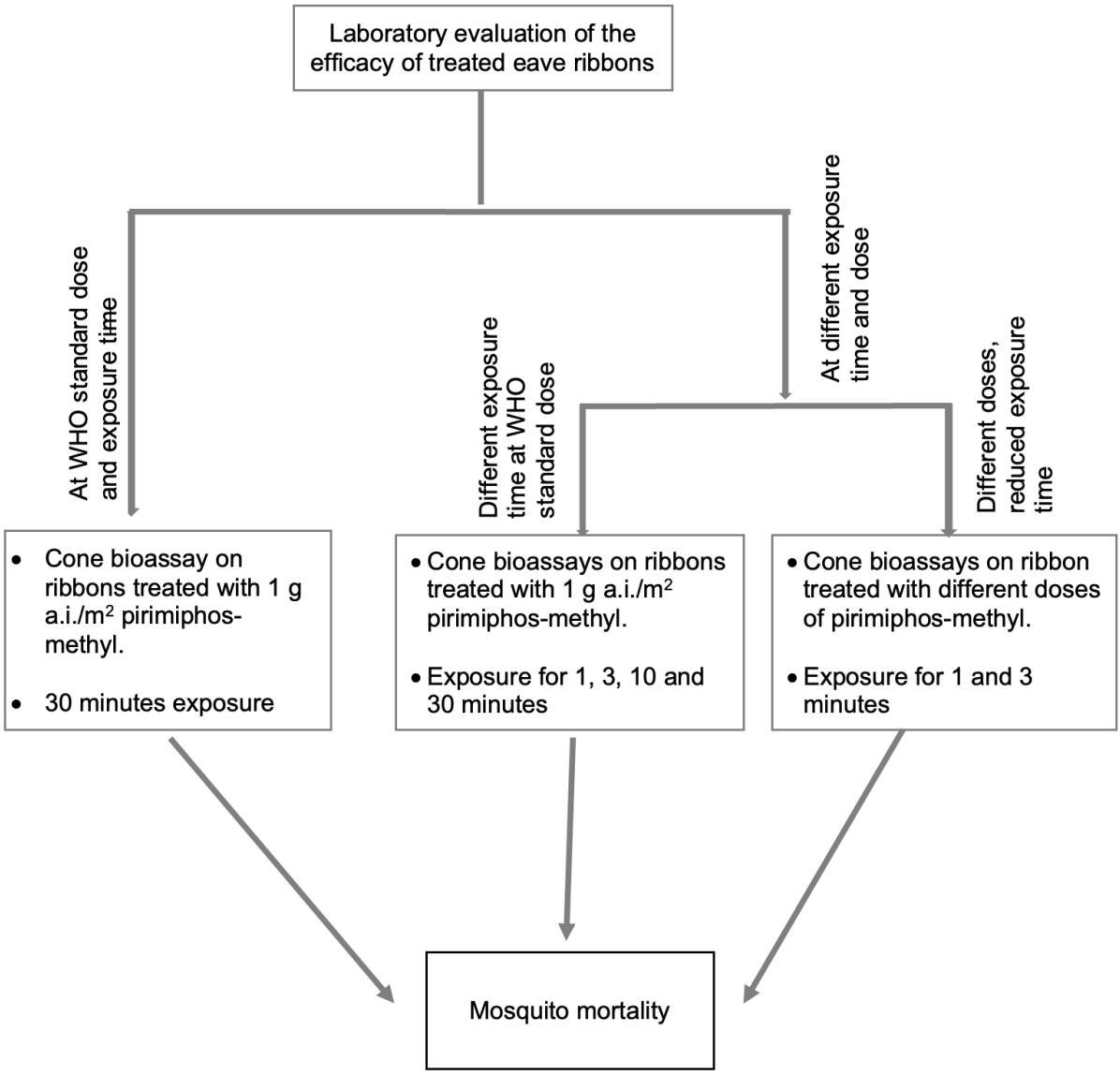

Figure 8: A diagram summarising the procedures for laboratory assessment of the efficacy of eave ribbons treated with pirimiphos-methyl.

2.7 Assessing the efficacy of insecticide-treated eave-ribbons against malaria vectors in and around human-occupied experimental huts within a semi-field system

This activity aimed at answering three questions:

i) To what extent do the insecticide treated eave ribbons reduce mosquito biting risk inside and outside human-occupied huts?

ii) To what extent do the insecticide treated eave ribbons reduce the densities of indoor and outdoor resting mosquitoes?

iii) To what extent do the insecticide-treated eave ribbons induce mortality of mosquitoes in the treatment chambers compared to control chambers?

To answer these questions, three different experiments were conducted, and three different doses of pirimiphos-methyl were evaluated (1 g a.i./m^2, 2 g a.i./m^2 and 4 g a.i./m^2) in a semi-field system, as follows:

- A semi-field study to assess the efficacy of eave ribbons treated with 1 g a.i./m^2 pirimiphos-methyl against *An. arabiensis*
- Two semi-field studies to assess the efficacies of eave ribbons treated with 2 g a.i./m^2 and 4 g a.i./m^2 pirimiphos-methyl against *An. arabiensis*
- A comparative study to assess the efficacies of eave ribbons treated with 2 g a.i./m^2 and 4 g a.i./m^2 pirimiphos-methyl against *An. arabiensis* and *An. funestus*

Mosquito collection procedures

Each activity involved releasing 200 laboratory-reared female mosquitoes every night inside each chamber with experimental huts fitted with treated or untreated or with no ribbon. The mosquitoes were released in two batches, the first 100 females at 06:30 pm and the remaining 100 at 10:00 pm. The mosquitoes were released at two intervals to ensure that mosquitoes were always available in the chamber and to avoid HLC collecting all mosquitoes and having no mosquitoes available for morning collections.

Adult volunteers performed human landing catches (HLC) from 07:00-10:00 pm within each experimental chamber while seated outside one meter away from the experimental huts inside the chambers. The volunteers entered the huts at 10:30 pm and slept under untreated bed nets until 06:00 am the next morning. Mosquitoes that entered the huts during the night and attempted to exit through the windows in the morning were collected in window exit traps at 06:00 am using a mouth aspirator. Additionally, indoor and outdoor resting mosquitoes were collected using a Prokopak® aspirator (Model 1419 supplied by John W Hock company) (John W, 2009). Collected mosquitoes were maintained with a 10% sugar water solution and mortality was recorded after 24, 48 and 72 hours. Primary outcomes were: (i) the number of mosquitoes caught indoors in the exit trap, ii) the number of mosquitoes captured outdoors by HLC, iii) resting mosquitoes collected by the Prokopak® aspirator and (iv) the percentage mortality of the recaptured mosquitoes. The mosquitoes that were not recaptured by any trapping method were not included in data analysis. During each experiment, environmental conditions were recorded using Tinytag Plus2 data loggers. Two data loggers were placed in the first and the last chamber outside the experimental

huts to record temperature and humidity each night of experimental except during the testing eave ribbons treated with 1 g a.i./^{2}.

2.7.1 Semi-field evaluation of the efficacy of eave ribbons treated with 1 g a.i./m^2 pirimiphos-methyl against *An. arabiensis*

Two eave ribbons were sprayed on both sides using standard IRS concentration of Actellic® 300CS (a.i. pirimiphos-methyl 1 g a.i./m^2) (IVCC, 2013; WHO, 2013)**,** and a second set of untreated ribbons were used as control. Although it had been demonstrated in the cone bioassays that this dose was not having significant mortality at the one and three minutes exposures, this dose was tested in the semi-field system to assess if it could reduce the biting risk as well as the number of mosquitoes resting indoors and outdoors overnight. The ribbons were fitted around the eave spaces of experimental huts inside separate different chambers (Figure 9). Two hundred laboratory-reared female *An. arabiensis* were released every night inside each chamber. Mosquitoes were released and re-collected indoors and outdoors as described in section 2.7 above. This was done for four days and stopped after observing that there was no difference between mosquito recaptures and mortality in control and treatment arm.

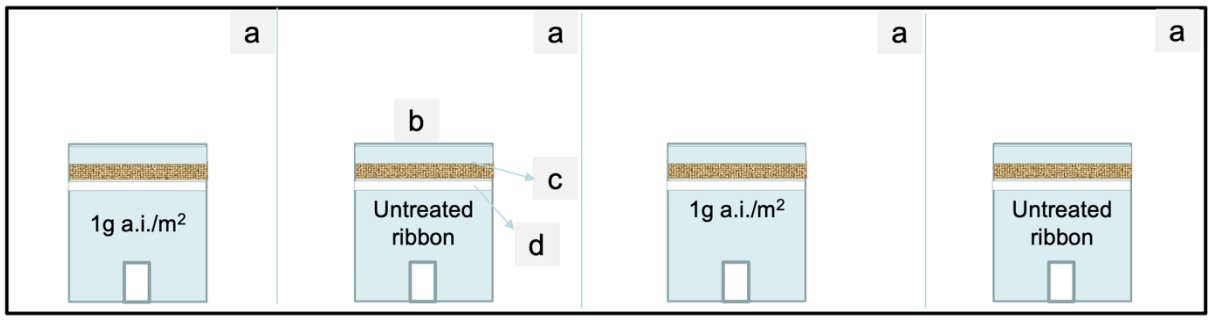

Figure 9: Arrangement of the interventions in the four experimental huts located inside the semi-field tunnel. The Figures show: **a**) the four semi-field chambers, **b)** an example of a single experimental hut, **c)** an eave ribbon fitted on eave space of the hut, and **d)** an open eave gap not blocked by eave ribbon to allow females to enter the house

2.7.2 A semi-field evaluation of eave ribbons treated with 2 g a.i./m^2 and 4 g a.i./m^2 pirimiphos-methyl against *An. arabiensis*

In the second set of experiments, the dosage of the insecticide treatment was increased to 2 g a.i./m^2 and 4 g a.i./m^2 pirimiphos-methyl, and the two doses were tested in parallel. This activity was done as a result of the low efficacy obtained in the

first experiment with the lower dose described in section 2.7.1 above. The eave ribbons were treated with 2 g a.i./m^2 and 4 g a.i./m^2 pirimiphos-methyl and placed on the eave spaces of the experimental huts as shown in Figure 10. The chambers and huts were cleaned with 70% alcohol and soap before introducing new interventions. Similar procedures for releasing and re-capturing the mosquitoes in the semi-field system were used for four days as described above in section 2.7.

The experiment was stopped after four days after realizing possible cross contamination due to similar mosquito mortalities in control and treatment chambers. The contamination might have been a result of cross contamination between chambers with experimental huts with treated eave ribbons into the control chambers with experimental huts with untreated eave ribbon or no eave ribbon. The experimental design explained below in section 2.7.3 was then adopted.

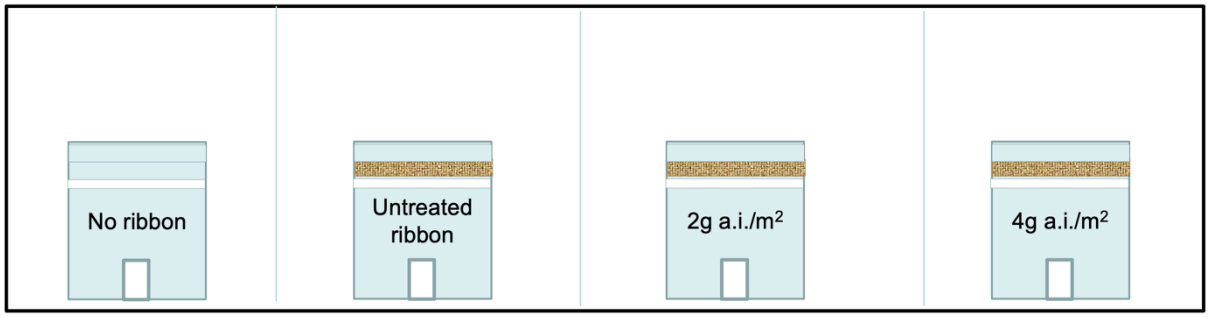

Figure 10: Arrangement of different interventions in the four huts located inside the semi-field tunnel (the doses are indicated as g a.i./m^2)

2.7.3 A comparative study to assess the efficacy of eave ribbons treated with 2 g a.i./m^2 and 4 g a.i./m^2 pirimiphos-methyl against *An. arabiensis* and *An. funestus*

This study was done after observing slightly higher control mortality in the above experiments. To ensure that there is no cross contamination between the control and treatment chambers, a *before-and-after* study design was implemented, where baseline control (No ribbon in the experimental huts) tests were conducted before introducing the treated eave ribbons. To evaluate the possibility of cross-contamination between chambers with experimental huts with treated eave ribbon and those with no ribbon, an additional control was included in parallel to the intervention phase.

Two doses of pirimiphos-methyl i.e., 2 g a.i./m^2 and 4 g a.i./m^2 were assessed separately starting with the lower dose followed by the higher dose (Figure 11). The

first experiment was done with all chambers acting as control with no ribbon for ten days. Following the control with no eave ribbon phase, the eave ribbons treated with 2 g a.i./m^2 pirimiphos-methyl were introduced into the experimental huts in the first and last chamber with the two middle chambers remaining as control with no ribbon. Two hundred mosquitoes of *An. arabiensis* females were released in each chamber for ten days.

After completing the experiment with a 2 g a.i./m^2 dose, the chambers and huts were cleaned with soap and 70% alcohol and a second baseline control with no ribbons was done for ten days this time using *An. arabiensis* and *An. funestus* released together each night per chamber. Two hundred *An. arabiensis* and one hundred *An. funestus* were jointly released per night per chamber. Fewer *An. funestus* were released compared to *An. arabiensis* due to the limited availability of the former species. These two species were jointly released to mimic natural settings where the species occur in sympatry.

After the second baseline control with no ribbons, eave ribbons treated with 4 g a.i./m^2 pirimiphos-methyl were introduced into the experimental huts in the first and last chambers while two middle chambers served as control as it was done in the first experiment with 2 g a.i./m^2 ribbons. Two hundred *An. arabiensis* and one hundred *An. funestus* were released together in each chamber.

Mosquitoes were collected and monitored for mortality as explained in section 2.7 above.

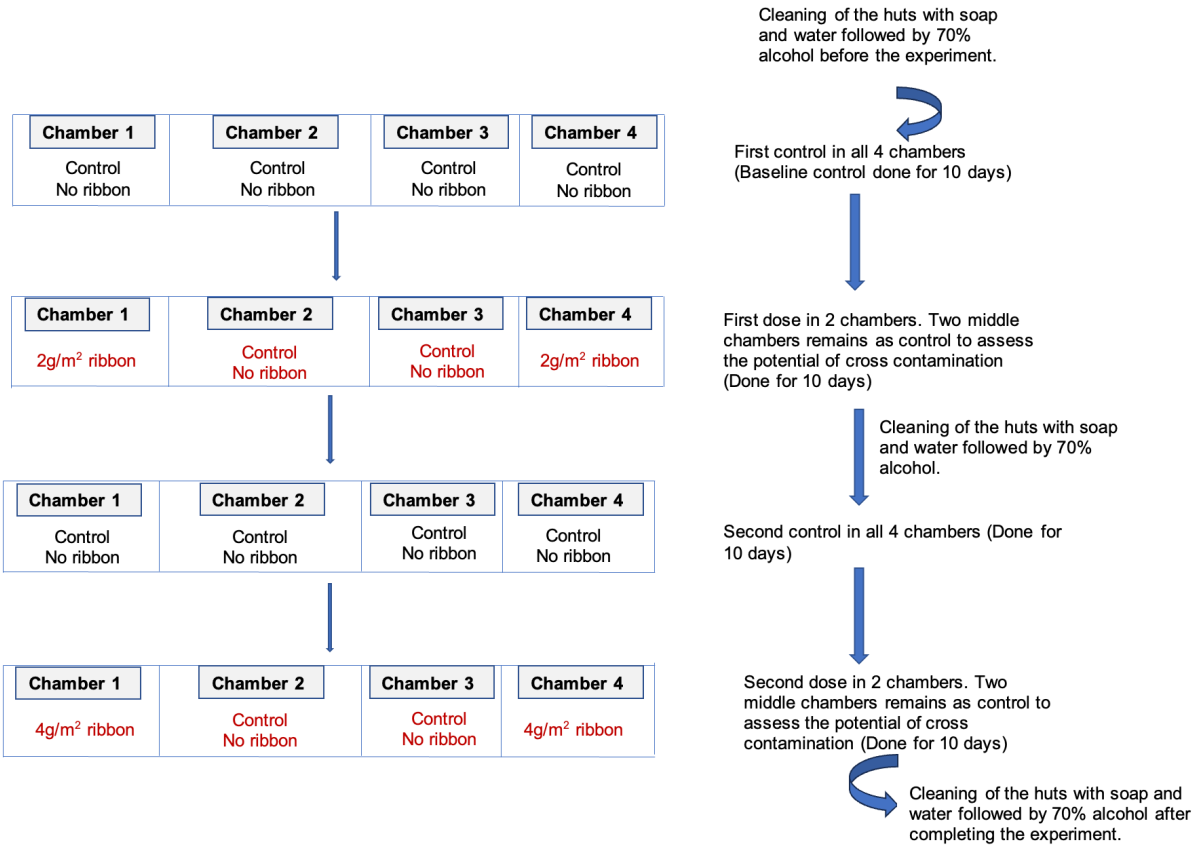

Figure 11: A schematic diagram showing a *before-and-after* study design with the arrangement of treatments and controls in the chambers

2.8 Data analysis

Analysis was done using R statistical software version 4. 1. 2 (R Core Team, 2017). Data for each species and insecticide dosage were analyzed separately. Graphics were generated using *ggplot2* (Wickham, 2011). The mean number of recaptured and dead mosquitoes was calculated in R using the *dplyr* package. The percentage mortality of mosquitoes was calculated as the proportion of exposed mosquitoes that died.

$$(\%\ \text{mortality} = \frac{\text{Dead mosquitoes}}{\text{Exposed mosquitoes}} * 100).$$

To compare the differences in percentages of mortalities of mosquitoes exposed at different exposure durations (one, three, 10 and 30 minutes) and holding time post-exposure (24, 48 and 72 hours), the Kruskal-Wallis test was done followed by Dunn's pairwise comparison test (Kruskal and Wallis, 1952). This was done after confirming by Shapiro–Wilk test that the bioassay data were not normally distributed therefore the

assumptions of ANOVA were not met. Probit analysis was used to assess the dose that can kill 99% of mosquito species tested (LC_{99}) using the ecotox package.

The impact of pirimiphos-methyl on biting risk and mortality of recaptured mosquitoes was modelled using a generalized linear mixed model (GLMMs) following binomial distributions. For biting risk, the number of recaptured mosquitoes as a fraction of those released was added as the dependent variable, while for the case of mortality, the proportion of recaptured mosquitoes that died was used as the dependent variable. Intervention was added as a fixed factor while Chamber.ID and the day of test were added as random factors. Percentage protective efficacy was calculated using the formula:

$$\frac{\text{Mean number captured in control} - \text{Mean number captured in treatment}}{\text{Mean number captured in control}} * 100.$$

Indoor biting risk was measured using a mean number of mosquitoes captured in exit traps, while outdoor-biting risk was measured using a mean number of mosquitoes recaptured by HLC. The impact of treated ribbons on resting mosquitoes indoors and outdoors was assessed by the number of mosquitoes captured by Prokopak® indoors and outdoors respectively.

2.9 Ethical approval and consent to participate

The study participants were recruited only upon providing written informed consent forms. All the information about the study, including objectives, benefits and potential risks was explained to the participants in the local language (Swahili) so that they can comprehend prior to consenting. They were informed of their right to end participation at any time without the need to provide reasons to the investigator. Those who voluntarily agreed to participate were recruited.

This study was approved by the Institutional Review Board of Ifakara Health Institute (IHI/IRB/No:56-2020), the Medical Research Coordinated Committee of the National Institute for Medical Research of the United Republic of Tanzania (Ref: NIMR/HQ/R.8a/Vol.IX/3637) and the Wits Ethics Clearance Board (M211014). All the clearance certificates are attached in the appendix section (Appendix 2-4)

CHAPTER THREE

3 RESULTS

3.1 Results of the cone bioassays

Mortality of mosquitoes exposed to 1 g a.i./m^2 pirimiphos-methyl for 30 minutes

In all three species tested namely *An. arabiensis*, *An. funestus* and *Ae. aegypti*, 100% 24 hours mortality was observed at the standard IRS dose of 1 g a.i./m^2 of pirimiphos-methyl and a standard exposure time of 30 minutes. The control mortalities did not exceed 10% (Table 2).

Table 2: Percentage mortalities of mosquitoes exposed to 1 g a.i./m^2 pirimiphos-methyl for 30 minutes

Species	Intervention	Nº exposed	No dead at 24h	% Mortality
Anopheles arabiensis	1 g a.i./m^2 pirimiphos-methyl	80	80	100
	Control (Untreated ribbon)	20	2	10
Anopheles funestus	1 g a.i./m^2 pirimiphos-methyl	80	80	100
	Control (Untreated ribbon)	20	0	0
Aedes aegypti	1 g a.i./m^2 pirimiphos-methyl	80	80	100
	Control (Untreated ribbon)	20	2	10

Effects of exposure duration on mosquito mortality

Host seeking females might have a short contact duration with the treated eave ribbons and this might have an impact on the 24 hours mortality. Therefore, the three species of mosquitoes were exposed to treated eave ribbons for one, three and 10 and compared to 30 of minutes exposure as a reference. The results are presented in Figure 12. The highest mortalities were recorded at 30 minutes exposure but declined with reduced exposure time (Figure 12). *Anopheles funestus* had the highest mortalities, exceeding 95% at 10 and 30 minute exposures followed by *An. arabiensis*, which had a mortality of 73% and 98% at 10 and 30 minutes exposure, respectively. For *Ae. aegypti* however, lower mortalities of 20% and 61% at 10 and 30 minutes of exposure respectively were observed. Exposure times of one and three minutes resulted in 24 hours mortalities of less than 31% for all three species.

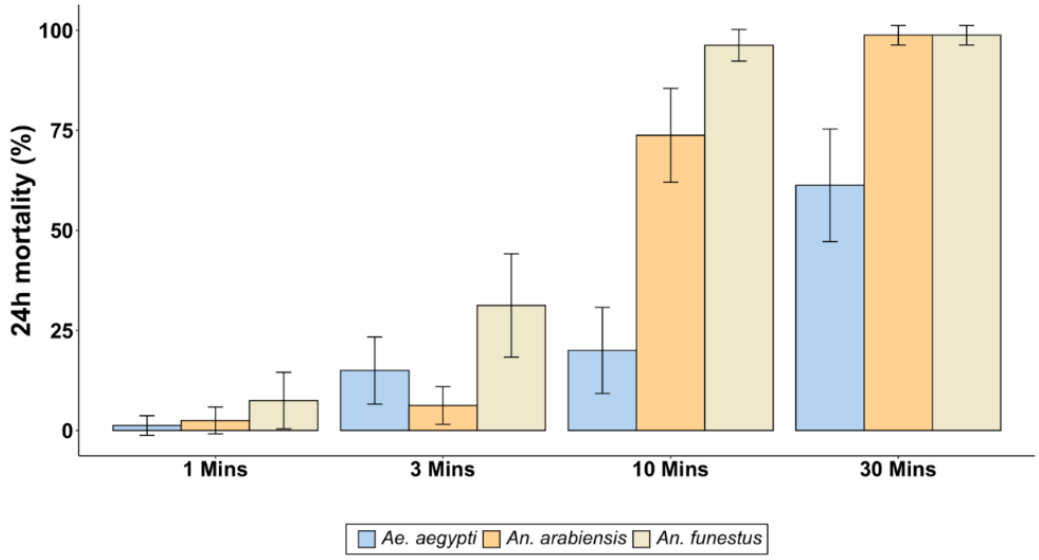

Figure 12: Mean percentage mortality, 24 hours post-exposure of mosquitoes exposed to eave-ribbons treated with 1 g a.i./m^2 of pirimiphos-methyl at different exposure times. The error bars represent 95% confidence interval (CI)

The Kruskal-Wallis test indicated that there was a significant difference in the median percentages of mortalities of mosquitoes exposed in different exposure durations in all three species tested (Figure 13). Dunn's pairwise comparisons of the 24 hours mortalities at different exposure times, indicated that 30 minutes of exposure induced higher mortalities than other exposure durations in each mosquito species tested. At 10 minutes of exposure, the impact was reduced significantly in *Ae. aegypti* (Median difference (MD) 40.00, p = 0.003)) while that of *An. funestus* and *An. arabiensis* remained similar to that of 30 minutes of exposure.

At one and three minutes of exposure, the impact of pirimiphos-methyl on 24 hours mortality was significantly reduced in all species compared to the impact observed at 30 minutes of exposure (p values <0.001) (Figure 13). The exposure of mosquitos for either three or 10 minutes resulted in a significant difference in the mortality of *An. arabiensis* (MD 80%, p value 0.001) and *An. funestus* (MD 60%, p value 0.001) but not in *Ae. aegypti*. The percentages of mortalities observed in mosquitoes exposed for one minute was similar to those observed for three minutes exposure in all three species.

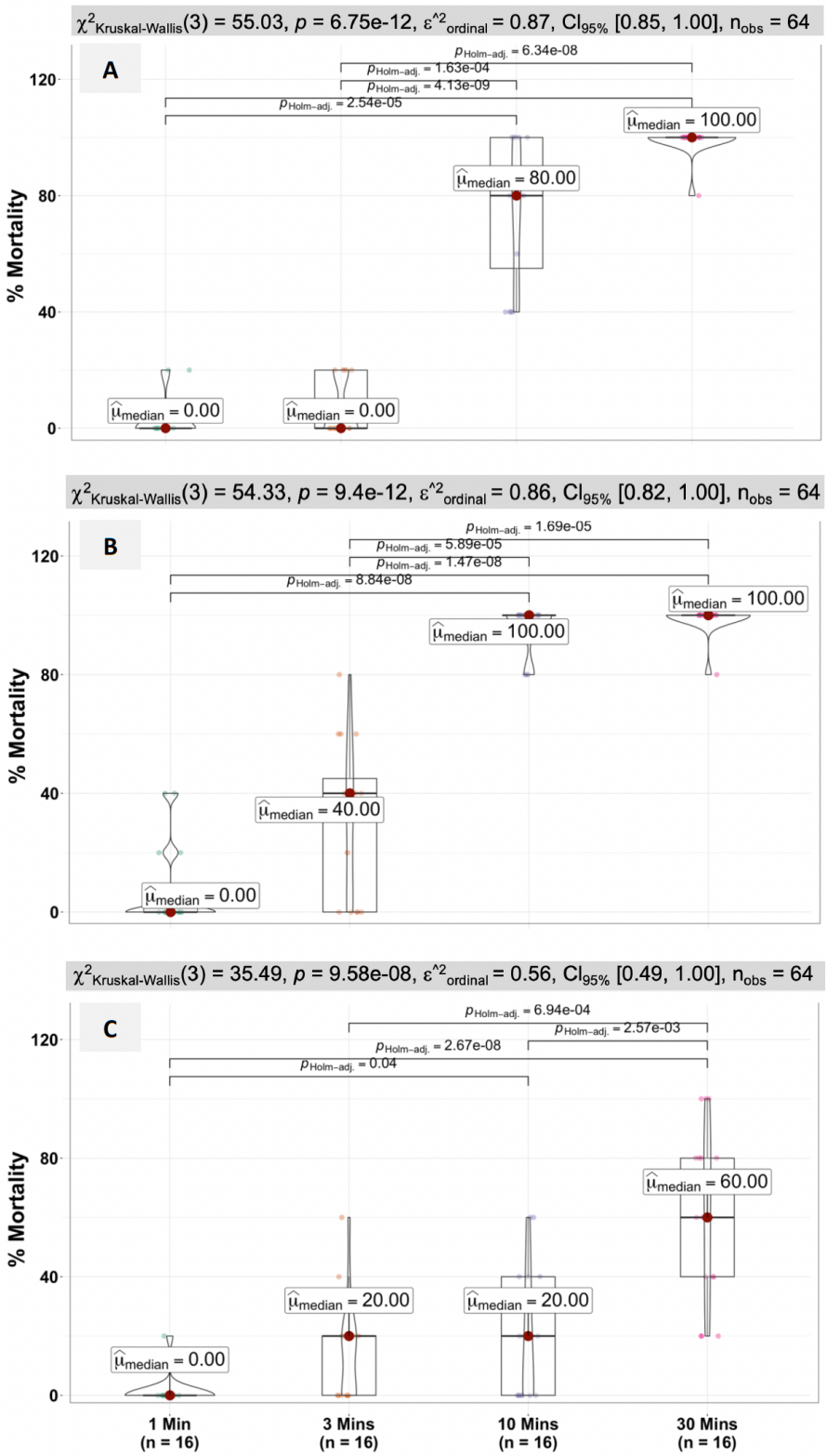

Figure 13: Pairwise comparison of the impact of exposure time on mosquito mortality. The top row in grey shows Kruskal-Wallis test results followed by the bars indicating significant differences between the groups with *p* values and the red dots denotes the median. "**A**" represents *An. arabiensis*, "**B**" represents *An. funestus*, and "**C**" represents *Ae. aegypti*.

For all three species tested, holding the mosquitoes at the insectary for 24, 48 and 72 hours post-exposure had no significant impact on per cent mortality ($p > 0.05$). This was the same at all exposure durations (Table 3)

Table 3: The impact of holding time (24, 48 and 72 hours) post-exposure on mortality of mosquitoes at different exposure durations

Exposure time	*An. arabiensis*		*An. funestus*		*Ae. aegypti*	
	$\chi^2_{Kruskal-Wallis}$	*p*	$\chi^2_{Kruskal-Wallis}$	*p*	$\chi^2_{Kruskal-Wallis}$	*p*
1 minute	4.19	0.12	4.31	0.12	3.37	0.19
3 minutes	2.72	0.26	1.09	0.58	1.09	0.58
10 minutes	0.19	0.91	0	1	1.15	0.56
30 minutes	0	1	2	0.37	0.39	0.82

Mortality of mosquitoes exposed to varying doses of pirimiphos-methyl at the reduced exposure durations

As mosquito mortality at 24 hours post-exposure reduced significantly at shorter exposure durations, the impact of higher concentration of pirimiphos-methyl on the 24 hours mortality was also investigated at one and three minute exposure durations. The mortality of mosquitoes increased with increasing doses and exposure duration. At concentrations of 0.5 g a.i./m^2 and 1 g a.i./m^2, less than 10% mortality was recorded for all species for one and three minutes exposures.

However, at a concentration of 2 g a.i./m^2, the mortality of *An. arabiensis* rose to 83% after one minute of exposure and 100% after three minutes of exposure. At the same concentration, the mortality of *Ae. aegypti* remained low (22.5%) after one minute of exposure but increased to 83% after three minutes of exposure. At concentrations of 4 g a.i./m^2 and 8 g a.i./m^2, the mortality of *An. arabiensis* was 100% after both one and three minutes of exposure. The mortality of *Ae. aegypti* was 83% after one minute exposure at 4 g a.i./m^2 and 100% for all remaining exposures. These tests were not done on *Anopheles funestus* (Table 4).

Table 4: Percentage mortalities of mosquitoes exposed to different concentrations of pirimiphos-methyl

Species	1 minute exposure				3 minutes exposure			
	Dose (g/m^2)	Nº exposed	Nº dead (24h)	% Mortality	Dose (g/m^2)	Nº exposed	Nº dead (24h)	% Mortality
An. arabiensis	****0**	200	6	3	**0**	200	8	4
	0.5	80	4	5	**0.5**	80	5	6.25
	1	80	5	6.25	**1**	80	13	16.25
	2	80	67	83.75	**2**	80	80	100
	4	80	80	100	**4**	80	80	100
	8	80	80	100	**8**	80	80	100
Ae. aegypti								
	****0**	200	1	0.5	**0**	200	1	0.5
	0.5	80	0	0	**0.5**	80	3	3.75
	1	80	1	1.25	**1**	80	5	6.25
	2	80	18	22.5	**2**	80	67	83.75
	4	80	67	83.75	**4**	80	80	100
	8	80	80	100	**8**	80	80	100

******The 0 g a.i./m^2 represent the control which was not treated with pirimiphos-methyl, **Nº** represent number.

Using Probit analysis, the lethal concentration that could induce mortality of 99% in *An. arabiensis* (LC_{99}) was estimated to be 3.8 g a.i./m^2 and 2.7 g a.i./m^2 for one and three minutes exposures respectively. For *Ae. Aegypti*, the LC_{99} was 6.8 g a.i./m^2 and 3.6 g a.i./m^2 for one and three minute exposures respectively (Table 5).

Table 5: LC_{99} for the *An. arabiensis* and *Ae. aegypti* two different exposure time

Species	Exposure time	Lethal conc	Dose (g a.i./m^2)	95% CI (g a.i./m^2)
An. arabiensis	1min	LC_{99}	3.8	2.7-8.2
	3min	LC_{99}	2.7	1.99-5.73
Ae. aegypti	1min	LC_{99}	6.8	4.9-14.6
	3min	LC_{99}	3.6	2.6-7.7

3.2 Results of the semi-field studies

To evaluate the efficacy of eave ribbons under semi-field conditions, the three concentrations (1 g a.i./m^2, 2 g a.i./m^2 and 4 g a.i./m^2) of pirimiphos-methyl were evaluated. Two malaria vectors *An. arabiensis* and *An. funestus* were assessed in the

semi-field. *Anopheles funestus* was assessed at the dose of 4 g a.i./m^2 only in a "*before and after"* study due to the limited availability of these species. *Aedes aegypti* was not evaluated in the semi-field after observing low efficacy in malaria vectors and also due to low mortality of *Ae. aegypti* observed at reduced exposure duration in the cone bioassay.

Each night 200 female mosquitoes were released in each semi-field chamber. Volunteers collected mosquitoes outdoors by HLC from 07:00 to 10:00 pm In the morning they collected mosquitoes in the window exit traps, resting mosquitoes indoors and outdoors using a Prokopak® aspirator. The recaptured mosquitoes were monitored at the insectary and mortality was recorded after 24 hours post-exposure. The outcome measures were (i) indoor and outdoor biting risk measured by the number of mosquitoes captured on exit trap and HLC respectively (ii) reduction in indoor and outdoor resting mosquitoes measured by the number of mosquitoes captured by Prokopak® aspirator indoors and outdoors (iii) 24 hours mortality.

Efficacy of eave ribbons treated with 1 g a.i./m^2 pirimiphos-methyl against *An. arabiensis*

Eave ribbons treated with 1 g a.i./m^2 pirimiphos-methyl did not reduce indoor biting by *An. arabiensis*, but there was an insignificant reduction in outdoor biting risk by 6.21% (Odds Ratio (OR)=0.93, 95% CI: 0.74-1.18, $p > 0.05$). Similarly, the eave ribbons also did not reduce outdoor resting by the same mosquitoes species but caused a small insignificant reduction of indoor resting of 13.29% (OR =0.87, 95% CI: 0.55-1.35, $p > 0.05$) (Table 6). The 24 hours mortality of recaptured mosquitoes in the treatment arm was also not significantly different from the 24 hours mortality observed in control arms (OR=1.08, 95% CI: 0.75-1.56, $p > 0.05$) (Table 7).

Efficacy of eave ribbons treated with 2 g a.i./m^2 and 4 g a.i./m^2 pirimiphos-methyl against *An. arabiensis*

The efficacy of eave ribbons treated with 2 g a.i./m^2 and 4 g a.i./m^2 pirimiphos-methyl was assessed in parallel in the semi-fields system. The results are presented in Table 8. The eave ribbons treated with either 2 g a.i./m^2 or 4 g a.i./m^2 pirimiphos-methyl significantly reduced *An. arabiensis* biting risk by 13.19% (OR = 0.85, 95% CI: 0.73-0.99, p =0.04) and 27.07% (OR = 0.70, 95% CI: 0.59-0.82, $p < 0.001$) respectively compared to untreated controls. There was no significant difference in biting risk in

chambers with experimental huts with untreated eave ribbon control compared to no eave ribbon control ($p > 0.05$). There was also no significant difference in 24 hours mortality observed in the presence of treated eave ribbons compared to untreated eave ribbon control or no ribbon control ($p > 0.05$). Untreated eave ribbon control and no ribbon control 24 hours mortality post-exposure were slightly high and did not significantly differ from that observed in treated eave ribbons. The average temperature recorded during the nights of the experiment was 22.08 degrees Celsius (°C), while the average relative humidity (RH) was 40.08%.

Efficacy of eave ribbons treated with 2 g a.i./m^2 and 4 g a.i./m^2 pirimiphos-methyl against *An. arabiensis* and *An. funestus* _ a "*before and after"* study

*Baseline: C*ontrol experiments (no eave ribbons) were conducted in the experimental huts before carrying out the treatment arms (treated eave ribbons). The aim of doing baseline control was to get the data at baseline in the absence of the intervention. This was done after observing slightly high control mortalities in the above experiments. To make sure the chambers are clean and free of contamination before introducing interventions we did a control experiment in all chambers first. The results of baseline control indicated low mortalities of less than 10% up to 72h post-exposure (Table 9). The nightly temperature and humidity during the baseline control were 21.67°C and 41.40% RH respectively. During the second baseline control, temperature was 18.90°C while relative humidity was 47.89%.

Indoor and outdoor biting risk: The effects of treated eave ribbons on indoor and outdoor biting risk are summarized in Table 10. Using eave ribbons treated with 2 g a.i./m^2 pirimiphos-methyl reduced indoor biting risk by 18.2% and outdoor biting risk by 5.8% compared to the control, though these reductions were not statistically significant ($p > 0.05$). The average nightly temperature and humidity recorded during this experiment were 20.13°C and 38.13% RH respectively. Using eave ribbons treated with 4 g a.i./m^2 pirimiphos-methyl significantly reduced outdoor biting *An. arabiensis* by 11.62% (OR = 0.80, 95% CI: 0.71-0.91, $p < 0.001$). However, there was no significant reduction in indoor biting risk. In the same settings, the use of eave ribbons treated with 4 g a.i./m^2 pirimiphos-methyl reduced indoor biting risk in *An. funestus* by 24.39% while the outdoor biting risk was reduced by 0.70%. Here too, the reductions were not statistically significant ($p > 0.05$). The nightly temperature was 18.48°C while relative humidity was 46.91%.

Indoor and outdoor resting mosquitoes: The effects of treated eave ribbons on indoor and outdoor resting by the mosquitoes are summarized in Table 11. The use of eave ribbons treated with 2 g a.i./m^2 pirimiphos-methyl significantly reduced the indoor resting *An. arabiensis* by 16.5% (OR = 0.80, 95% CI=0.67-0.95 p = 0.01) compared to control. However, there was no significant reduction in outdoor resting mosquitoes (p > 0.05). In the same settings, the use of eave ribbons treated with 4 g a.i./m^2 pirimiphos-methyl did not lead to any significant reductions in indoor and outdoor resting *An. arabiensis* and *An. funestus* (p > 0.05).

Table 6: Effects of eave ribbons treated with 1 g a.i./m^2 pirimiphos-methyl on biting reduction and reduction in indoor and outdoor resting *An. arabiensis*

	Location	Intervention	Nights	Nº released	Nº captured	Mean captured/night/chamber (95% CI)	Reduction (%)	OR (95% CI)	P value
Biting reduction	Indoor	Control	4	800	17	2.12 (0.99-3.26)	Ref	1	0.33
		1 g a.i./m^2	4	800	27	3.37 (1.99-4.76)	-58.96	1.62 (0.62-4.26)	
	Outdoor	Control	4	800	161	20.12 (15.46-24.79)	Ref	1	0.55
		1 g a.i./m^2	4	800	151	18.87 (14.41-23.34)	6.21	0.93 (0.74-1.18)	
Reduction in resting mosquitoes	Indoor	Control	4	800	68	8.50 (5.06-11.93)	Ref	1	0.53
		1 g a.i./m^2	4	800	59	7.37 (5.60-9.15)	13.29	0.87 (0.55-1.35)	
	Outdoor	Control	4	800	174	21.75 (15.93-27.57)	Ref	1	0.61
		1 g a.i./m^2	4	800	184	23.00 (19.13-26.87)	- 5.75	1.06 (0.83-1.36)	

Table 7: Summary of 24 hours mortalities of *An. arabiensis* recaptured in chambers with eave ribbons treated with 1 g a.i./m^2 pirimiphos-methyl.

Intervention	Nights	Nº Captured	Mean captured/night/chamber (95% CI)	No dead	Mean dead/night/chamber (95% CI)	OR (95% CI)	P value	% Mortality
Control	4	420	13.12 (9.64-16.60)	73	2.28 (0.93-3.63)	1	0.66	17.38
1 g a.i./m^2	4	421	13.16 (9.96-16.36)	69	2.16 (1.37-2.95)	1.08 (0.75-1.56)		16.41

Table 8: Protective efficacy and mortality offered by eave ribbons treated with 2 g and 4 g a.i./m^2 against *An. arabiensis*

	Night	Nº Captured	Mean captured/night/chamber (95% CI)	Biting reduction (%)	OR (95% CI)	P value	Nº dead	Mean dead/night/chamber (95% CI)	% Mortality	OR (95% CI)	P value
No ribbon	4	403	25.19 (16.89-33.49)	Ref	1		46	2.87 (0.38-5.36)	11.41	1	
Untreated	4	372	23.25 (15.21-31.29)	7.70	0.91 (0.78-1.06)	0.23	29	1.81 (0.84-2.78)	7.79	0.64 (0.39-1.05)	0.08
2 g a.i./m^2	4	350	21.87 ((13.53-30.21)	13.19	0.85 (0.73-0.99)	0.04	52	3.25 (1.11-5.39)	14.86	1.41 (0.92-2.16)	0.12
4 g a.i./m^2	4	294	18.37 (11.59-25.15)	27.07	0.70 (0.59-0.82)	<0.001	34	2.12 (0.98-3.26)	11.56	1.04 (0.65-1.67)	0.88

Table 9: Baseline control mortality done before introducing the treated eave ribbons into the experimental huts

Baseline control	Species	No. of nights	Mean captured/night/ Chamber (95% CI)	Mean dead 24 hours /night/ Chamber (95% CI)	% Mortality 24 hours	Mean dead 48 hours /night/ Chamber (95% CI)	% Mortality 48 hours	Mean dead 72 hours /night/ Chamber (95% CI)	% Mortality 72 hours
Control 1	*An. arabiensis*	10	22.54 (19.66-25.42)	0.32 (0.11-0.21)	1.42	0.68 (0.54-0.81)	3.02	1.11 (0.93-1.28)	4.92
Control 2	*An. arabiensis*	10	21.21 (18.59-23.82)	0.10 (0.04-0.16)	0.47	0.22 (0.13-0.22)	1.04	0.39 (0.26-0.51)	1.84
	An. funestus	10	11.44 (10.19-12.68)	0.17 (0.08-0.26)	1.49	0.36 (0.23-0.49)	3.15	0.72 (0.56-0.89)	6.30

Table 10: Effects of eave ribbons treated with 2 g a.i./m^2 or 4 g a.i./m^2 of pirimiphos-methyl on indoor and outdoor biting densities of *An. arabiensis* and *An. funestus* in the semi-field settings: results of a "before and after" study.

Species	Location	Intervention	Nights	Released	Nº captured	% Recaptured	Mean captured/night/ Chamber (95% CI)	Reduction (%)	OR (95% CI)	P value
An. arabiensis	Indoor	Control	10	4,000	77	1.92	3.85 (3.28-4.42)	Ref	1	
		2 g a.i./m^2	10	4,000	63	1.57	3.15 (2.31-3.99)	18.2	0.81 (0.57-1.16)	0.26
		Control	10	4,000	36	0.90	1.80 (1.49-2.10)	Ref	1	
		4 g a.i./m^2	10	4,000	28	0.70	1.40 (1.01-1.79)	22.2	0.78 (0.47-1.27)	0.32
	Outdoor	Control	10	2,000	1019	50.95	50.95 (46.77-55.13)	Ref	1	
		2 g a.i./m^2	10	2,000	960	48.00	48.00 (45.10-50.90)	5.8	0.89 (0.78-1.00)	0.06
		Control	10	2,000	921	46.05	46.05 (42.85-49.25)	Ref	1	
		4 g a.i./m^2	10	2,000	814	40.70	40.70 (37.68-43.72)	11.62	0.80 (0.71-0.91)	0.0006
An. funestus	Indoor	Control	10	2,000	41	2.05	2.05 (1.75-2.35)	Ref	1	
		4 g a.i./m^2	10	2,000	31	1.55	1.55 (1.11-1.90)	24.39	0.75 (0.47-1.20)	0.24
	Outdoor	Control	10	1,000	429	42.9	21.45 (20.26-22.64)	Ref	1	
		4 g a.i./m^2	10	1,000	426	42.6	21.30 (20.19-22.41)	0.70	0.99 (0.83-1.18)	0.89

**Indoor biting risk was measured by the number of mosquitoes captured on the exit trap while outdoor biting risk was measured by the number of mosquitoes captured by HL

Table 11: Effects of eave ribbons treated with 2 g a.i./m^2 or 4 g a.i./m^2 of pirimiphos-methyl on indoor and outdoor resting densities of *An. arabiensis* and *An. funestus* in the semi-field settings: results of a "before and after" study.

Species	Location	Intervention	Nights	Released	Nº captured	% Recaptured	Mean captured/night/ Chamber (95% CI)	Reduction (%)	OR (95% CI)	P value
An. arabiensis	Indoor	Control	10	4,000	321	8.02	16.05 (13.87-18.23)	Ref	1	
		2 g a.i./m^2	10	4,000	268	6.70	13.40 (11.64-15.16)	16.5	0.82 (0.69-0.97)	0.02
		Control	10	4,000	218	5.45	10.90 (9.56-12.24)	Ref	1	
		4 g a.i./m^2	10	4,000	210	5.25	10.50 (9.37-11.63)	3.67	0.96 (0.79-1.17)	0.69
	Outdoor	Control	10	4,000	526	13.15	26.30 (22.41-30.19)	Ref	1	
		2 g a.i./m^2	10	4,000	497	12.42	24.85 (11.22-27.59)	5.5	0.94 (0.82-1.07)	0.35
		Control	10	4,000	555	13.87	27.75 (25.49-30.01)	Ref	1	
		4 g a.i./m^2	10	4,000	550	13.75	27.50 (24.89-30.10)	0.90	0.99 (0.84-1.14)	0.90
An. funestus	Indoor	Control	10	2,000	153	7.65	7.65 (6.88-8.42)	Ref	1	
		4 g a.i./m^2	10	2,000	148	7.4	7.40 (6.38-8.42)	3.27	0.96 (0.76-1.22)	0.76
	Outdoor	Control	10	2,000	444	22.20	22.20 (20.33-24.07)	Ref	1	
		4 g a.i./m^2	10	2,000	447	22.35	22.35 (20.72-23.98)	- 0.67	1.01 (0.87-1.17)	0.91

**Indoor resting mosquitoes were measured by the number of mosquitoes captured by Prokopak® aspirator inside the experimental huts while outdoor resting mosquitoes were measured by the number of mosquitoes captured by Prokopak® aspirator outdoors.

Mortality of mosquitoes recaptured from experimental huts fitted with treated eave ribbons: The mortality of recaptured mosquitoes was consistently higher in huts fitted with the treated eave ribbons compared to control huts (Table 12, Figures 14 & 15).

Out of 1,788 recaptured *An. arabiensis* from huts having eave ribbons treated with 2 g a.i./m^2 pirimiphos-methyl, 8% had died after 24 hours, 14% after 48 hours and 22% after 72 hours. Increasing the dose to 4 g a.i./m^2 pirimiphos-methyl did not result in increased mortalities of recaptured *An. arabiensis*. The mortalities were 4% after 24 hours, 10% after 48 hours and 16% after 72 hours; out of the 1,602 recaptured (Table 12, Figure 14). The odds of mortality among the recaptured *An. arabiensis* after 24 hours were ten times higher in huts with 2 g a.i./m^2 ribbons (OR=10.65, 95% CI: 5.97-18.99, $p < 0.001$) compared to controls; and seven times higher in huts with 4 g a.i./m^2 ribbons (OR=7.36, 95% CI:3.92-13.83, $p < 0.001$) compared to control.

In tests with *An. funestus*, 1,052 females were recaptured from huts having treated eave ribbons. In huts having eave ribbons treated with 4 g a.i./m^2, mortality of the recaptured *An. funestus* was 10% after 24 hours, 20% after 48 hours and 34% after 72 hours while that of control remained below 5% (Figure 15). The odds that the recaptured mosquitoes would die after 24 hours were eleven times higher in huts with treated eave ribbons compared to control huts (OR=11.29, 95% CI: 5.35-23.81, $P < 0.001$)

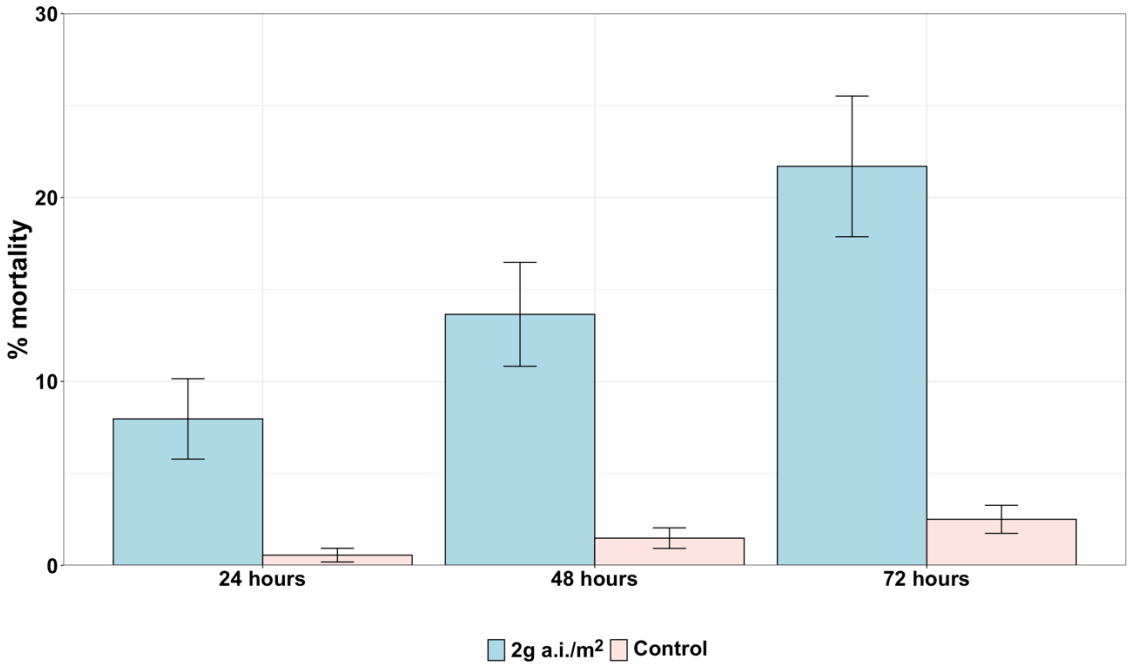

Figure 14: Percentage mortality of recaptured mosquitoes in experimental huts fitted with treated eave ribbons (2 g a.i./m^2) or control with no ribbons. These tests were conducted with only *An. arabiensis*

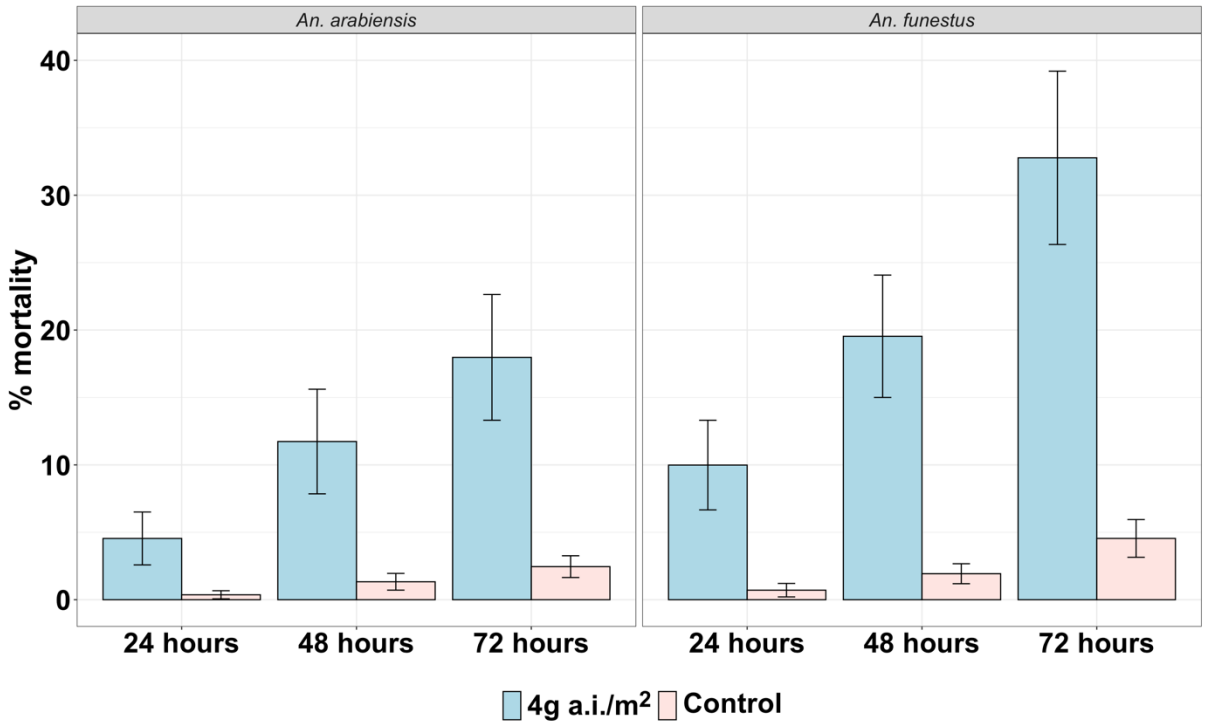

Figure 15: Percentage mortality of recaptured mosquitoes in experimental huts fitted with treated eave ribbons (4 g a.i./m^2) or control with no ribbons. These tests were conducted with *An. arabiensis* and *An. funestus*

Table 12: Summary of the 24 hours mortality of *An. arabiensis* and *An. funestus* mosquitoes recaptured from chambers with eave ribbons treated with 2 g & 4 g a.i./m^2 of pirimiphos-methyl.

Species	Intervention	Night	Nº Captured	Mean captured/night/chamber (95% CI)	Nº dead	Mean dead/night/chamber (95% CI)	Odds ratio (95% CI)	P value	% Mortality
An. arabiensis	Control	10	1,943	24.29 (20.18-28.39)	17	0.21 (0.09-0.34)	1	<0.001	0.86
	2 g a.i./m^2	10	1,788	22.35 (18.51-26.19)	147	1.84 (1.34-2.33)	10.65 (5.97-18.99)		8.23
	Control	10	1,730	21.62 (17.76-25.49)	11	0.14 (0.03-0.25)	1	<0.001	0.65
	4 g a.i./m^2	10	1,602	20.02 (16.53-23.52)	70	0.87 (0.61-1.14)	7.36 (3.92-13.83)		4.34
An. funestus	Control	10	1,067	13.33 (11.33-15.35)	11	0.14 (0.04-0.23)	1	<0.001	1.05
	4 g a.i./m^2	10	1,052	13.15 (11.10-15.19)	107	1.34 (0.98-1.69)	11.29 (5.35-23.81)		10.19

CHAPTER FOUR

4 DISCUSSION

ITNs and IRS are the cornerstones of malaria vector control, but their efficacy is threatened by the development and spread of resistance in key malaria vectors (Hemingway *et al.*, 2016). While most ITNs use pyrethroids, against which there are already very high levels of resistance, IRS uses different classes of insecticides, many of which are still effective against malaria vectors (WHO, 2022b). Unfortunately, the implementation of IRS is limited due to high costs and logistical challenges (Conteh *et al.*, 2021), as well as the stringent environmental regulations relevant to the management and disposal of pesticides. Other limitations of IRS include low acceptability from community members (Magaço *et al.*, 2019), modification of sprayed surfaces (Opiyo *et al.*, 2022) and changes in indoor mosquito resting surfaces (Msugupakulya *et al.*, 2020). This necessitates the development of new tools and delivery formats that are cost-effective, widely accepted, and scalable.

While some studies have attempted to reduce the costs of IRS by performing partial spraying (Gandahusada *et al.*, 1984; Dunbar *et al.*, 2019; Coleman *et al.*, 2021) most of the challenges mentioned above are still not addressed by this approach. Ifakara Health Institute in Tanzania has previously investigated eave ribbons treated with a spatial repellent, transfluthrin for protection against bites indoors and outdoors; and as a possible complementary tool to address some of the current limitations of vector control. The findings indicated that the transfluthrin treated eave ribbons could offer high protection against malaria and non-malaria vectors (Mmbando *et al.*, 2018; Mwanga *et al.*, 2019; Swai *et al.*, 2019; Tambwe *et al.*, 2021).

This study assessed the potential of using eave ribbons treated with pirimiphos-methyl, an organophosphate commonly used for IRS, against malaria and dengue vectors to address the challenges associated with IRS. To the best of our current understanding, this is the first scientific study to assess eave ribbons treated with insecticide other than transfluthrin. This tool is expected to reduce the cost by simplifying the logistics which can result in wider coverage and potentially address the challenges associated with IRS. The study involved laboratory cone bioassays on treated eave ribbons to assess their efficacy, effective exposure durations and dosages, followed by semi-field experiments in volunteer occupied experimental huts. The semi-field experiments assessed the human biting risk as well as mortality induced by treated eave ribbons.

Generally, in the cone bioassays, *An. funestus* and *An. arabiensis* had higher mortalities than *Ae. aegypti*. In the first objective, eave ribbons treated with 1 g a.i./m2 pirimiphos-methyl resulted in 100% mortality of all three species when exposed for 30 minutes. In the second activity, the exposure time was reduced. Reduced exposure durations resulted in a decline in mortality in all species, whereas the mortality increased with an increase in exposure duration. An increase in insecticide dose resulted in an increase in mortality and at higher doses, higher mortalities were observed even at short exposure durations. These findings are consistent with previous laboratory studies which assessed the mortality of insecticide treated eave tubes, which demonstrated an increase in mortality with an increase in exposure time (Oumbouke *et al.*, 2018). This is because increasing either dose or exposure time increases the possibility of mosquitoes picking a lethal dose of insecticide.

Surprisingly, in the first and second activity with eave ribbons treated with 1 g a.i./m^2 pirimiphos-methyl at 30 minutes exposure, *Ae. aegypti* had different percentages of mortalities. In the first activity, 100% mortality was observed but in the second activity, only 61% mortality was observed despite the dose and exposure time being the same. It is unclear why the mortality decreased; however, it is possible that differences in environmental conditions during the experiments contributed to this variation. Despite these variations, it generally required higher doses or long exposure durations to kill *Ae. aegypti* compared to the other species. The lethal dose required to kill 99% of the *Ae. aegypti* was determined to be almost two times higher than that of *An. arabiensis*. This could indicate that, *Ae. aegypti* have a higher tolerance to insecticides than other species tested. Although one study in Ifakara using wild *Ae. aegypti* confirmed its susceptibility against pirimiphos-methyl and pyrethroids (Kahamba *et al.*, 2020) future studies should confirm the susceptibility status of *Ae. aegypti* using WHO tube resistance bioassay (WHO, 2016b).

In the semi-field settings, treated eave ribbons resulted in only modest protective efficacy and mortality against *An. arabiensis* and *An. funestus.* There was only significant protection against *An. arabiensis* observed outdoors at the dose of 4 g a.i./m^2 while there was no significant protection against *An. funestus* observed indoors or outdoors. At the doses of 1 g a.i./m^2 and 2 g a.i./m^2, there was no significant indoor or outdoor bite prevention against *An. arabiensis* while *An. funestus* was not assessed at these doses due to the limited availability of the species. Previous studies targeting the eaves using insecticide treated or untreated screens (Lindsay, Emerson and

Charlwood, 2002; Kirby *et al.*, 2009; Msoffe *et al.*, 2022) and eave tubes (Snetselaar *et al.*, 2017; Sternberg *et al.*, 2021) demonstrated a significant reduction in indoor biting risk which are contrary to the findings observed in this study. This could be due to the design of those interventions which block the eaves thereby preventing mosquitoes from entering indoors unlike eave ribbons which do not completely block the eaves.

Moreover, moderate level of mortality against *An. funestus* and *An. arabiensis* was observed. *Anopheles funestus* had slightly higher mortality than *An. arabiensis* which is consistent with the cone bioassay findings where *An. funestus* had slightly higher mortality at low exposure duration compared to *An. arabiensis* (Figure 12). These findings are contrary to previous studies on window screens and eave baffles treated with pirimiphos-methyl which indicated high mortalities of *An. funestus* and *An. arabiensis* (Killeen *et al.*, 2017; Chinula *et al.*, 2018). This could be because the eave baffles are designed in such a way that, there is a high possibility that mosquitoes will contact the treated surface when entering indoors unlike eave ribbons where mosquitoes may or may not contact the treated material when entering indoors (Killeen *et al.*, 2017). Another study using insecticide treated eave tubes demonstrated mortalities of up to 64% of the recaptured mosquitoes after 24 hours post- exposure (Oumbouke *et al.*, 2018) which is contrary to the findings of this study. However, the study used pyrethroids which are fast acting insecticides unlike pirimiphos-methyl as used in this study.

Despite previous studies of IRS using pirimiphos-methyl in semi-field and experimental huts demonstrating high mortality and densities reduction against malaria and non-malaria vectors even at a lower dose of 1 g a.i./m^2 pirimiphos-methyl (Rowland *et al.*, 2013; Oxborough *et al.*, 2014; Sy *et al.*, 2018; Ngwej *et al.*, 2021), these observations are contrary to the observations of this study. The modest effects could be related to the limited surface area treated and hence limit the probability of the mosquitoes being exposed to the insecticide treated eave ribbon, compared to IRS. In typical IRS, the mosquitoes are exposed to a much wider surface area on the entire indoor surfaces. However, in this case, the surface area of contact was only 1.5m by 7.7m, equivalent to just 2.31 m^2 total surface area on both sides of the ribbon. In addition, IRS had the advantage that blood fed female mosquitoes rest on the treated walls for a much longer time, hence allowing females to be exposed to a lethal dose of the active ingredients. However, in this study, mosquitoes were expected to contact the treated eave ribbons as they enter indoors which may not allow them to be exposed to a lethal dose of

pirimiphos-methyl. Nevertheless, the modest mortality and bite reduction observed suggests that a vapor-phased intervention or one that has high knock-down effects may achieve greater mortality and bite prevention. Future evaluations of treated eave ribbons should therefore consider alternative insecticides or insecticide classes to demonstrate effectiveness. Indeed, work with transfluthrin (a vapor-phase) insecticide suggests that there can be high levels of both mortality and bite prevention (Mmbando *et al.*, 2018, 2019; Mwanga *et al.*, 2019; Swai *et al.*, 2019; Tambwe *et al.*, 2021). While vapor phase repellents have proven to offer high protection due to repellency effect, this was not a major impact observed with eave ribbons treated with pirimiphos-methyl. Videographic observations have indicated that mosquitoes do spend a short time (a few seconds) around the eaves and they contacted the treated eave tubes (Spitzen *et al.*, 2016; Sperling *et al.*, 2017; Oumbouke *et al.*, 2018). Future studies should therefore assess the behavior of mosquitoes around the eaves and determine the effective contact rates.

Whereas the main objective of the study was met, there were several limitations. Firstly, cone bioassays were done on treated eave ribbons only once and residual activity was not assessed which could have given a picture of the longevity of the insecticide on treated eave ribbons. Secondly, tests with *Ae. aegypti* in the semi-field setting were not conducted after observing the low efficacies in malaria vectors and also lower mortalities of this species in the bioassays. Although the LC99 for *Ae. aegypti* was determined to be 3.6g a.i./m^2 at 3 minutes of exposure and the highest dose assessed in the semi-field was 4g a.i./m^2, it was not feasible to include tests with *Ae. aegypti* in the semi-field experiment due to observed low efficacy with malaria vectors, which were more susceptible than *Ae. aegypti* in the cone bioassays, however, further assessment in the future is necessary. Thirdly, the limited availability of *An. funestus* resulted in some of the activities being done using only *An. arabiensis.* This species is notoriously difficult to rear under laboratory conditions and this is not unique to this study (Gillies and De Meillon, 1968; Ngowo *et al.*, 2021). Fourthly, the actual amount of pirimiphos-methyl absorbed by the eave ribbons was also not determined. Lastly, this study did not assess the behavior of mosquitoes around the eave spaces to confirm if the mosquitoes are contacting the treated eave ribbons. Future iterations of this study should address these gaps.

CHAPTER FIVE

5 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The study evaluated the use of insecticide-treated eave ribbons, which are pieces of hessian fabric treated with an insecticide and placed around the eaves of poorly constructed houses to kill or repel mosquitoes, as a potential tool for controlling mosquito-borne diseases such as malaria and dengue. Laboratory and semi-field experiments were conducted to assess the efficacy of the eave ribbons in killing or repelling the targeted mosquito species. The results showed that treated eave ribbons resulted in higher mosquito mortality compared to untreated ribbons, but the impact increased with increased exposure duration or dose. In the semi-field study, there was moderate protection against mosquito bites and mortality, but the level of protection varied depending on the insecticide dose and the mosquito species. In conclusion, while insecticide-treated eave ribbons may have the potential for controlling malaria and dengue vectors, more research is needed to validate their efficacy in field settings and to identify suitable insecticides or insecticide combinations that are safe and effective.

5.2 Recommendations

i. Further research is needed to determine the effectiveness and limitations of insecticide-treated eave ribbons, as well as suitable insecticides or insecticide combinations. This should include testing in both laboratory and experimental hut settings, with a focus on fast-acting insecticides. Pyrethroids for example are fast acting with high knock-down effect. Combinations of pyrethroids with other insecticide classes could be assessed in future studies.

ii. More studies should be conducted to understand mosquito behavior around the eaves, including whether they contact the eave ribbons as they enter houses.

iii. It is also recommended to conduct studies on the residual efficacy of the active ingredients on the eave ribbons, to determine how long they remain effective.

REFERENCES

Achee, N.L., Bangs, M.J., Farlow, R., Killeen, G.F., Lindsay, S., Logan, J.G., Moore, S.J., Rowland, M., Sweeney, K., Torr, S.J., Zwiebel, L.J. and Grieco, J.P. (2012) 'Spatial repellents: From discovery and development to evidence-based validation', *Malaria Journal*, 11(1), p. 164. Available at: https://doi.org/10.1186/1475-2875-11-164.

Ahmed, A., Khogali, R., Elnour, M.A.B., Nakao, R. and Salim, B. (2021) 'Emergence of the invasive malaria vector *Anopheles stephensi* in Khartoum State, Central Sudan', *Parasites and Vectors*, 14(1), p. 511. Available at: https://doi.org/10.1186/s13071-021-05026-4.

Ahorlu, C.S., Adongo, P., Koenker, H., Zigirumugabe, S., Sika-Bright, S., Koka, E., Tabong, P.T.N., Piccinini, D., Segbaya, S., Olapeju, B. and Monroe, A. (2019) 'Understanding the gap between access and use: A qualitative study on barriers and facilitators to insecticide-treated net use in Ghana', *Malaria Journal*, 18(1), p. 417. Available at: https://doi.org/10.1186/s12936-019-3051-0.

Akogbéto, M.C., Salako, A.S., Dagnon, F., Aïkpon, R., Kouletio, M., Sovi, A. and Sezonlin, M. (2018) 'Blood feeding behaviour comparison and contribution of *Anopheles coluzzii* and *Anopheles gambiae,* two sibling species living in sympatry, to malaria transmission in Alibori and Donga region, northern Benin, West Africa', *Malaria Journal*, 17(1), p. 307. Available at: https://doi.org/10.1186/s12936-018-2452-9.

Alegana, V.A., Macharia, P.M., Muchiri, S., Mumo, E., Oyugi, E., Kamau, A., Chacky, F., Thawer, S., Molteni, F., Rutazanna, D., Maiteki-Sebuguzi, C., Gonahasa, S., Noor, A.M. and Snow, R.W. (2021) '*Plasmodium falciparum* parasite prevalence in East Africa: Updating data for malaria stratification', *PLOS Global Public Health*, 1(12), p. e0000014. Available at: https://doi.org/10.1371/journal.pgph.0000014.

Amarasinghe, A., Kuritsky, J.N., William Letson, G. and Margolis, H.S. (2011) 'Dengue virus infection in Africa', *Emerging Infectious Diseases*, 17(8), pp. 1349–1354. Available at: https://doi.org/10.3201/eid1708.101515.

Assogba, B.S., Sillah, S., Opondo, K.O., Cham, S.T., Camara, M.M., Jadama, L., Camara, L., Ndiaye, A., Wathuo, M., Jawara, M., Diabaté, A., Achan, J. and

D'Alessandro, U. (2022) '*Anopheles gambiae s.l.* swarms trapping as a complementary tool against residual malaria transmission in eastern Gambia', *Scientific Reports*, 12(1), p. 17057. Available at: https://doi.org/10.1038/s41598-022-21577-7.

Badmos, A.O., Alaran, A.J., Adebisi, Y.A., Bouaddi, O., Onibon, Z., Dada, A., Lin, X. and Lucero-Prisno, D.E. (2021) 'What sub-Saharan African countries can learn from malaria elimination in China', *Tropical Medicine and Health*, 49(1), p. 86. Available at: https://doi.org/10.1186/s41182-021-00379-z.

Balabanidou, V., Grigoraki, L. and Vontas, J. (2018) 'Insect cuticle: A critical determinant of insecticide resistance', *Current Opinion in Insect Science*, 27, pp. 68–74. Available at: https://doi.org/10.1016/j.cois.2018.03.001.

Balabanidou, V., Kampouraki, A., Maclean, M., Blomquist, G.J., Tittiger, C., Juárez, M.P., Mijailovsky, S.J., Chalepakis, G., Anthousi, A., Lynd, A., Antoine, S., Hemingway, J., Ranson, H., Lycett, G.J. and Vontas, J. (2016) 'Cytochrome P450 associated with insecticide resistance catalyzes cuticular hydrocarbon production in *Anopheles gambiae*', *Proceedings of the National Academy of Sciences of the United States of America*, 113(33), pp. 9268–9273. Available at: https://doi.org/10.1073/pnas.1608295113.

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J.M., Brownstein, J.S., Hoen, A.G., Sankoh, O., Myers, M.F., George, D.B., Jaenisch, T., Wint, W.G.R., Simmons, C.P., Scott, T.W., Farrar, J.J. and Hay, S.I. (2013) 'The global distribution and burden of dengue', *Nature*, 496(7446), pp. 504–507. Available at: https://doi.org/10.1038/nature12060.

Bhatt, S., Weiss, D.J., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U., Battle, K.E., Moyes, C.L., Henry, A., Eckhoff, P.A., Wenger, E.A., Briët, O., Penny, M.A., Smith, T.A., Bennett, A., Yukich, J., Eisele, T.P., Griffin, J.T., Fergus, C.A., *et al.* (2015) 'The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015', *Nature*, 526(7572), pp. 207–211. Available at: https://doi.org/10.1038/nature15535.

Bøgh, C., Pedersen, E.M., Mukoko, D.A. and Ouma, J.H. (1998) 'Permethrin-impregnated bednet effects on resting and feeding behaviour of lymphatic filariasis

vector mosquitoes in Kenya', *Medical and Veterinary Entomology*, 12(1), pp. 52–59. Available at: https://doi.org/10.1046/j.1365-2915.1998.00091.x.

Carnevale, P. and Manguin, S. (2021) 'Review of issues on residual malaria transmission', *Journal of Infectious Diseases*, 223(Supplement_2), pp. S61–S80. Available at: https://doi.org/10.1093/infdis/jiab084.

Carter, T.E., Yared, S., Gebresilassie, A., Bonnell, V., Damodaran, L., Lopez, K., Ibrahim, M., Mohammed, S. and Janies, D. (2018) 'First detection of *Anopheles stephensi* Liston, 1901 (Diptera: culicidae) in Ethiopia using molecular and morphological approaches', *Acta Tropica*, 188, pp. 180–186. Available at: https://doi.org/10.1016/j.actatropica.2018.09.001.

CDC (2017) *Potential Range of Aedes Mosquitoes*. Available at: https://www.cdc.gov/mosquitoes/mosquito-control/professionals/range.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fzika%2Fvector%2Frange.html.

Chen-Hussey, V., Carneiro, I., Keomanila, H., Gray, R., Bannavong, S., Phanalasy, S. and Lindsay, S.W. (2013) 'Can topical insect repellents reduce malaria? A cluster-randomised controlled trial of the insect repellent N,N-diethyl-m-toluamide (DEET) in Lao PDR', *PLoS ONE*, 8(8), p. e70664. Available at: https://doi.org/10.1371/journal.pone.0070664.

Chinula, D., Sikaala, C.H., Chanda-Kapata, P., Hamainza, B., Zulu, R., Reimer, L., Chizema, E., Kiware, S., Okumu, F.O. and Killeen, G. (2018) 'Wash-resistance of pirimiphos-methyl insecticide treatments of window screens and eave baffles for killing indoor feeding malaria vector mosquitoes: An experimental hut trial, south-east of Zambia', *Malaria Journal*, 17(1), p. 164. Available at: https://doi.org/10.1186/s12936-018-2309-2.

Chipwaza, B., Mugasa, J.P., Selemani, M., Amuri, M., Mosha, F., Ngatunga, S.D. and Gwakisa, P.S. (2014) 'Dengue and Chikungunya fever among viral diseases in outpatient febrile children in Kilosa District Hospital, Tanzania', *PLoS Neglected Tropical Diseases*, 8(11), p. e3335. Available at: https://doi.org/10.1371/journal.pntd.0003335.

Chipwaza, B., Sumaye, R.D., Weisser, M., Gingo, W., Yeo, N.K.W., Amrun, S.N., Okumu, F.O. and Ng, L.F.P. (2021) 'Occurrence of 4 dengue virus serotypes and Chikungunya virus in Kilombero valley, Tanzania, during the dengue outbreak in 2018', *Open Forum Infectious Diseases*, 8(1), p. ofaa626. Available at: https://doi.org/10.1093/ofid/ofaa626.

Christie, J. (1872) 'Remarks on "Kidinga Pepo": A peculiar form of exanthematous disease', *The British Medical Journal*, 1(596), pp. 577–579. Available at: https://doi.org/10.1136/bmj.1.596.577.

Coetzee, M. (2000) 'Efficacy of three insect repellents against the malaria vector *Anopheles arabiensis*', *Medical and Veterinary Entomology*, 14(4), pp. 441–444. Available at: https://doi.org/10.1046/j.1365-2915.2000.00261.x.

Coetzee, M., Hunt, R.H., Wilkerson, R., Torre, A. Della, Coulibaly, M.B. and Besansky, N.J. (2013) '*Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex', *Zootaxa*, 3619(3), pp. 246–274. Available at: https://doi.org/10.11646/zootaxa.3619.3.2.

Coleman, S., Yihdego, Y., Sherrard-Smith, E., Thomas, C.S., Dengela, D., Oxborough, R.M., Dadzie, S.K., Boakye, D., Gyamfi, F., Obiri-Danso, K., Johns, B., Siems, L. V., Lucas, B., Tongren, J.E., Zigirumugabe, S., Dery, D., Fornadel, C., George, K., Belemvire, A., *et al.* (2021) 'Partial indoor residual spraying with pirimiphos-methyl as an effective and cost-saving measure for the control of *Anopheles gambiae s.l.* in northern Ghana', *Scientific Reports*, 11(1), p. 18055. Available at: https://doi.org/10.1038/s41598-021-97138-1.

Implications of Insecticide Resistance Consortium (2018) 'Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: Trends in pyrethroid resistance during a WHO-coordinated multi-country prospective study', *Parasites & Vectors*, 11(1), p. 550. Available at: https://doi.org/10.1186/s13071-018-3101-4.

Conteh, L., Shuford, K., Agboraw, E., Kont, M., Kolaczinski, J. and Patouillard, E. (2021) 'Costs and cost-effectiveness of malaria control interventions: A systematic literature review', *Value Health*, 24(8), pp. 1213–1222. Available at: https://doi.org/10.1016/j.jval.2021.01.013.

Cox, F.E. (2010) 'History of the discovery of the malaria parasites and their vectors', *Parasites and Vectors*, 3(1), p. 5. Available at: https://doi.org/10.1186/1756-3305-3-5.

Craig, M., Le Sueur, D. and Snow, B. (1999) 'A climate-based distribution model of malaria transmission in sub-Saharan Africa', *Parasitology Today*, 15(3), pp. 105–111. Available at: https://doi.org/10.1016/S0169-4758(99)01396-4.

Critchley, B.R. (1971) 'Insect repellents', *PANS Pest Articles & New Summaries*, 17(3), pp. 313–314. Available at: https://doi.org/10.1080/09670877109413276.

Curtis, C.F., Lines, J.D., Ijumba, J., Callaghan, A., Hill, N. and Karimzad, M.A. (1987) 'The relative efficacy of repellents against mosquito vectors of disease', *Medical and Veterinary Entomology*, 1(2), pp. 109–119. Available at: https://doi.org/10.1111/j.1365-2915.1987.tb00331.x.

Dadzie, S., Boakye, D., Asoala, V., Koram, K., Kiszewski, A. and Appawu, M. (2013) 'A community-wide study of malaria reduction: Evaluating efficacy and user-acceptance of a low-cost repellent in northern Ghana', *American Journal of Tropical Medicine and Hygiene*, 88(2), pp. 309–314. Available at: https://doi.org/10.4269/ajtmh.2012.12-0370.

Danasekaran, R., Mani, G., Annadurai, K. and Ramasamy, J. (2014) 'Small Bite, Big Threat: The burden of vector-borne diseases', *Iranian Journal of Public Health*, 43(7), pp. 1014–1015.

Davies, T.G.E., Field, L.M., Usherwood, P.N.R. and Williamson, M.S. (2007) 'DDT, pyrethrins, pyrethroids and insect sodium channels', *IUBMB Life*, 59(3), pp. 151–162. Available at: https://doi.org/10.1080/15216540701352042.

Diouf, E. hadji, Niang, E. hadji A., Samb, B., Diagne, C.T., Diouf, M., Konaté, A., Dia, I., Faye, O. and Konaté, L. (2020) 'Multiple insecticide resistance target sites in adult field strains of *Anopheles gambiae s.l.* from south-eastern Senegal', *Parasites and Vectors*, 13(1), p. 567. Available at: https://doi.org/10.1186/s13071-020-04437-z.

Dunbar, M.W., Correa-Morales, F., Dzul-Manzanilla, F., Medina-Barreiro, A., Bibiano-Marín, W., Morales-Ríos, E., Vadillo-Sánchez, J., López-Monroy, B., Ritchie, S.A., Lenhart, A., Manrique-Saide, P. and Vazquez-Prokopec, G.M. (2019) 'Efficacy of

novel indoor residual spraying methods targeting pyrethroid-resistant *Aedes aegypti* within experimental houses', *PLoS Neglected Tropical Diseases*, 13(2), p. e0007203. Available at: https://doi.org/10.1371/journal.pntd.0007203.

Durnez, L. and Coosemans, M. (2013) 'Residual transmission of malaria: An old issue for new approaches', in S. Manguin (ed.) *Anopheles mosquitoes: New insights into malaria vectors*. Rijeka: InTech, pp. 671–704. Available at: https://doi.org/10.5772/55925.

Fang, Y., Shi, W.Q., Wu, J.T., Li, Y.Y., Xue, J.B. and Zhang, Y. (2019) 'Resistance to pyrethroid and organophosphate insecticides, and the geographical distribution and polymorphisms of target-site mutations in voltage-gated sodium channel and acetylcholinesterase 1 genes in *Anopheles sinensis* populations in Shanghai,' *Parasites and Vectors*, 12(1), p. 396. Available at: https://doi.org/10.1186/s13071-019-3657-7.

Fantini, B. (1999) 'The concept of specificity and the Italian contribution to the discovery of the malaria transmission cycle', *Parassitologia*, 41(1/3), pp. 39–47.

Fathy Khater, H., M. Selim, A., A. Abouelella, G., A. Abouelella, N., Murugan, K., P. Vaz, N. and Govindarajan, M. (2019) 'Commercial mosquito repellents and their safety concerns', in F.H. Kasenga (ed.) *Malaria*. IntechOpen. Available at: https://doi.org/10.5772/intechopen.87436.

Faulde, M.K., Rueda, L.M. and Khaireh, B.A. (2014) 'First record of the Asian malaria vector *Anopheles stephensi* and its possible role in the resurgence of malaria in Djibouti, Horn of Africa', *Acta Tropica*, 139, pp. 39–43. Available at: https://doi.org/10.1016/j.actatropica.2014.06.016.

Fillinger, U., Kannady, K., William, G., Vanek, M.J., Dongus, S., Nyika, D., Geissbühler, Y., Chaki, P.P., Govella, N.J., Mathenge, E.M., Singer, B.H., Mshinda, H., Lindsay, S.W., Tanner, M., Mtasiwa, D., De Castro, M.C. and Killeen, G.F. (2008) 'A tool box for operational mosquito larval control: Preliminary results and early lessons from the Urban Malaria Control Programme in Dar es Salaam, Tanzania', *Malaria Journal*, 7(1), p. 20. Available at: https://doi.org/10.1186/1475-2875-7-20.

Fillinger, U. and Lindsay, S.W. (2006) 'Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya', *Tropical Medicine*

and International Health, 11(11), pp. 1629–1642. Available at: https://doi.org/10.1111/j.1365-3156.2006.01733.x.

Fillinger, U., Ndenga, B., Githeko, A. and Lindsay, S.W. (2009) 'Integrated malaria vector control with microbial larvicides and insecticide-treated nets in western Kenya: A controlled trial', *Bulletin of the World Health Organization*, 87, pp. 655–665. Available at: https://doi.org/10.2471/BLT.08.055632.

Fradin, M.S. (1998) 'Mosquitoes and mosquito repellants: A clinician's guide', *Annals of Internal Medicine*, 128(11), pp. 931–940. Available at: https://doi.org/10.7326/0003-4819-128-11-199806010-00013.

Gainor, E.M., Harris, E. and Labeaud, A.D. (2022) 'Uncovering the burden of dengue in Africa: Considerations on magnitude, misdiagnosis, and ancestry', *Viruses*, 14(2), p. 233. Available at: https://doi.org/10.3390/v14020233.

Gallup, J.L. and Sachs, J.D. (2001) 'The economic burden of malaria : Cross-country evidence', *American Journal of Tropical Medicine and Hygiene*, 64(Supplement 1-2), pp. 85–96. Available at: https://doi.org/10.4269/ajtmh.2001.64.85.

Gandahusada, S., Fleming, G.A., Sukamto, Damar, T., Suwarto, Sustriayu, N., Bang, Y.H., Arwati, S. and Arif, H. (1984) 'Malaria control with residual fenitrothion in Central Java, Indonesia: An operational-scale trial using both full and selective coverage treatments', *Bulletin of the World Health Organization*, 62(5), pp. 783–794.

Gautret, P., Simon, F., Askling, H.H., Bouchaud, O., Leparc-Goffart, I., Ninove, L., Parola, P. and EuroTravNet, C. for (2010) 'Dengue type 3 virus infections in European travellers returning from the Comoros and Zanzibar, February-April 2010', *Eurosurveillance*, 15(15), p. 19541.

Geissbühler, Y., Kannady, K., Chaki, P.P., Emidi, B., Govella, N.J., Mayagaya, V., Kiama, M., Mtasiwa, D., Mshinda, H., Lindsay, S.W., Tanner, M., Fillinger, U., de Castro, M.C. and Killeen, G.F. (2009) 'Microbial larvicide application by a large-scale, community-based program reduces malaria infection prevalence in urban Dar es Salaam, Tanzania', *PLoS ONE*, 4(3), p. e5107. Available at: https://doi.org/10.1371/journal.pone.0005107.

Gillies, M.T. and De Meillon, B. (1968) *The Anophelinae of Africa south of the Sahara (Ethiopian Zoogeographical Region)*. Johannesburg: South African Institute for Medical Research.

Githeko, A.K., Service, M.W., Mbogo, C.M., Atieli, F.K. and Juma, F.O. (1994) 'Origin of blood meals in indoor and outdoor resting malaria vectors in western Kenya', *Acta Tropica*, 58(3–4), pp. 307–316. Available at: https://doi.org/10.1016/0001-706x(94)90024-8.

Goodyer, L.I., Croft, A.M., Frances, S.P., Hill, N., Moore, S.J., Onyango, S.P. and Debboun, M. (2010) 'Expert review of the evidence base for arthropod bite avoidance', *Journal of Travel Medicine*, 17(3), pp. 182–192. Available at: https://doi.org/10.1111/j.1708-8305.2010.00402.x.

Govella, N.J., Chaki, P.P., Mpangile, J.M. and Killeen, G.F. (2011) 'Monitoring mosquitoes in urban Dar es Salaam: Evaluation of resting boxes, window exit traps, CDC light traps, Ifakara tent traps and human landing catches', *Parasites and Vectors*, 4(1), p. 40. Available at: https://doi.org/10.1186/1756-3305-4-40.

Govella, N.J. and Ferguson, H. (2012) 'Why use of interventions targeting outdoor biting mosquitoes will be necessary to achieve malaria elimination', *Frontiers in Physiology*, 3, p. 199. Available at: https://doi.org/10.3389/fphys.2012.00199.

Govella, N.J., Ogoma, S.B., Paliga, J., Chaki, P.P. and Killeen, G. (2015) 'Impregnating hessian strips with the volatile pyrethroid transfluthrin prevents outdoor exposure to vectors of malaria and lymphatic filariasis in urban Dar es Salaam, Tanzania', *Parasites and Vectors*, 8(1), p. 322. Available at: https://doi.org/10.1186/s13071-015-0937-8.

Gryseels, C., Uk, S., Sluydts, V., Durnez, L., Phoeuk, P., Suon, S., Set, S., Heng, S., Siv, S., Gerrets, R., Tho, S., Coosemans, M. and Peeters Grietens, K. (2015) 'Factors influencing the use of topical repellents: Implications for the effectiveness of malaria elimination strategies', *Scientific Reports*, 5(1), p. 16847. Available at: https://doi.org/10.1038/srep16847.

Hagenlocher, M. and Castro, M.C. (2015) 'Mapping malaria risk and vulnerability in the United Republic of Tanzania: A spatial explicit model', *Population Health Metrics*, 13(1), p. 2. Available at: https://doi.org/10.1186/s12963-015-0036-2.

Haji, K.A., Khatib, B.O., Smith, S., Ali, A.S., Devine, G.J., Coetzee, M. and Majambere, S. (2013) 'Challenges for malaria elimination in Zanzibar: Pyrethroid resistance in malaria vectors and poor performance of long-lasting insecticide nets', *Parasites and Vectors*, 6(1), p. 82. Available at: https://doi.org/10.1186/1756-3305-6-82.

Hancock, P.A., Hendriks, C.J.M., Tangena, J.A., Gibson, H., Hemingway, J., Coleman, M., Gething, P.W., Cameron, E., Bhatt, S. and Moyes, C.L. (2020) 'Mapping trends in insecticide resistance phenotypes in African malaria vectors', *PLoS Biology*, 18(6), p. e3000633. Available at: https://doi.org/10.1371/journal.pbio.3000633.

Hargreaves, K., Hunt, R.H., Brooke, B.D., Mthembu, J., Weeto, M.M., Awolola, T.S. and Coetzee, M. (2003) '*Anopheles arabiensis* and *Anopheles quadriannulatus* resistance to DDT in South Africa', *Medical and Veterinary Entomology*, 17(4), pp. 417–422. Available at: https://doi.org/10.1111/j.1365-2915.2003.00460.x.

Hargreaves, K., Koekemoer, L.L., Brooke, B.D., Hunt, R.H., Mthembu, J. and Coetzee, M. (2000) '*Anopheles funestus* resistant to pyrethroid insecticides in South Africa', *Medical and Veterinary Entomology*, 14(2), pp. 181–189. Available at: https://doi.org/10.1046/j.1365-2915.2000.00234.x.

Hawkes, C. and Ruel, M. (2006) 'The links between agriculture and health: An intersectoral opportunity to improve the health and livelihoods of the poor', *Bulletin of the World Health Organization*, 84(12), pp. 985–991.

Hayes, J.D. and Wolf, C.R. (1988) 'Glutathione transferase in drug resistance', in S. Helmut and B. Ketterer (eds) *Glutathione conjugation: Mechanisms and biological significance*. Cambridge: Academic Press Ltd, pp. 315–355.

Hemingway, J., Ranson, H., Magill, A., Kolaczinski, J., Fornadel, C., Gimnig, J., Coetzee, M., Simard, F., Roch, D.K., Hinzoumbe, C.K., Pickett, J., Schellenberg, D., Gething, P., Hoppé, M. and Hamon, N. (2016) 'Averting a malaria disaster: Will insecticide resistance derail malaria control?', *The Lancet*, 387(10029), pp. 1785–1788. Available at: https://doi.org/10.1016/S0140-6736(15)00417-1.

Ingham, V.A.V.A., Anthousi, A., Douris, V., Harding, N.J.N.J., Lycett, G., Morris, M., Vontas, J., Ranson, H. and Ranson, R. (2020) 'A sensory appendage protein protects malaria vectors from pyrethroids', *Nature*, 577(7790), pp. 376–380. Available at: https://doi.org/10.1038/s41586-019-1864-1.

IRAC (2011) *Prevention and management of insecticide resistance in vectors of public health importance*. IRAC.

IVCC (2013) *Actellic CS 300*. Available at: https://www.ivcc.com/market-access/actellic-300cs/.

Iwashita, H., Dida, G., Futami, K., Sonye, G., Kaneko, S., Horio, M., Kawada, H., Maekawa, Y., Aoki, Y. and Minakawa, N. (2010) 'Sleeping arrangement and house structure affect bed net use in villages along Lake Victoria', *Malaria Journal*, 9(1), p. 176. Available at: https://doi.org/10.1186/1475-2875-9-176.

John W, H. (2009) 'Improved Prokopack aspirator — Model 1419'.

Kahamba, N.F., Limwagu, A.J., Mapua, S.A., Msugupakulya, B.J., Msaky, D.S., Kaindoa, E.W., Ngowo, H.S. and Okumu, F.O. (2020) 'Habitat characteristics and insecticide susceptibility of *Aedes aegypti* in the Ifakara area, south-eastern Tanzania', *Parasites and Vectors*, 13(1), p. 102. Available at: https://doi.org/10.1186/s13071-020-3920-y.

Kaindoa, E.W., Matowo, N.S., Ngowo, H.S., Mkandawile, G., Mmbando, A., Finda, M. and Okumu, F.O. (2017a) 'Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in south-eastern Tanzania', *PLoS ONE*, 12(5), p. e0177807. Available at: https://doi.org/10.1371/journal.pone.0177807.

Kaindoa, E.W, Ngowo, H.S., Limwagu, A., Mkandawile, G., Kihonda, J., Masalu, J.P., Bwanary, H., Diabate, A. and Okumu, F.O. (2017b) 'New evidence of mating swarms of the malaria vector, *Anopheles arabiensis* in Tanzania', *Wellcome Open Research*, 2, pp. 1–20. Available at: https://doi.org/10.12688/wellcomeopenres.12458.1.

Kaindoa, E.W., Mmbando, A.S., Shirima, R., Hape, E.E. and Okumu, F.O. (2021) 'Insecticide-treated eave ribbons for malaria vector control in low-income communities', *Malaria Journal*, 20(1), p. 415. Available at: https://doi.org/10.1186/s12936-021-03945-2.

Kaindoa, E.W., Ngowo, H.S., Limwagu, A.J., Tchouakui, M., Hape, E., Abbasi, S., Kihonda, J., Mmbando, A.S., Njalambaha, R.M., Mkandawile, G., Bwanary, H.,

Coetzee, M. and Okumu, F.O. (2019) 'Swarms of the malaria vector *Anopheles funestus* in Tanzania', *Malaria Journal*, 18(1), p. 29. Available at: https://doi.org/10.1186/s12936-019-2660-y.

Kanyangarara, M., Hamapumbu, H., Mamini, E., Lupiya, J., Stevenson, J.C., Mharakurwa, S., Chaponda, M., Thuma, P.E., Gwanzura, L., Munyati, S., Mulenga, M., Norris, D.E. and Moss, W.J. (2018) 'Malaria knowledge and bed net use in three transmission settings in southern Africa', *Malaria Journal*, 17(1), p. 41. Available at: https://doi.org/10.1186/s12936-018-2178-8.

Kasai, S., Komagata, O., Itokawa, K., Shono, T., Ng, L.C., Kobayashi, M. and Tomita, T. (2014) 'Mechanisms of pyrethroid resistance in the dengue mosquito vector, *Aedes aegypti*: Target site insensitivity, penetration, and metabolism', *PLoS Neglected Tropical Diseases*, 8(6), p. e2948. Available at: https://doi.org/10.1371/journal.pntd.0002948.

Kibret, S. and Wilson, G.G. (2016) 'Increased outdoor biting tendency of *Anopheles arabiensis* and its challenge for malaria control in Central Ethiopia', *Public Health*, 141, pp. 143–145. Available at: https://doi.org/10.1016/j.puhe.2016.09.012.

Kienberger, S. and Hagenlocher, M. (2014) 'Spatial-explicit modeling of social vulnerability to malaria in East Africa', *International Journal of Health Geographics*, 13, p. 29. Available at: https://doi.org/10.1186/1476-072X-13-29.

Kigadye, E.S.P., Nkwengulila, G., Magesa, S. and Abdulla, S. (2012) 'Investigations on risk factors for malaria in Rufiji district, Tanzania', *Huria: Journal of the Open University of Tanzania*, 11, pp. 1–12.

Kigozi, R., Baxi, S.M., Gasasira, A., Sserwanga, A., Kakeeto, S., Nasr, S., Rubahika, D., Dissanayake, G., Kamya, M.R., Filler, S. and Dorsey, G. (2012) 'Indoor residual spraying of insecticide and malaria morbidity in a high transmission intensity area of Uganda', *PLoS ONE*, 7(8), p. e42857. Available at: https://doi.org/10.1371/journal.pone.0042857.

Killeen, G.F., Govella, N.J., Lwetoijera, D.W. and Okumu, F.O. (2016) 'Most outdoor malaria transmission by behaviourally-resistant *Anopheles arabiensis* is mediated by mosquitoes that have previously been inside houses', *Malaria Journal*, 15(1), p. 225. Available at: https://doi.org/10.1186/s12936-016-1280-z.

Killeen, G.F., Masalu, J.P., Chinula, D., Fotakis, E.A., Kavishe, D.R., Malone, D. and Okumu, F.O. (2017) 'Control of malaria vector mosquitoes by insecticide-treated combinations of window screens and eave baffles', *Emerging Infectious Diseases*, 23(5), pp. 782–789. Available at: https://doi.org/10.3201/eid2305.160662.

Kirby, M.J., Ameh, D., Bottomley, C., Green, C., Jawara, M., Milligan, P.J., Snell, P.C., Conway, D.J. and Lindsay, S.W. (2009) 'Effect of two different house screening interventions on exposure to malaria vectors and on anaemia in children in The Gambia: A randomised controlled trial', *The Lancet*, 374(9694), pp. 998–1009. Available at: https://doi.org/10.1016/S0140-6736(09)60871-0.

Koenker, H., Worges, M., Yukich, J., Gitanya, P., Chacky, F., Lazaro, S., Mwalimu, C.D., Aaron, S., Ibrahim, R., Abbas, F., Khamis, M., Mwingizi, D., Dadi, D., Selby, A., Serbantez, N., Msangi, L., Loll, D. and Kamala, B. (2023) 'Estimating population ITN access at council level in Tanzania', *Malaria journal*, 22(1), p. 4. Available at: https://doi.org/10.1186/s12936-022-04432-y.

Kruskal, W.H. and Wallis, W.A. (1952) 'Use of Ranks in One-Criterion Variance Analysis', *Journal of the American Statistical Association*, 47(260), pp. 583–621. Available at: https://doi.org/10.1080/01621459.1952.10483441.

Kyalo, D., Amratia, P., Mundia, C.W., Mbogo, C.M., Coetzee, M. and Snow, R.W. (2017) 'A geo-coded inventory of anophelines in the Afrotropical Region south of the Sahara: 1898-2016.', *Wellcome Open Research*, 2, p. 57. Available at: https://doi.org/10.12688/wellcomeopenres.12187.1.

Lapied, B., Pennetier, C., Apaire-Marchais, V., Licznar, P. and Corbel, V. (2009) 'Innovative applications for insect viruses: Towards insecticide sensitization', *Trends in Biotechnology*, 27(4), pp. 190–198. Available at: https://doi.org/10.1016/j.tibtech.2008.12.005.

Lefèvre, T., Gouagna, L.C., Dabiré, K.R., Elguero, E., Fontenille, D., Renaud, F., Costantini, C. and Thomas, F. (2009) 'Beyond nature and nurture: Phenotypic plasticity in blood-feeding behavior of *Anopheles gambiae s.s.* when humans are not readily accessible', *American Journal of Tropical Medicine and Hygiene*, 81(6), pp. 1023–1029. Available at: https://doi.org/10.4269/ajtmh.2009.09-0124.

LeGoff, G., Robert, V. and Pierre, Carnevale. (1994) 'Evaluation of a DEET-based repellent on 3 vectors of malaria in central Africa', *Santé*, 4(4), pp. 269–273.

Leroy, E.M., Nkoghe, D., Ollomo, B., Nze-nkogue, C., Becquart, P., Grard, G., Pourrut, X., Charrel, R. and Moureau, G. (2009) 'Concurrent chikungunya and dengue virus infections during simultaneous outbreaks, Gabon, 2007', *Emerging Infectious Diseases*, 15(4), pp. 591–593. Available at: https://doi.org/10.3201/eid1504.080664.

Lindsay, S.W., Emerson, P.M. and Charlwood, J.D. (2002) 'Reducing malaria by mosquito-proofing houses', *Trends in Parasitology*, 18(11), pp. 510–514. Available at: https://doi.org/10.1016/s1471-4922(02)02382-6.

Lindsay, S.W. and Snow, R.W. (1988) 'The trouble with eaves; house entry by vectors of malaria', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 82(4), pp. 645–646. Available at: https://doi.org/10.1016/0035-9203(88)90546-9.

Livadas, G.A. and Georgopoulos, G. (1953) 'Development of resistance to DDT by *Anopheles sacharovi* in Greece.', *Bulletin of the World Health Organization*, 8(4), pp. 497–511.

Lorenz, L.M., Keane, A., Moore, J.D., Munk, C.J., Seeholzer, L., Mseka, A., Simfukwe, E., Ligamba, J., Turner, E.L., Biswaro, L., Okumu, F.O., Killeen, G.F., Mukabana, W.R. and Moore, S.J. (2013) 'Taxis assays measure directional movement of mosquitoes to olfactory cues', *Parasites & Vectors*, 6(1), p. 131. Available at: https://doi.org/10.1186/1756-3305-6-131.

Lowassa, A., Mazigo, H.D., Mahande, A.M., Mwangonde, B.J., Msangi, S., Mahande, M.J., Kimaro, E.E., Elisante, E. and Kweka, E.J. (2012) 'Social economic factors and malaria transmission in lower Moshi, northern Tanzania', *Parasites and Vectors*, 5(1), p. 129. Available at: https://doi.org/10.1186/1756-3305-5-129.

Magaço, A., Botão, C., Nhassengo, P., Saide, M., Ubisse, A., Chicumbe, S. and Zulliger, R. (2019) 'Community knowledge and acceptance of indoor residual spraying for malaria prevention in Mozambique: A qualitative study', *Malaria Journal*, 18(1), p. 27. Available at: https://doi.org/10.1186/s12936-019-2653-x.

Maia, M.F., Onyango, S.P., Thele, M., Simfukwe, E.T., Turner, E.L. and Moore, S.J. (2013) 'Do topical repellents divert mosquitoes within a community? Health equity

implications of topical repellents as a mosquito bite prevention tool', *PLoS ONE*, 8(12), p. e84875. Available at: https://doi.org/10.1371/journal.pone.0084875.

Maia, M.F., Tenywa, F.C., Nelson, H., Kambagha, A., Ashura, A., Bakari, I., Mruah, D., Simba, A. and Bedford, A. (2018) 'Attractive toxic sugar baits for controlling mosquitoes: A qualitative study in Bagamoyo, Tanzania', *Malaria Journal*, 17(1), p. 22. Available at: https://doi.org/10.1186/s12936-018-2171-2.

Majambere, S., Pinder, M., Fillinger, U., Ameh, D., Conway, D.J., Green, C., Jeffries, D., Jawara, M., Milligan, P.J., Hutchinson, R. and Lindsay, S.W. (2010) 'Is mosquito larval source management appropriate for reducing malaria in areas of extensive flooding in the Gambia? A cross-over intervention trial', *American Journal of Tropical Medicine and Hygiene*, 82(2), pp. 176–184. Available at: https://doi.org/10.4269/ajtmh.2010.09-0373.

Mapua, S.A., Hape, E.E., Kihonda, J., Bwanary, H., Kifungo, K., Kilalangongono, M., Kaindoa, E.W., Ngowo, H.S. and Okumu, F.O. (2022) 'Persistently high proportions of *Plasmodium* infected *Anopheles funestus* mosquitoes in two villages in the Kilombero valley, south-eastern Tanzania', *Parasite Epidemiology and Control*, 18, p. e00264. Available at: https://doi.org/10.1016/j.parepi.2022.e00264.

Masalu, J.P., Finda, M., Killeen, G.F., Ngowo, H.S., Pinda, P.G. and Okumu, F.O. (2020a) 'Creating mosquito-free outdoor spaces using transfluthrin-treated chairs and ribbons', *Malaria Journal*, 19(1), p. 109. Available at: https://doi.org/10.1186/s12936-020-03180-1.

Masalu, J.P., Finda, M., Killeen, G.F., Ngowo, H.S., Pinda, P.G. and Okumu, F.O. (2020b) 'Creating mosquito-free outdoor spaces using transfluthrin-treated chairs and ribbons', *Malaria Journal*, 19(1), p. 109. Available at: https://doi.org/10.1186/s12936-020-03180-1.

Masalu, J.P., Okumu, F.O., Mmbando, A.S., Sikulu-Lord, M.T. and Ogoma, S.B. (2018) 'Potential benefits of combining transfluthrin-treated sisal products and long-lasting insecticidal nets for controlling indoor-biting malaria vectors', *Parasites and Vectors*, 11(1), p. 231. Available at: https://doi.org/10.1186/s13071-018-2811-y.

Matowo, N., Tanner, M., Munhenga, G., Mapua, S.A., Finda, M., Utzinger, J., Ngowi, V. and Okumu, F.O. (2020) 'Patterns of pesticide usage in agriculture in rural

Tanzania call for integrating agricultural and public health practices in managing insecticide-resistance in malaria vectors', *Malaria Journal*, 19(1), p. 257. Available at: https://doi.org/10.1186/s12936-020-03331-4.

Matowo, N.S., Tanner, M., Temba, B.A., Finda, M., Mlacha, Y.P., Utzinger, J. and Okumu, F.O. (2022) 'Participatory approaches for raising awareness among subsistence farmers in Tanzania about the spread of insecticide resistance in malaria vectors and the possible link to improper agricultural pesticide use', *Malaria Journal*, 21(1), p. 277. Available at: https://doi.org/10.1186/s12936-022-04289-1.

Mattingly, P.F. (1957) 'Genetical aspects of the *Aedes aegypti* problem', *Annals of Tropical Medicine and Parasitology*, 51(4), pp. 392–408. Available at: https://doi.org/10.1080/00034983.1957.11685829.

Mbogo, C.N.M., Baya, N.M., Ofulla, A.V.O., Githure, J.I. and Snow, R.W. (1996) 'The impact of permethrin-impregnated bednets on malaria vectors of the Kenyan coast', *Medical and Veterinary Entomology*, 10(3), pp. 251–259. Available at: https://doi.org/10.1111/j.1365-2915.1996.tb00739.x.

Mburu, M.M., Juurlink, M., Spitzen, J., Moraga, P., Hiscox, A., Mzilahowa, T., Takken, W. and McCann, R.S. (2018) 'Impact of partially and fully closed eaves on house entry rates by mosquitoes', *Parasites and Vectors*, 11(1), p. 383. Available at: https://doi.org/10.1186/s13071-018-2977-3.

Mmbando, A.S., Batista, E.P.A., Kilalangongono, M., Finda, M.F., Mwanga, E.P., Kaindoa, E.W., Kifungo, K., Njalambaha, R.M., Ngowo, H.S., Eiras, A.E. and Okumu, F.O. (2019) 'Evaluation of a push-pull system consisting of transfluthrin-treated eave ribbons and odour-baited traps for control of indoor- and outdoor-biting malaria vectors', *Malaria Journal*, 18(1), p. 87. Available at: https://doi.org/10.1186/s12936-019-2714-1.

Mmbando, A.S., Ngowo, H., Limwagu, A., Kilalangongono, M., Kifungo, K. and Okumu, F.O. (2018) 'Eave ribbons treated with the spatial repellent , transfluthrin , can effectively protect against indoor biting and outdoor biting malaria mosquitoes', *Malaria Journal*, 17(1), p. 368. Available at: https://doi.org/10.1186/s12936-018-2520-1.

Mmbando, A.S., Okumu, F.O., Mgando, J.P., Sumaye, R.D., Matowo, N.S., Madumla, E., Kaindoa, E., Kiware, S.S. and Lwetoijera, D.W. (2015) 'Effects of a new outdoor mosquito control device, the mosquito landing box, on densities and survival of the malaria vector, *Anopheles arabiensis*, inside controlled semi-field settings', *Malaria Journal*, 14(1), p. 494. Available at: https://doi.org/10.1186/s12936-015-1013-8.

MOH-Tanzania, Ministry of Health Zanzibar, National Bureau of Statistics Dar es Salaam, Office of Chief Government Statistician Zanzibar and ICF Rockville Maryland USA (2017) *Tanzania malaria indicator survey 2017*.

Moi, M.L., Takasaki, T., Kotaki, A., Tajima, S., Lim, C.-K., Sakamoto, M., Iwagoe, H., Kobayashi, K. and Ichiro, K. (2010) 'Importation of dengue virus type 3 to Japan from Tanzania and Cote d'Ivoire', *Emerging Infectious Diseases*, 16(11), pp. 1770–1772. Available at: https://doi.org/10.3201/eid1611101061.

Moiroux, N., Gomez, M.B., Pennetier, C., Elanga, E., Djènontin, A., Chandre, F., Djègbé, I., Guis, H. and Corbel, V. (2012) 'Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in benin', *Journal of Infectious Diseases*, 206(10), pp. 1622–1629. Available at: https://doi.org/10.1093/infdis/jis565.

Monroe, A., Moore, S., Koenker, H., Lynch, M. and Ricotta, E. (2019) 'Measuring and characterizing night time human behaviour as it relates to residual malaria transmission in sub - Saharan Africa : A review of the published literature', *Malaria Journal*, 18(1), p. 6. Available at: https://doi.org/10.1186/s12936-019-2638-9.

Moore, S.J., Davies, C.R., Hill, N. and Cameron, M.M. (2007) 'Are mosquitoes diverted from repellent-using individuals to non-users? Results of a field study in Bolivia', *Tropical Medicine and International Health*, 12(4), pp. 532–539. Available at: https://doi.org/10.1111/j.1365-3156.2006.01811.x.

Moyes, C.L., Athinya, D.K., Seethaler, T., Battle, K.E., Sinka, M., Hadi, M.P., Hemingway, J., Coleman, M. and Hancock, P.A. (2020) 'Evaluating insecticide resistance across African districts to aid malaria control decisions', *Proceedings of the National Academy of Sciences of the United States of America*, 117(36), pp. 22042–22050. Available at: https://doi.org/10.1073/pnas.2006781117.

Msoffe, R., Hewitt, M., Masalu, J.P., Finda, M., Kavishe, D.R., Okumu, F.O., Mpolya, E.A., Kaindoa, E.W. and Killeen, G.F. (2022) 'Participatory development of practical, affordable, insecticide-treated mosquito proofing for a range of housing designs in rural southern Tanzania', *Malaria Journal*, 21(1), p. 318. Available at: https://doi.org/10.1186/s12936-022-04333-0.

Msugupakulya, B.J., Kaindoa, E.W., Ngowo, H.S., Kihonda, J.M., Kahamba, N.F., Msaky, D.S., Matoke-Muhia, D., Tungu, P.K. and Okumu, F.O. (2020) 'Preferred resting surfaces of dominant malaria vectors inside different house types in rural south-eastern Tanzania', *Malaria Journal*, 19(1), p. 22. Available at: https://doi.org/10.1186/s12936-020-3108-0.

Musiime, A.K., Smith, D.L., Kilama, M., Rek, J., Arinaitwe, E., Nankabirwa, J.I., Kamya, M.R., Conrad, M.D., Dorsey, G., Akol, A.M., Staedke, S.G., Lindsay, S.W. and Egonyu, J.P. (2019) 'Impact of vector control interventions on malaria transmission intensity, outdoor vector biting rates and *Anopheles* mosquito species composition in Tororo, Uganda', *Malaria Journal*, 18(1), p. 445. Available at: https://doi.org/10.1186/s12936-019-3076-4.

Mwanga, E.P., Mmbando, A.S., Mrosso, P.C., Stica, C., Mapua, S.A., Finda, M.F., Kifungo, K., Kafwenji, A., Monroe, A.C., Ogoma, S.B., Ngowo, H.S. and Okumu, F.O. (2019) 'Eave ribbons treated with transfluthrin can protect both users and non-users against malaria vectors', *Malaria Journal*, 18(1), p. 314. Available at: https://doi.org/10.1186/s12936-019-2958-9.

Nájera, J.A., González-Silva, M. and Alonso, P.L. (2011) 'Some lessons for the future from the global malaria eradication programme (1955-1969)', *PLoS Medicine*, 8(1), p. e1000412. Available at: https://doi.org/10.1371/journal.pmed.1000412.

Ndenga, B.A., Mulaya, N.L., Musaki, S.K., Shiroko, J.N., Dongus, S. and Fillinger, U. (2016) 'Malaria vectors and their blood-meal sources in an area of high bed net ownership in the western Kenya highlands', *Malaria Journal*, 15(1), pp. 6–8. Available at: https://doi.org/10.1186/s12936-016-1115-y.

Ngowo, H.S., Hape, E.E., Matthiopoulos, J., Ferguson, H.M. and Okumu, F.O. (2021) 'Fitness characteristics of the malaria vector *Anopheles funestus* during an attempted laboratory colonization', *Malaria Journal*, 20(1), pp. 1–13. Available at: https://doi.org/10.1186/s12936-021-03677-3.

Ngwej, L.M., Mashat, E.M., Mukeng, C.K., Mundongo, H.T., Malonga, F.K., Kashala, J.C.K. and Bangs, M.J. (2021) 'Variable residual activity of K-Othrine® PolyZone and Actellic® 300 CS in semi-field and natural conditions in the Democratic Republic of the Congo', *Malaria Journal*, 20(1), p. 358. Available at: https://doi.org/10.1186/s12936-021-03892-y.

Nkya, T.E., Poupardin, R., Laporte, F., Akhouayri, I., Mosha, F., Magesa, S., Kisinza, W. and David, J.P. (2014) 'Impact of agriculture on the selection of insecticide resistance in the malaria vector *Anopheles gambiae*: A multigenerational study in controlled conditions', *Parasites and Vectors*, 7(1), p. 480. Available at: https://doi.org/10.1186/s13071-014-0480-z.

NMCP-Tanzania (2021) *National Malaria Strategic Plan 2021-2025*.

Ochomo, E.O., Milanoi, S., Abong'o, B., Onyango, B., Muchoki, M., Omoke, D., Olanga, E., Njoroge, L., Juma, E., Otieno, J.D., Matoke, D., Kamau, L., Rafferty, C., Gimnig, J.E., Shieshia, M., Wacira, D., Mwangangi, J., Maia, M., Chege, C., *et al.* (2023) 'Molecular surveillance leads to the first detection of Anopheles stephensi in Kenya', *Scientific Reports* [Preprint]. Available at: https://doi.org/doi.org/10.21203/rs.3.rs-2498485/v1.

Ogoma, S.B., Mmando, A.S., Swai, J.K., Horstmann, S., Malone, D. and Killeen, G.F. (2017) 'A low technology emanator treated with the volatile pyrethroid transfluthrin confers long term protection against outdoor biting vectors of lymphatic filariasis, arboviruses and malaria', *PLoS Neglected Tropical Diseases*, 11(4), p. e0005455. Available at: https://doi.org/10.1371/journal.pntd.0005455.

Ogoma, S.B., Moore, S.J. and Maia, M.F. (2012) 'A systematic review of mosquito coils and passive emanators: Defining recommendations for spatial repellency testing methodologies', *Parasites and Vectors*, 5(1), p. 287. Available at: https://doi.org/10.1186/1756-3305-5-287.

Ogoma, S.B., Ngonyani, H., Simfukwe, E.T., Mseka, A., Moore, J. and Killeen, G.F. (2012) 'Spatial repellency of transfluthrin-treated hessian strips against laboratory reared *Anopheles arabiensis* mosquitoes in a semi-field tunnel cage', *Parasites and Vectors*, 5(1), p. 54. Available at: https://doi.org/10.1186/1756-3305-5-54.

Okumu, F., Gyapong, M., Casamitjana, N., Castro, M.C., Itoe, M.A., Okonofua, F. and Tanner, M. (2022) 'What Africa can do to accelerate and sustain progress against malaria', *PLOS Global Public Health*, 2(6), p. e0000262. Available at: https://doi.org/10.1371/journal.pgph.0000262.

Okumu, F.O., Moore, J., Mbeyela, E., Sherlock, M., Sangusangu, R., Ligamba, G., Russell, T. and Moore, S.J. (2012) 'A modified experimental hut design for studying responses of disease-transmitting mosquitoes to indoor interventions: The Ifakara experimental huts', *PLoS ONE*, 7(2), p. e30967. Available at: https://doi.org/10.1371/journal.pone.0030967.

Oleribe, O.O., Momoh, J., Uzochukwu, B.S.C., Mbofana, F., Adebiyi, A., Barbera, T., Williams, R. and Taylor-Robinson, S.D. (2019) 'Identifying key challenges facing healthcare systems in Africa and potential solutions', *International Journal of General Medicine*, 12, pp. 395–403. Available at: https://doi.org/10.2147/IJGM.S223882.

Opiyo, M., Sherrard-Smith, E., Malheia, A., Nhacolo, Arsenio, Sacoor, C., Nhacolo, Ariel, Máquina, M., Jamu, L., Cuamba, N., Bassat, Q., Saúte, F. and Paaijmans, K. (2022) 'Household modifications after the indoor residual spraying (IRS) campaign in Mozambique reduce the actual spray coverage and efficacy', *PLOS Global Public Health*, 2(4), p. e0000227. Available at: https://doi.org/10.1371/journal.pgph.0000227.

Oumbouke, W.A., Tia, I.Z., Barreaux, A.M.G., Koffi, A.A., Sternberg, E.D., Thomas, M.B. and N'Guessan, R. (2018) 'Screening and field performance of powder-formulated insecticides on eave tube inserts against pyrethroid resistant *Anopheles gambiae s.l.*: An investigation into "actives" prior to a randomized controlled trial in Côte d'Ivoire', *Malaria Journal*, 17(1), p. 374. Available at: https://doi.org/10.1186/s12936-018-2517-9.

Oxborough, R.M., Kitau, J., Jones, R., Feston, E., Matowo, J., Mosha, F.W. and Rowland, M.W. (2014) 'Long-lasting control of *Anopheles arabiensis* by a single spray application of micro-encapsulated pirimiphos-methyl (Actellic® 300 CS)', *Malaria Journal*, 13(1), p. 37. Available at: https://doi.org/10.1186/1475-2875-13-37.

Padonou, G.G., Gbedjissi, G., Yadouleton, A., Azondekon, R., Razack, O., Oussou, O., Gnanguenon, V., Rock, A., Sezonlin, M. and Akogbeto, M. (2012) 'Decreased

proportions of indoor feeding and endophily in *Anopheles gambiae s.l.* populations following the indoor residual spraying and insecticide-treated net interventions in Benin West Africa', *Parasites and Vectors*, 5(1), p. 262. Available at: https://doi.org/10.1186/1756-3305-5-262.

Pates, H. and Curtis, C. (2005) 'Mosquito behaviour and vector control', *Annual Review of Entomology*, 50, pp. 53–70. Available at: https://doi.org/10.1146/annurev.ento.50.071803.130439.

Paupy, C., Ollomo, B., Kamgang, B., Moutailler, S., Rousset, D., Demanou, M., Hervé, J.P., Leroy, E. and Simard, F. (2010) 'Comparative role of *Aedes albopictus* and *Aedes aegypti* in the emergence of dengue and chikungunya in central Africa', *Vector-Borne and Zoonotic Diseases*, 10(3), pp. 259–266. Available at: https://doi.org/10.1089/vbz.2009.0005.

Pinda, P.G., Eichenberger, C., Ngowo, H.S., Msaky, D.S., Abbasi, S., Kihonda, J., Bwanaly, H. and Okumu, F.O. (2020) 'Comparative assessment of insecticide resistance phenotypes in two major malaria vectors, *Anopheles funestus* and *Anopheles arabiensis* in south-eastern Tanzania', *Malaria Journal*, 19(1), p. 408. Available at: https://doi.org/10.1186/s12936-020-03483-3.

Qualls, W.A., Müller, G.C., Revay, E.E., Allan, S.A., Arheart, K.L., Beier, J.C., Smithe, M.L., Scott, J.M., Kravchenko, V.D., Hausmann, A., Yefremova, Z.A. and Xue, R.-D. (2014) 'Evaluation of attractive toxic sugar bait (ATSB)—barrier for control of vector and nuisance mosquitoes and its effect on non- target organisms in sub-tropical environments in Florida', *Acta Tropica*, 131, pp. 104–110. Available at: https://doi.org/10.1016/j.actatropica.2013.12.004.

Qualls, W.A., Müller, G.C., Traore, S.F., Traore, M.M., Arheart, K.L., Doumbia, S., Schlein, Y., Kravchenko, V.D., Xue, R. De and Beier, J.C. (2015) 'Indoor use of attractive toxic sugar bait (ATSB) to effectively control malaria vectors in Mali, West Africa', *Malaria Journal*, 14(1), p. 301. Available at: https://doi.org/10.1186/s12936-015-0819-8.

R Core Team (2017) *R: A language and environment for statistical computing*. Vienna, Australia.

Ranson, H., Abdallah, H., Badolo, A., Guelbeogo, W.M., Kerah-Hinzoumbé, C., Yangalbé-Kalnoné, E., Sagnon, N., Simard, F. and Coetzee, M. (2009) 'Insecticide resistance in *Anopheles gambiae*: Data from the first year of a multi-country study highlight the extent of the problem', *Malaria Journal*, 8(1), p. 299. Available at: https://doi.org/10.1186/1475-2875-8-299.

Ranson, H. and Lissenden, N. (2016) 'Insecticide resistance in African *Anopheles* mosquitoes: A worsening situation that needs urgent action to maintain malaria control', *Trends in Parasitology*, 32(3), pp. 187–196. Available at: https://doi.org/10.1016/j.pt.2015.11.010.

Reddy, M.R., Overgaard, H.J., Abaga, S., Reddy, V.P., Caccone, A., Kiszewski, A.E. and Slotman, M.A. (2011) 'Outdoor host seeking behaviour of *Anopheles gambiae* mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea', *Malaria Journal*, 10(1), p. 184. Available at: https://doi.org/10.1186/1475-2875-10-184.

Reid, M.C. and McKenzie, F.E. (2016) 'The contribution of agricultural insecticide use to increasing insecticide resistance in African malaria vectors', *Malaria Journal*, 15(1), p. 107. Available at: https://doi.org/10.1186/s12936-016-1162-4.

Rek, J., Musiime, A., Zedi, M., Otto, G., Kyagamba, P., Rwatooro, J.A., Arinaitwe, E., Nankabirwa, J., Staedke, S.G., Drakeley, C., Rosenthal, P.J., Kamya, M., Dorsey, G. and Krezanoski, P.J. (2020) 'Non-adherence to long-lasting insecticide treated bednet use following successful malaria control in Tororo, Uganda', *PLoS ONE*, 15(12), p. e0243303. Available at: https://doi.org/10.1371/journal.pone.0243303.

Renggli, S., Mandike, R., Kramer, K., Patrick, F., Brown, N.J., McElroy, P.D., Rimisho, W., Msengwa, A., Mnzava, A., Nathan, R., Mtung'e, R., Mgullo, R., Lweikiza, J. and Lengeler, C. (2013) 'Design, implementation and evaluation of a national campaign to deliver 18 million free long-lasting insecticidal nets to uncovered sleeping spaces in Tanzania.', *Malaria journal*, 12(1), p. 85. Available at: https://doi.org/10.1186/1475-2875-12-85.

Riveron, J.M., Huijben, S., Tchapga, W., Tchouakui, M., Wondji, M.J., Tchoupo, M., Irving, H., Cuamba, N., Maquina, M., Paaijmans, K. and Wondji, C.S. (2019) 'Escalation of pyrethroid resistance in the malaria vector *Anopheles funestus* induces a loss of efficacy of piperonyl butoxide-based insecticide-treated nets in

Mozambique', *Journal of Infectious Diseases*, 220(3), pp. 467–475. Available at: https://doi.org/10.1093/infdis/jiz139.

Riveron, J.M., Ibrahim, S.S., Chanda, E., Mzilahowa, T., Cuamba, N., Irving, H., Barnes, K.G., Ndula, M. and Wondji, C.S. (2014) 'The highly polymorphic CYP6M7 cytochrome P450 gene partners with the directionally selected CYP6P9a and CYP6P9b genes to expand the pyrethroid resistance front in the malaria vector *Anopheles funestus* in Africa', *BMC genomics*, 15, p. 817. Available at: https://doi.org/10.1186/1471-2164-15-817.

Ross, R. (1923) 'Memoirs: With a full account of the great malaria problem and its solution', *Nature*, 112, pp. 3–5. Available at: https://doi.org/10.1038/112003a0.

Rossati, A., Bargiacchi, O., Kroumova, V., Zaramella, M., Caputo, A. and Garavelli, P.L. (2016) 'Climate, environment and transmission of malaria', *Le Infezioni in Medicina*, 24(2), pp. 93–104.

Rowland, M., Boko, P., Odjo, A., Asidi, A., Akogbeto, M. and N'Guessan, R. (2013) 'A new long-lasting indoor residual formulation of the organophosphate insecticide pirimiphos-methyl for prolonged control of pyrethroid-resistant mosquitoes: An experimental hut trial in Benin', *PLoS ONE*, 8(7), p. e69516. Available at: https://doi.org/10.1371/journal.pone.0069516.

Rowland, M., Downey, G., Rab, A., Freeman, T., Mohammad, N., Rehman, H., Durrani, N., Reyburn, H., Curtis, C., Lines, J. and Fayaz, M. (2004) 'DEET mosquito repellent provides personal protection against malaria : A household randomized trial in an Afghan refugee camp in Pakistan', *Tropical Medicine and International Health*, 9(3), pp. 335–342. Available at: https://doi.org/10.1111/j.1365-3156.2004.01198.x.

Runge, M., Thawer, S.G., Molteni, F., Chacky, F., Mkude, S., Mandike, R., Snow, R.W., Lengeler, C., Mohamed, A. and Pothin, E. (2022) 'Sub-national tailoring of malaria interventions in Mainland Tanzania: simulation of the impact of strata-specific intervention combinations using modelling', *Malaria Journal*, 21(1), p. 92. Available at: https://doi.org/10.1186/s12936-022-04099-5.

Russell, T.L., Beebe, N.W., Cooper, R.D., Lobo, N.F. and Burkot, T.R. (2013) 'Successful malaria elimination strategies require interventions that target changing

vector behaviours', *Malaria Journal*, 12(1), p. 56. Available at: https://doi.org/10.1186/1475-2875-12-56.

Russell, T.L., Govella, N.J., Azizi, S., Drakeley, C.J., Kachur, S.P. and Killeen, G.F. (2011) 'Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania', *Malaria Journal*, 10(1), p. 80. Available at: https://doi.org/10.1186/1475-2875-10-80.

SACIDS (2019) *Dengue outbreaks in Tanzania: recent trends and importance of research data in disease surveillance*. Available at: http://www.sacids.org/news/dengue-outbreaks-tanzania-recent-trends-importance-research-data-disease-surveillance/ (Accessed: 16 April 2021).

Sangbakembi-Ngounou, C., Costantini, C., Longo-Pendy, N.M., Ngoagouni, C., Akone-Ella, O., Rahola, N., Cornelie, S., Kengne, P., Nakouné, E.R., Komas, N.P. and Ayala, D. (2022) 'Diurnal biting of malaria mosquitoes in the Central African Republic indicates residual transmission may be "out of control"', *Proceedings of the National Academy of Sciences of the United States of America*, 119(21), p. e2104282119. Available at: https://doi.org/10.1073/pnas.2104282119.

Sangoro, O., Turner, E., Simfukwe, E., Miller, J.E. and Moore, S.J. (2014) 'A cluster-randomized controlled trial to assess the effectiveness of using 15% DEET topical repellent with long-lasting insecticidal nets (LLINs) compared to a placebo lotion on malaria transmission', *Malaria journal*, 13(1), p. 324. Available at: https://doi.org/10.1186/1475-2875-13-324.

Sangoro, O.P., Gavana, T., Finda, M., Mponzi, W., Hape, E., Limwagu, A., Govella, N.J., Chaki, P. and Okumu, F.O. (2020) 'Evaluation of personal protection afforded by repellent-treated sandals against mosquito bites in south-eastern Tanzania', *Malaria Journal*, 19(1), p. 148. Available at: https://doi.org/10.1186/s12936-020-03215-7.

Savage, H.M., Ezike, V.I., Nwankwo, A.C., Spiegel, R. and Miller, B.R. (1992) 'First record of breeding populations of *Aedes albopictus* in continental Africa: implications for arboviral transmission.', *Journal of the American Mosquito Control Association*, 8(1), pp. 101–103.

Sawadogo, S.P., Niang, A., Bilgo, E., Millogo, A., Maïga, H., Dabire, R.K., Tripet, F. and Diabaté, A. (2017) 'Targeting male mosquito swarms to control malaria vector density', *PLoS ONE*, 12(3), p. e0173273. Available at: https://doi.org/10.1371/journal.pone.0173273.

Sharma, V.P. (2012) '"Continuing challenge of malaria in India"', *Current Science*, 102(5), pp. 678–682.

Sinka, M.E., Bangs, M.J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C.M., Hemingway, J., Patil, A.P., Temperley, W.H., Gething, P.W., Kabaria, C.W., Burkot, T.R., Harbach, R.E. and Hay, S.I. (2012) 'A global map of dominant malaria vectors', *Parasites and Vectors*, 5(1), p. 69. Available at: https://doi.org/10.1186/1756-3305-5-69.

Siria, D.J., Batista, E.P.A., Opiyo, M.A., Melo, E.F., Sumaye, R.D., Ngowo, H.S., Eiras, A.E. and Okumu, F.O. (2018) 'Evaluation of a simple polytetrafluoroethylene (PTFE)-based membrane for blood-feeding of malaria and dengue fever vectors in the laboratory', *Parasites and Vectors*, 11(1), p. 236. Available at: https://doi.org/10.1186/1756-3305-5-69.

Sissoko, F., Junnila, A., Traore, M.M., Traore, S.F., Doumbia, S., Dembele, S.M., Schlein, Y., Traore, A.S., Gergely, P., Xue, R. De, Arheart, K.L., Revay, E.E., Kravchenko, V.D., Beier, J.C. and Müller, G.C. (2019) 'Frequent sugar feeding behavior by *Aedes aegypti* in Bamako, Mali makes them ideal candidates for control with attractive toxic sugar baits (ATSB)', *PLoS ONE*, 14(6), p. e0214170. Available at: https://doi.org/10.1371/journal.pone.0214170.

Smith, A. (1962) 'The preferential indoor resting habits of *Anopheles gambiae* in the Umbugwe area of Tanganyika.', *East African Medical Journal*, 39(11), pp. 631–635.

Snetselaar, J., Njiru, B.N., Gachie, B., Owigo, P., Andriessen, R., Glunt, K., Osinga, A.J., Mutunga, J., Farenhorst, M. and Knols, B.G.J. (2017) 'Eave tubes for malaria control in Africa: Prototyping and evaluation against *Anopheles gambiae s.s.* and *Anopheles arabiensis* under semi-field conditions in western Kenya', *Malaria Journal*, 16(1), p. 276. Available at: https://doi.org/10.1186/s12936-017-1926-5.

Snyman, K., Mwangwa, F., Bigira, V., Kapisi, J., Clark, T.D., Osterbauer, B., Greenhouse, B., Sturrock, H., Gosling, R., Liu, J. and Dorsey, G. (2015) 'Poor

housing construction associated with increased malaria incidence in a cohort of young Ugandan children', *American Journal of Tropical Medicine and Hygiene*, 92(6), pp. 1207–1213. Available at: https://doi.org/10.4269/ajtmh.14-0828.

Sombié, A., Saiki, E., Yaméogo, F., Sakurai, T., Shirozu, T., Fukumoto, S., Sanon, A., Weetman, D., McCall, P.J., Kanuka, H. and Badolo, A. (2019) 'High frequencies of F1534C and V1016I kdr mutations and association with pyrethroid resistance in *Aedes aegypti* from Somgandé (Ouagadougou), Burkina Faso', *Tropical Medicine and Health*, 47, p. 2. Available at: https://doi.org/10.1186/s41182-018-0134-5.

Sougoufara, S., Diédhiou, S.M., Doucouré, S., Diagne, N., Sembène, P.M., Harry, M., Trape, J.F., Sokhna, C. and Ndiath, M.O. (2014) 'Biting by *Anopheles funestus* in broad daylight after use of long-lasting insecticidal nets: A new challenge to malaria elimination', *Malaria Journal*, 13(1), p. 125. Available at: https://doi.org/10.1186/1475-2875-13-125.

Sperling, S., Cordel, M., Gordon, S., Knols, B.G.J. and Rose, A. (2017) 'Eave tubes for malaria control in Africa: Videographic observations of mosquito behaviour in Tanzania with a simple and rugged video surveillance system', *MalariaWorld Journal*, 8, p. 9.

Spitzen, J., Koelewijn, T., Mukabana, W.R. and Takken, W. (2016) 'Visualization of house-entry behaviour of malaria mosquitoes', *Malaria Journal*, 15(1), p. 233. Available at: https://doi.org/10.1186/s12936-016-1293-7.

Stanaway, J.D., Shepard, D.S., Undurraga, E.A., Halasa, Y.A., Coffeng, L.E., Brady, O.J., Hay, S.I., Bedi, N., Bensenor, I.M., Castañeda-Orjuela, C.A., Chuang, T.W., Gibney, K.B., Memish, Z.A., Rafay, A., Ukwaja, K.N., Yonemoto, N. and Murray, C.J.L. (2016) 'The global burden of dengue: An analysis from the Global Burden of Disease Study 2013', *The Lancet Infectious Diseases*, 16(6), pp. 712–723. Available at: https://doi.org/10.1016/S1473-3099(16)00026-8.

Sternberg, E.D., Cook, J., Alou, L.P.A., Assi, S.B., Koffi, A.A., Doudou, D.T., Aoura, C.J., Wolie, R.Z., Oumbouke, W.A., Worrall, E., Kleinschmidt, I., N'Guessan, R. and Thomas, M.B. (2021) 'Impact and cost-effectiveness of a lethal house lure against malaria transmission in central Côte d'Ivoire: A two-arm, cluster-randomised controlled trial', *The Lancet*, 397(10276), pp. 805–815. Available at: https://doi.org/10.1016/S0140-6736(21)00250-6.

Stevenson, B.J., Bibby, J., Pignatelli, P., Muangnoicharoen, S., O'Neill, P.M., Lian, L.Y., Müller, P., Nikou, D., Steven, A., Hemingway, J., Sutcliffe, M.J. and Paine, M.J.I. (2011) 'Cytochrome P450 6M2 from the malaria vector *Anopheles gambiae* metabolizes pyrethroids: Sequential metabolism of deltamethrin revealed', *Insect Biochemistry and Molecular Biology*, 41(7), pp. 492–502. Available at: https://doi.org/10.1016/j.ibmb.2011.02.003.

Stevenson, J.C., Simubali, L., Mudenda, T., Cardol, E., Bernier, U.R., Vazquez, A.A., Thuma, P.E., Norris, D.E., Perry, M., Kline, D.L., Cohnstaedt, L.W., Gurman, P., D'hers, S. and Elman, N.M. (2018) 'Controlled release spatial repellent devices (CRDs) as novel tools against malaria transmission: A semi-field study in Macha, Zambia', *Malaria Journal*, 17(1), p. 437. Available at: https://doi.org/10.1186/s12936-018-2558-0.

Strode, C., Donegan, S., Garner, P., Enayati, A.A. and Hemingway, J. (2014) 'The impact of pyrethroid resistance on the efficacy of insecticide-treated bed nets against African *Anopheline* mosquitoes: Systematic review and meta-analysis', *PLoS Medicine*, 11(3), p. e1001619. Available at: https://doi.org/10.1371/journal.pmed.1001619.

Swai, J.K., Mmbando, A.S., Ngowo, H.S., Odufuwa, O.G., Finda, M.F., Mponzi, W., Nyoni, A.P., Kazimbaya, D., Limwagu, A.J., Njalambaha, R.M., Abbasi, S., Moore, S.J., Schellenberg, J., Lorenz, L.M. and Okumu, F.O. (2019) 'Protecting migratory farmers in rural Tanzania using eave ribbons treated with the spatial mosquito repellent, transfluthrin', *Malaria Journal*, 18(1), p. 414. Available at: https://doi.org/10.1186/s12936-019-3048-8.

Sy, O., Niang, E.H.A., Ndiaye, M., Konaté, L., Diallo, A., Ba, E.C.C., Tairou, F., Diouf, E., Cissé, B., Gaye, O. and Faye, O. (2018) 'Entomological impact of indoor residual spraying with pirimiphos-methyl: A pilot study in an area of low malaria transmission in Senegal', *Malaria Journal*, 17(1), p. 64. Available at: https://doi.org/10.1186/s12936-018-2212-x.

Takken, W. and Verhulst, N.O. (2013) 'Host preferences of blood-feeding mosquitoes', *Annual Review of Entomology*, 58, pp. 433–453. Available at: https://doi.org/10.1146/annurev-ento-120811-153618.

Tambwe, M.M., Moore, S., Hofer, L., Kibondo, U.A. and Saddler, A. (2021) 'Transfluthrin eave-positioned targeted insecticide (EPTI) reduces human landing rate (HLR) of pyrethroid resistant and susceptible malaria vectors in a semi-field simulated peridomestic space', *Malaria Journal*, 20(1), p. 357. Available at: https://doi.org/10.1186/s12936-021-03880-2.

Tambwe, M.M., Moore, S.J., Chilumba, H., Swai, J.K., Moore, J.D., Stica, C. and Saddler, A. (2020) 'Semi-field evaluation of freestanding transfluthrin passive emanators and the BG sentinel trap as a "push-pull control strategy" against *Aedes aegypti* mosquitoes', *Parasites and Vectors*, 13(1), p. 392. Available at: https://doi.org/10.1186/s13071-020-04263-3.

Taylor, B. (1975) 'Changes in the feeding behaviour of a malaria vector, *Anopheles farauti Lav.,* following use of DDT as a residual spray in houses in the British Solomon Islands Protectorate.', *Transactions of the Royal Entomological Society of London*, 127(Pt 3), pp. 277–292.

Thawer, S.G., Chacky, F., Runge, M., Reaves, E., Mandike, R., Lazaro, S., Mkude, S., Rumisha, S.F., Kumalija, C., Lengeler, C., Mohamed, A., Pothin, E., Snow, R.W. and Molteni, F. (2020) 'Sub-national stratification of malaria risk in mainland Tanzania: A simplified assembly of survey and routine data', *Malaria Journal*, 19(1), p. 177. Available at: https://doi.org/10.1186/s12936-020-03250-4.

Thomsen, E.K., Koimbu, G., Pulford, J., Jamea-Maiasa, S., Ura, Y., Keven, J.B., Siba, P.M., Mueller, I., Hetzel, M.W. and Reimer, L.J. (2017) 'Mosquito behavior change after distribution of bednets results in decreased protection against malaria exposure', *Journal of Infectious Diseases*, 215(5), pp. 790–797. Available at: https://doi.org/10.1093/infdis/jiw615.

Thwing, J., Hochberg, N., Eng, J. Vanden, Issifi, S., James Eliades, M., Minkoulou, E., Wolkon, A., Gado, H., Ibrahim, O., Newman, R.D. and Lama, M. (2008) 'Insecticide-treated net ownership and usage in Niger after a nationwide integrated campaign', *Tropical Medicine and International Health*, 13(6), pp. 827–834. Available at: https://doi.org/10.1111/j.1365-3156.2008.02070.x.

Toé, K.H., Jones, C.M., N'fale, S., Ismai, H.M., Dabiré, R.K. and Ranson, H. (2014) 'Increased pyrethroid resistance in malaria vectors and decreased bed net

effectiveness Burkina Faso', *Emerging Infectious Diseases*, 20(10), pp. 1691–1696. Available at: https://doi.org/10.3201/eid2010.140619.

Traore, M.M., Junnila, A., Traore, S.F., Doumbia, S., Revay, E.E., Kravchenko, V.D., Schlein, Y., Arheart, K.L., Gergely, P., Xue, R. De, Hausmann, A., Beck, R., Prozorov, A., Diarra, R.A., Kone, A.S., Majambere, S., Bradley, J., Vontas, J., Beier, J.C., *et al.* (2020) 'Large-scale field trial of attractive toxic sugar baits (ATSB) for the control of malaria vector mosquitoes in Mali, West Africa', *Malaria Journal*, 19(1), p. 72. Available at: https://doi.org/10.1186/s12936-020-3132-0.

Urio, N.H., Pinda, P.G., Ngonzi, A.J., Muyaga, L.L., Msugupakulya, B.J., Finda, M., Matanila, G.S., Mponzi, W., Ngowo, H.S., Kahamba, N.F., Nkya, T.E. and Okumu, F.O. (2022) 'Effects of agricultural pesticides on the susceptibility and fitness of malaria vectors in rural south-eastern Tanzania', *Parasites & Vectors*, 15(1), p. 213. Available at: https://doi.org/10.1186/s13071-022-05318-3.

Vairo, F., Mboera, L.E.G., de Nardo, P., Oriyo, N.M., Meschi, S., Rumisha, S.F., Colavita, F., Mhina, A., Carletti, F., Mwakapeje, E., Capobianchi, M.R., Castilletti, C., di Caro, A., Nicastri, E., Malecela, M.N. and Ippolito, G. (2016) 'Clinical, virologic, and epidemiologic characteristics of dengue outbreak, Dar es Salaam, Tanzania, 2014', *Emerging Infectious Diseases*, 22(5), pp. 895–899. Available at: https://doi.org/10.3201/eid2205.151462.

Valerio, L., Marini, F., Bongiorno, G., Facchinelli, L., Pombi, M., Caputo, B., Maroli, M. and Torre, A. (2010) 'Host-Feeding Patterns of *Aedes albopictus*', *Vector-Borne and Zoonotic Diseases*, 10(3), pp. 291–294. Available at: https://doi.org/10.1089/vbz.2009.0007.

Vaughan, A., Rodriguez, M. and Hemingway, J. (1995) 'The independent gene amplification of electrophoretically indistinguishable B esterases from the insecticide-resistant mosquito *Culex quinquefasciatus*', *Biochemical Journal*, 305(Pt 2), pp. 651–658. Available at: https://doi.org/10.1042/bj3050651.

Verhaeghen, K., Van Bortel, W., Roelants, P., Backeljau, T. and Coosemans, M. (2006) 'Detection of the East and West African kdr mutation in *Anopheles gambiae* and *Anopheles arabiensis* from Uganda using a new assay based on FRET/Melt Curve analysis', *Malaria Journal*, 5(1), p. 16. Available at: https://doi.org/10.1186/1475-2875-5-16.

Wanjala, C.L., Mbugi, J.P., Ototo, E., Gesuge, M., Afrane, Y.A., Atieli, H.E., Zhou, G., Githeko, A.K. and Yan, G. (2015) 'Pyrethroid and DDT resistance and organophosphate susceptibility among *Anopheles* spp. mosquitoes, western Kenya', *Emerging Infectious Diseases*, 21(12), pp. 2178–2181. Available at: https://doi.org/10.3201/eid2112.150814.

Weill, M., Malcolm, C., Chandre, F., Mogensen, K., Berthomieu, A., Marquine, M. and Raymond, M. (2004) 'The unique mutation in *ace-1* giving high insecticide resistance is easily detectable in mosquito vectors', *Insect Molecular Biology*, 13(1), pp. 1–7. Available at: https://doi.org/10.1111/j.1365-2583.2004.00452.x.

WHO (1957) 'Expert Committee on Insecticide Seventh Report', p. 32. Available at: http://apps.who.int/iris/bitstream/handle/10665/40380/WHO_TRS_125.pdf?sequence=1&isAllowed=y.

WHO (2006) *Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets*, *World Health Organization*. Geneva. Available at: https://apps.who.int/iris/handle/10665/69296.

WHO (2009) *Dengue guidelines for diagnosis, treatment, prevention and control : New edition*. Geneva. Available at: https://apps.who.int/iris/handle/10665/44188.

WHO (2012a) *Global plan for insecticide management in malaria vectors*. Geneva. Available at: https://apps.who.int/iris/handle/10665/44846.

WHO (2012b) *The role of larviciding for malaria control in sub-Saharan Africa*. Geneva. Available at: https://apps.who.int/iris/handle/10665/337991.

WHO (2013) *WHO recommended insecticides for indoor residual spraying against malaria vectors*. Geneva. Available at: https://www.who.int/neglected_diseases/vector_ecology/vector-control/Insecticides_IRS_22_September_2018.pdf?ua=1.

WHO (2015) *Indoor residual spraying: An operational manual for indoor residual spraying (IRS) for malaria transmission control and elimination, 2nd ed*. Geneva. Available at: https://apps.who.int/iris/handle/10665/177242.

WHO (2016a) *Global report on insecticide resistance in malaria vectors: 2010-2016*. Geneva. Available at: https://apps.who.int/iris/handle/10665/272533.

WHO (2016b) *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2nd ed*. Edited by WHO. Geneva. Available at: https://apps.who.int/iris/handle/10665/250677.

WHO (2018) *WHO | High burden to high impact: A targeted malaria response*, *Geneva*. Geneva. Available at: https://www.who.int/publications/i/item/WHO-CDS-GMP-2018.25.

WHO (2022a) *Dengue and severe dengue*, *WHO*. Available at: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue.

WHO (2022b) *WHO - Prequalification of Medical Products (IVDs, Medicines, Vaccines and Immunization Devices, Vector Control)*. Available at: https://extranet.who.int/pqweb/vector-control-products/prequalified-product-list.

WHO (2022c) *World Malaria Report 2022*. Geneva. Available at: https://www.who.int/publications/i/item/9789240064898.

WHO Regional Office for South-East Asia (2014) *Vector-borne diseases. Report of an informal expert consultation*. Geneva. Available at: https://apps.who.int/iris/handle/10665/206531.

Wickham, H. (2011) 'Ggplot2', *Wiley Interdisciplinary Reviews: Computational Statistics*, 3(2), pp. 180–185. Available at: https://doi.org/10.1002/wics.147.

Wiebe, A., Longbottom, J., Gleave, K., Shearer, F.M., Sinka, M.E., Massey, N.C., Cameron, E., Bhatt, S., Gething, P.W., Hemingway, J., Smith, D.L., Coleman, M. and Moyes, C.L. (2017) 'Geographical distributions of African malaria vector sibling species and evidence for insecticide resistance', *Malaria Journal*, 16(1), p. 85. Available at: https://doi.org/10.1186/s12936-017-1734-y.

Wilson, A.L., Chen-Hussey, V., Logan, J.G. and Lindsay, S.W. (2014) 'Are topical insect repellents effective against malaria in endemic populations? A systematic review and meta-analysis', *Malaria Journal*, 13(1), p. 446. Available at: https://doi.org/10.1186/1475-2875-13-446.

Wilson, A.L., Courtenay, O., Kelly-Hope, L.A., Scott, T.W., Takken, W., Torr, S.J. and Lindsay, S.W. (2020) 'The importance of vector control for the control and

elimination of vector-borne diseases', *PLoS Neglected Tropical Diseases*, 14(1), p. e0007831. Available at: https://doi.org/10.1371/journal.pntd.0007831.

Wood, O.R., Hanrahan, S., Coetzee, M., Koekemoer, L.L. and Brooke, B.D. (2010) 'Cuticle thickening associated with pyrethroid resistance in the major malaria vector *Anopheles funestus*', *Parasites and Vectors*, 3(1), p. 67. Available at: https://doi.org/10.1186/1756-3305-3-67.

Yahouédo, G.A., Chandre, F., Rossignol, M., Ginibre, C., Balabanidou, V., Mendez, N.G.A., Pigeon, O., Vontas, J. and Cornelie, S. (2017) 'Contributions of cuticle permeability and enzyme detoxification to pyrethroid resistance in the major malaria vector *Anopheles gambiae*', *Scientific Reports*, 7(1), p. 11091. Available at: https://doi.org/10.1038/s41598-017-11357-z.

Yang, X., Quam, M.B.M., Zhang, T. and Sang, S. (2021) 'Global burden for dengue and the evolving pattern in the past 30 years', *Journal of travel medicine*, 28(8), p. taab146. Available at: https://doi.org/10.1093/jtm/taab146

APPENDICES

Appendix 1: Tank mix preparations

Eave ribbons with two different dimensions were treated.

i. The ribbons used in cone bioassays had final dimensions of 0.15 m wide and 11.6 m long, treatable surface area of 2 x 1.74 m^2 = 3.48 m^2

ii. The ribbons used in semi-field studies had final dimensions of 0.15 m wide and 7.7 m long, consisting of a treatable surface area of 2 x 1.155 m^2 = 2.31 m^2.

Test insecticide and doses

The doses of pirimiphos-methyl tested were 0.5 g/m^2, 1 g a.i./m^2, 2 g a.i./m^2, 4 g a.i./m^2, and 8 g a.i./m^2 for cone bioassay, and 1 g a.i./m^2, 2 g a.i./m^2 and 4 g a.i./m^2 for semi-field studies.

Tank mix preparations

Ribbons used in cone bioassays.

i. The ribbons used in cone bioassay had the surface area of 1.74 m^2 and total holding capacity of 1,044 mL.

ii. To get the target doses of pirimiphos-methyl for each dose, the required amount was as follows:

- To get the target doses of 0.5 g a.i./m^2, 2.9 mL pirimiphos-methyl mixed with 1,041.1 mL of water was required.
- To get the target doses of 1 g a.i./m^2, 5.8 mL pirimiphos-methyl mixed with 1,038.2 mL of water was required.
- To get the target doses of 2 g a.i./m^2, 11.6 mL pirimiphos-methyl mixed with 1,032.4 mL of water was required.
- To get the target doses of 4 g a.i./m^2, 23.2 mL pirimiphos-methyl mixed with 1,020.8 mL of water was required.
- To get the target doses of 8 g a.i./m^2, 46.3 mL pirimiphos-methyl mixed with 997.7 mL of water was required.

iii. Since we cannot prepare the exactly amount of spray solution, 2 L solution was prepared for each dose to ensure enough amount of mixture to spray the ribbons and for the sprayer to work well.

iv. The following mixtures were prepared for each dose.

- 5.5 mL of pirimiphos-methyl were mixed with 1,994.5 mL of water, to get the target dose of 0.5 g a.i./m^2 pirimiphos-methyl.

- 11.1 mL of pirimiphos-methyl mixed with 1,988.9 mL of water to get the target dose of 1 g a.i./m^2 pirimiphos-methyl.
- 22.2 mL of pirimiphos-methyl were mixed with 1,977.8 mL of water to get the target dose of 2 g/m^2 pirimiphos-methyl.
- 44.5 mL of pirimiphos-methyl were mixed with 1,955.5 mL of water to get the target dose of 4 g/m^2 pirimiphos-methyl.
- 88.7 mL of pirimiphos-methyl were mixed with 1,911.3 mL of water to get the target dose of 8 g a.i./m^2 pirimiphos-methyl.

v. Each ribbon was sprayed on both sides and the remaining amount of liquid after spraying each ribbon was discarded.

Ribbons used in semi-field experiment

i. For semi-field work three doses were prepared 1 g a.i./m^2, 2 g a.i./m^2 and 4 g a.i./m^2. The ribbons used were having a total surface area of 1.155 m^2 which could absorb 693 mL of liquid both sides.

ii. To get the target doses for ribbon treatment, 3.8 mL pirimiphos-methyl mixed with 689.2 mL of water, 7.7 mL pirimiphos-methyl mixed with 685.3 mL of water and 15.4 mL pirimiphos-methyl mixed with 677.6 mL of water were required to treat each ribbon to get 1 g a.i./m^2, 2 g a.i./m^2 and 4 g a.i./m^2 pirimiphos-methyl respectively.

iii. However, a total of 1,500 mL solution was prepared by mixing, 8.3 mL pirimiphos-methyl with 1,491.7 mL of water, 16.6 mL pirimiphos-methyl with 1483.4 mL of water and 33.3 mL pirimiphos-methyl with 1,766.7 mL of water to get 1 g a.i./m^2, 2 g a.i./m^2 and 4 g a.i./m^2 pirimiphos-methyl respectively.

iv. This amount was sprayed to each ribbon corresponding to each dose. The remaining amount of liquid was discarded.

Appendix 2: Ethics Review Certificate (Ifakara Health Institute)

F120-ILH-v20.0

Plot 463, Kiko Avenue, Mikocheni | P.o. Box 78,373 Dar es Salaam. Tanzania | Phone: +255222774756 Email: irb@ihi.or.tz

INSTITUTIONAL REVIEW BOARD

ISO 9001:2015 certified

IFAKARA HEALTH INSTITUTE
research | training | services

December 4, 2020

National Institute for Medical Research
P O Box 9653
Dar Es Salaam
Email; headquarters@nimr.or.tz

Dr Fredros Okomu
Ifakara Health Institute
P O Box 53
Ifakara

IHI/IRB/No: 56-2020

INSTITUTIONAL CLEARANCE CERTIFICATE FOR CONDUCTING HEALTH RESEARCH

On 27th November 2020, the Ifakara Health Institute Review Board (IHI-IRB) reviewed from study titled: ***"Assessment of mosquito biting reduction and mortality rates caused by eave-ribbons treated with different formulations of transfluthrin inside the semi-field system"*** submitted by the Co-Principal Investigator Ruth Shirima.

The following documents were reviewed:

1. Protocol
2. Informed Consent Forms
3. Budget
4. Tools
5. CVs

The study has been approved for implementation after IRB consensus. This certificate thus indicates that; the above-mentioned study has been granted an Institutional Ethics Clearance to conduct this study in Kilombero and Ulanga Districts.

The Principal Investigator of the study must ensure that, the following conditions are fulfilled during or after the implementation of the study:

1. PI should submit a six-month progress report and the final report at the end of the project
2. Any amendment, which will be done after the approval of the protocol, must be communicated as soon as possible to the IRB for another approval
3. All research must stop after the project expiration date, unless there is prior information and justification to the IRB.
4. There should be plans to give feedback to the community on the findings
5. Any publication needs to pass through the IRB and NIMR
6. The approval is valid until 27th November 2021

The IRB reserves the right to undertake field inspections to check on the protocol compliance

Chairperson
Prof. Esther Mwaikambo

IRB Secretary
Dr Mwifadhi Mrisho

0

Appendix 3: Ethics Review Certificate (Tanzania Government)

THE UNITED REPUBLIC OF TANZANIA

National Institute for Medical Research
3 Barack Obama Drive
P.O. Box 9653
11101 Dar es Salaam
Tel: 255 22 2121400
Fax: 255 22 2121360
E-mail: nimrethics@gmail.com

Ministry of Health, Community Development, Gender, Elderly & Children
University of Dodoma, College of Business Studies and Law
Building No. 11
P.O. Box 743
40478 Dodoma

NIMR/HQ/R.8a/Vol. IX/3351

06th February2019

Dr. Emmanuel Kaindoa
Ifakara Health Institute
P. O. Box 78373
Dar es Salaam

RE: ETHICAL CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Using repellent eave ribbons to protect low income families against malaria and other mosquito borne-infections in rural Tanzania (Kaindoa E. et al) has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Sites: Minepa and Mavimba Villages in Kilombero Distict, Morogoro Region

Approval is valid for one year: 6th February 2020 to 5th February 2021.

Name: Prof. Yunus Daud Mgaya

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY & CHILDREN

CC: Director, Health Services-TAMISEMI, Dodoma
RMO of Morogoro Region
DMO/DED of Kilombero District

Appendix 4: Ethics Review Certificate (University of the Witwatersrand)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Miss Ruth Shirima

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M211014

NAME: (Principal Investigator)	Miss Ruth Shirima
DEPARTMENT:	Wits Research Institute for Malaria School of Pathology Ifakara Health Institute Tanzania
PROJECT TITLE:	The use of insecticide treated eave ribbons as a protection tool against populations of mosquitoes that transmit malaria and dengue fever
DATE CONSIDERED:	29/10/2021
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof L. Koekemoer and Dr F. Okumu
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	21/01/2022

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions.Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. I **agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **October** and will therefore be due in the month of **October** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

04/02/2022
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 5: Waiver from the Animal Research Ethics Committee (University of the Witwatersrand)

ANIMAL RESEARCH ETHICS COMMITTEE

Registration number: AREC-101210-002

Date: July 1, 2019

Certificate reference: 20190701-7O
Category: O
Re: Waiver from the Animal Research Ethics Committee of the University of the Witwatersran

Reference : Animal Ethics to complete
Re: Waiver from the Animal Research Ethics Committee of the University of the Witwatersrand
HoD applicant : Lizette Koekemoer
Staff number : 09700304
Degree : PhD/Msc Candidates
Study Title : "Studies on malaria vectors".
Department: Wits Research Institute for Malaria;
laboratory
Supervisor: Lizette Koekemoer, Givemore Munhenga, Yael Dahan-Moss, Shune Oliver, Basil Brooke and Maria Kaiser
Email: lizette.koekemoer@wits.ac.za
Address: 7 York Road, Parktown, Johannesburg, 2001
Tel: + 011-717 2486;
Full clearance certificate from the AREC of the University of the Witwatersrand is NOT required.
Reason: This study uses mosquitoes (invertebrates).
Comment/Notes :
1) This is a General Waiver for the PI and listed Supervisors.
2) Individual researchers and MSc & PhD candidates may require Ethics Clearance in the own name according to applicable rules of the Post-Graduate and other Committees.
3) Feeding of the mosquitos should be specified.

Please contact me should you require further information.

GP Candy

Geoffrey Candy PhD
Chair : Animal Research Ethics Committee

Geoffrey P Candy PhD
Professor, Department of Surgery, Faculty of Health Sciences, University of the Witwatersrand, *7 York Road Parktown 2193, Johannesburg; Tel: 27-11-717-2574; Email: geoffrey.candy@wits.ac.za*

Appendix 6: Turn-it in similarity report

Ruth_Thesis_for_turn_it_in.docx

NR 22 February 2023

Fredros Okumu, Feb 22nd 2023

ORIGINALITY REPORT

12%	7%	10%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet Source	1%
2	eprints.gla.ac.uk Internet Source	1%
3	Njoroge, Margaret Mendi. "Host Seeking Behaviour of Anopheles Mosquitoes in Response to Olfactory and Visual Cues", Wageningen University and Research, 2021 Publication	1%
4	"Innovative strategies for vector control", Wageningen Academic Publishers, 2021 Publication	<1%
5	Hakizimana, Emmanuel. "Integrated Vector Management (Ivm) as a Tool for Community Empowerment Towards Malaria Elimination in Rwanda", Wageningen University and Research, 2021 Publication	<1%
6	Siyethemba L. Masikane, Precious Novela, Phillimon Mohale, Adéle McLeod. "Effect of phosphonate application timing and -strategy	<1%

Appendix 7: Consent form

CONSENT FORM

Title of Project: Using insecticide treated eave ribbons as a protection tool against populations of mosquitoes that transmit malaria and dengue fever.

Initial's box

1. I confirm that I have read the information sheet for the above study, and I have understood. ☐

2. I had a chance to ask questions and received satisfactory answers. ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without any negative consequence. ☐

4. I understand that data collected during the study may be looked at by investigators involved in this study. I give permission to these individuals to have access to my records. ☐

5. I agree to take part in the above study. ☐

By signing below, I am indicating my consent to participate in the research.
Participant's name:

_____

Participant's signature or thumbprint: _____ Date:

_____

Witness Name (As appropriate): _____

Witness signature (As appropriate): _____ Date: _____

Study team member's statement:
I, the undersigned, have explained to the participant in a language that s/he understands; the procedures to be followed in the study, the risks and benefits involved, and the obligations of the study team.

Name _____Signature _____ Date_____

When completely filled, one copy will be given to you and another copy for a researcher.