Dynamics of insecticide resistance in *Anopheles arabiensis* and *Culex pipiens* complex, and its associations to agricultural practices in southern Tanzania

Inauguraldissertation

zur

Erlangung der Würde eines Doktors der Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

Nancy Matowo

Basel, 2022

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät auf Antrag von Prof. Dr. Marcel Tanner, Prof. Dr. Jürg Utzinger, und Prof. Dr. Alexander Mathis.

Basel, 15.12.2020

Prof. Dr. Martin Spiess

Dekan der Philosophisch-Naturwissenschaftlichen Fakultät

Table of contents

List of figures

List of tables

List of Abbreviations

Ace	Acetylcholinesterase gene
AChE	Acetylcholinesterase
ACTs	Artemisinin-based combination therapy
Bti	*Bacillus thuringiensis* var. *israelensis* (microbial larvicides)
Bs	*Bacillus sphaericus (Bs)*
CAG	Controller and Auditor General
CDC	Center for Disease Control and Prevention
EIR	Entomological inoculation rate
DDE	Dichloro-diphenyl-dichloroethylene
DDT	Dichloro-diphenyl-trichloroethane (Organochlorine synthetic insecticide)
DEM	Diethyl maleate
DP	Dusting powder
EW	Emulsifiable concentrate
ELISA	Enzyme-linked immunosorbent assay
EC	Emulsifiable concetrate
ESKAS	Swiss Government Excellence Scholarship via the Federal Commission for Scholarships for Foreign Students FCS
FAO	United Nations Food and Agriculture Organisation
FFS	Farmer field schools
GDP	Gross domestic product
GMAP	Global malaria action plan
GPIRM	Global action plan for insecticide resistance management in malaria vectors
GSTs	Glutathione S-transferases
GTS	Global Technical Strategies for Malaria
IHI	Ifakara Health Institute
IRAC	Insecticide Resistance Action Committee
IRS	Indoor residual spraying
IVCC	Innovative Vector Control Consortium
IVM	Integrated vector management
ITNs	Insecticide-treated nets
IPM	Integrated pest management
IVPM	Integrated vector and pest management
KDR	Knock-down resistance
KDT50	Knocked down time
LF	Lymphatic filariasis
LLINs	Long-lasting insecticidal nets
LSM	Larval source management

MDA	Mass drug administration
MoHCDGEC	Ministry of Health, Community Development, Gender, Elderly and Children
MUHAS	Muhimbili University of Health and Allied Sciences
MT	Metric tons
NAOT	National Audit of Tanzania
NEMC	National Environment Management Council
NIMR	National Institute for Medical Research
NA	Not applicable
NMCP	National malaria control programme
PBO	Piperonyl butoxide
PCR	Polymerase chain reaction
PMI	US' Presidents Malaria Initiative
QR	Quick response (code)
RBM	Roll Back Malaria Partnership
RDT	Rapid diagnostic test
SD	Soluble dust
SUA	Sokoine University of Agriculture
s.l.	Sensu lato
s.s.	Sensu stricto
Swiss TPH	Swiss Tropical and Public Health Institute
SUFI	Scale-up for impact
TDR	Tropical Disease Research
TMIS	Tanzania Malaria Indicator Survey
TPRI	Tropical Pesticides Research Institute
TPHPA	Tanzania Plant Health and Pesticides Authority
TPP	Triphenyl phosphate
TFDA	Tanzania Food and Drugs Authority
OD	Thunder Oil Dispersion (OD)
UNEP	The United Nations Environment Programme
UNICEF	United Nations Children's Fund
USAID	United States Agency for International Development
VCAG	The WHO vector control advisory group
VGSC	Voltage gated sodium channel
WP	Wettable powders
WS	Wettable soluble
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme

Acknowledgements

First and foremost, I give all the praise and glory to the Almighty God, for His guidance, and strengths throughout every step of my PhD journey. Even at times when I failed, messed up and made mistakes over and over again, God strengthens me; Philippians 4:13.

Prof. Dr. Marcel Tanner, thank you so much for the inspiration and motivations and being a father to many students. I recall when I first proposed my PhD research work while sitting by the swimming pool at the rest house at Ifakara Health Institute. You had no doubts and encouraged me to pursue it and promised to support me. My sincere gratitude for allowing pursuing my studies though Swiss TPH and the University of Basel. Many thanks for the discussions we had on the progress of my research despite having a busy schedule. Thank you so much for your collaborations.

Many thanks to Prof. Dr. Jürg Utzinger for accepting being my co-supervisor. I truly appreciate your excellent collaborations, and for always finding time to review and proofread my thesis chapters despite your busy schedule. I would like to thank Prof. Dr. Brigit Obrist for sharing her knowledge and qualitative research skills that partly improved the design of this research.

Dr. Fredros O. Okumu, I am always incredibly inspired by your passion and efforts in supporting and mentoring the young generation in their research carrier development, regardless. I am profoundly grateful for seeing my potential, believing in me, and support my MSc programme at the University of Witwatersrand, South Africa, that opened many doors in my carrier development, including this PhD journey. Many thanks for supporting my dreams and visions. Thank you for challenging me not to settle for good enough, and for always reminding me to use the space between my ears effectively. I appreciate your endless support and my prayers have been that one day my son will inspire the world as you have been doing.

My endless gratitude to the amazing and supportive Christine Mensch. Thank you for your tireless administrative and moral support. Truly appreciated for taking care of me during my stay in Basel and overall being a lovely secret mommy to many students.

Dr. Givemore Munenga, thank you so much for supporting my PhD journey. Many thanks for the training and practical guidance on PCR and ELISA techniques, insecticide susceptibility bioassays, and molecular techniques for detecting resistance mechanisms in vectors at VCRU laboratory in South Africa. Indeed, I appreciate Prof. Lizette L. Koekemoer and Prof. Maureen Coetzee for the opportunity to learn and practises at your lab, and to Prof. Coetzee for your invaluable support and providing hands-on training on taxonomy and morphologically identification of *Culex* species.

To the vibrant entomology team and friends at Ifakara Health Institute, it has been my greatest pleasure to collaborate with you in my PhD research and other related projects. Thank you for the weekly journal club, multiple scientific talks, in-house training on statistical analysis and monthly project meetings we held. I thank all those who helped me through this journey. My sincere gratitude to my friends April Monroe, Corine Karema, Josephine Malinga, Yeromin Mlacha, Belinda Nimako, Apoorva Sharan, Isaac Lyatu, Daniel Msellemu, Aneth Tumbo, Khadija Saidi and Grace Mhalu for sharing their great moments with me in Basel. The dissertation uninterrupted writing camp with you during weekends was enjoyable and useful. I am grateful to the farmers, pesticides dealers, village leaders, field technicians in Ifakara and all who participated in this research. Thank you for sharing their time, experiences, contributions and co-operation during the study in the field. Moreover, I thank you the key officials from government sectors for sharing their opinions, advice and recommendations during the study.

To my late beloved dad Stephen Aggrey Obed Matowo, I can never thank you enough, you have been my greatest inspiration. Growing up under your guidance was the perfect gift in my life. I would not be what I am today without your constant support and encouragements. Thank you for seeing the best in me and encourage me always to do the right thing even when it is difficult to do so and when no one sees me. I miss you every day dad. I have been trying to read some books you have read, my favourite being “Positive Imaging by Norman Vincent Peale”. I will treasure the moments we had together, multiple discussions we had when you were still alive. Papa may you continue to enjoy eternal life in Heaven. To my prayer warrior, mother, Midllaster Stephen Matowo, thank you for sacrificing your dreams so that I was able to dream. Your constant prayers are the most amazing support for me, and I still need it for the rest of my life. To my mommy, sister Haika, Norah and my little brother Elikunda, many thanks for your encouragements, especially sometimes when I was overwhelmed with school and family.

My heartfelt thanks to my lovely wonderful sons Baraka, Ivan, Ian, Nathaniel and daughters Kimberly-Lulu, Tracey, and Neema. I’m incredibly grateful for their patient, especially during times when I was away for school in Basel and during the writing of my thesis that required long working day and night hours. I promise to support you as much as I can, God will. Thanks to my husband, Lameck Luwanda, for taking care of the kids while I was away.

To anonymous reviewers and Editors, my deepest thanks for critically reviewing my manuscripts that contributed to most of my thesis chapters and eventually got published. Many thanks for your helpful comments and suggestions that substantially improved the final product of the thesis.

My PhD programme would not have been possible without the generous financial support of the Swiss Government Excellence Scholarship via the Federal Commission for Scholarships for Foreign Students FCS (ESKAS), and the Swiss Tropical and Public Health Institute (Swiss TPH). I acknowledge The Wellcome Trust Intermediate Fellowship in Public Health and Tropical Medicine awarded to Dr. Fredros O. Okumu for finance part of the field research work.

Summary

Background

Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) remain the primary tools for the control of malaria and other vector-borne disease. However, mosquito vectors are increasingly developing resistance to public health insecticides, thus threatening the effectiveness of both interventions. Similarly, the use agricultural pesticides may enhance selection pressure and increase resistance in mosquitoes, but current vector control practices do not consider the intensive pesticides use in agriculture as a factor in the spread of resistance. Insecticide resistance monitoring is an integral part of pest management and should guide national programmers to implement effective and sustainable vector control methods.

The main aim of this thesis was to investigate the dynamics of insecticide resistance in *Anopheles arabiensis*, and *Culex pipiens* complex, and its associations to agricultural practices in southern Tanzania. The study examined the spatial temporal variations in phenotypic resistance and its mechanisms in the malaria vector, *An. arabiensis*, and the nuisance biting mosquitoes, *Cx. pipiens* complex in southern Tanzania. It then explored types of agricultural pesticides as well as the knowledge, views and practices linked to resistance development, among farmers, pesticide dealers and policy advisors. Lastly, it explored opportunities to engage and improve farmer's awareness on insecticide resistance in malaria vectors and relationships to agricultural pesticide use practices.

Methods

Phenotypic resistance was measured using standard World Health Organisation (WHO) tests against pyrethroid, carbamate, organophosphate and organochlorine insecticides. Bioassays were carried out with females and male *An. arabiensis*, and *Cx. pipiens* mosquitoes emerging from wild-collected larvae in three neighbouring wards, Minepa, Lupiro, and Mavimba during the dry (June-December 2015) and wet seasons (January-May 2016). Synergist assays were used to assess possible metabolic-based resistance mechanisms in adult female *An. arabiensis*, and *Cx. pipiens* complex, and hydrolysis probe assays used to screen for L1014F (kdr-w) and L1014S (kdr-e) mutations in a subsets of *An*. *arabiensis*. Biting rates of *Culex* species, relative to other taxa, were estimated from indoor surveillance data collected in 2012, 2013, and 2015. A sub-sample of *Cx. pipiens* complex were molecularly identified to distinguish between the two siblings, i.e. *Cx. pipiens pipiens* and *Cx. quinquefasciatus*, using PCR targets the acetyl-cholinesterase-2 locus (ace-2). An exploratory sequential mixed-methods were used to analysis the qualitative data. In-depth interviews were carried to assess general awareness and practices regarding agricultural pesticides among 17 retailers and 57 farmers, followed by a survey involving 427 farmers. Simultaneously, field observations were done to validate the findings. Additional in-depth interviews were done with key informants from public health, agriculture, and environment sectors to explore their views on the linkage between pesticide use in agriculture and resistance evolution in mosquito and options to mitigate/minimize the challenge. Lastly, participatory workshops and field trainings were conducted involving entomologists, farmers, and agricultural and veterinary officers, focusing on agro-ecosystem practices relevant to pesticides use for crop protection.

Findings

The study found substantial fine-scale heterogeneities in phenotypic resistance over time and space in both *An. arabiensis* and Culex species to pyrethroids, DDT and bendiocarb. The differences in insecticide resistance in local mosquito populations were observed even between nearby villages, only 5 km apart. Male mosquitoes were found equally resistant to the insecticides as females. While resistance was distributed across sites, higher frequencies were observed in both species in dry season and in Minepa ward. Resistance in mosquitoes corresponding to the time when insecticides were highly sprayed for pest control in dry season by farmers. Restoration of insecticide susceptibility in mosquito, following pre-exposure to synergists, along with a lack L1014F nor L1014S resistance mutations suggesting the metabolic mechanism is primarily contributing to insecticide resistance.

Though malaria is the most important mosquito-borne disease, 79% of biting density indoors was associated with *Cx. pipiens* mosquitoes, particularly *Cx. quinquefasciatus*. Unfortunately, most communities are not knowledgeable of basic information on mosquito biology thus cannot distinguish between *Anopheles* and other mosquito species. The increased biting densities of *Culex* mosquitoes combined with lack of knowledge on mosquito biology among the communities, is likely to be perceived that the LLINs or any interventions in place is not effective against malaria vectors even if they interventions work.

Most farmers (94%) considered pesticide use as an effective strategy for pest management and improvement of crop productivity. Lambda-cyhalothrin, cypermethrin (both pyrethroids) and imidacloprids (neonicotinoids) were the most common agricultural insecticides used by the farmers. The herbicide glyphosate (amino-phosphonates) (59.0%), fungicides, dithiocarbamate and acylalanine (54.5%), and organochlorine (27.3%) were also readily available in the agrovet shops and widely used by farmers. Most farmers and sellers recognized pesticides by their trade names, but they lacked knowledge on pest control or proper usage of these pesticides. One-third of the farmers disposed of their pesticide leftovers (30.0%) and most farmers discarded empty pesticide containers into rivers or nearby bushes (55.7%). A third of farmers had used non-chemical methods for pest control, but found them ineffective. Three quarters of farmers were not aware of mosquito larvae being present in their fields and only (7.0%) considered their fields as potential sources of mosquitoes. Two thirds were unaware of any effects that agricultural pesticides may have on mosquitoes, and three quarters had never heard of insecticide resistance in malaria mosquitoes. Participatory workshops, enhanced farmers understating on basic mosquito biology and ecology common crop pests and diseases, appropriate pesticides selection and use, and basic knowledge and procedures for detecting insecticide resistance in malaria vectors.

Conclusions

This study showed that resistance phenotypes in *An. arabiensis* and *Cx. pipiens* complex to pyrethroids, DDT and bendiocarb and the underlying mechanisms was widespread but varied considerably at fine geographical and temporal scales. Monooxygenases and esterases partly underlie the resistance phenotypes against pyrethroids, while glutathione-S-transferase (GSTs) play a significant role in DDT resistance. Despite the presence of insecticide resistance, communities should keep using the LLINs to minimize the risk of malaria transmission. Since the nets provide only physical barriers, complementary tools such the LLINs contain dual insecticides with different modes of action or incorporated with synergist may enhance the killing efficacy against vectors, and thus sustain and the gains made against malaria. The findings indicated the need to reform the current and future national insecticide resistance monitoring plan by expanding the sentinel sites within the districts/wards other than extrapolating resistance data from few representative sentinel sites that are far from each other, with varying selection pressures, for country-level decisions. Continued monitoring is crucial to ensure optimal resistance management and effectiveness of the malaria vector control. Similarities of active ingredients used in agriculture and malaria vector control, poor pesticide management practices and low-levels of awareness among farmers and pesticides retailers might enhance the selection of insecticide resistance in malaria vectors. The findings indicate the need to improve awareness among subsistence farmers on the ecology of mosquito vectors and to integrate pest management practices with those in public health. Further epidemiological studies are recommended to evaluate the operational impact of insecticide resistance in vector and disease control. Information on insecticide susceptibility of male mosquitoes could be useful when designing interventions primarily against males, e.g. sterile insect technique (SIT), spraying of male swarms with insecticides and use of attractive toxic sugar baits.

Key messages and policy implications for malaria other vector-borne disease control

The national malaria control programme (NMCP) of Tanzania has been implementing two main strategies as part of resistance management strategies in line with the GPIRM. These include rotations and mixtures of insecticides with different modes of action and target sites. For example, between 2007 and 2011 IRS programmes in all PMI-supported districts of Lake Zone with the highest malaria burden, were performed using pyrethroid lambda-cyhalothrin (ICON 10CS, Syngenta, Basel, Switzerland). In 2011 malaria vectors developed resistance to pyrethroids therefore the IRS policy switched to bendiocarb (Ficam 80% wettable powder, Bayer) in 2012 and later replaced by pirimphos-methyl in 2013 after detection of bendiocarb resistance. A mixtures approach has also been practiced in some regions including the use of Olyset® Plus in Muleba, Kagera region in North-West Tanzania, where metabolic resistance was confirmed. Among the oldest yet successful malaria vector control interventions include larval source management or larviciding with biolarvicides (*Bti)* and (*Bs)*, however these control approaches are less promoted and implemented by the NMCP programmes. It is clear that there is a lack of intersectoral collaboration for pesticide management used for public health and agriculture that could contribute in the selection of resistant vectors.

The findings of this thesis could have policy implications for malaria, other vector-borne disease control programmes, agriculture and environmental sectors as suggested below;

1) Revise the current NMCP insecticide resistance monitoring plan by expanding the sentinel sites within the districts/wards other than extrapolating resistance data from few representative sentinel sites that are far from each other, with varying selection pressures, for country-level decisions. Continued monitoring is crucial to ensure optimal resistance management and effectiveness of the malaria vector control.

2) The national IRS programmes should operate in line with agricultural pesticide spraying programmes, when selecting class of insecticide and spraying period. This will consider the observed spatial (correlation in space) and temporal variations (correlation in time) in resistance frequency in mosquitoes and ensure targeted and effective interventions are implemented. Moreover, agricultural pesticide applications could be strictly conducted by

trained experts similar to the ones perform IRS rather than using farmers who are not trained.

3) Review and strengthen the current national regulations and policies guiding pesticides manufactures, registration, quality and standards, trading, handling, supply/selling, use, disposal and overall management. Considering a limited options of insecticides are available for public health use, insecticides usage should be harmonized across public and agriculture sectors. Currently agriculture has a wide range of insecticides with more than 600 classes/active ingredients and different formulations unlike public health. One of possible approaches could be to allocate pyrethroids (permethrin, deltamethrin), organophosphates (pirimiphos-methyl, Actellic 300SC)) and carbamates (bendiocarb) solely for public health purposes (LLINs and IRS) and keep other classes of insecticides for agricultural pest control. Even though, management and disposal of obsolete pesticides has been centralized and carried out by the NEMC but remaining/unused pesticides and empty pesticide containers are still indiscriminately discarded into the environment.

   I. Possible solution could be creating a centralized legally controlled system at the district/regional level where all pesticide leftover from farms can be collected, later returned back to the supplier and get recycled, thus suppress pesticide waste and accumulation in the environment.

   II. To minimize re-packaging, manipulation and wastage of pesticides, manufactures and suppliers could pack and seal small quantities based on local needs.

   III. Establish a comprehensive pesticide monitoring and control system in the country by using additional bar-code label or quick response (QR) code on the pesticide products that can be scanned instantly using a mobile smartphone to authenticate their registration status, formulations, validity, dosage, and specific crop before use.

   IV. In addition, pesticide policy could impose restrictions of pesticides-buying practices that require a trained customer to seek prescription and an approval certificate from the agricultural/veterinary extension officers.

4) In coordination with the ministry of agriculture, increase the number of trained agricultural extension officers at village level to support farmers through empowering them with proper agricultural knowledge for an improved farming practices in line with the national agricultural policy of 2007. Strengthen the legislations system and mechanism in place to ensuring pesticides dealers are adequately trained on overall pesticide management before initiation of pesticides business. Increase human resources and finance support for regular pesticide inspection and spot checking at the market ensuring the adherence to the pesticide handling guidelines.

5) The current NMCP insecticide resistance and malaria control strategies should consider integrating with agricultural pesticide application practices and general pest management programmes. Possible opportunities could be creating a networks of subsistence farmers, agricultural experts, public health officials, and researchers and engage them through participatory workshops and field learning activities. This will improve community knowledge on mosquito biology linked with agricultural practices, awareness among the farming community on the proper usage and disposal of agricultural pesticide, and enhance community ownership of malaria control program and uptake of research findings. Trained farmers could support implementation of other supplementary vector control strategies such as larva source management or larviciding using biolarvicides (*Bti)* and (*Bs)* in few, detectable and permanent aquatic mosquito breeding sites possibly related to agricultural practices but with minimal pesticide use. Possible opportunity could be combining/apply biolarvicides with agricultural input such as fertilizers to save cost and improve productivity.

6) Incorporating *Culex* species into malaria vector management plans to ensure community acceptability and sustainability of the current and future mosquito control programmes. Additional studies are necessary to investigate the epidemiological importance of the species in transmission filariasis and arboviruses in the study area. Malaria vector control programmes should also consider monitoring the resistance profile of culicines as part of the routine resistance management plan.

7) Even though it might be challenging to change the agricultural land use and pesticide applications patterns as they are primarily driven by economic and livelihoods necessity. Thus this study recommends awareness improvements among subsistence farmers on the ecology of mosquito vectors, the use of non-chemical pest control tools such as crop

rotations and biological, pest-resistant crops. Besides, integrated vector and pest management methods could reduce mosquito populations destiny, risk of malaria transmission and minimize pesticide use/ensure insecticide are judiciously used among both sectors.

1. Chapter 1: General introduction

1.1 Epidemiology and global malaria burden

The world malaria map has shrinked [1] (Figure 1.1). Recent report showed a global decline in malaria cases by 18% and deaths by 28% between 2010 and 2017 [1], but this reduction has slowed since 2017 [2]. For example, in 2018, there were 228 million cases of malaria worldwide, compared with 251 million cases in 2010 and 231 million cases in 2017 [1]. Globally, 405,000 deaths from malaria were estimated in 2018, compared to 416,000 deaths in 2017, and 585,000 in 2010 [1]. Children under five years of age remain the most vulnerable population to infection and deaths, accounting for an estimated 67% (272,000) of all malaria deaths [1].

Despite the progress made and efforts in controlling malaria, the disease continue to be one of the most important public health problems, globally and it is a leading cause of morbidity and mortality, particularly in under five and pregnant women [1]. About 3.4 billion population in 92 countries are still at risk of contracting malaria [1]. the disease hits hardest in the tropical and sub-tropical countries and India due to the availability of the most efficient mosquito vectors, warm temperatures, humid and wet climate favouring the breading and survivals of the malaria vectors. Besides, malaria has been strongly linked with poverty, especially in the least developing African communities. About 94% of all global malaria deaths and illness occurred in the least developing WHO African countries [1].

The WHO goal has been to eradicate malaria worldwide by 2050 [3]. The Global Technical Strategy for malaria 2016–2030 (GTS), established the goals to reduce global malaria incidence and mortality rates by at least 40% by 2020, at least 75% by 2025, 90% by 2030 and eliminate malaria in at least 35 countries where there were transmissions occurred in 2015 [4]. The GTS goals and milestones have been monitored in 2020 and 2025 in comparisons to the progress made in 2015. Until recently, the WHO has declared 38 malaria-free countries. About 10 countries in sub-Saharan Africa, Uganda, the Democratic Republic of the Congo, United Republic of Tanzania, Niger, Mozambique, Mali, Nigeria, Cameroon, Burkina Faso and Ghana) and India in Asia remain the high-burden countries with 70% of the global malaria cases. Following the observed resurgence in global malaria cases with 217 and 219 million cases reported in 2016 and 2017, respectively [5,6], a new “high burden to high impact” response, a country-led strategy was proposed by the WHO and its partners 2018 [2]. The approach aims to sustain the gains in controlling malaria and accelerate global progress in fighting the disease in line with GTS and the global Sustainable Development Goals (SDGS) related to health [2]. This country- evidence-led approach calls for the government commitments, multisectoral coordination and maximise the use of local resources for maximum impact in reducing malaria burden especially in countries hardest hit by malaria [2].

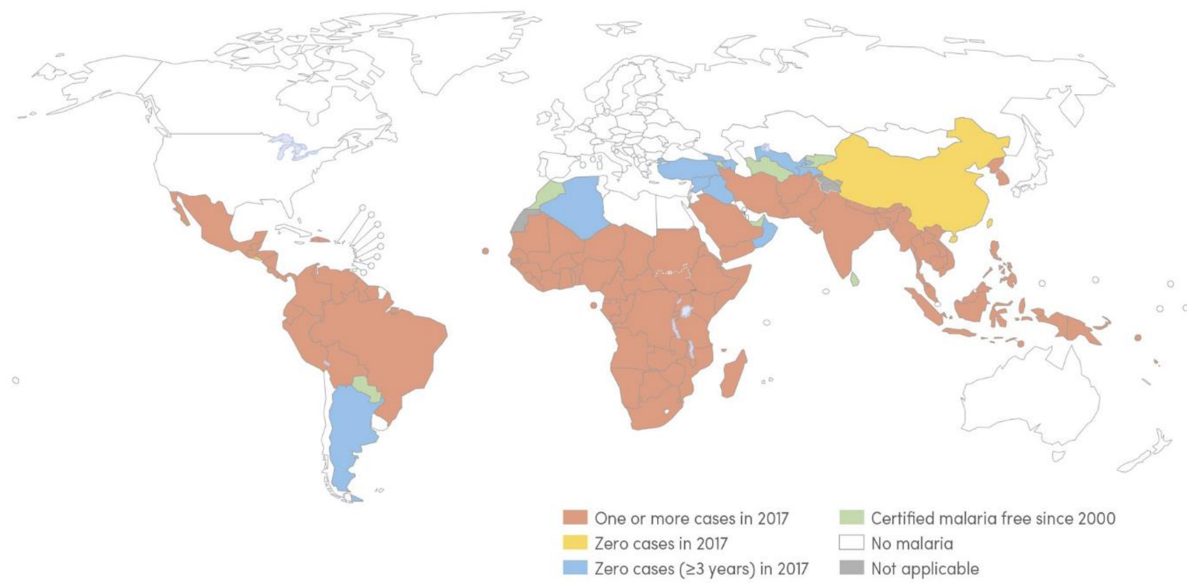

Figure 1.1: The global malaria map showing countries with malaria cases in 2000 and their status in 2017, adopted from the WHO database 2018 [1].

1.1.1 The current malaria status in Tanzania

Notwithstanding the success in malaria control in Tanzania, 93% of the population still lives in malaria-endemic areas [7]. Tanzania is among the 10 sub-Saharan African countries where malaria is hitting hardest [2]. A large part of the country (60%) is characterised by stable perennial transmission while 20% of the country experiences unstable malaria transmission but varies across the seasons, and the remaining 20% part of the country experiences stable malaria with seasonal variation [7]. Malaria infection in the country is predominantly (96%) mediated by *Plasmodium falciparum* with the remaining 4% contributed by *P. malariae* and *P. ovale*.

There had been an increase in malaria prevalence, with national average prevalence from 9.5% in 2012 to 14.8% in 2016 [8,9].The recent Tanzania Malaria Operational Plan highlighted malaria prevalence among under-five children varied by regions ranging from less than 1% in the Northern highland zones in Arusha to 41% in the lake zones of the country based on a rapid diagnostic test (RDT) [7]. The Tanzania Malaria Indicator Survey (TMIS) of 2017 found a decrease in an overall malaria prevalence, 7% among children with age between 6 months and 59 months using RDT [10]. The prevalence largely varied across regions from less than 1% in Arusha, northern highlands to the highest as 24% in the Kigoma region, northwestern Tanzania (Figure 1.2) [10]. The National Bureau of Statistics, however, indicated a significant decreased in malaria prevalence rate from 14.4% in 2015 to 7.3% in 2017 still far from the national target of less than 1% prevalence in 2020.

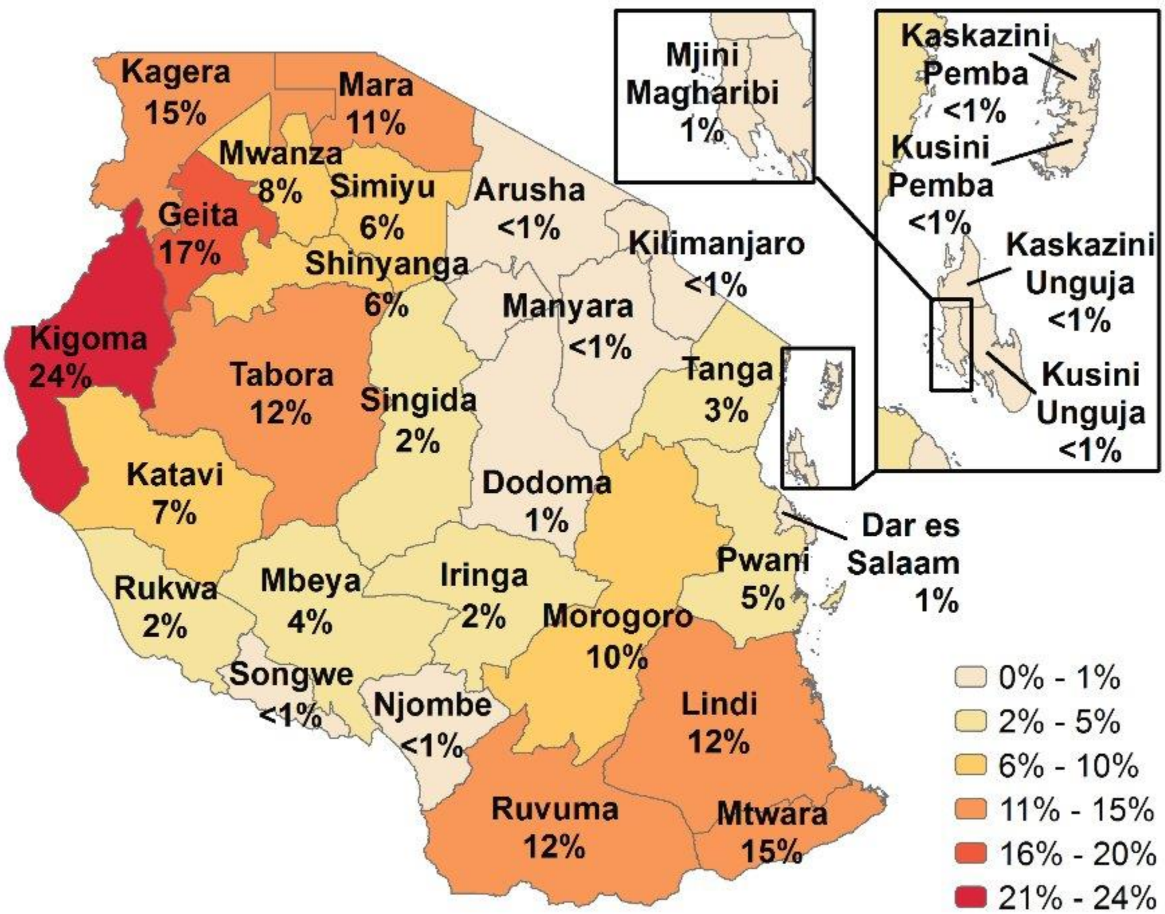

Figure 1.2: Tanzania map showing variations on malaria prevalence in children age between 6 months and 59 months across regions, estimated using a rapid diagnostic test (source TMIS 2017)

1.2 The biology of malaria infection and parasite lifecycle

Malaria is caused by a protozoan belonging to the genus *Plasmodium*. There are 200 *Plasmodium* species based on their morphology, host they infect, and geographical distribution. Five major *Plasmodium* species cause malaria infections in humans including *Plasmodium falciparum*, *P. malariae*, *P. ovale*, *P. vivax* and *P. knowlesi*. Among these species *P. falciparum* and *P. vivax* are the most efficient species [1]. About 98% of the malaria transmission in African regions and 50% of malaria cases in the WHO South-East Asia Region are due to *P. falciparum*. Similarly, *P. falciparum* accounted 71% of cases in the Eastern Mediterranean and 65% in the Western Pacific. About 75% of malaria transmission are due to *P. vivax* [1]. Malaria is mainly transmitted to human through an infections bite from a female *Anopheles* mosquito. In rare cases, a human may contract malaria infection through transfusion of parasites contaminated blood, or a mother to foetus infection during pregnancy or giving birth.

Malaria parasite development involves a complex lifecycle, including the asexual stage in the human host and sexual stage in the mosquito (Figure 1.3). The asexual phase of parasite development begins by the female *Anopheles* mosquitoes injecting sporozoites to the human host during a blood meal. The sporozoites migrate to the liver and later invade hepatocytes and undergo exoerythrocytic or pre-erythrocytic phase. The hepatocytes burst and release thousands of merozoites into the bloodstream. For *P. vivax* and *P. ovale* infections, the parasites can remain dormant in the liver for many months, difficulty to detect and if untreated can cause malaria relapse. The merozoites in the bloodstream invade red blood cells, multiply and cause the raptures of the cells and infect more erythrocytes, leads to fever and other symptoms. During blood meal, the female *Anopheles* mosquito ingests mature gametocytes, the sexual forms of the malaria parasites. In the mosquito gut, the gametocytes undergo sexual reproduction, growth and produce the infective sporozoites that move to the salivary glands of the mosquito.

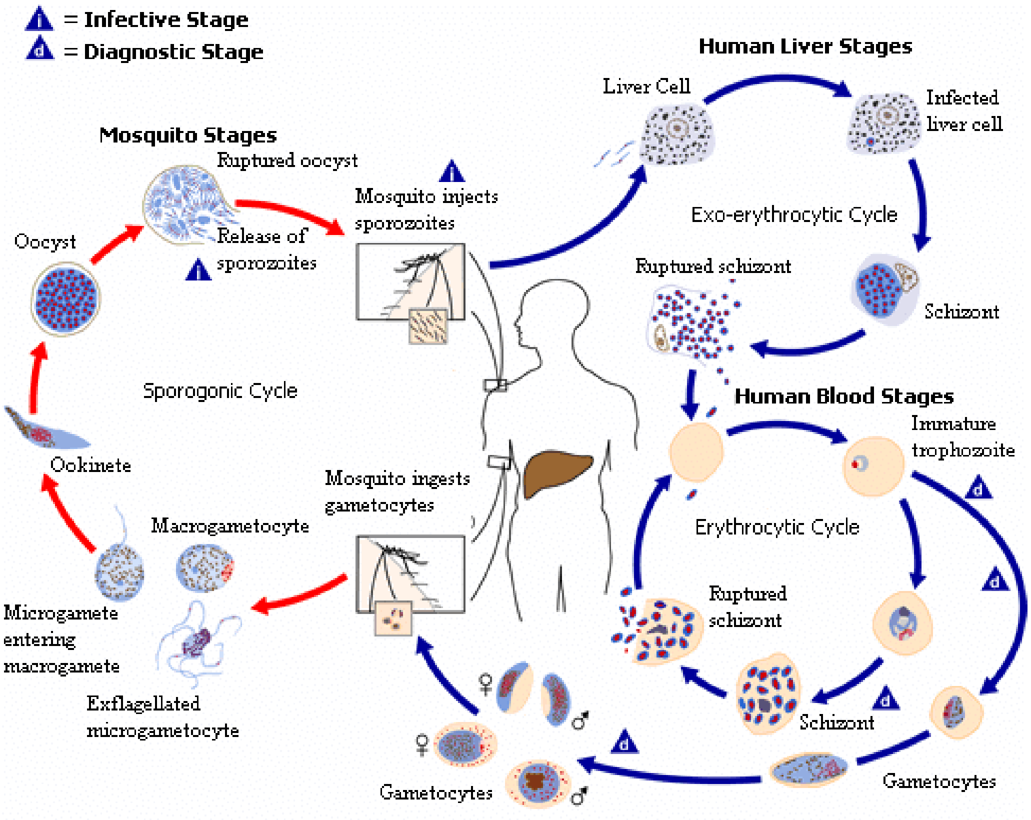

Figure 1.3: Schematic diagram showing the malaria parasite life cycle in both human and mosquito host, adopted from the Center for Disease Control and Prevention (CDC)

1.3 Other mosquito-borne diseases

Mosquitoes transmit numerous diseases, such as malaria, dengue fever, chikungunya, yellow fever, Zika virus, West Nile virus, filariasis, and Japanese encephalitis [11]. Transmission and risk of mosquito vector-borne diseases are rapidly increasing due to global climatic and environmental changes, rapid growth and unplanned urbanization, rising global travel of people, trade of goods and evolving new strains of pathogens [11]. The conditions also favour the proliferation and enhancement of the efficiency of the mosquito vectors.

Other than Anophelines, Culicine vectors such as *Aedes*, *Mansonia* and some members of *Culex* (*Cx*) *pipiens* complex and *Cx. univittatus* complex are the most widespread mosquito species and commonly known as “house mosquito” and contributes a larger proportion of the indoor population density that cause biting nuisances in both rural and urban areas [12-15]. Most of these vectors are neglected and less investigated; however, some members of these species may harbour arboviruses and filarial worms which affect more than 1 billion people globally

[16,17]. For example *Wuchereria bancrofti,* the causative agent of lymphatic filariasis (LF) [18,19]. LF is the second most common mosquito-borne disease after malaria [20]. LF disproportionately affects communities in sub-Saharan Africa and Asia, and is considered as one of the leading causes of long-term disability in the world [19]. Tanzania is the third country in Africa in terms of people at risk (34 million) and high cases, about 6 million people with disabilities due to LF [18,21], despite high coverage of mass drug administration (MDA) control strategies. The prevalence of the disease varies across the country, with a high prevalence of 45-60% along the coast to 2-4% in western Tanzania [18,21].

Cx. quinquefasciatus, a member of *Culex (Cx) pipiens* complex has become an increasingly important vector of LF transmission in East Africa [18]. A study on the transmission and prevalence of *Bancroftian filariasis* in coastal areas of Tanzania found *Cx. quinquefasciatus* was the second leading in LF transmission, and its biting density occurred throughout the year and was 22 times higher than that of *An. gambiae* s.l. [22]. Similarly*, Cx. pipiens* complex, primarily *Cx. quinquefasciatus*, were found to be the most abundant Culex species in rural southern Tanzania, and contributed to more than 79% of indoors human-biting risk [15].

Arboviruses include Rift Valley fever, dengue, chikungunya, yellow fever, Zika, Sindbis, Wesselsbron, O'nyong-nyong and West Nile [16,17]. Several sporadic outbreaks of arbovirus infections have been reported recently, including Rift Valley fever in Kenya and Tanzania as well as dengue fever [23-25] and chikungunya [26,27]. Moreover, the biting nuisances caused by the overwhelming number of *Culex* and *Mansonia* mosquitoes could jeopardize community acceptability of the primary malaria vector control methods, LLINs and IRS [16, 17]. Culicines are characterized by highly opportunistic feeding behaviours on both humans and animals, thus increasing chances of transmitting zoonotic diseases [28]. Understanding the diversity, distribution of culicine vector populations, their response to insecticides and implication in diseases transmission is of paramount importance in the establishment of a sustainable integrated vector surveillance and control programmes. However, Culicines are less studied compared to Anophelines as information on Culicine species composition, susceptibility to insecticides are lacking.

1.4 Malaria vector control methods

Vector control interventions along with access to early diagnosis and treatment as well as urbanization and improvements in living standards and health care have contributed significantly to the reduction of malaria morbidity and mortality in malaria and vector-borne disease endemic countries [1,29]. Between 2000 and 2015, LLINs alone has contributed to a 68% reduction in malaria cases in sub-Saharan Africa [29].

Malaria vector-control tools primarily aim to protect all populations at risk for malaria, by preventing infective mosquito bites, reduce vector abundance, and local malaria transmission intensity thus reduce disease incidence and prevalence [30]. In 2008, the Roll Back Malaria Partnership (RBM) developed the Global Malaria Action Plan (GMAP) for reducing malaria burden through “scaling up for impact” (SUFI) through core malaria interventions, including LLINs and IRS [31]. In sub-Saharan Africa, malaria vector control methods rely mainly on wide-scale coverage and usage of insecticidal-based interventions such as LLINs and IRS in which insecticides are applied indoors on the walls and roofs [6,32]. LLINs and IRS have been widely used as the front-line interventions against malaria vectors because they are cost-effective, highly effective, readily implemented and easily to be scaled up, especially in sub-Saharan African where there is the highest malaria burden with more than 80% of the global malaria cases and deaths. Between 2016 and 2018, about 297 million LLINs were delivered in 11 high-burden nations, and 93 million people globally were also protected by IRS targeting the focal areas in the country with high disease prevalence [1]. These core interventions also provide synergist benefit through integrated vector control strategies by controlling other vector-borne diseases, such as arboviruses and filarial worms transmitted by Culicine vectors [33,34]. Four primary classes of insecticides approved for public health purposes against vector and diseases are categorized according to their chemical composition/active ingredients and mode of action, including pyrethroids organophosphates, carbamates and organochlorines [35,36].

In Tanzania, during the early 2000s, the conventionally treated nets with deltamethrin were primarily used as indoor malaria vector control method. Currently, pyrethroids impregnated LLINs mainly permethrin-impregnated Olyset® (Sumitomo) nets and PermaNet 2.0 deltamethrin, have been widely scaled up and used as the major and most cost-effective method of malaria vector control [37,38]. At the same time, IRS coverage is limited to a few parts of Tanzania, principally in the mainland north-western regions, which have the highest malaria prevalence [7,38]. Over the past years, efforts in sustaining ≥80% countrywide universal LLINs coverage in the country have been through freely universal coverage mass distribution campaigns [37,39], discounted vouchers from antenatal care and routine immunisation visits [40], and keep up school children program (School Net Program) [41]. Generally, there has been a marginal increase in net coverage, access and usage in the country based on the 2017 TMIS compared to the data obtained in the 2015-16 TDHS-MIS [10]. The recent 2017 TMIS report found that three-quarters of households (78%) in the country own at least one insecticide-treated nets (ITNs) and only 45%

of the households have at least one ITN for every two people who slept in the house the night prior to the survey [10]. About 63% of the population in the country had access to the nets, while only 52% reported having slept under an ITN the night before the survey [10]. The first IRS campaign was implemented in Karega region in western Tanzania in 2006, using lambda-cyhalothrin (pyrethroids) and later extended to other lake zone regions of the country in 2011. In 2009 IRS operations were conducted with bendiocarb (carbamate). Unfortunately the vector populations reduced susceptibility to bendiocarb [42] thus later shifted to pirimiphos-methyl (Actellic 300SC), an oprganophoshates in 2014.

Historically, before the introduction of dichlorodiphenyltrichloroethane (DDT), larval source management (LSM) was the primary and most affordable malaria control method. This old method substantially contributed in malaria elimination efforts in Brazil [43], Egypt [44], Zambian copper mines [45], Italy, United States of America, Israel [46], and elsewhere [47-50]. LSM approaches target immature stages of mosquitoes (larvae and pupae) by preventing their development into adults through; (i) modification of mosquito aquatic breeding habitats such as drainage/filling of stagnant water [51] or compete for coverage of water bodies with materials that are impermeable to mosquitoes [49]; (ii) habitat manipulation involves temporal changes of the aquatic mosquito habitat such as temperature manipulation by providing shades on the aquatic habitats [52,53]; (iii) larviciding through regular application of biological/microbial larvicides such as *Bacillus thuringiensis israeliensis* (Bti), *Bacillus sphaericus* (Bs) [54], and insect-growth regulator such pyriproxyfen [55] into confirmed aquatic mosquito breeding sites; and (iv) biological control using natural enemies such as predatory fish (*Tilapia niloticaor*, *Gambusia affinis*), parasites that prey on mosquito larvae/pupae in the habitats [56]. The LSM is, however, effective in areas where the aquatic mosquito breeding sites are “few, permanent and findable”. A community-based LSM through larviciding has been successfully implemented as a supplementary vector control and substantially contributed in reducing malaria transmission by 31%, in urban areas in Dar es salaam, Tanzania [57]. In rural Kenya, larviciding with *Bti* reduced *Anopheles* larval density and human biting rates by 95% and 92% respectively [54].

1.5 WHO calls for integrated vector management

Historically, in the early 1980s the concept of integrated diseases control “the Blue Nile Health Project” was developed and successfully implemented in Sudan [58]. The project aimed to control malaria, diarrhoea and schistosomiasis simultaneously, along with the irrigated scheme of Sudan [58]. The WHO Global Vector Control Response strategy recognizes the need for a comprehensive and integrative evidence-based approach tackling pathogens transmitted by different vector species [59,60]. WHO firstly adopted the concept of IVM in 2014, and it advocates the use of a range of approved vector control tools either alone or combination in collaboration with other sectors to improve efficiency and sustainability of vector control programmes [60]. WHO simply defines IVM as a "rational decision-making process for the optimal use of resources for vector control" [59,60]. The five key elements of the IVM includes (i) evidence-based decision making; (ii) integrated approach; (iii) collaboration within the health sector and other sectors; (iv) advocacy, social mobilization and legislation; and (v) capacity building [60].

IVM could also be promoted in countries where disease co-exists thus provides a synergistic effect in controlling multiple diseases concurrently [61]. For example, malaria and LF diseases co-exist in most African communities [62]. Although MDA campaign supported by WHO remain the mainstay of the current strategy for LF elimination [19], IVM and control programmes could accelerate the interruption of LF through simultaneously targeting Anopheline and Culicine vector species transmitting malaria and LF [63,64]. Ogoma *et al*. found that house screening could reduce indoor densities of both Anophelines and Culicines, improve uptake of control interventions by the community, and offer great potential for integrated vector control of LF, malaria and arboviruses [65]. Chandra and colleagues described successful and comprehensive IVM activities implemented by the Zambian National Malaria Control Programme that also advocates community engagements and collaborations with other sectors, including research institutions and public sectors [66]. The implementation and uptake of the IVM approaches in most countries remain slow due to lack of political support, lack of commitments within the health sector, minimal intersectoral collaborations and limited understanding of IVM approach.

1.5.1 Integrated pest and vector management

Integrated Pest and Vector Management (IPVM) programme was recently developed by FAO and UNEP to address the dual contribution of agricultural practices in food production and nurturing vectors [67]. IPVM was built following a successful implementation of IPM practices that integrate non-pesticides and minimal pesticides usage in agriculture to prevent resistance development in crop pests as established by FAO through the Insecticide Resistance Action Committee (IRAC) and IVM strategies in the public health sector. The programme empowers the community through Farmer Field Schools (FFS) in a rational decision-making process on management of both vectors and crop pests, including environmental management, and reduces dependence on the use of pesticides. IPVM was successfully piloted in Sri Lanka between 2002 and 2006 for the reduction of malaria and Japanese encephalitis [67]. Insecticide resistance management in vector species could be a useful component of IPM.

1.6 Challenges to effective and sustainable malaria vector control

Despite the achievements made in malaria control, the current vector control interventions are limited based on the fact they rely on a limited number of insecticides approved for vector and disease control [1]. At present LLINs are treated only with pyrethroids insecticide and IRS programmes rely on pyrethroids, and a few of organophosphates, carbamates and organochlorine [1]. However, the major and inevitable challenge of continuous use of insecticides-based control interventions is the selection pressure it poses in targeted organisms, resulting in the development and spread of insecticide-resistant vector populations. Insecticide resistance in the mosquito is an evolutionary occurrence, in which the mosquito vector tolerate and survive a known sub-lethal dose of insecticide that would otherwise kill the susceptible populations [68]. An epidemiological translation of resistance in vector populations could mean failure of the control interventions and linked to an increasing malaria transmission/ resurgence in malaria cases, however, there is limited evidence [69-72], and the available ones are still contradictory [73-75].

Globally, the first reports on resistance in local mosquito vectors to DDT were reported in the 1950s in Culicine in the United States of America, and in 1951 in *An. sasharovi* in Greece 5 years after DDT was firstly introduced for malaria control and eradication in 1946 [76,77]. In Africa, DDT resistance was initially detected in *An. gambiae* in Burkina Faso in 1967 [78], and ever since insecticide resistance has been widespread across countries endemic to malaria [69,79-81]. Between 2010 and 2018, at least one malaria mosquito species in 73 malaria-endemic countries were found resistant to one of the four classes of insecticides [1]. Globally, pyrethroids and organochlorines resistance in malaria vectors is more prevalent reported in at least 68% and 63% of the study areas, respectively [1]. Carbamates and organophosphates resistance is, however, less widespread as it is only reported in 31% and 26% of the study sites, respectively [1]. About 26 malaria-endemic countries have confirmed resistance in malaria vectors to all four classes of public health insecticides [1]. However, the global magnitude of insecticide resistance challenge is still limited because many countries do not carry out sufficient routine insecticide resistance surveillance due to limited resources, and even when performed the data are not reported in a timely manner.

In Tanzania, resistance to all four classes of insecticides recommended for malaria vector control has been confirmed from the selected sentinel sites by 2017 [82,83]. At the species level, *An. gambiae* species complex, the primary malaria vectors have developed resistance to all recommended classes of insecticides [83,84]. Similarly, *An. funestus* s.s, the most efficient malaria vector in the country, has been confirmed highly resistant across pyrethroids (deltamethrin, permethrin lambda-cyhalothrin) and DDT [82]. Insecticide resistance has been reported to be highly prevalent in agricultural settings linked with extensive pesticide usage [85-87].

1.6.1 The underlying insecticides resistance mechanisms in mosquito vector species

Worldwide, insecticide resistance in vector species is mediated by four major resistance mechanisms [68], including;

i) **Target-site resistance** occurs when there is modification or mutation in the receptors of the nervous system of a mosquito which are main target sites of an insecticide. Target site mutations prevent the insecticides from binding the target sites, thus reduce its killing efficiency. Mutations on voltage-gated sodium channels of a mosquitoes is associated with pyrethroids and DDT resistance, while acetylcholinesterase (AChE) is linked to organophosphates and carbamates resistance. A common target-site resistance mechanism is knock-down resistance (kdr) caused by a single mutation in the sodium channel, due to substitution of leucine to phenylalanine (commonly called West Africa mutation/ L1014F kdr) or to serine (East Africa mutation/ L1014S) [88-90]. In Tanzania, both kdr Eastern (L1014S) and kdr Western (L1014F kdr) variants have been confirmed in *An. gambiae* s.s and *An. arabiensis* wild populations, which are resistant to pyrethroids [91]. However, L1014S is predominant in *An. gambiae* s.s, while Western variant (L1014F kdr) has been frequently confirmed in *An. arabiensis* populations [91].

   The target site resistance is considered as an effective resistance mechanism as it can influence cross-resistance to all insecticides that share target sites. Cross-resistance occurs when resistance to one insecticide confers resistance to a second insecticide even if the mosquito has not been exposed to the second insecticide [92].

ii) **Metabolic resistance** is due to overproduction of enzymes that are capable of metabolising/detoxifying foreign materials, including insecticides into non-toxic substances before they reach the target site of action [93]. The enhanced metabolism in mosquitoes can be due to gene duplications or mutations in the coding gene portion of the enzymes, thus producing the more efficient detoxification of the insecticide [92]. Three major enzymes families that can metabolise insecticides in mosquito body including, elevated levels of cytochrome P450 monooxygenases [94] leading to detoxification of pyrethroids, organophosphates and a lesser extent carbamates [95]. Metabolic resistance mechanism has been reported in local malaria vectors populations in Tanzania. Over-transcription of CYP6P3 and CYP9J5 genes family of P450s has been associated with pyrethroid and DDT resistance, in *An. arabiensis* in Dar es Salaam, Tanzania [96].

Over-production of esterases in mosquitoes have been implicated in pyrethroids and organophosphate resistance. For example, high levels of two carboxylesterases, Estα21 and Estβ21 esterases in *Cx. quinquefasciatus* populations have been associated with organophosphates resistance [97]. Esterases have been reported to be able to metabolise pyrethroids in *An. gambiae* species [98]. Glutathione S-transferases (GSTs) are members of a large family of intracellular multifunctional enzymes that plays a significant role in DDT resistance including dehydrochlorination of DDT into a non-toxic product DDE [99], and a secondary role in pyrethroids resistance [100].

iii) **Behavioural resistance** refers to any changes in mosquito behaviours that could influence the avoidance of lethal or sub-lethal doses of an insecticide [101]. The behavioural adaptations in mosquitoes could be due to either mosquito escape the insecticide-treated surfaces/materials after making physical contact or short contact duration “contact irritancy” or avoid insecticide exposure without making any contact “spatial repellency”[92]. The mosquito behaviour changes including a shift from indoor to outdoor feeding and resting [102,103], change in feeding time [104], deviation in blood preference host to cattle, and shift in vector dominance from *An. gambiae* s.s to *An. arabiensis* [102,105,106], following implementations of vector control interventions. These behavioural alterations in mosquitoes are likely to undermine the effectiveness of the indoor interventions [105,107]. A study conducted in Kilombero Valley, south-eastern Tanzania by Russel *et al*. in 2011 showed an increase in outdoor feeding patterns among *An. arabiensis* and *An. funestus*, during early evenings after the implementation of LLINs [108]. In southern Benin West Africa, Moiroux *et al*. found a large proportion (26%) of *An. funestus* populations being caught early morning hours of the day between 6 a.m. and 9 a.m. outdoors, three years after scaling up universal LLINs coverage [104].

1.7 The linkages between agriculture and malaria

Agriculture contributes to economic development and food security. In sub-Saharan Africa, agriculture is the backbone of the economy and major source of food and livelihood. However, some agricultural practices could increase the risk of malaria transmission, and prolonged use of agricultural pesticides are likely to influence insecticide resistance development in the mosquito [109]. Farming activities associates with irrigation systems provide potential aquatic breeding sites for mosquitoes thus increase human biting rates (Figure 1.4).

Figure 1.4: An example of irrigated rice farming practices where larvae mosquitoes breed, in Ulanga district, Tanzania (picture taken by Nancy Matowo, in dry farming season 2017)

Some agricultural, cultural practices increase risks to malaria and other mosquito-borne diseases transmission. For example, in rural Tanzania during farming seasons, especially in Kilombero and Ulanga districts where this study was carried out, the majority of rice farmers tend to migrate with young children from their houses to distant farms sites for long periods [110]. These farmers improvise temporary semi-open shelters for sleeping during farm preparation, planting, weeding and harvesting period [110]. The temporary farm huts are usually not protective against mosquito bites, poorly/incomplete constructed with wood and thatched materials difficulty to fix ITNs, and absence of proper building materials for IRS implementation, thus increased risks to infectious mosquito bites and malaria [111]. These farming communities, while in their remote farms, do not have access to the nearest health care facilities [112]. Other studies showing the correlation between agriculture and increase in mosquito density and risks of malaria transmissions included a study in urban settings of Côte d'Ivoire, the risk of contracting malaria was affected by specific crop systems and farming practices that created more aquatic breeding sites for *Anopheles* mosquitoes [113]. High (19.2) annual entomological inoculations rates (EIR), an estimated number of infectious bites per year an individual person received was reported in urban areas of Ghana, where irrigated farming practices are taking places compared with non-agricultural sites which were 6.6 [114]. Epidemiologically, malaria prevalence has been found significantly higher in children from the communities practising urban agriculture compared with children located far from agricultural areas in Ghana [115]

1.7.1 Pesticide usage in agriculture might accelerate selection of resistance in malaria vectors

The primary source of insecticide resistance in malaria vectors is through exposure to public health insecticide-based interventions [116-118]. However, agricultural pesticide use are likely to enhance selection pressure in the mosquito, thus development of resistant strain to the insecticide used in vector control [109]. Indeed, large agricultural expansions after World War II overlapped with most of malaria vector control campaigns [119]. Although establishing a definitive association between agricultural pesticide use and mosquito resistance has been challenging, multiple studies have shown the impact of prolonged use of agricultural pesticides on mosquitos' responses to the killing agents, through increasing selective pressure on larvae, thus driving the development of adult resistant mosquito populations [85,86,120-126]. Exposure to sub-lethal doses of pesticides, herbicides and other chemical pollutants causes metabolic stress to mosquito larvae that stimulates the mosquitos' detoxifying system mechanism resulting in intolerance of higher insecticides exposure levels [85,127-129]. High frequencies of kdr resistance mutation (L1014F and L1014S), and acetylcholinesterase (*ace.1 G116S)* mutation in *An. gambiae s.l,* were both associated with the use of organophosphate and carbamates by vegetable and cotton growers and IRS programme in Burkina Faso in West Africa [130]. Table 1.1 shows some studies across countries that assessed the effect of agricultural pesticide in driving selection pressure in local mosquito populations.

Most of the current agricultural insecticides have similar chemical active ingredients, similar target sites and mode of action as the ones recommended by WHO for public health against vector-borne diseases [120,122]. Many of public health insecticides are repurposed from a large and robust agricultural insecticide pipeline, thus easy practising resistance mitigation in crop pest while complicating insecticide resistance management programmes in malaria vectors [131,132].

Table 1.1: Example of studies that assessed the plausible association between agricultural pesticide use and selection of insecticide resistance in mosquito

Reference	**Country**	***Anopheles* species**	**Insecticide**	**Comment**
Koffi et al. [133]	Côte d'Ivoire	*An. gambiae* s.l.	Organochlorides, carbamates and pyrethroids	High resistance levels were detected in *An. gambiae* s.s collected from cotton, vegetable and horticulture cultivation sites
Nkya et al. [121]	Tanzania	*An. gambiae* s.s	Pyrethroids, organophosphates and organochlorides	Repeated exposure of mosquito larvae to agricultural pesticides select for cuticle, metabolic and synaptic transmission-based resistance mechanisms at adult stage
Nkya et al. [96]	Tanzania	*An. gambiae* s.l.	Pyrethroids	Deltamethrin resistance was higher in larvae than adults *An. gambiae* populations and highest in agricultural areas compared with the urban non-agricultural sites
Chouaïbo et al. [134]	Cameroon	*An. gambiae* s.l.	Pyrethroids, DDT	Reduced susceptibility in anopheles' populations to DDT and pyrethroids was significantly higher during insecticide application in cotton-growing regions than unsprayed regions. Pyrethroids were simultaneously used for agricultural pest management and vector control
Yadouléton et al. [124]	Benin	*An. gambiae* s.l	Pyrethroids, DDT	Knock-down resistance gene was highly spread at higher frequency in *An. gambiae* populations sampled from the cotton-growing areas where pyrethroids were extensively used compared with those sites with low or no use of insecticides

Abuelmaali et al. [135]	Sudan	*An. arabiensis*	Pyrethroids, organophosphates and carbamates	Highest level of resistance to bendiocarb in *An. arabiensis* was found in peri-urban areas linked with the intensive application of bendiocarb against crop pests, and poor disposal practise of pesticide leftovers
Mzilahowa et al. [136]	Malawi	*An. quadriannulatus* and *An. arabiensis*	DDT	Reduced susceptibility to DDT in *An. arabiensis and An. quadriannulatus* was attributed to selection pressure occurred on larvae mosquito populations likely from the pesticide residues used in agriculture
Talom et al. [137]	Benin	*An. coluzzii*	Non-insecticide (Copper) and lambda-cyhalothrin	The presence of lambda-cyhalothrin and non-insecticidal xenobiotic residues such as copper metal and in aquatic mosquito breeding habitats where vegetable cultivation had taken places, enhanced selection of lambda-cyhalothrin resistance in *An. coluzzii* populations
Hien et al. [138]	Burkina Faso	*An. gambiae* s.l.	Pyrethroids	Deltamethrin and permethrin residues were found in water samples from the convectional cotton growing areas which could be of the contributions to deltamethrin resistance in the wild adult *An. gambiae* s.l. populations
Mouhamadou et al. [139]	Côte d'Ivoire	*An. coluzzii*	Neonicotinoids	The observed neonicotinoids resistance in wild adults Anopheles mosquito was linked to imidacloprid and acetamiprid that were exclusively and widely used for cocoa and rice crop protection

1.8 Agricultural pesticide usage practices

All over the world, pesticides are extensively used in agriculture for crop protection and increasing productivity. In West Africa, an estimated 90% of insecticide has been used in cotton crop protection. For example, in Benin, insecticides applied against cotton pests twice a month after every three consecutive months each year [123]. Pesticides have also been used to combat pests and diseases of livestock. In Tanzania, the agricultural sector contributes 25% of the national GDP and serves as, primary sources of livelihood, food and employment for an estimated 80% of the population. However, pest infestations and diseases remain the main barrier to increased crop yields. Pesticide application has been the first-line measure of pests and diseases management and control by both small-scale subsistence and large-scale commercial farmers. Most of the pesticides in the country are imported products, and their formulation, repacking, and distributions is governed by law and regulations [140-142]. Pesticide dealers mainly agrovet dealers are required to sale only authorised products, while adhering to the safety and handling practices to minimize pesticide hazard to human and environment, according to the Plant Protection Act of 1997 [140] and Plant Protection Regulations of 1999 [141] (Figure 1.8). Nonetheless, there has been weak government enforcement of pesticide legislation and regulations and poor surveillance system, thus lead to unregulated pesticide importation and sales in the country [143].

Figure 1.5: Example of an agrovet store and pesticide truck suppling pesticides in rural southern Tanzania (Picture taken by Nancy Matowo)

A significant increase in pesticides and formulations importation into the country has been reported [144], between July 2013 and June 2014, whereby 11,482 metric tons (MT) of pesticides formulated products were imported to Tanzania compared to the 2,500 products imported in 2003 [144]. Among this 12.1% (1,390.4 MT) were insecticides, 21.1% (2,424.9 MT) were herbicides, 56.8% (6,528.1 MT) were fungicides, and the remaining 9.1% (1,058.8 MT) were other restricted categories including technical materials, fumigants and high-risk products [144]. A total of 1,182 pesticide products with various active ingredients were registered and approved by Tropical Pesticides Research Institute (TPRI) in Tanzania, with the majority being insecticides 41.7% (493) and herbicides 289 [145]. The lion share (81%) of these pesticides are used for pest control in agriculture and livestock sector while only 18% for public health measures against vectors and diseases and the remaining 1% for other purposes such as protecting house structures from insect pest damage [146].

A wide range of pesticides is used for crop protection, but insecticides, herbicides and fungicides account for over 90% of all agricultural pesticides used in the country against pests and diseases on cotton, rice, maize, coffee, vegetables and fruits cultivation. For example, in the northern part of Tanzania, small-scale farmers applied different pesticide formulations, including insecticides (59%), fungicides (29%), herbicides (10%) and a small proportion of rodenticides (2%) against pest and diseases attacking tomatoes, onions and cabbages [147]. Common classes insecticides that have been used for crop protection including pyrethroids, neonicotinoids, organophosphates, carbamates or a combination of more than one class were used for vector control [87,148]. Chouaïbou et al. found over 90% of the insecticides applied by rice and vegetable farmers in Côte d'Ivoire belonging to pyrethroids [149]. Recently, in rural southern Tanzania, various classes of agricultural insecticides and fungicides with similar compounds as those used for vector control were extensively used for pest and disease control on different crops [87].

1.8.1 General knowledge, practices of agricultural pesticide and management strategies among farmers and retailers

With increasing pesticide demand and usage in Tanzania, unfortunately, most farmers and pesticide dealers who are the principal users of pesticides are not equipped with the necessary knowledge on proper application, safe handling and storage of pesticides [150]. Training programmes to farmers and pesticide dealers on proper pesticide usage are typically facilitated by the TPRI, and Ministry of Agriculture through agricultural extension workers, however, it has not been adequately done. Most farmers are still exposed to pesticide hazards due to lack of knowledge, uncontrolled pesticide usage practices and limited support from the agricultural extension officers [145].

Figure 1.6: An example of improper pesticide handling and disposal practices among farmers in Ulanga district, southern Tanzania (taken by Nancy Matowo)

1.8.2 Farmers' perspectives and understanding of the links between agricultural pesticide usage and insecticide resistance in malaria vectors

In malaria-endemic settings, where agricultural pesticides are widely used, only few studies have investigated the plausible effect of agricultural pesticides usage and insecticide resistance devolvement in mosquito vectors. Studies have documented farmers' perspectives on malaria in relation to mosquito vectors. Most of the studies found that farmers were aware of the association between mosquitoes and malaria; however, the majority could not link mosquito ecology, biology and breeding sites and with their agricultural activities. This means they cannot associate any possible effect of the pesticides used in agriculture on mosquito ecology. A study in Ghana found that all farmers who participated in the study had never seen mosquito larvae before and were not aware that water from the agricultural practices supports mosquitoes breeding habitats in Ghana [151]. In southern part of Côted'Ivoire, most people were also found using agricultural pesticides, but could not associate with any possible effect on mosquito, including influence in insecticide resistance development [152]. The lack of awareness on mosquito ecology and biology and the concept of insecticide resistance in mosquito among farmers could have negative implications in the implementation of vector control programmes.

1.9 The Global Action Plan for insecticide resistance management in malaria vectors

Monitoring the development and distribution of insecticide resistance in mosquito vectors is one of the key components in insecticide resistance management [68]. In response to insecticide resistance threat in malaria vectors, the WHO Global Malaria Programme and its partners developed a Global Action Plan for insecticide resistance management in malaria vectors (GPIRM) which was launched in 2012 [68]. The comprehensive GPIRM, highlights essential strategies in tackling the ongoing insecticide resistance challenges in disease-endemic countries, while maintaining the effectiveness of existing malaria and disease control tools [68]. The GPIRM includes five key actions “five pillars”, classified in short-, medium- and long-term to guide all stakeholders in controlling insecticide resistance in malaria vectors (Figure 1.6).

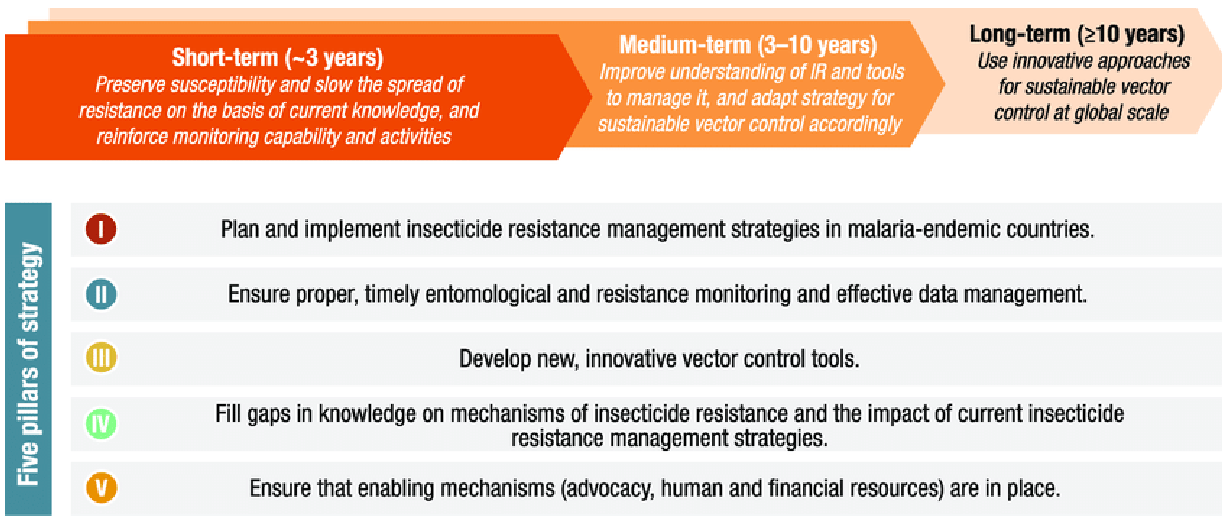

Figure 1.7: An illustration of five pillars of the Global Plan for insecticide resistance management in malaria vectors. Excerpted from the GPIRM [68].

The ultimate goal of resistance management has been to sustain the effectiveness of vector control interventions, despite the resistance challenge. Various strategies adopted from agriculture sector [153] have been recommended in managing resistance in malaria vectors to public health insecticides including (i) rotation of at least two insecticides with different modes of action at a different year; (ii) combination of pyrethroids-based LLINs and IRS with a different class of insecticide on walls in a house so that the survivor vectors are likely to contact the second insecticide; (iii) mosaic spraying of two different classes of insecticides, in different geographical locations; and (iv) mixture of two or more different classes of insecticides into a single product so that the vector is assured to make contact on the two insecticidal classes at the same time. The later approach, which involves a mixture of two or more different classes of insecticides in one product, is limited because these products are not yet in the market. Most of these strategies are, however, feasible for IRS while for LLINs is limited to a combination approach.

1.9.1 The current insecticide resistance monitoring bioassays

Detection of phenotypic resistance and resistance intensities in vector populations is commonly done by exposing mosquitoes to a known diagnostic concentration using standard WHO bioassays and Center for Disease Control and Prevention (CDC) bottle assays [154,155]. In most malaria-endemic countries, monitoring and management of insecticide resistance are, however, currently centralized and primarily done by public health officials [68,156,157]. Unfortunately, current insecticide resistance management policies and programmes are not integrated into the agricultural practices, and many do not take into account the collective contributions by various sectors in the spread of insecticide resistance [154].

The existing standard WHO procedures for monitoring insecticide resistance in malaria vectors relies, on central country-wide programmes to assess the susceptibility of common malaria vectors to insecticides in use in public health [154]. The information generated during insecticide resistance monitoring is a useful guide in making decisions on selection, allocation and implementation of appropriate vector control interventions in the geographical area concerned. In diseases endemic countries in Africa, insecticides resistance is monitored at a large scale at country or district levels (Figure 1.7A) [158,159]. In Tanzania, insecticide resistance monitoring is carried out annually in selected districts of regions assumed as a representative of the different ecological and epidemiological settings (Figure 1.7B) [156,160].

Reports obtained from insecticide resistance assessments provide essential data for country-level decision making and have immensely improved the understanding of resistance phenotypes in many countries. This global approach to insecticide resistance management has resulted in the stratification of malaria transmission zones within countries. It is now evident that as malaria transmission declines, transmission patterns become increasingly variable over space, time and demographic sub-groups [161-163]. Within a country some parts record high malaria transmission levels, while others experience low transmissions [164]. The occurrence of geographically and demographically distinct pockets of malaria transmission is becoming more and more apparent. This is likely driven by numerous factors, including the differences in how mosquito populations in different areas respond to the insecticidal interventions being implemented and the presence of agricultural contaminants in the mosquito breeding habitats. It is likely that this fine-scale variability is associated with the occurrence of residual mosquito biting hotspots, and could be contributing significantly to persistent residual malaria transmission. There is a need to investigate further the links between residual malaria transmission and spatial and temporal distributions of insecticide resistance in malaria vectors, also in south-eastern Tanzania.

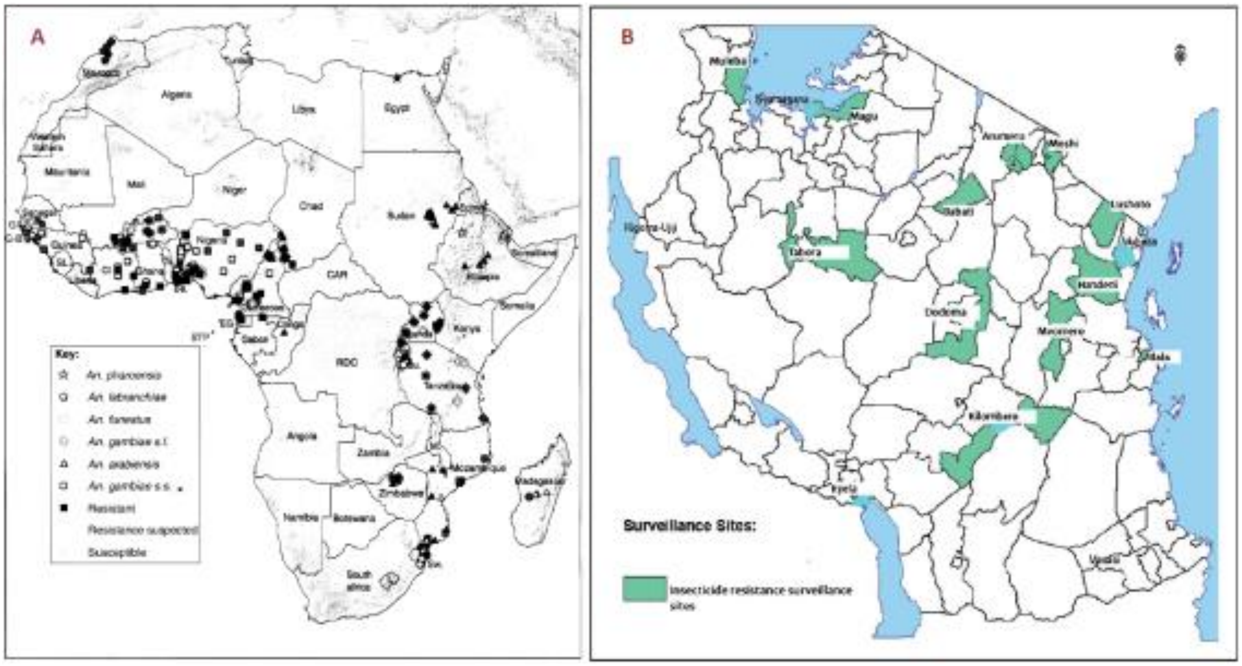

Figure 1.8: Maps showing resistance monitoring sites at the country level in Africa (A) [158] and at the regional level in Tanzania (B) [156]. Adapted from Ranson et al. and Kabula et al. [156,158].

1.9.2 Detection of insecticide resistance mechanisms

While the WHO tubes and CDC bottle standard bioassays offer a simple method to detect phenotypic resistance and resistance intensities in vector populations, these bioassays lack sensitivity and do not provide data on associated resistance mechanisms. Molecular and biochemical assays provide robust tools for detecting the cause of resistance/resistance mechanisms, thus allow the implementation of adequate resistance management strategies. Investigations of insecticide resistance mechanisms have been, however, mainly focused on target sites mutations using TaqMan assays technique in *An. gambiae* s.l. populations after being identified into sibling species compositions [165]. Relatively few studies on Taqman assays have been conducted in *An. funestus* populations [81]. Biochemical assays have been the most widely molecular technique used to measure the activity of detoxification enzymes or quantify the levels of these enzymes such as acetylcholinesterase, P450 monooxygenases, glutathione S-transferases (GST), and esterase mediating metabolic resistance in local wild *An. gambiae* s.l.

and *An. funestus* s.l. populations with a reference susceptible strain [166,167]. Other than biochemical assays, synergists bioassays serves as the simplest and most cost-effective method for assessing the metabolic resistance in wild mosquito populations [154,168]. Synergists are non-chemical molecules that block the activity of enzymes potentially involved in resistance [154,169]. Synergists increase the penetration of the insecticides into the mosquito body and counteracting the metabolic pathways that would otherwise metabolize the insecticides, thus restoring susceptibility to varying degrees [154,168]. Common synergists assays comprised the use of 20% triphenyl phosphate (TPP), an inhibitor of esterases, 20% diethyl maleate (DEM) an inhibitor of GSTs and 4% pyperonyl butoxide (PBO), an inhibitor of monooxygenase.

2 Chapter 2: Thesis rationale

2.1 Motivation

The general motivation of the study was due to the growing concern of the ongoing malaria transmission and unevenly distributions of the disease and biting rates in the communities despite the universal coverage with LLINs and improved access to health services in rural Tanzania. One possible reasons could be the mosquitoes are becoming less susceptible to the insecticidal interventions, and this situation varies among local vector populations and likely to be driven by the presence/absence of contaminants in the environment over a small geographical areas and period. This thesis focus on dynamics of insecticide resistance in *Anopheles arabiensis*, and *Culex pipiens* complex, and its associations to agricultural practices in southern Tanzania. We investigated the overall trends and changes in phenotypic resistance and the underlying resistance mechanisms in *Anopheles* and *Culex* mosquito vectors to public health insecticides. It also explored pesticide use practices in agriculture, awareness and views among farmers, pesticide dealers in relations to insecticide resistance in mosquitoes. It assessed opinions from the policy advisors on pesticides management, alternative pest control and management practices and possible ways to adopt and integrate in NMCP.

Data generated from this research will strengthen the NMCPs insecticide resistance monitoring and management strategy to sustain the achievements made against malaria and preserve efficacy of the current and new vector control tools. The findings could clarify the steps needed for an integrated pest and vector control and IRM community based programs that farmers, researchers, and experts from both agriculture and public health sectors. Such a clarification will help to improve communities' engagement with malaria research and control in line with the Global Technical Strategy for Malaria (GTS) 2016-2030 [4].

2.2 Main goal

The overriding goal of the research was to investigate spatial, temporal variations in insecticide resistance and resistance mechanisms in *Anopheles* and non-anopheles vector, and explore farming practices likely to influence insecticide resistance selection in mosquitoes. The study was also aimed at strengthening the role of farming communities in eco-health, and more specifically, in the management of insecticide resistance in malaria mosquitoes and malaria control through IVPM.

2.3 Specific objectives

The specific objectives of this study are as follows:

1) Assess fine-scale spatial and temporal heterogeneities in insecticide resistance profiles of the primary malaria vector *An. arabiensis* in rural south-eastern Tanzania

2) Investigates the fine-scale spatial and temporal differences in insecticide resistance, and resistance mechanism in the *Culex* species, and characterise *Culex* species diversity in rural southern Tanzania

3) Assess farmer's knowledge and practices on pesticide usage in agriculture and its likelihood to influence the development of insecticide resistance in disease vectors rural southern Tanzania

4) Develop and test a participatory field-based learning approach involving farmers, researchers, and experts from both agriculture and public health sectors in managing pests, mosquitoes and pesticides rural southern Tanzania

2.4 Thesis outline

Chapter 1 reviews the literature on insecticide resistance in mosquito vectors. It describes the epidemiology and global burden of malaria, the current malaria status in Tanzania, the biology of malaria infection and parasite lifecycle, other vector-borne diseases other than malaria, malaria vector control methods, the World Health Organization (WHO) call for integrated vector management (IVM) and challenges to IVM implementation, challenges of the existing vector control tools, underlying insecticide resistance mechanisms in the mosquito, agricultural pesticide usage in Kilombero, general knowledge and practices of agricultural pesticide among pesticide dealers and farmers, the role of agricultural pesticide in accelerating insecticide resistance in malaria vectors, farmer's perspectives and understanding on the linkage between agricultural pesticides usage practices and insecticide resistance in vectors, the WHO global action plan for insecticide resistance management, and challenges with the existing insecticide resistance management in malaria vectors.

Chapter 2 describes the overall motivation, main objective and specific objectives of this research.

Chapter 3 describes fine-scale spatial and temporal heterogeneities in insecticide resistance and resistance mechanisms of the primary malaria vector *An. arabiensis* in rural south-eastern Tanzania.

Chapter 4 investigates the fine-scale spatial and temporal differences in insecticide resistance, resistance mechanism in the *Culex* species, and characterise *Cx.* species diversity in rural southern Tanzania.

Chapter 5 assess farmer's knowledge and practices on pesticide usage in agriculture and its likelihood to influence the development of insecticide resistance in disease vectors rural southern Tanzania.

Chapter 6 develop and test a participatory field-based learning approach involving farmers, researchers, and experts from both agriculture and public health sectors in managing pests, mosquitoes and pesticides rural southern Tanzania.

Chapter 7 discusses the key findings of the thesis, study limitations, policy implications of the research and way forward.

3 Chapter 3. Fine-scale spatial and temporal heterogeneities in insecticide resistance profiles of the malaria vector, *Anopheles arabiensis* in rural south-eastern Tanzania

Nancy S. Matowo[1,2*], Givemore Munhenga [2,3], Marcel Tanner[4,5], Maureen Coetzee[2,3], Wim F. Feringa[6], Halfan S. Ngowo[1,7], Lizette L. Koekemoer[2,3] and Fredros O. Okumu[1,7,8]

Environmental Health and Ecological Sciences Department, Ifakara Health Institute, Ifakara, Tanzania.

Wits Research Institute for Malaria, MRC Collaborating Centre for Multi-disciplinary Research on Malaria, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Diseases, Johannesburg, South Africa.

Swiss Tropical and Public Health Institute, Basel, Switzerland.

University of Basel, Basel, Switzerland.

Faculty of Geo-Information Science and Earth Observation, University of Twente, The Netherlands.

Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK.

School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

Nancy S. Matowo*: nstephen@ihi.or.tz (corresponding author)

Published in Wellcome Open Research, 2017, 2:96
https://doi.org/10.12688/wellcomeopenres.12617.1

3.1 Abstract

Background: Programmatic monitoring of insecticide resistance in disease vectors is mostly done on a large scale, often focusing on differences between districts, regions or countries. However, local heterogeneities in residual malaria transmission imply the need for finer-scale data. This study reports small-scale variations of insecticide susceptibility in Anopheles arabiensis between three neighbouring villages across two seasons in Tanzania, where insecticidal bed nets are extensively used, but malaria transmission persists.

Methods: WHO insecticide susceptibility assays were conducted on female and male An. arabiensis from three proximal villages, Minepa, Lupiro, and Mavimba, during dry (June-December 2015) and wet (January-May 2016) seasons. Adults emerging from wild-collected larvae were exposed to 0.05% lambda-cyhalothrin, 0.05% deltamethrin, 0.75% permethrin, 4% DDT, 4% dieldrin, 0.1% bendiocarb, 0.1% propoxur, 0.25% pirimiphos-methyl and 5% malathion. A hydrolysis probe assay was used to screen for L1014F (kdr-w) and L1014S (kdr-e) mutations in specimens' resistant to DDT or pyrethroids. Synergist assays using piperonly butoxide (PBO) and triphenol phosphate (TPP) were done to assess pyrethroid and bendiocarb resistance phenotypes.

Results: There were clear seasonal and spatial fluctuations in phenotypic resistance status in *An. arabiensis* to pyrethroids, DDT and bendiocarb. Pre-exposure to PBO and TPP, resulted in lower knockdown rates and higher mortalities against pyrethroids and bendiocarb, compared to tests without the synergists. Neither L1014F nor L1014S mutations were detected.

Conclusions: This study confirmed the presence of pyrethroid resistance in *An. arabiensis* and showed small-scale differences in resistance levels between the villages, and between seasons. Substantial, though incomplete, reversal of pyrethroid and bendiocarb resistance following pre-exposure to PBO and TPP, and absence of kdr alleles suggest the involvement of P450 monooxygenases and esterases in the resistant phenotypes. We recommend, for effective resistance management, further bioassays to quantify the strength of resistance, and both biochemical and molecular analysis to elucidate specific enzymes responsible in resistance.

Keywords: Spatial and temporal variations, insecticide resistance, metabolic resistance, kdr detection, malaria control, Anopheles arabiensis, Tanzania.

3.2 Introduction

In sub-Saharan Africa, malaria vector control relies predominantly on insecticide-based, methods, namely long-lasting insecticide-treated bed nets (LLINs) and indoor residual spraying (IRS) of households. In Tanzania, LLINs are widely distributed and used as the primary and most affordable protective measure against diseases vectors [37,170,171]. The country has also recently implemented IRS, as a complementary vector control intervention in the north-western regions, with 11.6% - 14% of households currently covered by IRS [172,173]. Globally, implementation of LLINs and IRS, coupled with improved case diagnosis and treatment, as well as urbanization, improved living standards, and overall improvements in health systems, have contributed to 37% and 60% reduction of malaria morbidity and mortality respectively, between 2000 and 2015 [174]. In Tanzania, high malaria transmission remains, with an average prevalence of 14.8% in children under five years [175]. Nevertheless, the National Malaria Control Program currently has a strategic goal of reducing malaria prevalence to 1% by 2020 [176].

Despite the recent successes, the efficacy of current malaria interventions is hampered by numerous challenges, particularly insecticide resistance in malaria vectors [157-159]. This has necessitated continuous insecticide resistance monitoring, and periodic changes of insecticides used [156,160,177,178]. Some countries have put in place mechanisms to monitor the susceptibility of malaria vectors to insecticides using guidelines provided by the World Health Organization (WHO) Global Plan for Insecticide Resistance Monitoring (GPIRM) [68]. However, due to limited resources, insecticide resistance monitoring is mainly carried out only at large scale, often focusing on differences between districts or regions [156,157]. In Tanzania, insecticide susceptibility monitoring in mosquito populations is conducted at the district level, relying on designated sentinel sites in regions, considered to be representative of the whole country [156,160]. Such a generalized approach to insecticide resistance monitoring is not very effective to capture local variations, where there might be pockets of high and low malaria transmission areas [179,180]. The variations may be due to, among other factors, impacts of interventions or genetic differences in mosquito populations, in turn resulting in physiological differences in response to insecticidal pressures [179,180].

Different mosquito populations respond differently to insecticide pressure, depending on presence or absence, and type of resistance genes prevalent in the population [85,128,181]. This results in the occurrence of geographically distinct populations, which might result in transmission variability over space and time. It is likely that these fine scale-variabilities are associated with the occurrence of residual mosquito biting “hotspots”, contributing to persistent residual malaria transmission in areas where LLINs and IRS are already widely used [180]. Despite this, most vector surveillance programs still use global approaches without taking population variability into consideration. Furthermore, insecticide resistance studies have mainly focused on adult female mosquitoes, with limited studies on male populations.

The present study aimed at evaluating insecticide susceptibility of the dominant malaria vector, *An. arabiensis*, at a fine-scale between nearby villages in south-eastern Tanzania, where insecticides have been widely used for public health and agriculture, but where malaria transmission still persists.

3.3 Methods

3.3.1 Study villages

A sampling of mosquito larvae was carried out in three proximal villages of Minepa (-8.2665°S, 36.6775°E), Lupiro (- 8.3857º S, 36.6791º E), and Mavimba (- 8.3163°S, 36.6810°E), located in Ulanga district, south-eastern Tanzania (Figure 3.1). The minimum distance between villages was ~4km from Minepa to Mavimba, while the maximum distance was 9km from Minepa to Lupiro. All the villages lie between 120 and 350 meters above sea level and are located in the flood plains of the Kilombero river, between the Udzungwa mountain ranges to the north, and Mahenge hills to the south [37,170,171]. The main economic activity of the area is irrigated rice farming. The irrigation leaves rice paddies continuously flooded, creating permanent water bodies favourable for mosquito breeding habitats. It is also a perennially meso-endemic malaria area, where transmission is predominantly by *An. funestus s.s and An. arabiensis* [182-185]. Recent multiple assessments conducted in the same area have revealed that 100% of the *An. gambiae s.l* mosquitoes in this study area were *An. arabiensis* sibling species [185,186]. As such, all field-collected *An. gambiae s.l* mosquitoes are henceforth referred to as *An. arabiensis.* The main malaria vector control intervention in the area is LLINs [37,170,171].

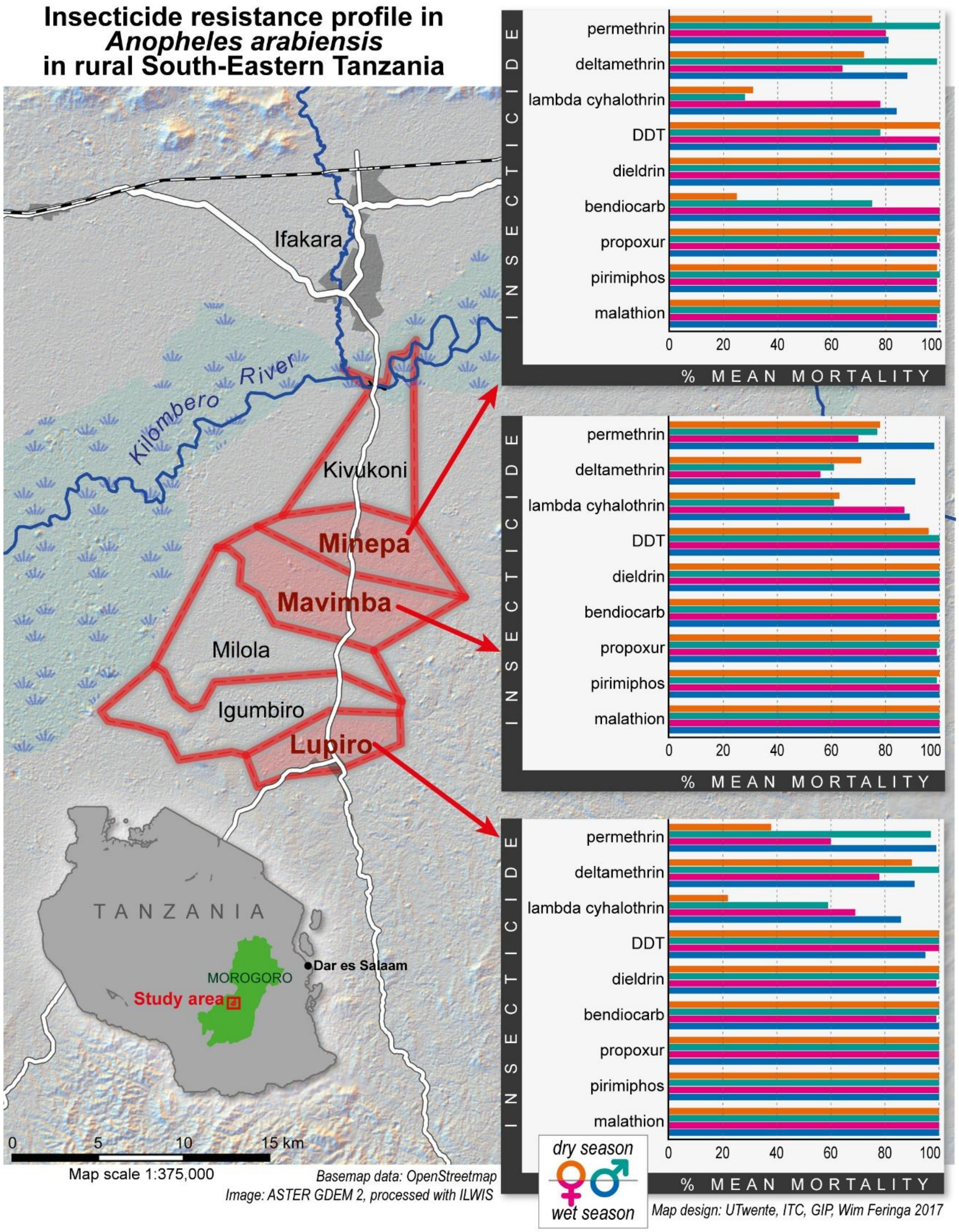

Figure 3.1: Geographic positions of the three study villages in south-eastern Tanzania. Embedded charts represent the fine-scale spatial and temporal variations of insecticide resistance profiles in both male and female malaria mosquitoes between the study villages.

3.3.2 Mosquito sampling and rearing

Larval collections were carried out in the dry season between June and December 2015, and in the wet season between January and May 2016. For each village, between seven and nine breeding sites were identified, geo-referenced, and permanently established as larval sampling points for resistance monitoring during this study. Immediately after sampling, larvae were separated into anophelines and culicines to prevent cannibalism, and for easier adult morphological identifications. After morphological identification, larvae were pooled by village and reared into adults under standard insectary conditions (temperature of 27 ± 3°C and relative humidity 70-90%) in a semi-field screen house [187]. During rearing, larvae were fed on mud, and algae collected from the respective breeding sites and supplemented with Tetramin® fish food (Tetra, Melle, Germany). Each morning, pupae were transferred into a plastic cup and placed in a net-covered cage for adult emergence. After emergence, adults were separated by sex, transferred into individual small cages with the provision of 10% glucose solution and maintained at 27-28°C and relative humidity of 70-90% for subsequent bioassays.

3.3.3 Insecticide susceptibility tests

Phenotypic resistance tests on adults were conducted following WHO guidelines [154]. Prior to susceptibility tests, the efficacy of insecticide-impregnated papers was verified against a known laboratory-reared susceptible *An. gambiae s.s.* strain (Ifakara strain) [188,189]. A group of 20 - 25 non-blood fed wild female and male mosquitoes aged three to five days were exposed for an hour to the diagnostic concentrations of 0.05% lambda-cyhalothrin, 0.05% deltamethrin, 0.75% permethrin, 4% DDT, 4% dieldrin, 0.1% bendiocarb, 0.1% propoxur, 0.25% pirimiphos-methyl, and 5% malathion. Controls consisted of mosquitoes exposed to oil-impregnated papers. During the one-hour exposure to insecticides, knockdown rates were recorded at 10, 15, 20, 30, 40, 50, and 60-minute intervals. After the exposure period, mosquitoes were transferred to holding tubes and maintained on 10% glucose solution. The final mortalities were recorded 24 hours post-exposure. Dead and surviving mosquitoes were kept separately, under preservation using silica in 1.5 ml Eppendorf tubes, for a further molecular examination of resistance genes.

3.3.4 Synergist bioassays

Synergist bioassays using piperonyl butoxide (PBO), an inhibitor of monooxygenase, and triphenyl phosphate (TPP), an inhibitor of esterases, were performed on the adult mosquitoes, to assess whether the pyrethroid resistance phenotypes observed during WHO susceptibility assays could be reversed by the synergistic activity of these insecticides, which would indicate a biochemical basis for the resistance [154,168]**.** Prior to the synergist assays, the bio-efficacy and quality of PBO and TPP synergist papers were validated against a reference laboratory colony, whose pyrethroid resistance and DDT resistance is mediated by high monooxygenases (FUMOZ-R) [190] and elevation of esterases (MBN-DDT) [191], respectively.

Due to the limited number of mosquito sample, the PBO and TPP assays were performed only on female *An. arabiensis* collected from Minepa village, and PBO test only in female *An. arabiensis* sampled from Mavimba village between the months of September and December 2016. Non-blood fed, 2-3 day old wild female *An. arabiensis* mosquitoes were used, each test consisting of 20 to 25 mosquitoes per tube with two controls. Five replicates were performed for each exposure set. Mosquitoes were pre-exposed to (either 4% PBO or 20% TPP) for 60 minutes, followed by exposure to WHO test papers impregnated with discriminatory doses of candidate insecticides (0.75% permethrin, 0.05% deltamethrin, 0.05% lambda-cyhalothrin, or 4% DDT) for another 60 minutes. To assess the effect of insecticides alone, another group of mosquitoes without pre-exposure to the synergists were concurrently exposed to each candidate insecticide only. At the same time, the same number of mosquitoes was exposed to either 4% PBO or 20% TPP only. Another group of mosquitoes was also exposed to control filter papers treated with a mixture of olive oil and acetone, and to plain filter papers with no chemicals that were used as environmental controls. During the one-hour exposure to synergist and to insecticides, the knock-down rates were recorded at 5,10,15,20,25,30,40,50 and 60-minute intervals. Mosquitoes were fed on 10% glucose solution, and mortalities from assays conducted with and without exposure to synergist were scored 24 hours post-exposure [168].

3.3.5 Knockdown resistance (kdr) detection using hydrolysis probe analysis

A hydrolysis probe assay was used to screen for L1014F (*kdr-w*) and L1014S (*kdr-e*) mutations in 220 randomly selected dead and alive female specimens, which had shown resistance to both DDT or pyrethroids, using procedures previously described [33]. DNA was extracted from the legs of each specimen using the ZyGEm prepGEM insect DNA extraction kit (Cat: PIN141106, ZyGEM NZ Ltd, Ruakura, New Zealand), following the manufacturer's guidelines, except that the reaction volume was quartered. DNA extracted (10-50ng) from each individual mosquito was then used to detect the presence of *kdr-w* and *kdr-e* in two PCR master mixtures in a CFX 96 real-time PCR machine (Biorad, Hercules, CA, USA). In each instance, positive controls comprised of a DNA template from mosquitoes with known West African (*kdr-w*) genotype sampled from Sudan (SENN-DDT, homozygous for the L1014F mutation) [192], and DNA from Burundi mosquitoes, which had been previously genotyped as homozygous for the East African (*kdr-e*) mutation, L1014S (unpublished study, Vector Control Reference Laboratory, Johannesburg, South Africa). Other positive controls were DNA templates from a homozygous susceptible colony originating from Kanyemba, Zimbabwe (KGB). The heterozygous controls were made up by mixing equal aliquots of susceptible and resistant DNA templates. A final control consisted of a master mix containing of PCR components, except the DNA template that was set up to monitor any contamination during reaction preparation.

3.4 Data analysis

Data analysis was done using R version 3.0 [193]. Susceptibility bioassay data was first summarised as mean percentage (%) mortality per insecticide per village and per season. Population susceptibility was classified according to the WHO criteria [154]. Data for the synergist tests were summarized as mean % mortality of the four replicates, and the 95% confidence intervals were calculated to estimate the probability that population means lie within the given ranges. Following an average of four replicates of each synergist test, final mortality observed 24 hours post-exposure was compared between samples with and without pre-exposure to synergists, using paired sample t-test. The time at which 50% of the experimental populations were knocked down (KDT_{50}) was determined using log-probit analysis [194]. Resistance reduction was obtained by dividing the KDT_{50} obtained from insecticide exposure with no synergist by the KDT_{50} obtained from insecticide plus the synergist ($KDT_{50\ \text{Insecticide alone}}$/ $KDT_{50\ \text{Insecticide plus Synergist}}$). The differences in mortality was considered statistically significant when $P< 0.05$. For *kdr* detection assays, the fluorescent signals detected in the experimental reactions were compared to those of the controls, and genotyping of each mosquito was done using the CFX manager software version 2.1 (Bio-Rad, Hercules, CA, USA).

3.5 Ethical statement

Permission to conduct larva sampling was obtained from the owners of the farms, after the researchers provided a description of the study aims and procedures. A brief description of the study was delivered in the local language, Kiswahili. Upon agreement, participants were asked to sign written informed forms. The proposed research went through an ethical review and obtained approval from the institutional review board of Ifakara Health Institute (Ref: IHI/IRB/NO: 34-2014) and the Medical Research Coordinating Committee at the National Institute for Medical Research in Tanzania (Ref: NIMR/HQ/R.8a/Vol.IX/1903). Permission to publish this manuscript was obtained from the National Institute for Medical Research in Tanzania (Ref: NIMR/HQ/P.12 VOL. XXII/27). Printed copies and online links to the manuscript will be provided to NIMR upon publication.

3.6 Results

3.6.1 Spatial and seasonal variability in phenotypic resistance in male and female *An. arabiensis* mosquitoes

The reference insectary-reared *An. gambiae ss* were fully susceptible (100% mortality) to all the insecticides tested, confirming the quality and bio-efficacy of the insecticide-impregnated papers used. The observed mortality in control groups was consistently below 5%, so no statistical correction was required. The WHO susceptibility test findings are summarized in Figure 2.1 and Table 2.1. There was marked seasonal and spatial variations in phenotypic resistance in both female and male *An. arabiensis* to three pyrethroids, permethrin, deltamethrin, lambda-cyhalothrin, but also to bendiocarb and DDT in the study villages.

Table 3.1: Mean percentage mortalities following exposure to insecticides for samples of 2-5 day old *Anopheles arabiensis* adults emerging from larval collections done in the dry season (June-December 2015) and the wet season (January-May 2016) from three neighbouring villages. Mortalities were recorded 24 hours post-exposure. Tests showing seasonal or spatial variability in susceptibility status are marked with symbols, ++ or ^^.

		Minepa Village % mean mortality (95% CI)		**Mavimba Village** % mean mortality (95% CI)		**Lupiro Village** % mean mortality (95% CI)	
	Insecticides	**Dry Season (n=1298)**	**Wet Season (n= 1200)**	**Dry Season (n=1345)**	**Wet Season (n= 1082)**	**Dry Season (n= 1318)**	**Wet Season (n=1115)**
Female Mosquitoes	0.75% Permethrin	75.4 (64.1-84.0)^{RR}	80.6 (69.4-88.4)^{RR}	79.2 (65.4-88.5)^{RR}	70.1 (58.7-79.4)^{RR}	37.7 (26.8-50.1)^{RR}	60.0 (48.9-70.1)^{RR}
	0.05% Deltamethrin	72.4 (60.8-81.6)^{RR}	64.4 (51.2-75.6)^{RR}	72.1 (56.8-83.5)^{RR}	56.3 (44.7-67.2)^{RR}	90.3 (80.8-95.4)^{RS}	77.5 (67.1-85.3)^{RR}
	0.05% Lambda-cyhalothrin	31.1 (21.2-43.0)^{RR}	63.3 (50.2-74.8)^{RR}	63.2 (47.0-76.8)^{RR}	87.4 (78.3-89.8)^{RR}	21.6 (13.5-32.5)^{RR}	69.0 (57.8-77.9)^{RR}
	4% Dieldrin	100^{SS}	100^{SS}	100^{SS}	100^{SS}	100^{SS}	98.8 (91.7-99.8)^{SS}
	4% DDT++^^	100^{SS}	100^{SS}	96.5 (89.7-98.8)^{RS}	98.8 (91.6-99.8)^{SS}	100^{SS}	83.5 (72.6-89.4)^{RR}
	0.1% Propoxur	100^{SS}	100^{SS}	100^{SS}	98.8 (91.6-99.8)^{SS}	100^{SS}	100^{SS}
	0.1% Bendiocarb ++^^	24.6 (16.0-35.9)^{RR}	100^{SS}	100^{SS}	100^{SS}	100^{SS}	100^{SS}
	0.25% Pirimiphos methyl	99.0 (93.0-99.9)^{SS}	99.1 (93.4-99.9)^{SS}	100^{SS}	100^{SS}	100^{SS}	100^{SS}
	5% Malathion	100^{SS}	100^{SS}	100^{SS}	100^{SS}	100^{SS}	100^{SS}
	Control (untreated paper)	1.6 (0.7-3.4)	3.1 (1.3-7.0)	0.6 (0.1-2.7)	1.3 (0.5-3.2)	3.9 (2.2-7.2)	1.9 (0.9 – 3.9)
		(n= 1326)	**(n=1198)**	**(n=1276)**	**(n=1080)**	**(n=1298)**	**(n=1110)**

Male Mosquitoes	0.75% Permethrin++^^	100^{SS}	80.5 (69.4-88.3)^{RR}	77.4 (62.4-87.6)^{RR}	97.5 (90.6-99.4) ^{SS}	97.2 (88.8-99.3)^{RS}	98.8 (91.8-99.8)^{SS}
	0.05% Deltamethrin^^	98.5 (92.7-99.7)^{SS}	87.5 (78.0-93.2)^{RR}	60.8 (43.8-75.5)^{RR}	91.3 (82.8-95.8) ^{RS}	100 ^{SS}	90.6 (81.6-95.4)^{RS}
	0.05% Lambda-cyhalothrin++^^	28.1 (14.2-48.0)^{RR}	83.5 (73.0-90.5)^{RR}	60.6^{RR}	89.4 (18.8-38.3)^{RR}	58.9 (32.4-81.1)^{RR}	85.7 (75.1-92.3) ^{RR}
	4% Dieldrin	100^{SS}	100^{SS}	100^{SS}	100^{SS}	100^{SS}	100^{SS}
	4% DDT++^^	78.4 (60.2-89.7)^{RR}	99.1 (93.4-99.9)^{SS}	100^{SS}	100^{SS}	100^{SS}	95.3 (87.2-98.4)^{RS}
	0.1% Propoxur	99.2 (93.9-99.9)^{SS}	99.1 (93.4-99.9)^{SS}	100^{SS}	100^{SS}	100^{SS}	100^{SS}
	0.1% Bendiocarb ++**^^**	75.3 (56.2-87.9)^{RR}	100^{SS}	100^{SS}	100^{SS}	99.3 (93.7-99.9)^{SS}	100^{SS}
	0.25 % Pirimiphos methyl	100 ^{SS}	99.1 (93.4-99.9)^{SS}	99.1 (93.6-99.9)^{SS}	100^{SS}	100^{SS}	100^{SS}
	5% Malathion	100 ^{SS}	99.1 (93.4-99.9)^{SS}	100^{SS}	100^{SS}	100^{SS}	100^{SS}
	Control (untreated paper)	1.4 (0.5-4.0)	1.4 (0.5-4.2)	1.2 (0.5-3.2)	2.8 (1.5-5.1)	3.8 (1.1-12.4)	0.9 (0.3-2.7)

SS: Mosquitoes were susceptible to the test insecticide (WHO assays mortality between 98% and 100%).

RS: Mosquitoes had reduced susceptibility indicating possible resistance and need for further investigation (WHO assays mortality of 90% to 97%).

RR: Mosquitoes were confirmed resistant to the test insecticide (WHO assays mortality below 90%).

++ Insecticides for which we observed differences in susceptibility of *Anopheles arabiensis* mosquitoes between dry and wet seasons, i.e. where mosquitoes were fully susceptible in one season and fully resistant in a different season in the same village.

^^ Insecticides for which we observed differences in susceptibility of *Anopheles arabiensis* mosquitoes between (nearby) villages, i.e. where mosquitoes were fully susceptible in one village and fully resistant in another.

For example, in Minepa village, the female mosquitoes were fully susceptible to bendiocarb in the wet season (mean mortality of 100%), yet highly resistant in the dry season (24.6%). Bendiocarb resistance also varied across different locations. While females collected from Minepa village in the dry season were resistant to bendiocarb, samples of the same species collected from the nearby villages of Mavimba and Lupiro during the same season were fully susceptible to the same chemical (100%). It was also observed that female *An. arabiensis* mosquitoes collected from Minepa village were fully susceptible to DDT (100%) in both seasons, while those collected from the nearby Mavimba village during dry season showed reduced susceptibility to DDT (96.5%), and resistance to the same insecticide in Lupiro village in the wet season (83.5%). Wild female mosquito populations from Minepa, Mavimba and Lupiro villages displayed variable levels of deltamethrin resistance across both seasons, but reduced susceptibility to this insecticide (90.3%) in the dry season in Lupiro. Throughout the study, female *An. arabiensis* were resistant to permethrin and lambda-cyhalothrin (mortality rates between 21.6% and 87.4%) in both seasons across the study villages.

As shown in Table 3.1, insecticide resistance variation in the male *An. arabiensis* was greater both by season and by locality and was observed for pyrethroids, DDT and bendiocarb. Males collected from Minepa were fully susceptible to permethrin in the dry season (100% mortality), but resistant to the same chemical in the wet season (80.5% mortality); those collected from Mavimba village, on the other hand, were susceptible to permethrin in the wet season (97.5%), but resistant in the dry season (77.4%). In Lupiro village, the males were fully susceptible to permethrin in wet seasons (98.8%), though there were also signs of weakening susceptibility among mosquitoes collected in the dry season (97.2%). Deltamethrin resistance in male *An. arabiensis* was observed in the wet season in Minepa (87.5%) and in the dry season in Mavimba (60.8%). There was also reduced susceptibility to deltamethrin in the male mosquito population sampled from Mavimba (91.3%) and Lupiro (90.6%) in wet season, but complete susceptibility was observed in Minepa (98.5%) and Lupiro in dry season (100%).

During the study, male mosquito samples from the three villages across both seasons displayed various levels of resistance to lambda-cyhalothrin (mortality rates between 28.1% and 89.4%). For both DDT and bendiocarb, male mosquitoes from Minepa were resistant in dry season 78.4% and 75.3% respectively, but susceptible in wet season (mortalities between 99.1% and 100%), while the males from both Mavimba and Lupiro were consistently susceptible to these two insecticides in both seasons (100%). A minor exception was specimens collected in wet season from Lupiro, where reduced susceptibility was observed against DDT (95.3%).

As illustrated in Figure 3.1, both male and female mosquito populations across the study villages and during both seasons remained fully susceptible to propoxur, dieldrin and all organophosphates tested (mortality rates between 98.8% and 100%).

3.6.2 Results of the synergist bioassays conducted with samples from Minepa village

Tests with PBO

There was a reduction in time to 50% knockdown (KDT_{50}) in mosquito cohorts pre-exposed to PBO followed by deltamethrin, permethrin, lambda-cyhalothrin and bendiocarb), compared to cohorts directly exposed to each of the candidate insecticides without PBO pre-exposure (Table 2). Resistance reduction levels of 1.4, 3.1, 1.9 and 1.5 fold were recorded in tests of deltamethrin, permethrin, lambda-cyhalothrin and bendiocarb, respectively. The resistance reduction ratios for all tested insecticides are shown in Table 2.2.

Table 3.2: Knockdown rates (KDT_{50}) and degree of resistance reduction of *Anopheles arabiensis* from two study villages after being exposed to various insecticides with and without pre-exposure to synergists.

Study sites	**Insecticide**	**KDT_{50} (min)**	**(95% CI)**	**Resistance reduction[¥]**
Minepa village	0.05% Deltamethrin	50.24	35.71 – 64.77	-
	4% PBO + 0.05% Deltamethrin	35.90	27.56 – 44.24	1.40
	0.75% Permethrin	70.20	34.42 – 105.98	-
	4% PBO +0.75% Permethrin	22.72	17.82 – 27.61	3.09
	0.05% Lambda cyhalothrin	54.88	34.62– 75.13	-
	4% PBO + 0.05% Lambda cyhalothrin	29.61	22.55 - 36.66	1.85
	0.05% Deltamethrin	60.87	38.84 – 82.89	-
	20% TPP + 0.05% Deltamethrin	65.23	35.46 – 94.99	0.93
	0.75% Permethrin	38.69	30.60 – 46.77	-
	20% TPP +0.75% Permethrin	27.65	20.59 – 34.70	1.40
	0.1% Bendiocarb	53.14	37.00 – 69.28	-
	4% PBO + 0.1% Bendiocarb	35.25	27.51 – 42.99	1.51
	0.1% Bendiocarb	56.14	40.04 – 72.24	-

	20% TPP +0.1% Bendiocarb	43.71	33.44 – 53.98	1.28
Mavimba village	0.05% Deltamethrin	46.35	32.64 – 60.06	-
	4% PBO + 0.05% Deltamethrin	23.33	17.98 – 28.68	1.99
	0.75% Permethrin	39.78	29.16 – 50.39	-
	4% PBO + 0.75% Permethrin	21.09	15.60 – 26.59	1.89
	0.05% Lambda cyhalothrin	68.65	35.10 – 102.20	-
	4% PBO +0.05% Lambda cyhalothrin	34.87	26.82 – 42.93	1.97

¥ Resistance reduction = $KDT_{50\ \text{insecticide alone}} / KDT_{50\ \text{insecticide plus synergist}}$

There was also a significant difference in 24-hr post-exposure mortality between mosquito cohorts (Table 3.3). Our tests revealed significant increases in mortalities when the mosquito populations were pre-exposed to PBO followed by deltamethrin compared to when the same populations were exposed to deltamethrin alone (paired t-test, df = 3, t = 18.4, and P < 0.001). Pre-exposure to PBO followed by permethrin also resulted in a significant increase in mortality relative to exposure to permethrin alone (paired t-test, df = 3, t = 9.80, and P = 0.002). Similarly, pre-exposure to PBO followed by lambda-cyhalothrin yield a significant increase in mortality compared to cohorts exposed to lambda-cyhalothrin alone (paired t-test, df = 3, t = 10.3, and P = 0.002). In tests for bendiocarb resistance, it was observed that pre-exposure to PBO created substantial synergism, resulting in higher mortality compared to exposure to bendiocarb with no synergist (paired t-test, df =3, t = 22.46, and P < 0.001).

Table 3.3: Mortality *Anopheles arabiensis* from Minepa village exposed to insecticides and the synergists, PBO or TPP.

Treatment	No. replicates done	Sample size *	% mean mortality (95% CI) Minepa village: 0.05% Deltamethrin	0.05% Lambda cyhalothrin	0.75% Permethrin	0.1% Bendiocarb
Environmental control	4	375	0	NA	0	0
Solvent control	4	375	0	NA	0	0
20% TPP only	4	375	0	NA	0	0
20% TPP & Test insecticide	4	375	27.0 (18.3 – 35.7)[b]	NA	29.5 (20.3 – 38.7)[b]	72.0 (62.9 – 81.0)[a]
Test insecticide only	4	374	24.0 (13.4 – 34.6)[b]	NA	26.5 (21.1 – 31.9)[b]	55.5 (46.4 – 64.6)[a]
Environmental control	4	370	0.2 (-0.2 – 0.6)	0	0	0
Solvent control	4	370	0.2 (-0.2 – 0.6)	0	0	0
4% PBO only	4	370	0	0	0	0
4% PBO & Test Insecticide	4	370	73.0 (63.5 – 82.5)[b]	97.5 (94.7 – 100)[a]	56.8 (46.9 – 66.6)[a]	76.0 (60.4 – 91.6)[a]
Test Insecticide only	4	370	45.0 (35.5 – 54.5)[b]	20.0 (5.6 – 34.4)[a]	8.8 (3.0 – 14.1)[a]	33.0 (23.5 – 42.5)[a]

NA=No assay was performed on this insecticide.

[a] There are significant differences in mean mortalities between exposure to insecticides with and without synergists.

[b] No significant difference in mean mortalities between exposure to insecticides with and without synergists.

Tests with TPP

There was a slight decrease in KDT_{50} when mosquitos were pre-exposed to TPP followed by either deltamethrin, permethrin or bendiocarb, compared to when the same population of mosquitoes was exposed to the candidate insecticides alone (Table 2.3). Resistance to deltamethrin, permethrin, and bendiocarb was reduced by 0.9, 1.4, and 1.3 fold, respectively, with TPP (Table 3.3). However, there was no difference in mortalities in mosquitoes exposed to deltamethrin with or without pre-exposure to TPP (paired t-test, df = 3, t = 0.73, and P = 0.520). Also, there was no statistical difference in mean mortalities of mosquitoes exposed to TPP plus permethrin compared to when they were exposed to permethrin alone (paired t-test, df = 3, t = 0.88, and P = 0.444). On the other hand, there were differences in the mean mortality between bendiocarb and TPP + bendiocarb (paired t-test, df = 3, t = 19.12, and P = 0.006).

3.6.3 Results of the synergist bioassays conducted with samples from Mavimba village

Prior exposure to PBO partially restored susceptibility to deltamethrin by 2.0 fold and decreased the KDT_{50} from 46.35min for deltamethrin alone to 23.33 min for deltamethrin and PBO (Table 2.3). The time required for 50% of the mosquitoes to be knocked down was also reduced from 39.78min for permethrin alone to 21.09 min after being exposed for permethrin and PBO. Resistance reduction level for permethrin following PBO pre-exposure was 1.9 fold (Table 3.3). Similarly, the resistance to lambda-cyhalothrin was reduced by 2.0 fold with PBO, with a shift in KDT_{50} from 68.65min to 34.87min (Table 3.3). There was a significant increase in mortality in mosquito populations pre-exposed to PBO followed by deltamethrin compared to when the same populations were exposed to deltamethrin alone (paired t-test, t = 18.4, df =3, p < 0.001) (Table 4). Similarly, when the mosquito populations were pre-exposed to PBO followed by lambda-cyhalothrin, this resulted in a significant increase in mean mortality compared to when the same population was exposed to lambda-cyhalothrin alone (paired t-test, t = 17.9, df = 3, p < 0.001) (Table 3.4).

Table 3.4: Mortality *Anopheles arabiensis* from Mavimba village exposed to insecticides and the synergists, PBO.

Treatment	**No. replicates done**	**Sample size ***	**% mean mortality (95% CI)**		
			Mavimba village		
			0.05% Deltamethrin	**0.05% Lambda cyhalothrin**	**0.75% Permethrin**
Environmental control	4	260	0.4 (-0.4 – 1.2)	0.4 (-0.4 – 1.2)	0
Solvent control	4	262	0.3 (-0.3 – 0.9)	0	0
4% PBO only	4	262	0	0	0
4% PBO & Test Insecticide	4	241	92.5 (86.2 – 98.8)[a]	85.2 (74.6 – 95.8)[a]	91.3 (82.9 – 99.6)[a]
Test Insecticide only	4	240	27.5 (24.7 – 30.3)[a]	20.0 (03.5 – 36.5)[a]	67.5 (54.5 – 80.5) [a]

[a] There are significant differences in mean mortalities between exposure to insecticides with and without synergists.

[b] No significant difference in mean mortalities between exposure to insecticides with and without synergists

3.6.4 Results of the molecular assays to detect knockdown resistance (kdr) alleles

A total of 74 adult female *An. arabiensis* mosquitoes from Minepa, 66 from Mavimba and 80 from Lupiro were assayed for *kdr* allele mutations L1014F (*kdr-west*) and the L1014S (*kdr-east*). All specimens were negative for both mutations.

3.7 Discussion

The increasing spread of insecticide resistance in malaria vectors jeopardizes control and elimination efforts [156-159,177,178], thus necessitating regular resistance monitoring to design setting-specific and successful resistance management programmes [68,154,195]. Overall, this study detected widespread resistance against pyrethroids, bendiocarb, and DDT; but not against propoxur, dieldrin, and the two organophosphates, pirimiphos-methyl and malathion, for which there was full susceptibility across all the villages and seasons. This study also found marked temporal and fine-scale fluctuations of insecticide resistance profiles in both male and female *An. arabiensis* against three insecticides in the pyrethroid class, DDT, and bendiocarb. In all the three villages, deltamethrin, permethrin, lambda-cyhalothrin, DDT and bendiocarb resistance of male *An. arabiensis* mosquitoes fluctuated between seasons and villages. The resistance of female *An. arabiensis* mosquitoes against DDT and bendiocarb also fluctuated between seasons and villages. The most resistant populations were observed in Minepa for bendiocarb, lambda-cyhalothrin and DDT and in Lupiro for lambda-cyhalothrin and permethrin. In Minepa, bendiocarb resistance was detected in the dry season, but completely diminished in wet seasons for both male and female populations, and DDT resistance followed a similar trend in the male population. However, in Lupiro village, DDT resistance was observed during the wet season only.

The seasonal and spatial variation in insecticide resistance detected in this study is not unique. Variations in both phenotypic and genotypic insecticide resistance in both *Anopheles* and *Aedes* mosquitoes over small spaces and time have been reported previously [22, 38-40]. A recent report in Chad found significant spatial changes in insecticide resistance in *An. arabiensis* population [196]. Similarly, there was a significant difference in phenotypic and genotypic resistance at a fine geographical scale in *Ae. aegypti* populations to chlorpyrifos-ethyl and deltamethrin sampled from nearby study sites in Mexico [197]. The seasonal and spatial fluctuations in insecticide resistance might be attributed to differences in the biology and genetics of the vector populations in particular ecological settings, as reported in a previous study by Verhaeghen *et al.* [198].

Perhaps the presence of chemical contaminants in a particular environment, possibly due to leached agricultural chemicals and other pollutants at a particular time might cause selection pressure in mosquitoes, and subsequent resistance to insecticides. Also, the existence of phenotypic resistance in the study areas to lambda-cyhalothrin, bendiocarb and DDT that are not used for LLINs or IRS, suggest cross-resistance between classes or alternative sources of insecticide resistance pressure, most likely from agriculture. The impact of agricultural pesticides in the selection of resistant mosquitoes has already been reported extensively [85,86,120-126]. This hypothesis is also supported by our preliminary observations that the majority of farmers in the study villages reported applying more pesticides in dry seasons than in wet seasons [87]. The differences in insecticide resistance between adjacent study villages suggest that other than variations that have been reported between districts and regions [156,158,160], there might also be fine-scale differences even within the villages that require further investigations. All these variations signify an important challenge to the vector control programs that might require proper consideration in the timing/season and choosing different insecticides for application even in a particular small area.

Male mosquitoes are considered to be more delicate and susceptible to insecticides as they have a shorter life expectancy than their females counterparts [154]. In this study, males were found to be resistant to the same insecticides as the females, but at a lower level. These observations are consistent with previous studies that have reported that adults male *An. arabiensis*, with previous exposure to insecticides, could also experience resistance similar to females [199]. For example, a high level of glutathione-S-transferase (GSTs) activity was found in both male and female *An. arabiensis* selected for resistance to DDT, but only elevated esterases was found in the male-DDT selected strain [199]. Resistance in male mosquitoes was reported previously to adversely affect their mating competiveness, as shown in *Culex pipiens* and *An. gambiae* [200-202]. This suggests the need for regular monitoring of susceptibility status of male mosquitoes, particularly in interventions targeting male mating behaviour, such as the sterile insect technique, which involves mass-rearing, sterilization, and release of sterile male mosquitoes into the wild population to prevent females from reproducing [203,204]. Other interventions that have been proposed for mosquito-borne disease elimination includes targeting male swarming behaviour [205], sugar-seeking behaviour through the use of attractive toxic sugar baits [206,207] and larval control [208]. In summary, our findings and the current evidence suggest the need for regular monitoring of susceptibility status of both males and females, especially for end-game scenarios where LLINs and IRS have already been widely used, but malaria transmission still persists.

As revealed in the synergist assays, the reduction in knockdown rates and increase in mortalities was due to synergistic action of piperonal butoxide (PBO), as an inhibitor of P450 monooxygenases, and triphenol phosphate (TPP), as an inhibitor of the esterases activity. Synergists have an effect by augmenting the penetration of the insecticides into the mosquito body and counteracting the metabolic pathways that would otherwise metabolize the insecticides, thus restoring susceptibility to varying degrees [168,169,209,210]. The observed effects in the present study suggest involvement to a significant degree of one or both of the two enzyme classes in conferring pyrethroid and bendiocarb resistance within the mosquito populations sampled from the study sites. However, esterases seem not to be involved in deltamethrin and permethrin resistance in the mosquito population sampled from Minepa village. Susceptibility to lambda-cyhalothrin was completely restored by 4% PBO in the mosquito population sampled from Minepa village, indicating that the resistance is metabolic mediated by monooxygenases. However, the inability of PBO and TPP to completely reverse the deltamethrin, permethrin and bendiocarb resistance across the study sites indicates that either other enzymes might be playing a role in the metabolic resistance, or there is presence of other mutations that require further investigation. These questions will need to be further explored through biochemical and genetic analyses. Our findings agree with previous studies that have consistently reported the combining effect of synergists and insecticides against resistant disease-transmitting mosquitoes and incomplete suppressions of pyrethroids resistance due to the synergists action [168,169,179,211-213].

The absence of L1014F and L1014S resistance alleles in the field-collected adult female mosquito populations suggests that the phenotypic resistance to pyrethroid and DDT was not associated with target site insensitivity of the voltage-gate sodium channel. The findings supports an earlier study by Okumu *et al*, who also showed absence of *kdr* mutations in wild population of *An. arabiensis* from Lupiro village, five years before this current study [214]. Similarly, a recent multi-region study in Tanzania by Kabula *et al* [215] reported absence of both L1014F and L1014S mutations in *An. arabiensis* populations from Kilombero district, which neighbours Ulanga district where our study was conducted. However, these gene mutations were detected in both *An. arabiensis* and *An. gambiae s.s.* from other sentinel districts of Tanzania where studies were carried out [215].

3.8 Conclusions

This study revealed multiple spatial and temporal fluctuations of insecticide resistance profiles in the *An. arabiensis* populations from the three neighbouring villages in south-eastern Tanzania, and confirmed the presence of pyrethroid, DDT and bendiocarb resistance in each of these three villages. The substantial, though not absolute reversal of pyrethroid and carbamate resistance when mosquitoes were pre-exposed to PBO or TPP, coupled with the absence of *kdr* resistance alleles, suggests involvement of P450 monooxygenases and esterases as key determinants conferring the resistance phenotypes. We recommend further intensity bioassays to determine the strength of phenotypic resistance, as well as biochemical and molecular analysis to elucidate various enzymes involved in the resistance. Such additional tests are essential for an effective resistance management programmes in this or similar areas. Overall, these results highlight the importance of periodic and continuous insecticide susceptibility surveillance and emphasize the need to consider fine-scale variations in insecticide resistance levels, even in small geographical locations, when implementing insecticidal-based interventions.

Data availability

Raw datasets for this study are available from the Ifakara Health Institute (IHI) data repository. Please follow the DOI number 10.17890/ihi.2017.09.99, and the link http://data.ihi.or.tz/index.php/catalog/71/get_microdata.

Competing interests

No competing interests were disclosed.

Grant information

This work was supported by the Wellcome Trust [102350], Intermediate Research Fellowship awarded to FOO, and [104029], Masters Fellowship in Public Health and Tropical Medicine awarded to the lead author NSM under the mentorship of MC, LLK, and FOO. MC is funded by the DST/NRF South African Research Chairs Initiative.

Acknowledgements

We highly appreciate the support received from the community from the study villages during the selection of the breeding sites for sampling mosquito larvae. We thank Dr. Maria Kaiser from the

National Institute of Communicable Diseases (NICD) in Johannesburg, for providing guidance during *kdr* assays, and Dr. Sherif Amer of the University of Twente in The Netherlands for assisting in the mapping of insecticide resistance. We extend much appreciation to Neema Nombo and Paulina Kasanga for their enormous work in larvae collections, rearing and assisting with the WHO and synergist bioassays, and Salum Mapua also for assisting with WHO bioassays. We sincerely thank Prof. George Corliss of Marquette University for his valuable time reviewing earlier versions of this manuscript and providing helpful comments. All persons acknowledged have been informed and have consented to the acknowledgement.

4 Chapter 4: Fine-scale spatial and temporal variations in insecticide resistance in *Culex pipiens* complex mosquitoes in rural south-eastern Tanzania

Nancy S. Matowo[1,2,3,4*], Said Abbasi[1], Givemore Munhenga[5,6], Marcel Tanner[2,3], Salum A. Mapua[1], David Oullo[7], Lizette L. Koekemoer[5,6], Emanuel Kaindoa[1,7], Halfan S. Ngowo[1,8], Maureen Coetzee[5,6], Jürg Utzinger[2,3] and Fredros O. Okumu[1,8,9]

[1]Environmental Health and Ecological Sciences Department, Ifakara Health Institute, Ifakara, Tanzania

[2]Swiss Tropical and Public Health Institute, Basel, Switzerland

[3]University of Basel, Basel, Switzerland

[4]Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK

[5]Wits Research Institute for Malaria, Wits/SAMRC Collaborating Centre for Multi-Disciplinary Research on Malaria, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

[6]Centre for Emerging Zoonotic & Parasitic Diseases, National Institute for Communicable Diseases, Johannesburg, South Africa

[7]US Army Medical Research Directorate-Africa, Kisumu, Kenya

[8]Institute of Biodervisity, Animal Health and Comparative Medicine, University of Glasgow, UK

[9]School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

*Correspondence: nstephen@ihi.or.tz

Published in Parasites & Vectors 2019 12, 413

https://doi.org/10.1186/s13071-019-3676-4

The contents in this this chapter have been slightly modified for clarity

4.1 Abstract

Background: *Culex* mosquitoes cause considerable biting nuisance and sporadic transmission of arboviral and filarial diseases.

Methods: Using standard World Health Organization procedures, insecticide resistance profiles and underlying mechanisms were investigated during dry and wet seasons of 2015 and 2016 in *Culex pipiens* complex from three neighbouring administrative wards in Ulanga District, Tanzania. Synergist tests with piperonyl butoxide, diethyl maleate, and triphenyl phosphate, were employed to investigate mechanisms of the observed resistance phenotypes. Proportional biting densities of *Culex* species, relative to other taxa, were determined from indoor surveillance data collected in 2012, 2013, and 2015.

Results: Insecticide resistance varied significantly between wards and seasons. For example, female mosquitoes in one ward were susceptible to bendiocarb and fenitrothion in the wet season, but resistant during the dry season, while in neighbouring ward, the mosquitoes were fully susceptible to these pesticides in both seasons. Similar variations occurred against bendiocarb, DDT, deltamethrin, and lambda-cyhalothrin. Surprisingly, with the exception of one ward in the wet season, the *Culex* populations were susceptible to permethrin, commonly used on bednets in the area. No insecticide resistance was observed against the organophosphates, pirimiphos-methyl and malathion, except for one incident of reduced susceptibility in the dry season. Synergist assays revealed possible involvement of monooxygenases, esterases, and glutathione S-transferase in pyrethroid and DDT resistance. Morphology-based identification and molecular assays of adult *Culex* revealed that 94% were *Cx. pipiens* complex, of which 81% were *Cx. quinquefasciatus*, 2% *Cx. pipiens*, and 3% hybrids. About 14% of the specimens were non-amplified during molecular identifications. Female adults collected indoors were 100% *Cx. pipiens* complex, and constituted 79% of the overall biting risk.

Conclusions: The *Cx. pipiens* complex constituted the greatest biting nuisance inside people's houses, and showed resistance to most public health insecticides possible. Resistance varied at a fine geographical scale, between adjacent wards, and seasons, which warrants some modifications to current insecticide resistance monitoring strategies. Resistance phenotypes are partly mediated by metabolic mechanisms, but require further evaluation through biochemical and molecular techniques. The high densities and resistance in *Culex* could negatively influence the acceptability of other interventions such as those used against malaria mosquitoes.

Keywords: *Culex pipiens* complex, Fine spatial scale and temporal differences, Insecticide resistance, Metabolic resistance, Tanzania.

4.2 Background

Culicine mosquitoes, including *Aedes*, *Mansonia* and members of the *Culex pipiens* and *Cx. univittatus* complexes, are common across East Africa [14,65,216,217]. Of particular importance is the *Cx. pipiens* complex, generally referred to as the “house mosquito”[218]. It is not only a major cause of biting annoyance to humans but is also a primary vector of many arboviruses and filarial worms, that affect more than 1 billion people globally [6-8]. The diseases of concern include Rift Valley fever, dengue, chikungunya, yellow fever, Sindbis, Wesselsbron, o’nyong-nyong and West Nile arboviruses, filarial worms causing Bancroftian filariasis, [16,17,219], and avian *Plasmodium* species [220,221]. Most of these pathogens are maintained in zoonotic cycles with humans being incidental hosts [28]. Culicines are adapted and dominate human habitats, increasing their risks to act as bridge vectors in transmitting pathogens between humans and animals [222-225]. In Africa there have been several sporadic outbreaks of arbovirus infections such as Rift Valley fever in Kenya and Tanzania as well as dengue fever [23-25] and Chikungunya [26,27].

The World Health Organization (WHO) Global Vector Response Strategy, recognizes the need to integrate surveillance and control of pathogens transmitted by different vectors species [34]. Surveillance and management of insecticide resistance are two crucial components [34,226] for effective decision-making on selection, allocation and implementation of appropriate integrated vector control interventions.

Current vector control interventions in Africa are primarily designed to target malaria vectors, with limited efforts to control other mosquito-borne disease vectors. This is also true for insecticide resistance monitoring [227]. Information on susceptibility of culicine species to insecticides used in public health is limited [228,229]. Yet these species contribute to the greatest human-biting densities. Culicine densities are usually high, because of the presence of numerous favourable aquatic breeding sites that include man-made stagnant water bodies (e.g. small multipurpose dams, rice paddies), waste disposal sites, open pit latrines and septic tanks and flooded vegetation [12-14]. Lack of resources in many countries has limited expansion of surveillance to non-malaria vectors including the culicines.

Previous studies showed spatial and temporal dynamics of insecticide resistance in mosquito vector populations, and influence of environmental contamiants such as agricultural pesticide residues, and such information has been used to plan resistance monitoring efforts [179,230,231].

For exampe in Tanzania, insecticide resistance monitoring is carried out at district level in selected sentinel sites in regions assumed to represent different eco-epidemiological settings [156]. Reports from these assessments provide essential data for country-level decision making. However, such a simplistic approach is inadequate for understanding insecticide resistance, which often varies geographically at finer scales other than at the unit of either district or country level [84].Besides, data on insecticides resistance and associated mechanisms in *Culex* species is also lacking in Tanzania. In addition, data on insecticide resistance in males mosquito populations are limited inspite of both mlaes and females being exposed to insecticides during vector control interventions. Males mosquito population substantially contribute in the reproduction and increasing population density and their response to insecticides is also a crucial component. In addition new novel vector control interventions such as spraying of swarms with insecticides directly target male mosquitoes. This suggest the need to monitor insecticide resistance on regular basis in male mosquitoes.

Knowledge on the resistant status of a population though important is not adequate and need to be supplemented with information regarding the mechanisms underlying the resistance phenotype. There a number of tools that can be used to determine insecticide resistance mechanisms. Synergist assays have been deployed as quick and simple method to assess metabolic resistance in mosquito vectors [168,169,209,210]. Synergists act by enhancing insecticides penetration into the mosquito body and inhibit the metabolic enzymes that would otherwise digest the insecticides, hence partially/fully restoring susceptibility [168,169,209,210].

Here, we investigated the spatial and seasonal variations in susceptibility to insecticides of *Cx. pipiens* complex mosquitoes from rural south-eastern Tanzanian villages where there is high LLINs coverage [37], and regular usage of agricultural pesticides (Matowo et al, unpublished data). The main objectives of the study were to: (i) fill important knowdge gaps on insecticide resistance and species diversity of *Culex* mosquitoes in the study area and, (ii) investigate fine-scale spatial and temporal differences in resistance and resistance mechanism in the *Culex* species.

4.3 Methods

4.3.1 Study area

Three neighbouring wards, i.e. Minepa (8.271° S, 36.677° E), Lupiro (8.385º S, 36.670º E) and Mavimba (8.312° S, 36.677° E), in Ulanga district, south-eastern Tanzania were selected (Figure 4.1). These villages have high LLINs coverage [37] and high agricultural pesticide use. Minimum and maximum distances between the wards was ~4 km (Minepa to Mavimba) and ~9 km (Minepa to Lupiro), respectively. All three wards lie between 120m and 350m altitude. Average annual rainfall ranges between 1,200 mm and 1,800 mm, with dry season between June and October, short rainy season in November and December, and wet season between January and May. Mean daily temperatures over the year vary from 20°C to 32°C, while the relative humidity is 70-90%. Residents practice rice farming [232], which is irrigated during dry season, so that the area is continuously favourable for mosquito breeding [111]. A national insecticide susceptibility survey in 2011 across 14 districts, including the nearby Kilombero district, reported widespread pyrethroid and DDT resistance in *Anopheles* mosquitoes [156], but no data on *Culex* was reported. Recent studies indicated that the two malaria vectors *An. arabiensis* and *An. funestus* are highly resistant to pyrethroids, bendiocarb, and DDT, thus compromising vector control efforts [84,233,234].

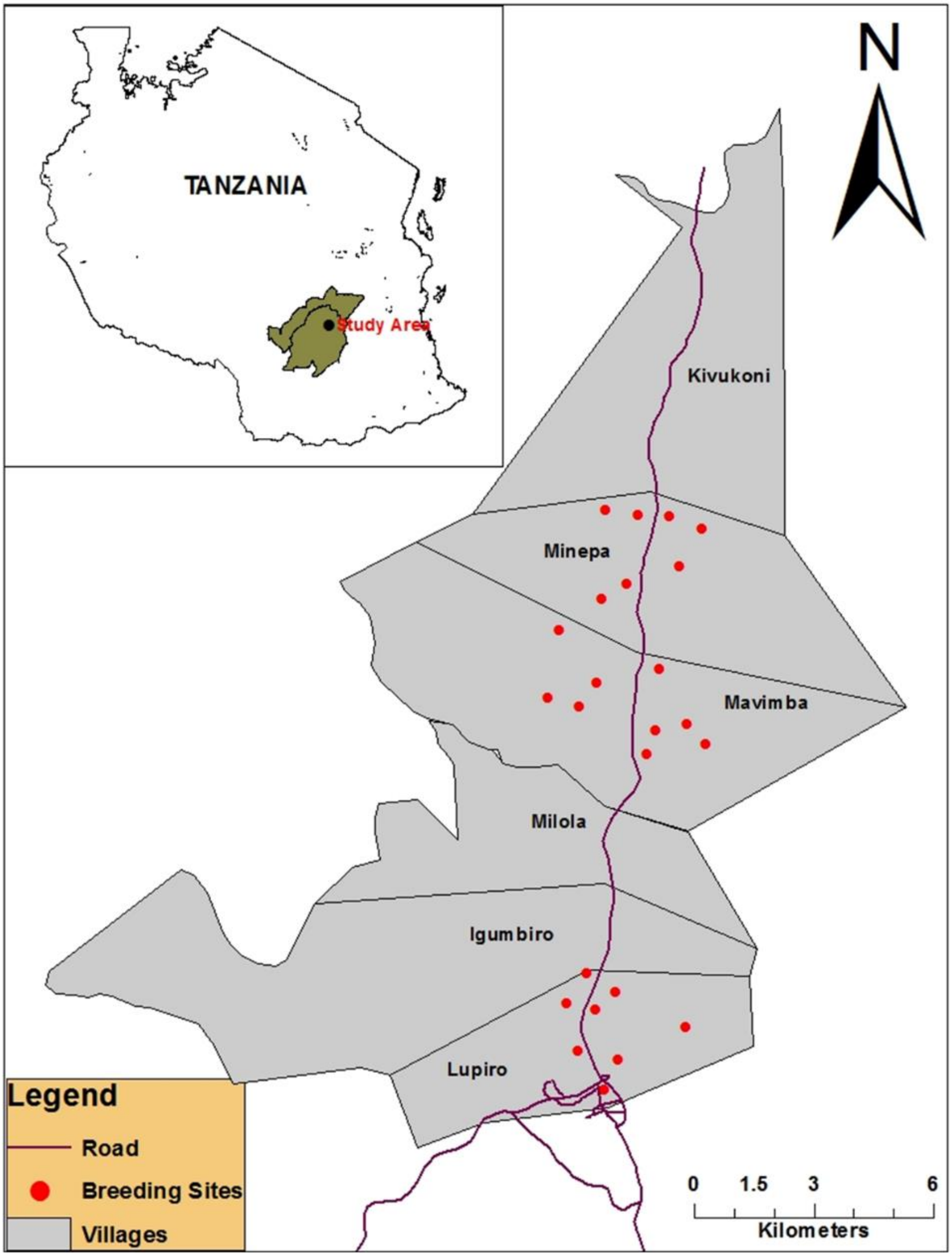

Figure 4.1: Locations of mosquito aquatic breeding sites in Minepa, Mavimba and Lupiro, south-eastern Tanzania, where larvae sampling was conducted between June 2015 and June 2016.

4.3.2 Mosquito sampling and larval rearing

Mosquito larvae were collected between June 2015 and June 2016, using standard larval dipping method [235] in three wards, during the dry season (June to December 2015) and wet season (January to May 2016). In each instance, seven to nine randomly selected and georeferenced aquatic habitats were sampled. Larvae were separated into anophelines and culicines to ensure easy adult morphological identifications. To assess spatial variations in insecticide resistance, collected larvae were separated per collection site for WHO insecticide resistance assays.
Collected larvae were transferred to the medical entomology laboratory, the “Vector Sphere”, at Ifakara Health Institute (Ifakara, Tanzania), and reared to adults at temperatures of 27 ± 2 °C and relative humidity of 70-90%). Larvae were fed on mud and algae from their original habitats, supplemented with Tetramin fish food (Tetra; Melle, Germany). Emergent adults were separated by gender and taxa, and provided with 10% glucose solution.

4.3.3 Insecticide susceptibility tests

Phenotypic insecticide resistance in *Culex* species in the three study villages was assessed in dry and wet seasons using using standard WHO test kits (Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia). Adult males and females (3 - 5 days old) were exposed in batches of either 20 or 25 individuals according to the discriminating doses of 0.05% lambda cyhalothrin, 0.05% deltamethrin, 0.75% permethrin, 4% DDT, 4% dieldrin, 0.1% bendiocarb, 0.1% propoxur, 0.25% pirimiphos methyl and 5% malathion [154]. The same number of mosquitoes were exposed to oil-impregnated papers as controls. Due to unavailability of reference susceptible *Culex* mosquitoes, a susceptible colony of *An. gambiae s.s.* (Ifakara strain), was used to validate efficacy of test papers. Knockdown was recorded after 10, 15, 20, 30, 40, 50 and 60 min. After the 60 min exposure, mosquitoes were transferred to holding tubes and offered 10% glucose. Final mortality was recorded 24 hours post-exposure [154], after which the mosquitoes were preserved in 1.5 ml Eppendorf tubes with silica for species identification, using polymerase chain reaction (PCR) assays.

4.3.4 Synergist assays

Synergist assays were performed using 4% piperonyl butoxide (PBO), a known inhibitor of monooxygenase, 20% diethyl maleate (DEM), an inhibitor of glutathione-*S*-transferases (GSTs) and 20% triphenyl phosphate (TPP), an inhibitor of esterases, as a quick and simple method to assess whether the observed phenotypic resistance had a metabolic enzymes basis [168]. The bio-efficacy of synergist papers was tested against a reference laboratory colony *(An. funestus)* with resistance phenotype mediated by monooxygenases and GSTs [190]. Due to resource limitations, the synergist tests were performed on only female mosquitoes in the dry season in Minepa and Mavimba wards. For each synergist, five cohorts of adults (n=125) were used. The first group was exposed to a synergist (either 4% PBO, 20% DEM or 20% TPP) for 60 min, and thereafter immediately exposed to WHO test papers impregnated with either 0.75% permethrin, 0.05% deltamethrin, 0.05% lambda-cyhalothrin or 4% DDT for another 60 min. The second group was exposed only to the respective WHO test papers, and the third group exposed to the synergist only. Fourth and fifth groups consisted of controls, i.e. filter papers treated with olive oil used to prepare the synergist papers (solvent control), and plain filter papers (environmental control).

4.3.5 Estimating relative densities of *Culex* mosquitoes and associated biting risk

The proportional biting population densities of *Culex*, relative to other mosquito species, was estimated from indoor night collections in 2012, 2013 and 2015 at Minepa, Mavimba and Kivukoni wards [186,236], using CDC light traps indoors [237] in 96 randomly-selected houses. The mosquitoes were segregated as *Anopheles, Culex*, *Aedes, Mansonia* and other species.

4.3.6 Morphological identification of *Culex* species

A sub-sample of female *Culex* mosquitoes (n = 430) from the resistance bioassays and (n = 1,053) female *Culex* mosquitoes from indoor collections were morphologically identified to determine composition of prevailing species and species complexes using the taxonomic keys of Edwards [238], under a stereo-zoom microscope, SZM-LED2 (digital Optika® Microscopes; Ponteranica, Italy). To improve identification, the mosquito images were enhanced using OptikalSview software version 3.6.6, and captured using a digital camera (Optika®; Ponteranica, Italy) attached to the microscope.

The diagnostic features used for species identification were: (i) presence and number of mesepimeral bristles; (ii) presence or absence of a pale band on proboscis; (iii) presence or absence of white scales on abdomen; (iv) presence or absence of white scales on femur or tibia; and (v) presence or absence of pale-ringed tarsi. The main morphological features for each Culex taxa are summarised in Figure 3.2.

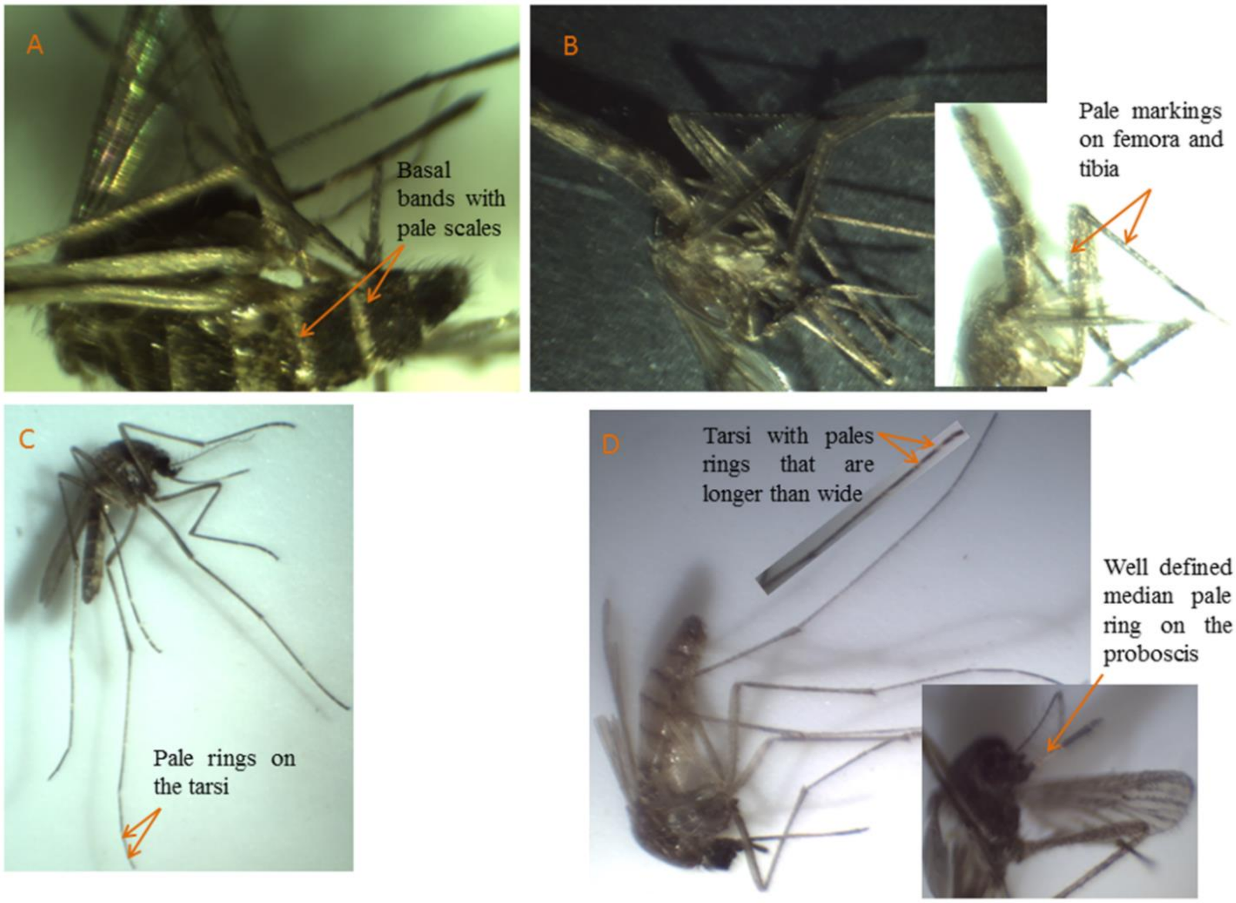

Figure 4.2: Key morphological identification features of *Culex* mosquitoes in the study area. The images depict: **a)** *Cx. pipiens* L. complex, with distinct basal bands of pale scales on dorsal part of the abdomen; **b)** *Cx.* (*Lutzia*) *tigripes* De Grandpre & De Charmoy, with pale markings on the femora and tibiae; **c)** *Cx.* (*Culex*) *duttoni* Theobald, with clear pale rings on the tarsi; and **d)** *Cx.* (*Culex*) *poicilipes* Theobad, with well-defined middle pale ring on the proboscis.

To complete the assessment, we first adapted the 1941 morphological keys [238], to focus on just the general diagnostic features and specific features known to occur in Tanzania *Culex* mosquitoes (Table 4.1).

Table 4.1 Identification keys showing main morphological features to distinguish among female *Culex* collected in three rural wards (Minepa, Mavimba and Lupiro) in Ulanga district, south-eastern Tanzania. Adapted from the morphological keys by F.W. Edwards ("Mosquitoes of the Ethiopian Region; culicine adults and pupae published in 1941") [238].

Taxa	Main morphological features for identification of *Culex* species collected in the three study areas
***Culex pipiens* complex**	1. Generally smaller size compared to other *Culex* species 2. Abdominal tergite with pale basal bands, sternite pale and not banded 3. Proboscis without a well- defined ring in the middle but pale beneath 4. Legs and tarsi mostly or entirely dark but hind tibia with a small pale spot at tip. 5. Presence of one lower mesepimeral bristle 6. Halters are yellowish
Culex* (*Lutzia*) *tigripes	1. One of the largest *Culex* species 2. About 10 small prominent palespots on a dark ground marking on femora and tibiae 3. Abdominal bands, 6 and 7 are broad, sometimes occupying almost half of the tergites, while sternites are all pale-scaled un-banded 4. Mainly dark proboscis 5. Dark-scaled wings 6. 3-10 bristles on lower half of the mesepimeron in a more or less regular row
Culex* (*Culex*) *poicilipes	1. Sharply-defined median pale yellowish ring on proboscis 2. Presence of 7-10 distinct small pale spots on anterior surfaces of front femora and tibiae 3. Tarsi with pale rings at joints, which are scarcely longer than wide; on joint 4-5 of hind tarsi, pale ring scarcely noticeable 4. No post-spiracular or pre-alar scales 5. Wings with all dark scales
Culex* (*Culex*) *duttoni	1. Distinctly pale rings on the tarsi and indefinitely ringed proboscis but with whitish scales on the palp almost half 2. Middle tibia with narrow pale anterior stripe 3. Presence of 2-4 lower mesepimeral bristles 4. Presence of few post-spiracular scales 5. Dark thorax with no pale scales 6. Head with pale scales

4.3.7 Molecular identification of sibling species in the *Cx. pipiens* complex

Morphological identification showed that the *Cx. pipiens* complex was the most common of all *Culex* species in the study area. Further molecular identification was conducted using PCR amplification to differentiate two members of the *Cx. pipiens* complex (i.e. *Cx. pipiens pipiens* and *Cx. quinquefasciatus*). This PCR targets the acetyl-cholinesterase-2 locus (*ace-2*). The *ace-2* locus was amplified using primers, B126, *ACEquin* and *ACEpip*, as previously described by Smith and Fonseca [239].

DNA was extracted from 280 specimens, randomly selected from the morphologically identified *Cx. pipiens* complex. A total of 5 µl of extracted genomic DNA per sample was amplified in a 20 µl reaction mix containing 1X PCR buffer, 250 µM dNTP, 2 mM $MgCl_2$, 0.4 µM of universal primer and *ACEquin*, 0.2 µM of *ACEpip* and 1 unit of Taq DNA polymerase over-laid by a drop of mineral oil. After PCR amplification, 10 µl of the DNA fragments were separated by electrophoresis on a 2.5% agarose gel stained with 0.5 µg/ml ethidium bromide and compared against a 100bp DNA marker included in the gel. Separated DNA fragments were photographed under ultraviolet light using Kodak Gel Logic 100 imaging system and scored as *Cx. pipiens pipiens* (610bp) or *Cx. quinquefasciatus* (274bp).

4.4 Statistical analysis

The data on susceptibility to insecticides were interpreted following WHO thresholds established in 2016 [154], where: (i) mean mortality ranging between 98% and 100% indicates susceptibility; (ii) mean mortality between 90% and 97% indicate possible resistance or presence of resistant genes in the vector populations, but requiring confirmation by repeat bioassays or by molecular assay; and (iii) mean mortality less than 90%,indicates confirmation of resistance in the test populations. Percentage mean mortality for controls were also calculated, and any tests with mortality greater than 5%, but less than 20%, were corrected using Abbott's formula [240]. Further analysis was done using R statistical software version 3.0 [193]. Mean mortalities in mosquitoes collected between dry and wet seasons were compared using t-test and any differences considered statistically significant at $P < 0.05$. In the synergist tests, the observed 24 hours post-exposure, was compared between synergised and un-synergised exposures using t-test and any differences considered statistically significant at $P < 0.05$. The relative densities of *Culex* mosquitoes were summarised in tabular format.

4.5 Results

4.5.1 Morphological identification of *Culex* mosquitoes

A sub-sample of 430 specimens reared from larvae from the three wards (Table 4.2) were identified as belonging to four *Culex* species or species complexes as follows: 94% (n = 405) *Cx. pipiens* complex, 2% (n = 8) *Cx.* (*Lutzia*) *tigripes*, 1% (n = 3) *Cx.* (*Culex*) *poicilipes* and 3% (n = 14) *Cx.* (*Culex*) *duttoni*. The 1,053 Culex mosquitoes sub-sampled from indoor collections were also identified as members of the *Cx. pipiens* complex. Given the dominance of *Cx. pipiens* complex, results of insecticide resistance tests are considered most representative of this species complex.

Table 4.2: Number of adults *Culex* of different species or species complexes identified from sub-samples emerged from larvae collected in three study wards in Ulanga district, Tanzania in 2015 and 2016.

Taxa	Number of specimens identified			
	Minepa ward	Mavimba ward	Lupiro ward	**Totals**
Cx. pipiens complex	160	112	133	**405**
Cx. (*Lutzia*) *tigripes*	4	1	3	**8**
Cx. (*Culex*) *poicilipes*	1	2	0	**3**
Cx. (*Culex*) *duttoni*	11	0	3	**14**
Total	**176**	**115**	**139**	**430**

4.5.2 Molecular identifications

About 94% of *Culex* belonged to *Cx. pipiens* complex, of which 81% were verified by PCR as *Cx. quinquefasciatus*; 2% as *Cx. pipiens pipiens* and 3% as hybrids of *Cx. pipiens pipiens* and *Cx. quinquefasciatus.* A small proportion of samples (14%) did not amplify.

4.5.3 Insecticides resistance status of *Culex* mosquitoes in different wards and seasons

Table 4.3 summarizes results for standard WHO susceptibility tests [32] on adult male and female *Culex* in the three study wards. The reference colony (*An. gambiae s.s.*) used to test insecticidal activity of the test papers was fully susceptible (100%) to all candidate insecticides. No mortality was observed upon exposure of wild-caught *Culex* to untreated papers. The *Culex* mosquitoes sampled displayed differences in resistance to each insecticide by ward, time of year (dry or wet season), sex (male or female mosquitoes) and insecticides tested.

Overall, lower mortality was observed in Minepa ward than the other two wards, and females had lower mortalities than males. In addition, resistance to bendiocarb, deltamethrin, lambda-cyhalothrin and DDT, was higher in the dry season than the wet season. There was complete resistance or reduced susceptibility to the pyrethroids, except permethrin, against which the mosquitoes (both males and females) from Minepa and Mavimba wards were fully susceptible regardless of the season. In Lupiro ward, however, *Culex* were susceptible to permethrin in dry season, but resistant to it during the wet season. In Minepa ward, both male and female *Culex* were resistant to bendiocarb in dry season, but fully susceptible in wet season. Those collected from Mavimba and Lupiro wards remained fully susceptible to bendiocarb during both seasons. Similar spatial-temporal variations in resistance were observed for male *Culex* exposed to deltamethrin, lambda-cyhalothrin and bendiocarb.

Table 4.3: Fine-scale spatial and seasonal variations in insecticide susceptibility of *Culex* mosquitoes[§§] collected in three neighbouring wards in the Ulanga district, Tanzania, in dry season (June-December 2015) and wet season (January-May 2016). Adult mosquitoes exposed for each insecticide were either 20 or 25 per replicate. Results expressed as % mean mortality 24 hours' post-exposure.

		Minepa (8.271°S, 36.677°E)		**Mavimba (8.312°S, 36.677°E)**		**Lupiro (8.385º S, 36.670º E)**	
	Insecticide	**Dry season**	**Wet season**	**Dry season**	**Wet season**	**Dry season**	**Wet season**
Female mosquitoes	**0.75% permethrin**	**100.0**^{SS}	**100.0**^{SS}	**100.0**^{SS}	**100.0**^{SS}	**100.0**$^{SS\ \breve{Y}}$	**72.0**$^{RR\ \breve{Y}}$
	0.05% deltamethrin	86.0$^{RR\ \breve{Y}}$	56.3$^{RR\ \breve{Y}}$	87.0^{RR}	90.0^{RS}	8.0$^{RR\ \breve{Y}}$	87.5$^{RR\ \breve{Y}}$
	0.05% lambda cyhalothrin	60.0^{RR}	82.5^{RR}	76.3$^{RR\ \breve{Y}}$	91.3$^{RS\ \breve{Y}}$	80.0$^{RR\ \breve{Y}}$	87.5$^{RR\ \breve{Y}}$
	4% dieldrin	94.0^{RS}	98.8^{SS}	98.8^{SS}	100.0^{SS}	100.0^{SS}	100.0^{SS}
	4% DDT	92.0^{RS}	95.0^{RS}	87.5^{RR}	91.3^{RS}	78.0^{RR}	71.0^{RR}
	0.1% propoxur	94.0^{RS}	100.0^{SS}	91.3$^{RS\ \breve{Y}}$	100.0^{SS}	100.0^{SS}	98.0^{SS}
	0.1% bendiocarb ++^^	**29.0**$^{RR\ \breve{Y}}$	**99.0**$^{SS\ \breve{Y}}$	**98.0**^{SS}	**100.0**$^{SS\ \breve{Y}}$	**100.0**^{SS}	**99.0**^{SS}
	0.25% pirimiphos methyl	100.0^{SS}	100.0^{SS}	90.0$^{RS\ \breve{Y}}$	100.0$^{SS\ \breve{Y}}$	100.0^{SS}	100.0^{SS}
	5% malathion	100.0^{SS}	100.0^{SS}	100.0^{SS}	97.5^{SS}	99.0^{SS}	100.0^{SS}
	Control (untreated paper)	4.6	2.4	2.2	1.8	1.2	2.9
Male mosquitoes	0.75% permethrin	100.0^{SS}	100.0^{SS}	98.8^{SS}	100.0^{SS}	100.0^{SS}	100.0^{SS}
	0.05% deltamethrin^^	**90.0**^{RS}	**93.0**^{RS}	**97.5**^{RS}	**98.8**^{SS}	**99.0**^{SS}	**95.0**^{RS}
	0.05% lambda-cyhalothrin++^^	**88.0**$^{RR\ \breve{Y}}$	**99.0**$^{SS\ \breve{Y}}$	**100.0**^{SS}	**98.8**^{SS}	**71.0**^{RR}	**92.0**^{RS}
	4% dieldrin	100.0^{SS}	100.0^{SS}	100.0^{SS}	100.0^{SS}	100.0^{SS}	100.0^{SS}
	4% DDT ++^^	**97.0**^{RS}	**95.0**^{RS}	**77.0**^{RR}	**98.8**^{SS}	**99.0**^{SS}	**100.0**^{SS}
	0.1% propoxur	93.0^{RS}	99.0^{SS}	97.0^{RS}	100.0^{SS}	100.0^{SS}	100.0^{SS}
	0.1% bendiocarb ++^^	**58.0**$^{RR\ \breve{Y}}$	**100.0**$^{SS\ \breve{Y}}$	**100.0**^{SS}	**98.0**^{SS}	**100.0**^{SS}	**100.0**^{SS}
	0.25 % pirimiphos methyl	100.0^{SS}	98.0^{SS}	97.0^{RS}	100.0^{SS}	100.0^{SS}	100.0^{SS}
	5% malathion	100.0^{SS}	100.0^{SS}	100.0^{SS}	100.0^{SS}	100.0^{SS}	100.0^{SS}
	Control (untreated paper)	1.1	11.2	4.8	2.5	2.6	3.9

[§§]Morphological identification of the *Culex* mosquitoes revealed 94% were *Cx. pipiens* complex. Of these, PCR assays revealed that 81% were *Cx. quinquefasciatus,* 2% were *Cx. pipiens pipiens* and 3% were hybrids of the two species. About 14% of the specimens were non-amplified. These test results can therefore be considered primarily representative of *Cx. pipiens* complex or more specifically for *Cx. quinquefasciatus*.

SS: Mosquitoes were susceptible to the test insecticide (WHO assays mortality between (98% and 100%).

RS: Mosquitoes had reduced susceptibility indicating possible resistance and need for further investigation (mortality of 90-97%).
RR: Mosquitoes were confirmed resistant to the test insecticide (WHO assays mortality below 90%).
++ Chemicals for which we observed differences in susceptibility of *Culex* mosquitoes between dry and wet seasons, i.e. where mosquitoes were fully susceptible in one season and fully resistant in a different season in same ward.
^^ Chemicals for which we observed differences in susceptibility of *Culex* mosquitoes between (nearby) wards, i.e. where mosquitoes were fully susceptible in one ward and fully resistant in another ward during the same season.
Ÿ: There is statistically significant difference in mortality between dry and wet seasons (t-test, $p<0.050$)

4.5.4 Effects of synergists on pyrethroid and DDT resistance phenotypes

Results of synergist tests on the different resistance phenotypes are detailed in Tables 4.4 and 4.5. In Minepa ward, samples synergized with 4% PBO exhibited mean mortality of 57.5% on exposure to 0.05% lambda-cyhalothrin, compared to 35.0% in un-synergized cohorts. The difference in mean mortality was marginal, when examined using two-sample *t*-test (degree of freedom (df) = 6; t = 2.50; P = 0.047). Conversely, synergizing the same population with 20% TPP did not change the mortality after exposure to lambda cyhalothrin (df = 6; t = 0.23; P = 0.827). Resistant phenotype pre-exposure to 20% TPP followed by exposure to deltamethrin, resulted in 1.6-fold increase in mortality, relative to exposure to deltamethrin alone (81.3% *versus* 51.3%). This difference was statistically significant (df = 3; t = 2.84; P = 0.03). Similarly, there was a statistical difference in mortalities after exposure to 0.05% deltamethrin with or without pre-exposure to 4% PBO (93.8% *versus* 73.8%; df = 4; t = 2.99; P = 0.042). However, there was no difference in mortalities in mosquitoes exposed to 4% DDT (90%) with or without pre-exposure to 20% DEM (95% *versus* 90%, df =6; t = -1.73, P = 0.134).

In Mavimba ward, there was a significant increase in mortality when mosquitoes were pre-exposed to 20% TPP, followed by lambda-cyhalothrin, as opposed to exposure to lambda-cyhalothrin alone (83.8% *versus* 72.5%; df =6; t = 2.80, P = 0.03). Similarly, pre-exposure to 4% PBO, followed by lambda-cyhalothrin increased mortality relative to exposure to lambda cyhalothrin alone (66.3% *versus* 28.8%; df = 6; t = 6.60, P <0.001). There was a marginal increase in mortality when mosquitoes were pre-exposed to 20% TPP, followed by exposure to deltamethrin, compared to exposure to deltamethrin alone (86.0% *versus* 75%; df = 6; t = 3.42; P = 0.014). Pre-exposure to 4% PBO, followed by deltamethrin also resulted in higher mortality relative to cohorts exposed to deltamethrin only (60.0% *versus* 41.3%; t = 3.17; P = 0.019). Lastly, pre-exposure to 20% DEM, followed by 4% DDT increased mortality in the synergized cohorts, compared to their un-synergized counterparts (82.5% *versus* 48.8%; df =6; t = 5.89, P = 0.001).

Table 4.4 Mean % mortality recorded 24 hours after exposure to lambda cyhalothrin and deltamethrin, with and without synergist, TPP (triphenyl phosphate) or PBO (piperonyl butoxide). The mosquitoes tested were 3- to 5-day-old adult *Culex* mosquitoes reared from wild-collected larvae from Minepa and Mavimba wards in Ulanga district, Tanzania in 2015 and 2016 [§§].

Treatment	No. replicates	Sample size	Mean % mortality (95% CI): Mavimba (8.312°S, 36.677°E) 0.05% deltamethrin	Mavimba 0.05% lambda cyhalothrin	Minepa (8.271°S, 36.677°E) 0.05% deltamethrin	Minepa 0.05% lambda cyhalothrin
Tests with triphenyl phosphate (TPP)						
Environmental control[&]	4	80	0	1.3 (-2.7 -5.2)	0	0
Solvent control	4	80	0	0	0	0
20% TPP only	4	80	0	1.3 (-2.7- 5.2)	0	0
20% TPP and test insecticide	4	80	86.0 (77.8-94.2)[a]	83.8 (76.1-91.4)[a]	81.3 (63.6-98.9)[a]	73.8 (48.3-99.2)[a]
Test insecticide only	4	80	75.0 (68.9-81.1)[b]	72.5 (62.2-82.8)[b]	51.3 (23.7-79.8)[b]	71.3 (47.5-95.2)[a]
Tests with piperonyl butoxide (PBO)						
Environmental control[&]	4	80	0	0	0	0
Solvent control	4	80	0	1.3 (-2.7-5.2)	0	0
4% PBO only	4	80	0	1.3 (-2.7-5.2)	0	0
4% PBO and test Insecticide	4	80	60.0 (42.8-77.2)[a]	66.3 (54.3-78.2)[a]	93.8 (86.1-101.4)[a]	57.5 (32.8-82.2)[a]
Test insecticide only	4	80	41.3 (33.6-48.9)[b]	28.8 (15.2-42.3)[b]	73.8 (53.9-93.6)[b]	35.0 (20.5-49.5)[b]

[§§]Morphological identification of the *Culex* mosquito populations revealed 94% were *Cx. pipiens* complex. Of these, PCR assays revealed that 81% were *Cx. quinquefasciatus,* 2% were *Cx. pipiens pipiens* and 3% were hybrids of the two species. About 14% of the specimens were non-amplified. These test results can therefore be considered primarily representative of *Cx. pipiens* complex or more specifically for *Cx. quinquefasciatus*.

[&] Environmental control refers to a control where mosquitoes are exposed to non-treated papers, and is used to assess any contamination in the test environment or during the procedures.

[a,b] The letters a and b signify statistically significant differences between % mortalities obtained in tests with or without the synergists.

Table 4. 5 Mean % mortality recorded 24 hours after exposure to 4% DDT, with and without the synergist, diethyl maleate (DEM), The mosquitoes tested were 3- to 5-day-old adult *Culex* mosquitoes reared from wild collected larvae from Minepa and Mavimba wards in Ulanga district, Tanzania in 2015 and 2016[§§].

			Mean % mortality (95% CI)	
Treatment	**No. replicates done**	**Sample size**	**Mavimba (8.312°S, 36.677°E)**	**Minepa (8.271°S, 36.677°E)**
Environmental control[&]	4	80	0	0
Solvent control	4	80	0	0
20% DEM only	4	80	0	0
20% DEM and 4% DDT	4	80	82.5 (67.3-97.7)[a]	90.0 (83.5-96.5)[a]
4% DDT only	4	80	48.8 (38.7-58.8)[b]	95.0 (88.5-101.5)[a]

[§§]Morphological identification of the *Culex* mosquitoes revealed 94% were *Cx. pipiens* complex. Of these, PCR assays revealed that 81% were *Cx. quinquefasciatus,* 2% were *Cx. pipiens pipiens* and 3% were hybrids of the two species. About 14% of the specimens were non-amplified.

[&] Environmental control refers to a control where mosquitoes are exposed to non-treated papers, and is used to assess any contamination in the test environment or during the procedures.

[a,b] The letters a and b signify statistically significant differences between % mortalities obtained in tests with or without the synergists.

4.5.5 Estimated biting densities of *Culex,* relative to other mosquito species

Of the 387,318 mosquitoes collected indoors during the sampling period Culex constituted 77% (n = 299,841) of the total catches. Of these, 79% were females (n = 236,484) and 21% males (n = 63,375). In total, 1,053 Culex mosquitoes were sub-sampled for species-specific identification; of these all were identified as members of the *Cx. pipiens* complex (Table 4.6).

Table 4.6: Relative abundance and indoor distribution of mosquitoes, across three study wards (including Minepa and Mavimba wards, from where *Culex* larvae were also obtained for the resistance tests). Data obtained from an annual mosquito surveillance programme conducted by the Ifakara Health Institute in Ulanga district, south-eastern Tanzania in 2012, 2013, and 2015

	Mosquito species	**2012**	**2013**	**2015**	**Total**
Minepa Ward	Total mosquitoes collected	57,393	23,448	39,359	120,200
	An. arabiensis, females ¥	15,305 (26.6%)	9,224 (39.3%)	10,950 (27.8%)	35,479
	An. funestus group, females	7,713 (13.4%)	1,582 (6.7%)	3,097 (7.9%)	12,392
	Cx. pipiens complex, males	6,469 (11.2%)	2,062 (8.7%)	4,160 (10.5%)	12,691
	Cx. pipiens complex, females €	27,906 (48.6%)	10,580 (45.1%)	21,152 (53.7%)	59,638
Mavimba ward	Total mosquitoes collected	44,378	14,673	23,540	82,591
	An. arabiensis, females ¥	4,292 (9.6%)	3,158 (21.5%)	2,101 (8.9%)	9,551
	An. funestus group, females	2,460 (5.5%)	894 (6.0%)	793 (3.4%)	4,147
	Cx. pipiens complex, males	8,608 (19.3%)	1,418 (9.6%)	3,034 (12.8%)	13,060
	Cx. pipiens complex, females €	29,018(65.4%)	9,203 (62.7%)	17,612 (74.8%)	55,833
Kivukoni ward	Total mosquitoes collected	98,902	34,374	51,251	184,527
	An. arabiensis, females ¥	9,572 (9.6%)	4,416 (12.8%)	7,070 (13.7%)	21,058
	An. funestus group, females	3,327 (3.3%)	663 (1.9%)	860 (1.6%)	4,850
	Cx. pipiens complex, males	18,905 (19.1%)	7,546 (21.9%)	11,155 (21.7%)	37,606
	Cx. pipiens complex, females €	67,098 (67.8%)	21,749 (63.2%)	32,166 (62.7%)	121,013

¥Sub-samples of *An. gambiae* complex mosquitoes collected in this area during this period have consistently been 100% *An. arabiensis*.

€A sub-sample of 1,053 *Culex* mosquitoes were subjected to further morphological examination and identified as *Cx. pipiens* complex

4.6 Discussion

Until this study, the insecticide susceptibility status of non-malaria vectors such as Culex was widely unknown in Tanzania, despite abundance of these mosquito species. The present study investigated susceptibility of *Cx. pipiens* complex, to insecticides approved by WHO for vector control. WHO insecticide susceptibility bioassays were conducted separately for female and male *Culex* mosquitoes collected in different seasons and different wards, for both male and female mosquitoes.

Generally, *Culex* mosquitoes were found resistant to a wide range of pyrethroids, lambda-cyhalothrin and deltamethrin, DDT and the carbamate, bendiocarb. However, these species showed susceptibility to organophosphates, such as pirimiphos-methyl and malathion except for populations from Mavimba ward, which were resistance to these insecticide classes.

While resistance was widespread across the study sites, lowest mortalities were observed against bendiocarb in Minepa (mortalities of 29% in females and 58% in males *Culex* mosquitoes) and against deltamethrin in Lupiro (8% mortality in females *Culex*). Unlike most resistance surveys which focus on female mosquitoes, we also assessed susceptibility status of males to insecticides. Previous studies have reported that resistance in male *Culex pipiens* and *An. gambiae* could potentially affect mating competitiveness in nature [200-202]. Besides, information on insecticide susceptibility of male mosquitoes could be useful when designing interventions primarily against males, e.g. sterile insect technique (SIT) [203,204], spraying of males swarms with insecticides [205] and use of attractive toxic sugar baits [206,207].

In the study areas LLINs impregnated with permethrin remain as the primary vector and diseases control interventions [37]. This study confirmed phenotypic resistance to this and also other public health insecticides that are currently not used in the study area. This suggests alternative sources of insecticide resistance selective pressure, most likely from agricultural pesticides [85,127-129]. Indeed, direct observation in the communities revealed an array of chemical classes widely sold and used for crop protection (Matowo et al unpublished). Therefore, for effective vector control, an integrated approach with agricultural pest control programmes in the allocation of insecticides is recommended. With reference to *Culex*, which also causes considerable biting nuisance in these communities where most people are small-holder farmers [111], the need for integrated pest and vector management across public health and agriculture is particularly important.

The significant differences in resistance between the neighbouring wards, as depicted in this study, has also been reported for *Anopheles* [84], and clearly suggest that there are finer-scale differences, e.g. between small administrative wards, other than variations previously reported between districts and regions [156,158,160]. It may also indicate that there could be some environmental barriers that temporarily prevent spread of resistance across seasons and from one locality to another. These variations signify an important challenge to control programmes when choosing insecticides for particular time periods and locations. For example, susceptibility was generally higher in the wet season than dry season. Thus, the possibility that insecticide-based interventions aimed at this time may have greater impact on mosquito densities than those in the dry season, should be investigated.

These fine-scale spatial and temporal variations are increasingly being reported across multiple sites. In one study in one area in Mexico, both resistance phenotypes and genotypes were markedly varied at a fine spatial scale and time, in *Aedes aegypti* populations against chorpyrifos-ethyl and deltamethrin, driven by fine-scale pressure from the household insecticides use [197]. In Uganda, there were monthly variations in *kdr* allele frequency in *Plasmodium*-infected *An. gambiae s.s.* and the resistance was significantly higher in dry compared to the wet season which is likely to be caused by seasonal changes in insecticide pressure [198]. A recent report by Jones *et al*. on insecticide resistance in *Cx. quinquefasciatus* from Zanzibar showed variability of resistance levels between nearby study sites, though the results were incomparable due to differences of *Culex* species at these sites [229]. Niang *et al.* also reported spatial variations of the L1014F *kdr* allele found to dominate in *An. arabiensis* compared to *An. coluzzii,* and *An. gambiae* sampled from 20 different study sites in the south-eastern part of Senegal [241].

Mechanisms for resistance appear to be mixed. The partial suppression of pyrethroid resistance by synergist PBO and TPP exposures suggests that both P450 monooxgenases and esterases might be contributing to the pyrethroid resistance phenotypes observed in mosquito populations sampled from both Minepa and Mavimba wards. However, esterases seemed not to be involved in lambda-cyhalothrin resistance in mosquitoes from Minepa ward, as only minimal change in mortality was observed upon pre-exposure to the synergist. In addition, DDT resistance was significantly restored after being exposed to DEM, suggesting a role for GSTs in DDT resistance in Mavimba ward. However, DDT was not affected by DEM in Minepa samples, suggesting no role for metabolic resistance mechanisms here. Besides metabolic resistance, other resistance mechanisms, such as *kdr* mutation, could play a role and further research is required to identify the mechanisms of resistance. These observations are consistent with previous studies on incomplete suppressions of pyrethroids and DDT resistances due to pre-exposure to synergists [168,229,242]. Nonetheless, the multiplicity of resistance mechanisms in these mosquito populations is a major concern and should be considered by control programmes.

In line with the WHO Global Vector Response Initiative [34], it is important to integrate control of different arthropod vectors. In this area, where malaria is certainly the most important mosquito-borne disease, 79% of biting risk indoors was associated with *Cx. pipiens* mosquitoes. Despite long-term use of the permethrin-based Olyset® nets, which are regularly distributed via the national government's mass distribution campaigns [37], *Culex* mosquitoes were fortunately found susceptible to permethrin, except in Lupiro ward in the wet season (Table 3.3). Nonetheless, as resistance continues to spread, additional approaches, such as improved housing, larval source management or IRS with non-pyrethroids and non-carbamates, may be considered as alternatives against both *Culex* and malaria vectors.

The most abundant *Culex* species in tropical and subtropical countries, including East Africa, belong to the *Cx. pipiens* complex, which contains *Cx. quinquefasciatus, Cx. pipiens pipiens, Cx. pipiens torrentium* and *Cx. pipiens molestus* [14,217]. From our findings, 94% of *Culex* belonged to *Cx. pipiens* complex, of which 81% were verified by PCR as *Cx. quinquefasciatus*; 2% as *Cx. pipiens pipiens* and 3% as hybrids of *Cx. pipiens pipiens* and *Cx. quinquefasciatus.* A small proportion 14% of samples were non-amplified suggesting other Culex species of which they were no primers to separate. The presence of hybrids of *Cx. pipiens pipiens* and *Cx. quinquefasciatus* suggests that these species cross-mate in the wild. *Cx. quinquefasciatus* was previously documented through morphological identification as the dominant *Culex* species in Kilombero valley, where it occurred alongside a few *Cx. theileri* and *Cx. univittatus* [65]. However, none of these species were confirmed by PCR [65].

One of the limitations of this study is that we could not analyse many *Culex* samples, to identify other possible *Culex* sibling species using PCR techniques due to the lack of appropriate primers. Also, synergists findings which are presented here came from only females Culex species sampled in dry season in Minepa nd Mavimba wards as synergist papers are not easily accessible and not produced in bulk.

4.7 Conclusions

Cx. pipiens complex, which mostly consists of *Cx. quinquefasciatus,* are the most abundant *Culex* species in the study area, and contributes more than 79% of all biting risk experienced in houses. There is widespread resistance to carbamates and pyrethroids commonly used in public health inside houses, and also to DDT. The organophosphate, pirimiphos-methyl, which is also available for indoor spraying however remains effective. This study has also demonstrated that insecticide resistance phenotypes and the underlying mechanisms can vary significantly at fine geographic scales, such as between neighbouring wards, and between dry and wet seasons. Monooxygenases and esterases partly underlie the resistance phenotypes against pyrethroids, while GSTs plays an important role in DDT resistance. Further investigations are required to identify more drivers and other mechanisms of resistance in *Culex* species across the wards. Overall, the extent of resistance here indicates that additional approaches, such as improved housing, larval source management or IRS with non-pyrethroids and non-carbamates, should be considered as alternative vector control strategies. Lastly, resistances against insecticides not currently used for vector control in the villages, suggests possible linkages to agricultural pesticides use. Hence, multi-sectorial approaches should be encouraged to improve management of resistance.

Ethics statement

Following a detailed introduction of the aims, study procedures, potential risks and benefits, written informed consents were obtained from all the owners of the farms where larvae sampling were carried out. This study received ethical review and approval from institutional review board of the Ifakara Health Institute, Tanzania (reference no. IHI/IRB/NO: 34-2014) and the Medical Research Coordinating Committee at the National Institute for Medical Research in Tanzania (reference no. NIMR/HQ/R.8a/Vol.IX/1903). This work is published upon the permission of the Director General of the National Institute for Medical Research in Tanzania (reference no. NIMR/HQ/P.12 VOL XXVII/1).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declared no potential conflict of interests.

Funding

This work was financially supported by Wellcome Trust Masters Fellowship (grant no. WT104029/Z/14/Z) awarded to the lead author (NSM) and Wellcome Trust Intermediate Research Fellowship (grant no. WT102350/Z/13/Z) awarded to FOO. NSM receives training support from Swiss Government Excellence Scholarship via the Federal Commission for Scholarships for Foreign Students FCS (ESKAS-Nr: 2017.0786), and FOO was supported by WHO/TDR (grant no. B40445). MC and LLK are supported by a DST/NRF SA Research Chair grant.

Authors' contributions

NSM and FOO conceived the study. NSM, EWK and FOO designed the experiments. NSM, SAM and EWK performed the field experiments under the mentorship of MT, LLK, MC, JU and FOO. GM facilitated the training on synergist tests and DO and MC supported in the morphological identifications of Culex species. NSM, SA, and GM performed molecular analysis. NSM, GM, HSN and FOO analysed the data. NSM drafted the original manuscript. NSM, GM, MT, DO, LLK, MC, JU and FOO reviewed the initial draft, and all authors approved the final version of the manuscript.

Acknowledgements

We are grateful to the communities of Ulanga wards for giving us a permission to collect larvae from their farms. We highly acknowledge Paulina K. Kasanga and Neema K. Nombo for assisting with the mosquito larvae sampling and adults rearing.

5 Chapter 5: Patterns of pesticide usage in agriculture in rural Tanzania call for integrating agricultural and public health practices in managing insecticide-resistance in malaria vectors

Nancy S. Matowo[1,2,3,4]*, Marcel Tanner[2,3], Givemore Munhenga[5,6], Salum A. Mapua[1], Marceline Finda[1,7], Jürg Utzinger[2,3], Vera Ngowi[8], Fredros O. Okumu[1,7,9,10]

[1]Environmental Health & Ecological Sciences, Ifakara Health Institute, Ifakara, Tanzania
[2]Swiss Tropical and Public Health Institute, Basel, Switzerland
[3]University of Basel, Basel, Switzerland
[4]Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK
[5]Wits Research Institute for Malaria, School of Pathology, University of the Witwatersrand, Johannesburg, South Africa
[6]Centre for Emerging Zoonotic and Parasitic Diseases, National Institute for Communicable Diseases, Johannesburg, South Africa
[7]School of Public Health, University of the Witwatersrand, Johannesburg, South Africa
[8]Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania
[9]Institute of Biodiversity, Animal Health and Comparative Medicine, Glasgow, University of Glasgow, UK
[10]School of Life Science and Bioengineering, Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania

*corresponding author
Email: nstephen@ihi.or.tz

Published in Malaria Journal 2020 19, 257

https://doi.org/10.1186/s12936-020-03331-4

Abstract

Background

Unrestricted use of pesticides in agriculture is likely to increase insecticide resistance in mosquito vectors. Unfortunately, strategies for managing insecticide resistance in agriculture and public health sectors lack integration. This study explored the types and usage of agricultural pesticides, and awareness and management practices among retailers and farmers in Ulanga and Kilombero districts in south-eastern Tanzania, where *Anopheles* mosquitoes are resistant to pyrethroids.

Methods

An exploratory sequential mixed-methods approach was employed. First, a survey to characterise pesticide stocks was conducted in agricultural and veterinary (agrovet) retail stores. Interviews to assess general knowledge and practices regarding agricultural pesticides were performed with 17 retailers and 30 farmers, followed by a survey involving 427 farmers. Concurrently, field observations were done to validate the results.

Results

Lambda-cyhalothrin, cypermethrin (both pyrethroids) and imidacloprids (neonicotinoids) were the most common agricultural insecticides sold to farmers. The herbicide glyphosate (amino-phosphonates) (59.0%), and the fungicides dithiocarbamate and acylalanine (54.5%), and organochlorine (27.3%) were also readily available in the agrovet shops and widely used by farmers. Although both retailers and farmers had at least primary-level education and recognised pesticides by their trade names, they lacked knowledge on pest control or proper usage of these pesticides. Most of the farmers (54.4%, n=316) relied on instructions from pesticides dealers. Overall, 93.7% (400) farmers practised pesticides mixing in their farms, often in close proximity to water sources. One-third of the farmers disposed of their pesticide leftovers (30.0%, n=128) and most farmers discarded empty pesticide containers into rivers or nearby bushes (55.7%, n = 238).

Conclusion

Similarities of active ingredients used in agriculture and malaria vector control, poor pesticide management practices and low-levels of awareness among farmers and pesticides retailers might enhance the selection of insecticide resistance in malaria vectors. This study emphasises the need for improving awareness among retailers and farmers on proper usage and management of pesticides. The study also highlights the need for an integrated approach,

including coordinated education on pesticide use, to improve the overall management of insecticide resistance in both agricultural and public health sectors.

Keywords Malaria Vector, Agricultural practices, Lambda-cyhalothrin, Chlorpyrifos, Chlorothalonil, Imidacloprid, Glyphosate, Pesticides Knowledge, Insecticide Resistance, Malaria

5.1 Background

The control of malaria and other vector-borne diseases relies primarily on insecticide-based interventions, such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) [1,34]. The effectiveness of these interventions is being compromised by the increased geographical spread of insecticide resistance in the targeted mosquito populations [159,243]. Insecticide-resistance by mosquito populations to the limited number of insecticides approved for vector control has been implicated as the key driver of persistent malaria transmission [108,244].

Insecticide resistance in malaria vectors is predominantly attributed to exposure of mosquitoes to public health insecticides [159,243]. However, agricultural pesticides also exert strong selection pressures, thus contributing to resistance in vector species [85,86,96,120,121,123,125,245]. This is because of similarities in chemicals used, applications of these chemicals simultaneously, and their indiscriminate use in agriculture [109]. This phenomenon was observed in West Africa where *Anopheles gambiae sensu lato* (*s.l.*) populations sampled from farmlands characterised by high agriculture pesticide usage showed higher levels of resistance to insecticides compared to populations sampled in areas with limited or no agricultural pesticide usage [120,123-125]. Similarly, in Sudan agricultural usage of organophosphate and carbamates was linked to insecticide resistance in *Anopheles arabiensis* [135]. Aquatic exposures of mosquito larvae to sub-lethal doses of pesticides, herbicides and other pollutants have also been linked to higher tolerance to insecticides in malaria vectors [85,127-129]. Furthermore, Chouaïbou and colleagues found that over 90% of the insecticides used by vegetable and rice farmers in the southern part of Côte d’Ivoire were pyrethroids similar to those approved for vector control [149].

In many malaria endemic countries, agriculture is the main economic activity. To improve crop yields in these regions there is the rampant use of pesticides, fungicides and herbicides [246-248]. For example, in Tanzania, approximately 81% of pesticides are deployed in both agricultural and veterinary sectors [146]. Concurrently, pyrethroid impregnated LLINs are also widely used against disease vectors in these regions.

The World Health Organization (WHO) Global Malaria Programme has developed a global action plan for insecticide resistance management in malaria vectors to preserve the effectiveness of LLINs and IRS [68]. The principal recommended resistance management approaches, mostly adopted from agriculture include: (i) annual rotation of insecticides with different modes of action; (ii) combination of pyrethroid-based LLINs and IRS with non-pyrethroids; (iii) mosaic spraying of two different insecticide classes in different geographical locations; and (iv) mixtures of different classes of insecticides into a single product [68]. However, resistance management policies have yet to be integrated into agricultural and disease control programmes. As a result, the programmes do not account for the collective contributions by both public health and agricultural sectors to the spread of insecticide resistance.

The purpose of this study was to explore agricultural pesticides, pesticide usage practices, awareness, and management practices among retailers and farming communities from a rural malaria endemic area in south-eastern Tanzania, where mosquito vectors are resistant to public health insecticides [84,233]. The findings are expected to guide practical recommendations for collaboration between agriculture and public health sectors in insecticide resistance management in mosquito vectors and disease control.

5.2 Methods

5.2.1 Study area

The study was conducted in six wards, in Kilombero and Ulanga districts, south-eastern Tanzania (altitude ~300 m; annual precipitation: 1,200-1,800 mm; temperatures: 20-32 ºC), purposefully selected to represent different agro-ecological areas (Figure 5.1). Rice farming is the main economic activity of the area [232]. Vegetable and fruit cultivation is also quite common. Farmers here widely use synthetic pesticides and chemical fertilisers. During the dry season, rice production is maintained by irrigation (locally known as *"Ngapa"*) rendering the area continuously favourable for mosquitoes [249]. Malaria burden remains significant, with the heaviest burden experienced in children below five years [250,251]. *Anopheles funestus* s.s. and *An. arabiensis* are the predominant malaria vectors [84,233]. Additionally, non-malaria vectors, such as *Culex* and *Mansonia,* constitute biting nuisances [15,65]. Though pyrethroid-based LLINs are the main malaria intervention [37], mosquito populations are resistant to pyrethroids, bendiocarb (carbamates), and DDT [15,84,233].

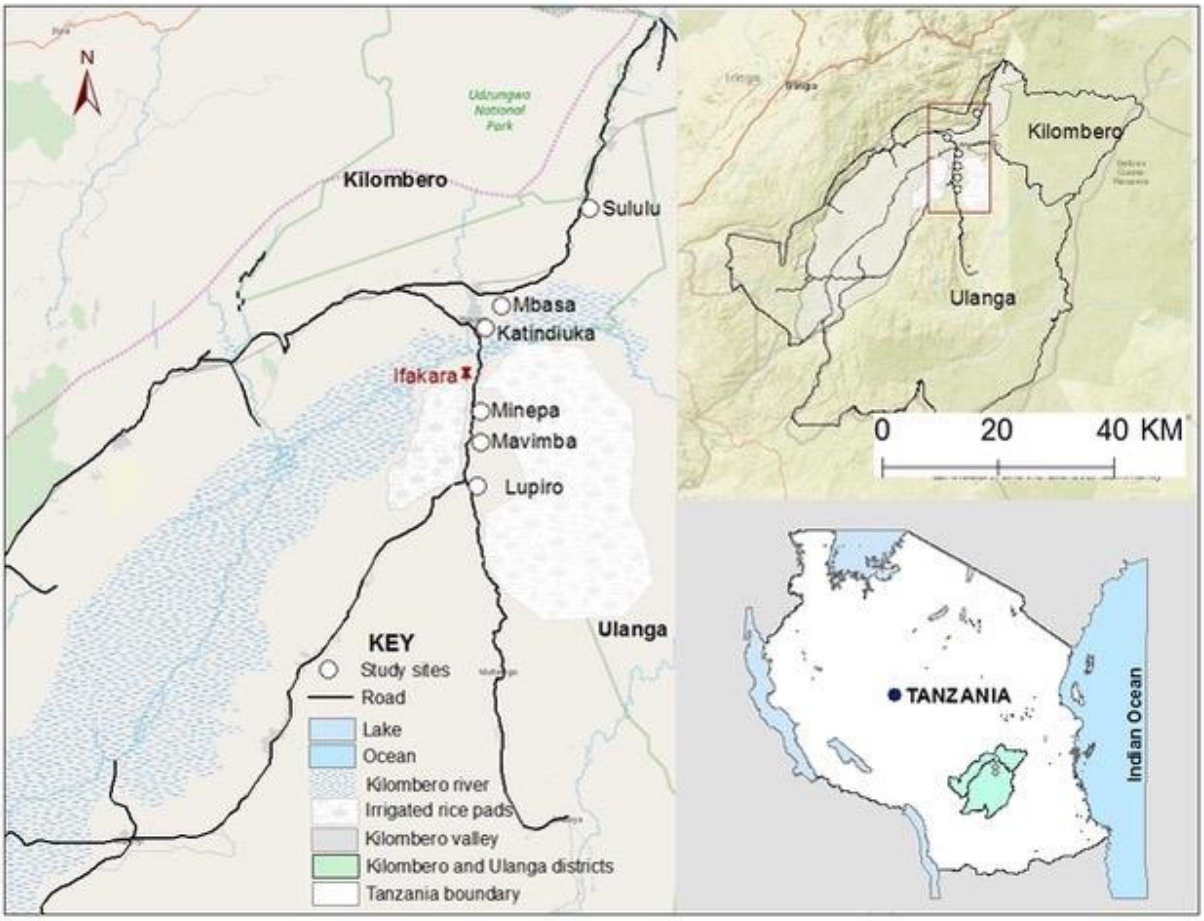

Figure 5.1: Map showing the study wards in the districts of Kilombero and Ulanga in the Kilombero Valley in south-eastern Tanzania.

5.2.2 Study design and data collection

An exploratory sequential mixed-methods study design was employed. In-depth interviews were done for collecting qualitative data and structured questionnaires were used to collect quantitative data. Both data collection tools were prepared in English, translated and used in Kiswahili the local native language. The questionnaires were pre-tested on a few participants (who were not otherwise enrolled in the actual study) to ensure clarity before the actual study. Direct observations were made and photographs taken of the pesticides in the stores to identify their active ingredients, and handling practices. In the farms prior observations were validated on pesticides usage and handling practices. Data collection was conducted between February 2017 and November 2017.

Exploration of awareness and perceptions of pesticides use, storage and disposal: In-depth interviews were conducted with agricultural and veterinary (agrovet) retail stores (n=17) and with famers in the six wards (n=30). With the retailers, the interviews aimed to explore awareness of pesticide prescription and handling practices. Interview guides explored the retailers' awareness and perceptions of (i) types of agricultural pesticides, knowledge of pesticides sold at their shops; and (ii) source of knowledge on using the pesticides, pesticides preferences, frequency of purchases and seasonal use of the pesticides/frequency of applications. With the community members, the interviews explored awareness and perceptions regarding different agricultural pesticides, use and storage methods, and challenges faced. Direct observations of agricultural practices in the farms, including handling and disposal practises of the pesticides were also done. Initial findings from these qualitative studies informed subsequent quantitative studies. All interviews were audio-recorded and field notes taken by the data collector.

Assessment of knowledge and practices regarding pesticide use: A cross-sectional survey using an electronic questionnaire form in an Open Data Kit (ODK) [252] was conducted with 427 randomly selected farmers from the six wards. The questionnaire assessed the farmers' awareness and practices of agricultural pesticides use, storage and disposal. Findings from the qualitative and quantitative study and direct observations were triangulated.

Assessment of types and classes of agricultural pesticides: Direct observations of the agricultural pesticides were done at all of the 17 agrovet retail stores. Information collected included pesticide types, classes and active ingredients.

5.3 Analysis of qualitative and quantitative data

Audio recorded interviews with the retailers of agricultural pesticides and farmers were transcribed verbatim and translated to English. The transcripts were imported into MAXQDA software for coding and analysis [253]. Systematic review and analysis of key issues, concepts, and repeated themes were done following framework analysis steps as described by Gale and colleagues [254]. For the data from farmers, a weaving approach was used, in which both quantitative and qualitative components were presented together [255]. Quantitative findings from the survey were presented, and further explanations drawn from the in-depth interviews. Selected participant's narratives from each theme are presented.

Quantitative data generated through surveys from agrovet stores were analysed descriptively, using Stata version 15 (Stata Cooperation; College Station, TX, USA). Pictures of all of the insecticides were individually reviewed and active chemical ingredients recorded to summarise their frequencies by insecticide class.

5.4 Results

5.4.1 Characteristics of pesticide retailers and farmers

More than half (58.8%, n=10) of the agrovet stores were in Kilombero district, while the remaining 41.2% (n=7) were in Ulanga district. Two-thirds of participants (65.2%, n=11) were females with age ranging between 18 and 43 years.

Table 4.1 summarises the demographic characteristics of the farmers who participated in the survey. Males comprised of 51.5% (n=220) and females 48.4% (n=207). Most farmers practised both small-scale subsistence farming 51.3%, (n=219) and large-scale cultivation 48.5% (n =207) for food and business, and had worked on their farms for at least five years 89.2% (n=381). The main farm crops farmed were rice, maize, different types of vegetables and fruits.

Table 5.1: Socio-demographic characteristics of farmers involved in the survey

Variable	Category	Percentage (n)
Gender	Males	51.5% (220)
	Females	48.5% (207)
Age (years)	18-30	16.9% (72)
	31-40	31.1% (133)
	41-50	28.3% (121)
	51-60	17.6% (75)
	>60	6.1% (26)
Education attainment	Primary school	85.2% (364)
	Secondary school	9.6% (41)
	College/university	0.7% (3)
	Professional training	0.5% (2)
	No formal training	4.0% (17)
Main economic activities¥	Small-scale subsistence farming activities	51.3% (219)
	Large-scale farming for food and business	48.5% (207)
	Livestock keeping	9.8% (12)
	Small-scale business	41.7% (178)
	Large-scale business	2.8% (3)
	Private employment	0.7% (2)
	Others	0.5% (42)

¥ (farmers with more than one sources of income, multiple responses)

5.4.2 Types and classes of agricultural pesticides

The agricultural pesticides (supplementary file 5.1), chemical classes and the active ingredients observed in the agrovet stores are summarised in Table 5.2. Most of the agricultural pesticides (87.5%, n=91) were approved plant protection substances under full registration category (6.7%, n =7) or had restricted registration or provisional registration according to Tanzania regulations [140,141]. A small proportion (2.9%, n=3) were unregistered. Insecticides accounted for (59.6%, n =62) of the pesticides, followed by herbicides (27.9%, n=29) and fungicides (10.6%, n=11). The highest proportion of agricultural insecticides surveyed were organophosphates (34%), followed by pyrethroids (30%). Herbicides from the amino-phosphonates class were the most popular (59%). The two main fungicide classes were dithiocarbamate (54.5%) and acylalanine organochlorine (27.3%), widely used by most vegetable growers (Table 5.2). The insecticide formulations were emulsifiable concentrate (EC) (63%), while (66%) herbicides, and (64%) fungicides were formulated as soluble (liquid) concentrate (SL) and wettable powders (WP), respectively.

Table 5.2: Common active ingredients found in the agricultural pesticides in the study locality

Pesticide type	Active ingredient (s)	N	%	Chemical class
Insecticides (N=62)	Abamectin	4	6.5	Macrocyclic lactones
	Alphacypermethrin	3	4.8	Pyrethroids
	Carbaryl and permethrin	1	1.6	Carbamates and pyrethroids
	Carbofuran	1	1.6	N-methyl carbamate Ib
	Carbryl and lambda-cyhalothrin	2	3.2	Carbamates and pyrethroids
	Chlorpyrifos	5	8.1	Organophosphates
	Cypermethrin	1	1.6	Pyrethroids
	Cypermethrin and chlorpyrifos	1	1.6	Pyrethroids and organophosphates
	Cypermethrin and imidacloprid	3	4.8	Pyrethroids and neonicotinoids
	Deltamethrin	1	1.6	Pyrethroids
	Diazinon	2	3.2	Organophosphates
	Dichlorvos	3	4.8	Organophosphates
	Dimethoate	1	1.6	Organophosphates
	Fenitrothion and deltamethrin	3	4.8	Organophosphates and pyrethroids
	Fipronil	1	1.6	Phenylpyrazole
	Imidacloprid	3	4.8	Neonicotinoids
	Imidacloprid and beta-cyfluthrin	2	3.2	Neonicotinoids and pyrethroids
	Lambda-cyhalothrin	11	17.7	Pyrethroids
	Lambda-cyhalothrin and acetamiprid	1	1.6	Neonicotinoids and pyrethroids
	Malathion	1	1.6	Organophosphates
	Permethrin	1	1.6	Pyrethroids
	Pirimiphos-methyl	2	3.2	Organophosphates
	Pirimiphos-methyl and permethrin	3	4.8	Organophosphates and pyrethroids
	Pirimiphos-methyl and thiamethoxam	1	1.6	Organophosphates and neonicotinoids
	Profenofos	5	8.1	Organophosphates
Herbicide (N = 29)	Bispyribac sodium	1	3.5	Bispyribac sodium
	S-metolachlor and atrazine	1	3.5	Triazines
	Amine salt	4	13.8	Aryloxyacides

	Atrazine	1	3.5	Dinitroanilines
	Glyphosate	17	58.6	Amino-phosphonates
	Paraquat	4	13.8	Pyridines
	Triclopyr	1	3.5	Pyridines
Fungicide (N= 11)	Monopotassium and dipotassium phosphonates	1	9.1	Phosphonic acid
	Chlorothalonil	3	27.3	Organochlorine
	Mancozeb	1	9.1	Dithiocarbamate
	Mancozeb and cymoxanil	1	9.1	Acylalanine and dithiocarbamate
	Metalaxyl and mancozeb	5	45.5	Dithiocarbamate and acylalanine
Insecticide + fungicide (N=2)	Imidacloprid, metalaxyl and carbendazim	2	100	Neonicotinoids, acylalanine and benzimidazole

Most insecticides had a single active ingredient (72.6%, n= 45), while fewer were mixed products with two different active ingredients at different doses (27.4%, n=17), as shown in Tables 5.2 and 5.3. The most common pyrethroid was lambda-cyhalothrin, while chlorpyrifos and profenofos were the predominant organophosphates (Table 5.2). Most of the insecticides are non-systemic broad-spectrum insecticides with contact and stomach actions against crop pests. Over half of the herbicides (59%) were based on glyphosates that were frequently used by most of the rice farmers (76.8%). The principle active ingredients in most fungicide were metalaxyl and mancozeb (45%) and chlorothalonil (27%) (Table 5.2). Table 5.3 summarizes some of the commonly used pesticide products with more than one active ingredients. A wide range of insecticide classes and active ingredients used in crop protection had similar target sites and modes of action with the limited public health insecticides (Table 5.4).

Table 5.3: Example of pesticide products with more than one active ingredient (as obtained from the factory)

WHO class/family	Brand name	Active ingredient(s)
Organophosphates and pyrethroids	Simba powder 113 DP	10 g/kg of fenitrothion and 1.3 g/kg of deltamethrin
	Duduba 450 EC	350 g/l of chlorpyrifos and 100 g/l of cypermethrin
	Mupa dust	1.0% of fenitrothion and 0.13% of deltamethrin
	Stocal super dust	16 g/kg of pirimiphos-methyl and 3 g/kg of permethrin
	Shumba super dust	1% of fenitrothion and 0.13% of deltamethrin
	Actellic Gold Dust	16 g/kg of pirimiphos-methyl and 3.6 g/kg of thiamethoxam
	Haigram 90 dusting powder (DP)	6 g/kg of pirimiphos-methyl and 3 g/kg of permethrin
	Actellic super dust	16 g/kg of pirimiphos-methyl and 3 g/kg of permethrin
Pyrethroids and neonicotinoids	Amekan C344 SE	144 g/l of cypermethrin and 200 g/l of imidacloprid
	Rapid-attack 344 SE	144 g/l of cypermethrin and 200 g/l of imidacloprid
	Blast 60 EC	3% g/l lambda-cyhalothrin and 3% g/l of aceptamiprid
	Buffalo 450OD	2.5% of betacyfluthrin and 7.5% of imidacloprid
	Thunder Oil Dispersion (OD) 145	45 g/l of beta-cyfluthrin and 100 g/l of imidacloprid
	Farmguard 344SE	144 g/l of cypermethrin and 200 g/l of imidacloprid
Carbamates and pyrethroids	Bakiller	5% w/w of carbaryl and 0.1% w/w of lambda cyhalothrin
	Akheri Powder	5% w/w carbryl and 0.1% w/w lambda-cyhalothrin
	Ultravin® Dudu dust	5% w/w of carbaryl, 1% w/w of permethrin and 94% w/w of inert carriers
Neonicotinoids, acylalanine and benzimidazole	Seed plus 20 wettable soluble (WS)	10% imidacloprid, 5% metalaxyl and 5% carbendazim WS

Table 5.4: Similarities between agricultural and public health insecticide classes and reported resistance mechanisms in disease vectors

Class of insecticide	Trade name (active ingredient (s)	Primary site/mode of action in an insect/vector	Agricultural use	Public health use	Known resistance and resistance mechanism in disease vectors
Pyrethroids	Karate 5 EC (lambda-cyhalothrinin)	Voltage-gated sodium channels/neurotoxic	Control of bollworms and aphids in vegetables and cotton [256]	Disease and vector control (IRS and LLINs) [257,258]	Knock-down mutation [88] Metabolic resistance [259] Cuticle thickening [260]
Organophosphates	Dasba 40 EC (chloropyrifos)	Acetylcholinesterase (AChE) inhibitors	Insecticide against insect pests in fruits, beans, tomatoes, cotton, coffee and green vegetables [261]	Disease and vector control (IRS and LLINs) [262]	Metabolic resistance [263]
Neonicotinoids	Amekan C344 SE (200 g/l of imidacloprid and 144 g/l of cypermethrin)	Nicotinic acetylcholine receptors (n AChRs)	Systemic insecticides with contact and stomach action against sucking and chewing pests on cotton, vegetables and flowers [264].	Prequalified vector and disease control products [265,266]	Metabolic resistance and target-sites [139,267]
Carbamates	Farmerzeb 80 WP (80% WP of mancozeb)	Acetylcholinesterase (AChE) inhibitors	A broad spectrum protectant and preventive fungicide for the control of fungal diseases on vegetables	Disease and vector control (IRS and LLINs) [268]	Metabolic resistance [42,269]

5.4.3 Awareness and perceptions of pesticide use among agrovet store retailers

Most retailers stated that their customers were mostly rice farmers or horticulture farmers, particularly those relying on the irrigation system. The frequency of purchasing particular pesticides depended on the season. A majority of retailers reported to have no formal training on the pesticides they were selling, and poor knowledge on the type of crop pests, disease and relevant pesticides to be used for each. They were only able to recommend the use (dilution and frequency of application) based on experiences, or based on recommendations from the store owners and pesticide suppliers:

> *"I have been selling pesticides for a long time. I started to work in Ifakara town shops. Also, the owner of the shop understands pesticides, and she does assist with information whenever needed"* (male retailer).

A majority of the retailers also reported giving instructions to their customers on pesticide usage, dosage and application time. However, upon examining the pesticide labels, the dosage suggested by the retailers was sometimes higher or lower than those recommended by the manufacturers on the product label. The handling of pesticides was commonly practised without protective measures. However, the retailers also occasionally provided information on use of protective measures such as wearing long-sleeve shirts and boots during preparation and spraying of pesticides:

> *"Most of my customers do not know the dosage of chemicals to use. I tell them that quantity of chemicals depends on the size of the farm, amount and type weeds, and particular for insecticides it depends on the pest problem, if they ask me I always ask them how big their problem is, then I tell them to add 250 mls of Agroround (480 g/l of glyphosate) to a 15-liter bucket"* (female retailer).

A total of 18 (17.5%) pesticides were commercially found repacked into small quantities in small unlabelled bottles. Decanted pesticide products were mainly targeting average income farmers who were able to afford small amounts.

5.4.4 Crop calendar and pesticide usage practices

Most of the farmers reported cultivating more than one type of crop. Overall, 64.8% (421) of the farmers grew cereal crops, predominantly rice and maize, 25.8% (168) cultivated vegetables and fruits, such as spinach, cabbages and watermelon, 5.2% (34) cultivated legumes such as beans and 3.2% (27) grew other crops such as cashew nuts and peanuts. Most farmers owned 1 to 20 ha of land. In the wet season, rice farmers prepared their land in November and December, planted in January and harvested in May or June. For the dry season (assisted by irrigation) they prepared farms starting in May, planted in June and harvested in October [232]. The irrigated farming practices used short-duration rice seeds, maturing in 4 months, while the non-irrigation farming method that depends on rainfall during wet season used long-duration rice seeds that mature within 5-6 months. The irrigated rice agro-ecosystem was reported to be prone to pest infestations, and hence, required regular insecticide applications. The farming methods also corresponded to the application patterns of various pesticides:

> *"Normally in the rain season there are few pests and can easily be destroyed by rainwater. From my experience, the rice seed cultivated in rainy season is not vulnerable to pests, thus different from the swamp rice farming that relies on irrigation, without pesticides application you will not have good produces"* (female farmer).

5.4.5 Knowledge and practices of farmers regarding pesticides and pesticide application

The majority of farmers (89.3%, n=381) had no awareness of pesticides. Most farmers (54.4%, n=316) sprayed doses of pesticides based on instructions received from the pesticide dealers, while (18.2%, n=106) relied on personal experiences or direct observations based on the estimation of farm sizes and incidence of pests and weeds. Only (15.5%, n=90) farmers reported that they read product labels, and only if written in the local language, Kiswahili. The rest of the farmers (11.5%, n=67) relied on experts, such as agricultural officers or other knowledgeable sources of information about pesticide usage:

> "*I always get instructions from the seller of the pesticides at the agrovet shop, but sometimes I read from the leaflet on the pesticide bottle only those written in Swahili"* (female farmer).

Only 27% of farmers believed it was necessary to use recommended pesticide doses as stipulated by the manufacturer for each pesticide, though there is no evidence that they followed those instructions. On the other hand, 62.1% perceived the right pesticide dosage as any amount enough to kill all the pests in the farm. Mixing of the pesticides was mostly done in a Knapsack® Sprayer tank, traditionally recognised as "*Solo*". Overall, 400 farmers (93.7%) performed pesticide dilutions and mixing at the farms, nearby water sources, such as irrigation canals or rivers (Figure 5.2). Most of the pesticides come with the measuring equipment, but farmers typically used empty soda bottles/syringe pipe to measure liquid pesticides.

Figure 5.2: Pesticides mixing, application and disposal practices among farmers observed in rice paddies, in the study area

Pesticide dose rates also varied among farmers (Table 5.5).

Table 5.5: Example of pesticide spray dosages as reported by farmers compared to the recommended dosage on the product label

Pesticide class	Trade name	Active ingredient (s)	Class of the pesticide	Knapsack spray dilution by farmers ml/l, g/l of water	Recommended knapsack dilution rate ml/l, g/l of water	Recommended dose (ml/ha)	Target crop
Insecticide	Karate 5EC	50 gm/l of lambda-cyhalothrin	Pyrethroids	15-40 ml/20 l	12 ml/20 l	300-400 ml/ha	Rice, maize, vegetables, fruits, green pepper, watermelon, beans green peas and tomatoes
	Amekan C344 SE	144 g/l of cypermethrin and 200 g/l of imidacloprid	Pyrethroids and Neonicotinoids	30 ml/20 l	8-10 ml/15 l	500 ml/ha	Tomatoes, watermelon, okra, potatoes, rice, spinach, maize, green pepper and cabbages
	Duduba 450EC	100 g/l of cypermethrin and 350 g/l of chlorpyrifos	Pyrethroids and organophosphates	30-50 ml/20 l	10 ml/20 l	400 ml/ha	Rice, cucumber, tomatoes, green pepper, cereals crops and fruits
	Buffalo 100OD	75 g/l of imidacloprid and 25 g/l of beta-cyfluthrin	Neonicotinoids and pyrethroids	35-60 ml/20 l	10ml/20 l	500 ml/ha	Tomatoes, maize, green peas potatoes, green pepper, beans and onions
	Ninja 5EC	50g/l of lambda-cyhalothrin	Pyrethroids	25 ml/15 l	40-60 ml/20 l	150-400 ml/ha	Rice, fruits, green peas vegetables and maize
	KungFu 5EC	50 gm/l of lambda-cyhalothrin	Pyrethroids	15-40 ml/20 l	12 ml/20 l	300-400 ml/ha	Tomatoes, watermelon, cucumber, rice, onions, vegetables, fruits and green pepper
	Suracron 720 EC/720/ Profecron 720 EC	720 g/l of profenofos	Organophosphates	200-350 ml/20 l	20-40 ml/15 l	500-800 ml/ha	Cabbage and tomatoes, okra, eggplant, cucumber and watermelon

	Nogozone 60 EC	600 g/l diazinon	Organophosphates	20-40 ml/20 l	5-30 ml/15 l	150-700 ml/ha	Watermelon and cucumber
Herbicide	2,4 D Amine	720 g/l of 2, 4 D-dimethyl amine salt	Aryloxyacides	150-300 ml/16 l	200 ml/20 l	2,000 ml/ha	Rice and maize
	Roundup	360 g/l of glyphosate	Amino-phosphonates	300-350 ml/15 l	200-300 ml/20 l	2,000-3000 ml/ha	Rice and maize
	Parapaz 200 SL	200g/l of paraquat dichloride	Pyridines	300-350 ml/15 l	100-200 ml/20 l	800-1,600 ml/ha	Maize, rice, sugarcane and tomatoes
Fungicide	Farmerzeb 800 WP	800g/kg of mancozeb	Dithiocarbamate	60 g/15 l	40-60 g/20 l	1,000-3,000 g/ha	Tomatoes, African eggplant, green pepper and potatoes
	Linkonil 500 SC	500g/l of chlorothalonil	Organochlorine fungicide	20-50 ml/20 l	46 ml/20 l	1,000-3,500 ml/ha	Tomatoes, okra, eggplant, watermelon and cucumber
	Victory 72 WP	640 g/kg of mancozeb and 80 g/kg of metalaxyl	Dithiocarbamate and acylalanine	60-80 g/20 l	50 g/20 l	2000-2500 g/ha	Tomatoes, okra, and potatoes, cucumber, watermelon and cabbage

5.4.6 Frequency and spraying patterns of pesticides

Most rice farmers reported re-applying insecticides at least twice every week, or anytime there were pests to achieve maximum control (Table 4.6). Other farmers reported pre-emptively re-spraying their farms to prevent pests coming from unsprayed neighbouring farms. Farmers also frequently sprayed herbicides to prevent or delay weeds:

> *"Since most of the insecticides are not as effective as they used to be, for instance, I have to re-apply Karate (lambda-cyhalothrin) two times after every week. I think it is time the effectiveness of the insecticide has depleted and cannot kill or repel pests anymore. Sometimes, I re-apply more often because there are a lot of insect-pests coming from neighbouring farms, especially those where spraying was delayed"* (male farmer).

Table 5.6: Farmers' responses about insecticide spray frequency

Application frequency	No. of farmers	Percentage (%)
Twice every week	120	28.1
Once every two weeks	61	14.3
2-4 times per growing season	71	16.6
Any time I find pests in the farm	111	26.0
I do not remember	64	15.0

Insecticides and fungicides were mostly used during the dry season for irrigated rice cultivation and vegetable farming. Most of the non-selective, systemic, post-emergence herbicides such as Roundup (glyphosate) were, however, sprayed before farming and planting of rice seeds, shortly before rains start during farm preparation. The selective herbicides such as 2,4-D Amine (2,4-D amine salt) were commonly used during weeding to control soft weeds in rice farms:

> *"I spray Kung-fu (lambda-cyhalothrin) in the dry and wet season but mostly in the dry season because this is the period there are a lot of pests. In the wet season, there are few or no pests because of rainfall. Pest does not survive when there is a lot of water, unlike in dry season"* (female farmer).

5.4.7 Challenges faced by the farmers regarding the usage of pesticides

Farmers reported multiple challenges when using pesticides. Half of the farmers (51.3%) claimed to have experienced adverse health events, such as skin irritation or coughing after spraying pesticides. The most common challenge and concern reported by about two-third of the farmers (64.6%) was that pesticides lost their killing efficiency against weeds and pests as they have had pests rebound after pesticides application. About 7.7% of the farmers suspected some pesticides are counterfeit, and 3.3% had experienced some pesticides being more diluted than expected. Switching to different classes of insecticide or mixing pesticides was a common practice (75.6% of the farmers):

> *"You will find in few days sometimes even the following day after spraying there are still some pests in the farms. I surveyed and tried to spray different pesticides other than the ones I'm used to. I realised rapid attack (a mixture of cypermethrin and imidacloprid) and Amekan (a mixture of cypermethrin and imidacloprid) are far better and effective insecticides than Duduba (a mixture of cypermethrin and chlorpyrifos) alone against most of the pests affecting vegetables, watermelons and rice"* (male farmer).

5.4.8 Use of pesticide mixtures

Tank mixing of more than one pesticide with the same or different active ingredients before spraying was commonly practised (Table 4.7), which was also observed at the farms, despite being against label instructions. Sometimes pesticides were combined with fertilisers before application following retailers' recommendations (Table 4.7). The popular pesticide mixtures were: (i) two herbicides (38.7%); (ii) two insecticides (16.1%); (iii) one fertilizer and one insecticide (16.1%); (iv) one insecticide and one fungicide (12.9%); and (v) one herbicide and one insecticide (9.7%), and other mixtures (6.5%). Most farmers (86.4%) perceived cocktail sprays are more efficient than when sprayed as a single product. They also perceived that mixing two or more pesticides into a single spray solution simplified work and saved time. For example, a cocktail of KungFu (lambda-cyhalothrin) and Duduba (cypermethrin, chlorpyrifos) was used on fruits and vegetables such as watermelon, tomatoes, cabbages, okra and spinach.

Table 5.7: Pesticide combination practices by farmers at the study sites

Pesticides cocktail	Type of pesticides	Pesticide class
KungFu and Duduba	Two insecticides	Two pyrethroids and one organophosphate
2,4-D and Roundup	Two herbicides	One aryloxyacide and one amino-phosphonates
Booster + Supercron	One fertiliser and One insecticide	Nitrogen, phosphorous, potassium and trace elements and one organophosphate

Karate and KungFu	Two insecticides	Two pyrethroids
Rapid attack and Amekan	Two insecticides	Two (pyrethroids and neonicotinoids)
Echlonil and Karate	One fungicide and one insecticide	One organochlorine fungicide and one pyrethroid
Rapid attack and Farmerzeb	One insecticide and one fungicide	One (pyrethroids and neonicotinoids) and one dithiocarbamate

5.4.9 Handling and disposal practices of left-over pesticides and pesticide containers

Most farmers practised unsafe handling and disposal of pesticides. About half of the farmers (51.8%, n=221) reported storing pesticide leftovers in their homes for either re-spraying rebounding pests or use in the next farming season. One third (n=128) dumped out leftover pesticides into either rivers or nearby bushes. A small minority reported burying the left-over pesticides underground (6/427) or using the pesticides to kill domestic insects such as cockroaches and houseflies in their houses (2/427). Regarding disposal of containers, the majority of farmers (55.7%, n = 238) reported that they discarded empty pesticide containers into either running water in the rivers or bushes on the farms, while approximately one fifth (22.0%) considered burning the empty pesticides bottles. Some (18.5%) of the farmers, however, buried the containers in the ground, and a small minority (3.7%) reported washing and re-using the empty bottles for either repacking pesticides or other domestic activities.

5.5 Discussion

Agricultural pesticides can drive selection pressure for resistance in wild mosquito vector populations breeding in agro-ecosystems [85,86,96,120,121,123,125,245], thus threatening the effectiveness of public health interventions, such as LLINs and IRS. The WHO global action plan for insecticide resistance management in malaria vectors recommends several strategies for preventing the spread of resistance, while sustaining the effectiveness of vector control interventions [68]. However, there is a lack of harmonization and integration with agricultural pesticides usage practices [8].

The current study found multiple formulations of synthetic agricultural pesticides sold at agrovet stores in the districts of Ulanga and Kilombero in south-eastern Tanzania. More than 90% of the farmers interviewed reported using either pyrethroids, organophosphates, neonicotinoids, carbamates, organochlorines or product mixtures with at least two of these classes. The active ingredients include alpha-cypermethrin, carbaryl, chlorpyrifos, chlorothalonil, cymoxanil, cypermethrin, deltamethrin, diazinon, dichlorvos, fenitrothion, imidacloprid, lambda-cyhalothrin, malathion, mancozeb, permethrin, pirimiphos-methyl, and profenofos. These insecticide groups for crop protection attack the same target sites and have similar modes of action as public health insecticides [35,36,153]. Most of the insecticide compounds found in use exhibit a broad spectrum of activity, indiscriminately killing even beneficial insects. These broad-spectrum insecticides are likely to be used more frequently than narrow-spectrum insecticides, thus exerting resistance selection pressure even on non-target insects, such as mosquitoes [270]. Other studies have reported extensive use of similar pesticide compounds by farmers for crop protection against pests and diseases in malaria-endemic regions [42]. For example, Philbert and colleagues found 48 pesticide formulations used by farmers in northern Tanzania, where malaria is endemic [148].

There are several similarities in insecticide active ingredients used in agriculture and those in public health in Tanzania. Nets impregnated with pyrethroids, mostly deltamethrin and permethrin, are widely used for malaria prevention [37]. Both lambda-cyhalothrin and bendiocarb were recently used for IRS, but have now been replaced with pirimiphos-methyl on Zanzibar Island and in some districts in north-western Tanzania [7]. Neonicotinoid-based interventions have also been tested and could be used [266]. Alpha-cypermethrin, which was found in most agricultural pesticides, is coated on Interceptor ® nets, which have been under evaluation for malaria control [271]. Beyond the basic chemical similarities, public health and agricultural pesticides also share modes of actions. For example, the voltage-gated sodium channels are targeted by pyrethroids and organochlorides, while acetylcholinesterase is targeted by both organophosphates and carbamates [35,36].

This study also revealed the presence of candidate compounds, chlorpyrifos emulsifiable concentrate (EC) and imidacloprid for both pest control on the farms and cereal preservation under storage. Chlorpyrifos, an organophosphate, was earlier recommended by WHO Pesticide Evaluation Scheme (WHOPES) for the control of juvenile mosquitoes [208] and has been evaluated for net impregnation against mosquitoes [262]. Additionally, imidacloprid (neonicotinoids) a nicotinic acetylcholine receptor stimulator, is also being considered as an alternative or in combinations with the commonly used pyrethroids [266].

Selection pressures are experienced when mosquitoes in their aquatic stages are exposed in their breeding habitats, where most farming activities are taking place [86]. In turn, this might cause insecticide tolerance, as part of defence mechanisms that lead to insecticide resistance to a subsequent new generation of emerged adult mosquitoes [96,121,123,125]. Metabolic resistance is one of the principal mechanisms in mosquitoes [93], and has been linked to the massive use of pesticides in irrigated rice plantations that enhanced the overproduction of detoxifications enzymes [272]. The over-expression of metabolic genes included four CYP6P3 and one CYP325 cytochrome P450s, two delta class GSTs, one peroxiredoxin and two cuticular pre-cursor genes in adults *An. gambiae sensu stricto* (*s.s*.) collected from different breeding habitats in Benin and Nigeria was reported to be influenced by the presence of xenobiotics and agricultural pesticides in their agro-ecological sites [245,273]. The detoxification genes and cuticular precursor genes were linked to pyrethroid resistance and reduction of insecticide penetration, respectively [273]. A study performed by Nkya and colleagues found that frequent exposure of *An. gambiae* larvae to agricultural pollutants influenced an over-expression of multiple genes responsible for the selection of target-site mutation resistance, cuticle resistance, metabolic-based resistance and nervous and synaptic-transmission based resistance in adult mosquitoes [96,121]. Similarly, bioassays revealed that a high level of pyrethroid resistance in *An. gambiae s.l.* was associated with DDT and pyrethroid residues from cotton-growing farms in West Africa [124].

Glyphosate was the most common active ingredients found in most of the herbicides. However, there were also herbicides containing 2,4-dichloro phenoxy acetic acid, s-metolachlor, atrazine, paraquat and 2,4 D-amine as active ingredients. Though herbicides are generally non-toxic to insects, many of them, and also several xenobiotics, could cause metabolic stress with the potential of modifying the insecticide detoxification systems in insects, hence causing insecticide tolerance and eventual resistance [127,129]. In one study, *Aedes aegypti* larvae exposed to glyphosate were significantly tolerant to permethrin, due to the stimulation of multiple detoxification genes, including P450s and GSTs [127].

Even though most of the agricultural pesticides found were on the list of pesticides approved in Tanzania [140,141], there were several versions deemed of less quality but with the same brand stamp as those found in the market. These findings are in line with Shao and colleagues, who reported the magnitude of counterfeit agro-inputs in Tanzania to be as high as 46.8%, that could pose a serious risk to the ecosystem [274]. In a similar study, repacking and decanting of pesticide products in un-labelled containers was done by a quarter of pesticide dealers in six study towns in Tanzania [150]. Farmers who participated in the current study reported having experienced reduced efficacy of some pesticides, hence sprayed their crops repeatedly or at a higher quantity. Previous reports have shown the reduced effectiveness of lambda-cyhalothrin against two species of rice stem borers, mainly *Chilo* species and *Sesania calamistis* in irrigated lowland rice ecosystems in the same study area [275].

Most of the retailers of agricultural pesticides and farmers lacked formal knowledge of the proper usage of pesticides, including pesticide dosages. The majority had never been trained on agricultural pesticide usage and had a lack of knowledge of crop pest biology and disease. The retailers prescribed informal instructions to the farmers on how to apply and at what amount agricultural pesticides are required based on their experiences. The findings agree with a recent study by Lekei and colleagues, which found that most of the retailers of pesticides in Tanzania are not qualified to provide professional instructions to the end-users [150]. Similarly, most of the farmers were not knowledgeable on crop pests and diseases, pesticide usage and management of agricultural pesticides, instead relying on information received from the retailers and personal work experience. Pesticide dilution rates were confused with application dosages and in most cases were used in larger volumes than the recommended dosage. These findings are in line with reports from southern Côte d'Ivoire, where less than half of the 208 vegetable and rice farmers who participated in a study adhered to the recommended pesticide dosage [149].

In the current study, pesticides application patterns and frequencies were observed and informed mostly by experience or perception and only to a limited extent by professional advice. Previous studies conducted in Tanzania revealed an increase in pesticide applications per season as a common practice in most farmers [147]. While the use of agricultural pesticides was influenced by the farming calendar, insecticides and fungicides were heavily used in the dry season by farmers practising irrigated rice cultivation and vegetables. Though no clear association was found on how the farming calendar influences resistance, studies in rural southern Tanzania have demonstrated clear seasonal and spatial variations in phenotypic resistance to public health pesticides in both Anopheles and *Culex* mosquito vectors, with the most resistant mosquito populations in dry seasons in areas where irrigated rice cultivations are concentrated [31,32]. The seasonal use of agricultural pesticides might provide an opportunity for vector control programmes to partner with agriculturalists in designing a coordinated resistance management plan.

Combining two or more pesticides or with fertilizer in a spray tank was routinely practiced among farmers, mainly to enhance efficacy and to save application time (Table 4.7). This practice has been reported in Tanzania [148] and elsewhere [276]. Usually, different pesticide formulations are incompatible and mixing them could induce toxicity of the plant and likely influence resistance selection pressure in crop pests and even in disease vectors [148,149].

Unsafe storage and disposal practices of left-over agricultural pesticides were reported and observed during the cross-sectional survey. Left-over pesticides were hanged on the roof or kept under the beds. Some farmers kept left-overs for the next season. However, small quantities of pesticide left-overs (i.e. generally less than a litre) were considered unwanted and were disposed either in the farms or washed off in the running water. One participant from Lupiro sprayed the left-over pesticides on the walls and the roof of the house or discarded it in the pit latrine to abate mosquitoes. The farmers also practiced unsafe disposal of empty pesticide containers. Poor storage and disposal practices of agricultural pesticides have also been reported elsewhere [145], which might pollute the ecosystem, contaminate breeding sites of mosquitoes and influence selection pressure for insecticides resistance.

This study recommends coordinated efforts between public health and agricultural sectors to prevent or delay insecticide resistance in disease vectors, while preserving the effectiveness of agricultural pesticides. The main challenge in managing insecticide resistance is not the unavailability of appropriate methods, but ensuring their adoption by farmers and pest control operators. Hence, raising awareness among pesticide retailers and farming community of the links between agricultural pesticide usage practices and insecticide resistance development in mosquitoes is urgently needed, through regular field engagement educational activities and participatory workshops and dialogues. An integrated pest and vector management (IPVM) approach could be adopted through farmer field school's empowerment programme, in the current and future mosquito vector insecticide resistance management strategies. The adoption of principles for IPVM provides opportunities to bridge the gap between agriculture and public health. Farmers could, therefore, make rational decisions on good agricultural practices, while minimising the use of pesticides by adopting other potential pest management options that include cultural and physical control, biocontrol and the use of biopesticides.

5.6 Study limitations

This study did not quantify the effect of agricultural pesticides in the selection of insecticide resistance in malaria vectors. Hence, there was no direct measure of association between agricultural pesticides exposure and resistance selection in malaria vectors. The study instead relied on an inventory of agricultural pesticides as well as the knowledge and practices among farmers and pesticides dealers. This research was nested in a larger study that investigated possible drivers of residual malaria transmissions [277], including insecticide resistance and resistance mechanisms in malaria vectors [84,233], in communities where insecticidal nets are widely used, and pesticides are heavily applied in agriculture.

5.7 Conclusions

The similarity of active ingredients in agricultural insecticides and insecticides for malaria vector control, coupled with a lack of awareness among pesticide dealers and users, might accelerate the intensity and spread of resistance in malaria vectors, thereby compromising the effectiveness of insecticide-based interventions, such as LLINs and IRS. This study emphasises the need for improving awareness among retailers and farmers on proper usage and management of agricultural pesticides. To ensure the judicious use of pesticides and preserve the effectiveness of public health insecticides, while improving crop yields, there is a pressing need for coordinated efforts between public health and agricultural sectors in the selection, timing of application and management of pesticides. One way of achieving this goal is to initiate coordinated education programmes in elementary farmer field schools on appropriate pesticide usage in both public health and agriculture sectors. Future studies should quantify pesticide residues from the soil and water, as to better estimate the magnitude of mosquito exposures to agricultural pesticides and the impact with a view to considering integrating agricultural practices for sustainable insecticide resistance management strategies in mosquito vector populations.

5.8 Ethics approval and consent to participate

Written informed consent was sought from the retailers of agrovet stores and farmers upon their agreement to be involved in the study. Ethical review and approval were granted by the Institutional Review Board (IRB) of the Ifakara Health Institute (IHI) (reference no. IHI/IRB/NO: 35-2015) and the Medical Research Coordinating Committee at the National Institute for Medical Research (NIMR) in Tanzania (reference no. NIMR/HQ/R.8a/Vol.IX/2162).

5.9 Consent for publication

The permission to publish this work was obtained from the Director of Research Information, Technology and Communication from NIMR in Tanzania (reference no. NIMR/HQ/P.12 VOL XXX/). Farmers provided consents for the photos to be taken and used for research dissemination.

Availability of data and materials

All data generated or analysed during this study are included in this published article (and its supplementary information files).

Competing interests

The authors declare that they have no competing interests.

Funding

The study was financially supported by a Wellcome Trust Intermediate Fellowship in Public Health and Tropical Medicine (grant no.: WT102350/Z/13/Z) and the World Health Organization's Tropical Disease Research (TDR) group (reference no.: 2015/590235-0) awarded to the senior author FOO. NSM is a recipient of a Swiss Government Excellence Scholarship via the Federal Commission for Scholarships for Foreign Students FCS (ESKAS) (reference no: 2017.0786). The funders had no role in the design of the study, data collection, analysis and interpretation, and in the writing of the manuscript.

Acknowledgements

We are grateful to the retailers of agrovet shops at Ifakara town in the Kilombero district and at Mavimba, Minepa and Lupiro wards in the Ulanga district for permitting us to conduct this study at their stores. We greatly appreciated the cooperation received from the farmers during the interviews. Noelia Pama and Tumpe Mwandiyana are gratefully acknowledged for their assistance during the interviews and Alex Limwagu for preparing a study area map. We thank the editor and anonymous reviewers for their careful reading of our work and providing valuable comments and suggestions that further improved the quality of this paper.

Authors' contributions

NSM and FOO conceived the study. NSM, MT, GM and FOO contributed to study design and development of data collection tools. MT, JU, VN and FOO reviewed the data collection tools. NSM and SAM conducted interviews with the support of field technicians. NSM led data analysis and interpretation. NSM drafted the manuscript. MT, GM, SAM, MF, JU, VN and FOO critically reviewed the manuscript. All authors read and approved the final version of the manuscript prior to submission.

6 Chapter 6: Participatory approaches to raise awareness among subsistence farmers in Tanzania about the spread of insecticide resistance in malaria vectors and the possible link to improper agricultural pesticide use

Nancy Stephen Matowo[1,2,3,4*], Marcel Tanner[2,3], Benigni A. Temba[5], Marceline Finda[1], Yeromin P. Mlacha[1,2,3], Jürg Utzinger[2,3], Fredros O. Okumu[1,6,7]

[1]Environmental and Ecological Sciences Department, Ifakara Health Institute, Ifakara, Tanzania

[2]Swiss Tropical and Public Health Institute, Allschwil, Switzerland

[3]University of Basel, Basel, Switzerland

[4]Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK

[5]Department of Veterinary Physiology, Pharmacology and Toxicology, Sokoine University of Agriculture, Morogoro, Tanzania

[6]School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

[7]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK

*Corresponding author

Email: nstephen@ihi.or.tz

In-press, Malaria Journal 2022

https://doi.org/10.21203/rs.3.rs-1602018/v1.

Abstract

Background

Insecticide resistance is a key barrier to long-term malaria control, and it may be exacerbated by poor agricultural pesticide use. Current practices, however, do not link public health and agricultural pesticide use. This study investigated the perspectives of farmers and other stakeholders regarding the integration of agricultural and public health measures to address resistance. Additionally, the feasibility of participatory workshops to increase the farmers' understanding and participation in pesticide stewardship was assessed.

Methods

Four themes were investigated: pesticide awareness, practices, and opinions of; insecticide resistance in malaria vectors; the effectiveness of current malaria prevention tools; and the links between agricultural and public health pesticide usage. Participatory workshops and field training were held with entomologists, farmers, and agricultural specialists, focusing on agro-ecosystem practices related to pest control; and local farmers were involved in live-testing for insecticides resistance of local Anopheles mosquitoes.

Results

Most farmers (94%) considered pesticides effective, and nearly half of them (n = 198, 46.4%) could identify and name crop pests and diseases, mostly using local names. Three quarters were unaware of mosquito larvae in their fields, and only 7% considered their fields as potential sources of mosquitoes. Two thirds were uninformed of any effects that agricultural pesticides may have on mosquitoes, and three quarters had never heard of resistance in malaria mosquitoes. Experts from various sectors acknowledged that agricultural pesticides might impact malaria control through increasing resistance. They did, however, emphasize the importance of crop protection and advocated for the use of pesticides sparingly and non-chemical approaches. Farmers learnt how to discriminate between malaria vectors and non-vectors, identify agricultural pests and diseases, choose and use pesticides effectively, and conduct resistance tests during the participatory workshops.

Conclusion

This study emphasizes the significance of enhancing subsistence farmers' awareness of mosquito ecology as well as merging public health and agricultural pest management measures. Participatory techniques have the potential to raise stakeholder awareness and engagement, resulting in more effective resistance management.

Keywords: Agricultural pesticides, Agricultural practices, Anopheles mosquitoes, Crop pests, Insecticide resistance, Malaria, Participatory learning, Tanzania

6.1 Background

In sub-Saharan Africa, malaria prevention relies primarily on long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) [29,278]. In Tanzania, LLINs are distributed throughout the country [37,38], whereas IRS is primarily used in the north-western regions of the country around Lake Victoria and on the islands of Zanzibar [7,38]. Despite considerable progress made over the past 20 years, the burden of malaria in Tanzania remains high. In 2017, the national prevalence among children under the age of 5 years was 7.3% [279]. Recent data suggest considerable spatial heterogeneity in malaria transmission [280,281]. Furthermore, the continuing COVID-19 pandemic might stall and revert prior successes in malaria control [282,283].

Persistent malaria transmission is partly attributed to the recent changes in malaria vector populations, notably behavioural or physiologically resistance after prolonged use of LLINs and IRS [284,285]. Indeed, both the strength and distribution of resistance have increased in Africa [286], and most countries had to change the classes of insecticides over time for effective vector control [178,287-289]. While LLINs continue to rely predominantly on pyrethroids, IRS now includes insecticide classes previously used in agriculture, notably organophosphates and neonicotinoids [265].

Agriculture is critical to the economies and livelihoods of the majority of African countries. However, the resulting agro-ecosystems provide favourable environments for mosquito vectors to breed. Besides, crop pest management relies on synthetic pesticides, which are frequently the same classes as those used in public health [290]. According to Tanzania estimates, a large share (i.e., 81% of synthetic pesticides) are used for agricultural purpose by small-scale farmers to protect crop from pests and diseases [291]. Unfortunately, selection pressures associated with widespread agricultural pesticides may influence the evolution of insecticide resistance in malaria vectors [292], as farmers are frequently unaware of potential impact their actions on disease transmission. A recent study in rural Tanzania demonstrated overlap between insecticide classes used in public health and agriculture in an environment where agricultural pesticide use was largely uncontrolled [293]. These challenges corroborate previous findings that small-holder farming communities may face the highest risk of malaria as a result of occupational exposures [294-297], cultural and behavioural practices (e.g., migratory farming practices) [298,299], and limited access to malaria prevention and prompt treatment services [298,299]. Regrettably, the World Health Organization (WHO) global action plan to control the spread of insecticide resistance in malaria vectors, did not include practical recommendations for addressing gaps in agricultural practices related to malaria control [68]. Current pest management practices do not consider the relationship between public health and agricultural pesticide use.

Tanzania's current National Malaria Strategic Plan 2014–2020 emphasizes the critical role of inter-sectoral coordination in malaria vector control [38]. The double-edged contributions of agriculture in food production and promoting resistance in disease vectors, however, must be recognized [38]. While community members are considered primary partners in vector control, they are not adequately empowered or involved in the implementation of resistance management programmes [38]. However, active collaboration and participation are required to improve malaria control in agriculturally dominated areas, particularly irrigated rice farming [300,301].

The purpose of this study was to explore the opinions of key stakeholders on potential approaches for integrating agricultural and public health practices to address resistance in malaria vectors. Additionally, the feasibility of participatory workshops for increasing awareness and participation of subsistence farmers in pesticide stewardship was determined. The study began with an assessment of current pest management practices, public awareness of the connection between agriculture and malaria, and perceptions of insecticide resistance in malaria vectors. The study also explored the perspectives of key stakeholders on the need for, and potential approaches to integrating agricultural practices into pests and disease vectors management strategies.

6.2 Methods

6.2.1 Study area

The study was carried out in six wards (i.e., Katindiuka, Lupiro, Mavimba, Mbasa, Minepa, and Sululu) in the districts of Kilombero and Ulanga in Tanzania's south-eastern region, rising 120-350 m above sea level on the flood plains of the Kilombero River valley (Figure 6.1). Rice farming and fishing are the primary sources of food and income. During the dry season, rice production is sustained by an irrigated system locally known as "Ngapa", which also supports local mosquito populations [298]. There is a stable transmission of *Plasmodium falciparum* throughout the year [250], mediated primarily by *Anopheles arabiensis* and *Anopheles funestus sensu stricto* (*s.s.*) [185]. In the study area, there is also a high density of *Culex* mosquitoes, generating significant biting nuisances [302]. Malaria control is mainly by LLINs treated with pyrethroids (mainly deltamethrin and permethrin) [37]. In this setting, the farmers also use a variety of pesticides (i.e., pyrethroids, carbamates, neonicotinoids, and organophosphates) to boost crop yields [293]. There is evidence, however, of mosquito resistance to the pyrethroids, DDT, and bendiocarb, which is most likely mediated by metabolic enzymes [185,303]. The study was conducted at different time periods between 2016 and 2018.

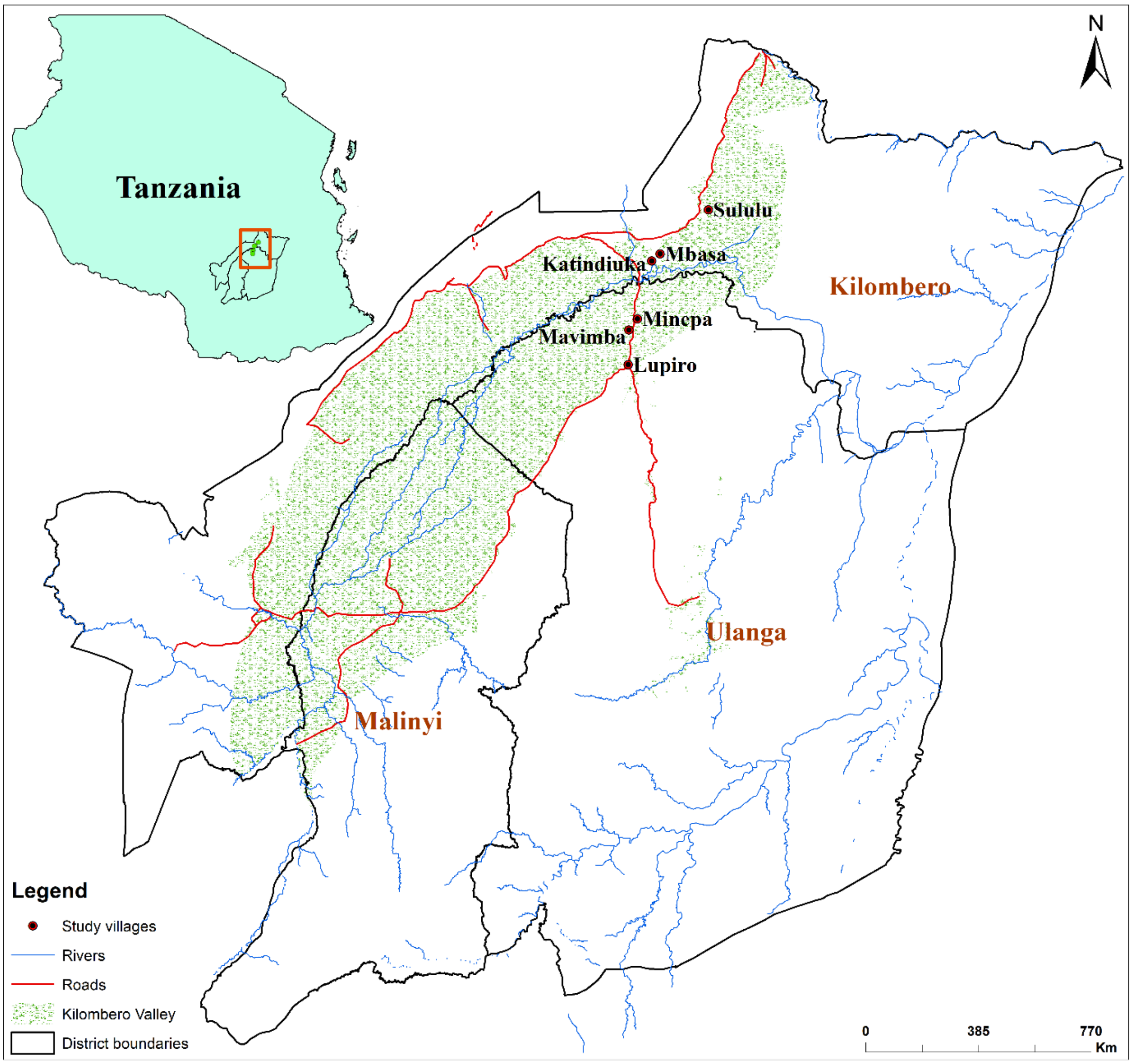

Fig 6.1. Map showing the study wards in the districts of Kilombero and Ulanga in the south-eastern parts of Tanzania, where the current investigation was carried out.

6.2.2 Study design and data collection

This study used an exploratory sequential mixed methods design. This involved in-depth interviews with 57 small-holder farmers, followed by a questionnaire survey enrolling 427 farmers. In-depth interviews were conducted with nine key informants from the public health, agriculture, and environmental sectors. Key findings were used to develop participatory workshops and training sessions with farmers and agricultural experts to better understand their perspective on the interactions between agricultural practices and mosquito control.

In-depth interviews. In-depth interviews were carried out with 57 farmers (30 from Ulanga district and 27 from Kilombero district). The interview guide was developed in English, translated into Kiswahili and used in the latter language. For confidentiality of respondents, unique identification numbers were assigned to match audio-recorded data. Open-ended questions with sub-questions for probing were administered to the farmers who participated in the study. The interview questions were classified and focused on six themes, namely (i) knowledge on pests, pest management practices, and knowledge of alternative non-chemical pest management; (ii) general knowledge on malaria, mosquito ecology, and linkage with agricultural practices; (iii) opinions on malaria trends in their village; (iv) benefits and challenges of current malaria control methods; (v) views on agrochemical usage and perceived effects of the chemicals on malaria vectors; and (vi) awareness and views on insecticide resistance in mosquitoes. All of the interviews were audio-recorded. Additionally, field notes were taken to complement the interviews.

Survey questionnaire. Findings from the qualitative study guided the development of questions for quantitative research. In total, 427 farmers were chosen at random and consented to participate in the survey. The questionnaire was translated from English into Kiswahili and administered in Kiswahili, using electronic forms on a free-access software programme, KoBoToolbox [304]. The questionnaire survey covered similar topics, but with adaptations based on preliminary findings of the aforementioned in-depth interviews. Data triangulation was conducted by integrating, interpreting, and comparing findings from both methods to improve understanding of the research questions.

Key informant interviews. In-depth interviews were conducted with nine purposively selected stakeholders from public health, agriculture, and environmental sectors, who had a direct or indirect impact on malaria and vector control. The key informants included two ward agricultural officers, one ward veterinary officer, one acting director general of the government institute, two university lecturers, two research scientists with leadership positions at departmental level, and two malaria control programme managers. The organizations represented included (i) the National Malaria Control Programme that is managed by the Ministry of Health; (ii) the National

Institute for Medical Research (NIMR); (iii) the National Environment Management Council; (iv) the Ministry of Agriculture delegates the Tanzania Plant Health and Pesticides Authority (TPHPA), previously known as Tropical Pesticides Research Institutes (TPRI) under the Ministry of Agriculture; (v) the Muhimbili University of Health and Allied Sciences; and (vi) the Sokoine University of Agriculture. These interviews explored participants' views regarding (i) malaria vector control interventions, progress, and challenges; (ii) linkages between agricultural practices and malaria control; (iii) effects of agricultural pesticides in resistance development in malaria mosquitoes; (iv) government policies and guidelines for regulating the use of agricultural pesticides; and (v) integrated vector management and inter-sectoral collaborations. The interview's responses were audio-recorded, and specific notes were taken.

Participatory workshops and practical sessions with farmers and agricultural experts. Four field-based participatory workshops and three on-site field visits were conducted for the researchers, farming communities, and agricultural and veterinary officers to meet and share knowledge, experiences, and challenges related to pesticide usage practices and crop pest management. These activities also provided opportunities to engage the farming communities and agricultural experts in understanding interactions between agricultural practices and mosquito control. A total of 26 farmers and three ward agricultural officers participated in the first two workshop sessions and practical learning in the farms, while 22 farmers, two ward agricultural officers, and one ward veterinary officer were involved in the last two sessions of the workshops and field visits.

The participatory workshops were partly motivated by a curriculum developed based on the recommendations raised by the participants during earlier data collection (Table 6.1). Video filming, photographs, and note-taking guided the collection of relevant information generated during workshop discussions and practical field visits. During the workshops, researchers presented summary feedback of previous investigations of resistance in local malaria vectors. They also presented a summary table showing WHO-approved insecticides for malaria vector control. At the same meetings, the researchers, together with the agricultural and veterinary experts, displayed and described common pesticides used and agricultural insecticides available in nearby agrovet stores. Of note, agrovet stores are shops where agricultural and veterinary supplies, including pesticides, are sold. In this study, the farmers were invited to share their experiences on use of agricultural pesticides and any challenges encountered. Similarities between active ingredients in both public health insecticides and agricultural pesticides were discussed and clarified. The concept of insecticide resistance in mosquito was explained in lay terms using simulated pictures on a video presentation. Additional open discussions were conducted with the farmers and agricultural experts, focusing on the same topics as in the aforementioned interviews.

Additional sessions were organized where participants conducted practical field activities and discussions on the interactions between agro-ecosystem and mosquito ecology. These included (i) sampling and identification of anopheline and culicine mosquito larvae; (ii) direct observations of different life-cycle stages of mosquitoes; (iii) demonstration of farming practices aimed at minimizing mosquito breeding; (iv) sampling and identification of adult crop pests and predators; (vi) demonstrations of proper handling, storage, and disposal of agricultural pesticides; and (vii) observations of non-insecticidal methods against crop pests, diseases, and weeds. Pesticides commonly used by the farmers were borrowed from the local agrovet stores and used for demonstration. These including Karate 5EC (lamba-cyahalothrin), Duduba 450EC (cypermethrin and chlorpyrifos), and Actellic 50EC® (pirimiphos-methyl).

Table 6.1: Topics and learning objectives pursued during the participatory workshops and practical field sessions during the study

Category	**Workshop/topic**		**Learning objective**
Basic knowledge and skills on malaria mosquitoes, breeding sites, and control approaches	1	Known associations between agricultural practices and mosquitoes	• Participants should be able to identify potential aquatic habitats for mosquitoes in or near their farms • Participants should understand the effects of agricultural practices on mosquitoes
	2	Sampling and identification of mosquito larvae and pupae	• Participants should be able to sample larvae and pupae from habitats • Participants should be able to identify and distinguish *Anopheles* from other larvae • Participants should be able to distinguish between male and female mosquitoes • Participants should be able to identify adult *Anopheles* and non-*Anopheles* mosquitoes
	3	Larval source management through improved agricultural practices	Farmers should be able to identify and destruct (clearing of ditches, soil filling, and draining) of suitable aquatic mosquito breeding sites linked to agricultural practices
Agricultural pesticides, public health pesticides, and malaria mosquitoes	4	Exploring the linkage between public health insecticides and agricultural insecticides	• Encourage farmers to share their farming experiences, including demonstrating the use of agricultural pesticides • Experts to share the experiences with the farmers on the performance of various agricultural pesticides

			• Researchers to demonstrate to the farmers and experts on insecticides used in public health against malaria and its link to agricultural insecticides • Farmers to be able to read labels, understand agricultural pesticides and its chemical ingredients before spraying
		Demonstrating the effects of agricultural pesticides on malaria vectors	Farmers should be able to understand the possible link of agricultural pesticides sprayed in the farms and their consequences, such as insecticide resistance in mosquito vectors
Management of crop pests and diseases	5	Collection and identification of common crop pests at the field	• Farmers should be able to identify individual common crop pests and diseases • Farmers should understand appropriate insecticides to spray against particular pests and diseases
Other options for crop pests management	6	Alternative crop pests and diseases management practices other than using agricultural pesticides	• Farmers should be able to suggest alternative pest-, disease-, and weed-control in crops other than using pesticides • Agricultural pesticides as the last option in controlling pests and diseases in crops
Good agricultural practices for pesticide management	7	Proper storage and disposals of agricultural pesticides	Experts and farmers should demonstrate good and safe agricultural practices for handling, keeping, or disposing of leftover pesticides, and empting containers

Participatory testing of insecticide susceptibility in local malaria vectors. During the participatory sessions, individuals were co-opted to participate in investigations of phenotypic resistance of malaria vectors. After initial training, the tests were pursued jointly with farmers, agricultural experts, and researchers, using female *An. arabiensis* mosquitoes raised from larvae that had been collected by the same farmers from their farms. The susceptibility tests were carried out in accordance with standard WHO guidelines [154]. The efficacy of insecticide-impregnated test papers was first validated against a laboratory-reared susceptible strain of *Anopheles gambiae s.s.* (Ifakara strain) prior to the actual bioassays. A minimum of 20 and a maximum of 25 non-blood-fed female mosquitoes, aged 3-5 days, were exposed for 60 min to the diagnostic concentrations of 0.75% permethrin, 0.05% deltamethrin, or 0.25% pirimiphos-methyl. Similar numbers of mosquitoes were exposed to oil-impregnated papers as controls. Knockdown rates were recorded at 10, 15, 20, 30, 40, 50, and 60 min intervals. After the exposure period, mosquitoes were transferred to holding tubes and maintained on 10% glucose solution. Finally, 24 hours post-exposure mortalities were recorded and compared [154].

6.3 Statistical analysis

Data reviews and discussions with the research team were done weekly. Audio data from the in-depth and key informant interviews were transcribed verbatim and then translated from Kiswahili into English. Data were first coded, explored, and interpreted following framework analysis steps described by Gale and colleagues [305]. The transcripts were coded and analysed using MAXQDA® software (VERB; Berlin, Germany) [306,307]. Codes were generated based on the study questions and through comprehensive and repeated reading of the transcripts. Similar codes were conceptualized, merged, categorized and, finally, developed into themes. Both peculiar and common views supporting themes were observed and recorded. An integration weaving approach was employed, in which both quantitative and qualitative data from the farmers were presented together [308].

Descriptive findings from the quantitative survey were summarized and presented as percentages, and representative direct quotes from different participants are presented to further illustrate the findings. Susceptibility test findings were analysed across the four replicates for each insecticide and percentage mean mortalities, 24-hour post-exposures were interpreted following WHO criteria for interpreting insecticide resistance [154].

6.4 Results

6.4.1 Demographic characteristics of farmers

Out of 57 farmers who participated in the qualitative in-depth interviews, 30 were females. Of the 420 farmers who participated in the questionnaire survey, there were slightly more males than females (220 *vs.* 200). The most common crops produced were rice, maize, tomato, vegetables, and fruits for both sale and home consumption. Overall the age of the participants ranged between 21 and 57 years.

6.4.2 Knowledge and practices related to pests and pest management

The majority of farmers (n=401, 93.9%) utilized synthetic pesticides in their farms; 374 sprayed herbicides, 285 sprayed insecticides, 66 sprayed fungicides, and 21 sprayed rodenticides on a regular basis. The pesticides were mostly used for fear of yield loss and desire to improve productivity. Most of the farmers (70.3%) believed pesticides were effective for pest control (Table 6.2). Pesticides were widely subsidized and easily accessible on the local market.

"*In the past, we were buying pesticides only from a big city such as Dar es Salaam, and they were costly, but these times you can purchase most of the pesticides even here in my village*" (male farmer, 53 years).

Pesticides selection and preference were commonly based on experiences and instructions received from the pesticides dealers (Table 6.2). Overall, slightly fewer than half (n=198, 46.4%) of the farmers reported being able to identify and name pests on the farms, but most could describe different pests based on their morphological features, including colour, size, and ability to fly. Insects were also described based on the damage they caused (e.g., holes on plant leaves), while some were named using local Kiswahili terms. Weed pests were also described based on physical features, such as soft and hard weeds. Local names were used to identify crop diseases. For example, fungal infections were generally grouped together and referred to as "*Ukungu*", and bacterial diseases were confused with rust fungal infections on the leaves, and termed as "*Kutu*".

"*(…) there are insect pests that destroy rice, especially during dry hot season, these pests have hard skin and are black-spotted. They primarily destroy the rice plant by cutting the roots. Unfortunately, I don't know the specific name of the pest*" (female farmer, 32 years).

Up to 80% of the farmers (n=341) were uninformed of any non-chemical pest management methods and only a small proportion of those who tried them found such methods effective. Commonly mentioned traditional methods for pest control were use of wood ashes, papaya leave extracts, and a mixture of onion and garlic extract solution against insect pests and fungal infection, as well as mechanical/hand weeding rather than using herbicides. About one quarter (n=102) knew about using natural enemies for pest control but had never practiced (Table 6.2).

The majority of farmers (n=411, 96.3%), had never heard about integrated pest management.

"*I had issues of "finyi" (referred to as caterpillar) in my small Chinese-lettuce garden, I tried to dust wood ashes twice a day and it worked. I will try to use it in my rice farm but I doubt its effectiveness on a large farm*" (female farmer, 28 years).

Table 6.2: Knowledge, practices, and opinions of rural farmers on pest management

Variable assessed	Response	N (%)
*Reasons for using synthetic pesticides***	Control pests infestations and improve agriculture production	391 (91.6)
	Easy and effective method to control crop pests and diseases	300 (70.3)
	Increased pests incidence and damage on the crop	21 (4.9)

	Availability of pesticides, subsidized agro input, and initiation of "*kilimo kwanza*" (agriculture first)	123 (28.8)
Ability to identify pests in the farm	Able	198 (46.4)
	Not able	229 (53.6)
Criteria used when selecting and using pesticides	Estimate the size of the farmland	174 (40.7)
	Rely on how extensive the insect pest have infested agricultural land	124 (29)
	Identify the type of weeds (hard and soft weeds)	267 (62.5)
	Spray any pesticide as long as it was effective previously	289 (67.7)
	Others (specify)	26 (6.1)
Awareness of non-chemical pest management methods	Aware	341 (79.9)
	Not aware	86 (20.1)
Use of non-chemical control methods against pests	Yes	153 (35.8)
	No	274 (64.2)
Perceived effectiveness of non-chemical pest management methods	Effective	41 (26.9)
	Not effective	61 (39.6)
	Don't remember	51 (33.5)
*Cultural pest management practices ever used***	Intercropping	178 (41.7)
	Crop rotation	5 (1.2)
	Mulching	6 (1.4)
	Don't remember	19 (4.4)
	None	234 (54.8)
	Don't know	23 (5.4)
Awareness of natural enemies/predators for pests control	Aware	102 (23.9)
	Not aware	318 (74.5)
	Don't know	7 (1.6)
Awareness of integrated pest management	Aware	16 (3.7)
	Not aware	411 (96.3)

***Questions with multiple responses options*
"Kilimo kwanza" resolution was referred to transformation of agriculture from subsistence into a modern and commercial sector.

6.4.3 Awareness of the associations between malaria transmission, mosquitoes, and agriculture

Most of the participants of the in-depth interviews were knowledgeable about *Anopheles* mosquitoes being vectors of malaria. Some participants knew that there are other mosquito species of medical importance, but they could not differentiate these from malaria vectors. The majority of participants believed malaria mosquitoes breed in stagnant and clean water, however they were unable to link their agricultural activities such as irrigated rice farming (locally known as "*Ngapa*") to mosquito densities in the agro-ecosystem.

> "*I know there are female Anopheles mosquitoes transmitting malaria, but I could not imagine malaria mosquitoes can lay eggs and grow in my rice paddies*" (female farmer, aged 27 years).

6.4.4 Knowledge and opinions regarding effects of agricultural pesticides on malaria vectors

About one third of the farmers had varying opinions on the effects of pesticides on mosquito vectors (Table 6.3), while the remaining two thirds (65.2%) had no idea on any such effects.

> "*I think agricultural pesticides might have an impact on mosquitoes in the farm but I don't know the details. From my experience, when I use Roundup (glyphosate) chemicals, the surface of the land turns black like rotten materials, perhaps it could support growth and development of insects such as earthworms and maybe malaria mosquitoes*" (male farmer, 31 years).

Three quarters (n=318, 74.5%) of the farmers had never heard about insecticide resistance in malaria mosquitoes and could not relate with their use of agricultural pesticides. Among those who had heard of resistance, 35.0% (49/140) believed it meant always having high mosquito population densities in the villages.

> "*Insecticide resistance in malaria mosquitoes is a tendency whereby there is always high mosquito population density that means too many mosquitoes each year causing malaria*" (female farmer, 29 years).

Table 6.3: Farmers' knowledge, views, and perceived effects of agricultural pesticides in malaria mosquitoes

Variable assessed	Participant responses	N (%)
Opinions about the effect of agricultural chemicals on malaria mosquitoes	Kill malaria mosquito Chase away malaria mosquitoes Do not have any effect in malaria mosquitoes Influence the increase of mosquito population density Influence insecticide resistance in malaria mosquitoes I don't know	80 (18.4) 18 (4.1) 42 (9.7) 10 (2.3) 1 (0.2) 283(65.2)
Awareness of insecticide resistance in malaria vectors	Aware Not aware	109(25.5) 318 (74.5)
Opinion on what insecticide resistance in malaria vectors means$	Increase in mosquito population density Mosquitoes cannot be killed or repelled by the insecticides Mosquitoes cause more malaria in the study village Mosquitoes are no longer responsive to the insecticidal interventions I don't know Others	57 (52.3) 9 (8.3) 15 (13.8) 11 (10.1) 10 (9.210) 7 (6.4)
Opinions on methods to prevent/delay insecticide resistance in malaria vectors	Minimize the use of agricultural chemicals Minimize the use of public health pesticides against mosquitoes Establish integrated pest and vector management (IPVM) Alternative use of biological and environmental methods for controlling pests and mosquitoes Others I don't know	1 (0.9) 1 (0.9) 2 (1.82) 17 (15.6) 56 (51.4) 32 (29.4)

**Most questions had options for multiple responses.

6.4.5 General knowledge of malaria, mosquito biology, and mosquito control methods

Most farmers were informed about malaria and its mode of transmission, and most believed that malaria mosquitoes bite and transmit disease at night ("*usiku wa manane*"). Farmers also claimed that they do experience many mosquito bites during the day, while in the farms and at evening hours when they are cooking, eating, and socializing outdoors, though they were not sure whether these bites were also infectious. Interestingly, none of the farmers could recognize the actual malaria vectors or distinguish them from other mosquitoes. Some believed that malaria vectors are larger than other mosquito species, are coloured, and hide during the day in the bushes then show up at midnight, and hence, challenging to see them.

> "*Malaria is a disease transmitted by a special mosquito that bites at 2 a.m. However, honestly speaking, I see lots of mosquitoes moving inside and outside my house, but I cannot identify the mosquito that transmits malaria*" (male farmer, 22 years).

Another male farmer expressed his experiences as follows:

> "*I really do not know how the mosquito that transmits malaria looks like. Since they bite at midnight around 2 a.m., it's difficult to catch, see, and understand the malaria mosquitoes*" (male farmer, 35 years).

A few farmers were also aware of other diseases transmitted by mosquitoes, and frequently mentioned lymphatic filariasis, yellow fever, and dengue.

Bed nets were the most widely used preventive measure against malaria, as reported by the farmers. The most common net brands were those without insecticides (Safi Polyester Bed Net©), purchased from the local stores or freely distributed LLINs. In addition, the respondents reported spraying insecticide aerosols indoors during evening hours before bedtime as additional control strategy against hiding mosquitoes that enter houses through open eaves and doors. Other approaches used by a few of the participants interviewed included environmental management (e.g., cleaning the environment, clearing bushes, elimination of breeding sites by filling unwanted ponds, and removing stagnant waters), fanning mosquitoes away with a piece of cloth, dressing babies in long-sleeved clothes, and applying repellent lotions in the evenings when spending time outdoors. Below are some of the responses from farmers.

> "*I have been using a bed net against malaria transmission which I purchased from the local shops here in Lupiro, and I do treat it with an insecticide called 'Zuia mbu' after every 3 months. But this year we received free bed nets, and we were told they are already treated with chemicals from the industry*" (female farmer, 36 years).

Some farmers who participated in the survey had contrasting responses regarding insecticide resistance in mosquito. Even though they appreciated the benefits of using bed nets, some claimed that the nets, especially those that were freely distributed, do not offer enough protection because they have big holes, are of poor texture, are less durable, and are easily stretched after washing. Some farmers also claimed that current bed nets do not have enough chemicals compared to the previous ones, as the mosquitoes could even rest for long periods on them.

> "*Even though experts say that the bed nets are impregnated with insecticides, still, I could find mosquitoes inside the net with blood in the body, in the morning*" (female farmer, 24 years).

> "*In the past years, bed nets were very heavy, strong, and durable as they could last for years but these days bed nets, especially the ones freely distributed, are easily stretched and malaria mosquitoes could get in and feed on us*" (male farmer, 44 years).

6.4.6 Results of the participatory workshops and field visits

Farming practices relevant to mosquitoes and their ecology. During the participatory workshops and field visits, farmers explored and learned potential sources of mosquitoes linked to agricultural practices, such as the establishment of rice paddies and irrigation channels. The local practice of irrigating rice fields (i.e., *Ngapa*), and vegetable irrigation in close proximity to river shores were the commonest sources of mosquitoes observed (Figure 6.2). Most farmlands had small pools and water-filled animal footprints favourable for malaria mosquitoes. Repairing broken rice paddies and maintaining the drainage system were identified as options to reduce mosquito breeding. Other approaches discussed included regular draining and/or replacing water in the flooded rice paddies.

Figure 6.2 Typical flooded rice paddies with irrigated channels as potential breeding sites for mosquitoes

Farmers expressed interests in learning some key morphological features to distinguish between *Anopheles* and other mosquitoes (Figure 6.3). Using larvae collected from rice paddies, farmers learned how to distinguish the mosquitoes based on their resting position to the water surface (anopheline larvae rest parallel to the water surface, while culicine larvae rest at an angle). Some of the larvae were raised to adult stage and used for training on how to distinguish *Anopheles* from culicines, using features such as wing spots. Some participants referred to *Aedes* as being the most beautiful mosquitoes (given its black body and white spots). The interviewees also referred to male mosquitoes as “bearded” (providing reference to their feathery antennae) just like human males.

> “*Ooh! Now I understand that not every mosquito in my house is Anopheles and can transmit malaria, there are other mosquitoes such as Culex which also dominate in our village*” (male farmer, 34 years).

Figure 6.3 Participatory workshops and practical sessions on the linkage between pesticides usage and susceptibility status of *Anopheles* mosquito

Improved knowledge on agricultural pesticides, crop pests, and good agricultural practices. During the visits, it was also observed that farmers were regularly mixing pesticides at the farms, in close proximity to water bodies, and lacked proper disposal of remnant pesticides and empty bottles, which were instead scattered around or emptied into rivers or irrigation channels. However, the farmers interacted with agricultural experts and learned safe and proper methods for handling, spraying, and disposal of pesticides. They were shown how to read pesticide labels, and how to identify pesticides by both common names and active ingredients. Farmers acknowledged that they had been spraying various pesticides at various dosages based on their experiences without considering potential negative impacts on mosquito ecology and the general ecosystem. Experts also outlined the general safety and management of pesticides and the use of recommended dosages and encouraged farmers to seek additional advice when necessary.

Both farmers and agricultural experts learned the associations between various public health pesticides and agricultural pesticides. The similarities in terms of active ingredients were discussed with the participants. Agricultural experts were concerned with the fact that most of the pesticides available on the market are broad-spectrum and could kill even beneficial insects, which support pollination and some feed on harmful insects.

> "*Unfortunately, the majority of the current agricultural pesticides have broad-spectrum/non-selective features and with the limited knowledge among users poses health risks to the community and beneficial insects in the environment*" (male agricultural expert, 28 years).

Extension officers engaged the farmers and scientists in discussion on how to identify crop pests and diseases. Beneficial insects were classified and learned among farmers. With the input from the extension officers, farmers collected crop pests and learned to identify them, and describing diseases affecting vegetables, tomatoes, maize, and rice, best practices for managing pests and diseases in crops, and selection of effective pesticide for a particular crop problem (Figure 6.4). Among the devastating insect pests and diseases collected and identified, including rice stem borer that infect rice crop, leafhoppers, maize stalk borers, cutworms that infect maize, leaf beetles and aphids that affect beans, spider mite and African bollworms that can infect tomatoes, maize, and vegetables. Common diseases detected in the farms included yellow virus, leaf blight bacterial wilt in tomato farms, leaf rust, and brown leaf spots. Most farmers, however, acknowledged that sometimes they were misidentified and describing the pests using the local names, thus incorrectly apply pesticides. Farmers recommended that the extension officers could create an archive of images of all common pests and diseases on a poster and their corresponding pesticides. Farmers were able to exchange contacts with the experts for further technical support and information on best pest and disease management practices.

Figure 6.4 Practical field-based sessions with the extension officers (A), discussing common pests and diseases (B), affecting crops in their farms

Results of the insecticide susceptibility bioassays. Researchers briefly described possible effects of agricultural pesticides on mosquitoes, including killing adult mosquitoes and likely influence in the development of insecticide resistance after prolonged use. Insecticide resistance findings were also presented and discussed with the participants. All participants jointly observed the results of the susceptibility tests and were guided through the interpretation.

Following WHO criteria [154], female adult *An. arabiensis* were found resistant to the two pyrethroids, permethrin (mean 24-hour mortality of 62%) and deltamethrin (mean 24-hour mortality of 60%) and even to the organophosphate, pirimiphos-methyl (mean 24-hour mortality of 58%).

6.4.7 Feedback from key-informant stakeholders

Malaria vector control interventions, benefits, and challenges. Most stakeholders acknowledged the overall decline in malaria prevalence over the past 10 years in Tanzania, and attributed this mostly to wide-coverage of LLINs, improved health-seeking behaviour among communities, and improved case management.

> "*At least everyone has access to the long lasting insecticidal net, that could have been significantly contributed in malaria cases and deaths reduction, especially in remote areas where health facilities are limited*" (male programme manager, age not disclosed).

However, the ward health officers, who mostly reside in the study area, indicated that they had been experiencing high mosquito densities indoors, coupled with increased outdoor-biting, making them doubt whether the LLINs still kill mosquitoes.

> "*In the past, you will only get bitten at midnight, thus the idea of the government promoting sleeping under an insecticidal bed net, but now even in the evening hours you struggle with the mosquito bites*" (female agricultural expert, 32 years).

Linkages between agricultural practices and malaria. Most of the stakeholders were aware of the linkage between agricultural and public health. Some associated transmission risks of diseases, such as malaria and schistosomiasis, to agricultural practices. Most of the interviewees identified farming practises that might create aquatic sites for mosquito vectors (e.g., rice flooded paddies).

> "*So yes, there is an association between agriculture and health, very clear association and at the moment I'm aware of several initiatives which are trying to bridge between agriculture and health, looking mostly at parasitic infections which are directly linked to agricultural activities, such as malaria and schistosomiasis*" (male director of sciences, age not disclosed).

The interviewees also acknowledged potential effects of agricultural pesticides on mosquitoes. It was emphasized that continuous use of pesticides could create pressure on malaria vectors breeding in the study area and thus govern resistance to insecticides. Interviews were also concerned that public health and agricultural sectors still operate independently, each with its own vision. Off-label use of pesticides was identified as common among farmers, due to the lack of awareness and poor communication between agricultural experts and farmers.

> "*There are quite some positive and beneficial effects of using agricultural pesticides in crop protection, increasing productivity and some for veterinary purposes. However, there has been little consideration among users on how these pesticides used in*

agriculture intersects with public health insecticides and have effects in malaria mosquitoes" (director public sector, 51 years).

Controls and regulations relevant to pesticide use. There are multiple laws and regulations in place, overseeing pesticide use in Tanzania, with three different regulatory bodies being responsible. Agricultural pesticides manufacturing, registration, distribution, handling, and usage are governed by the Plant Protection Act no. 13 issued in 1997 [309] and the Plant Protection Regulations of 1999 [141] under the Ministry of Agriculture. In this act "plant protection substances" are referred to as pesticides. The Ministry of Agriculture delegates the Tropical Pesticides Research Institute as the competent authority with the full mandate of registering, approving the quality of pesticides, and licensing of stores selling pesticides. In contrast, Tanzania Food and Drugs Authority (TFDA) law regulates veterinary pesticides.

> "*(…) if you look at the law, the law concerning pesticides is fragmented, there used to be a law which was comprehensive and was under the Tropical Pesticide Research Institute Act which it's part 5 was pesticide control. But then someone came along and gave support to the government saying, "Why are you giving regulatory responsibilities to autonomous institutions, it has to be directly under the government. So the Ministry of Agriculture is the one that is supposed to oversee these regulations." So they wrote another law on pesticides which they called Plant Protection Substances act in 1997.*" (female senior lecturer at public sector, age not disclosed).
>
> "*So when you look at the different laws that touch upon the use of pesticides, they do not provide a very solid border and there is always an overlap of activities, for example in the control of livestock chemicals this should be done by TFDA*" (male senior lecturer at public sector, 49 years).

The officials recognized that the majority of distributors and retailers do not comply with the pesticides management laws and regulations, and that there are inadequate pesticide surveillance practices. For example, according to laws, all retailers of agricultural chemicals are required to be trained and certified by the TPHPA prior to the opening of an agrovet store. However, in many cases, licensed agrovet store owners acquire training but do not practice; instead, they employ untrained personnel, either a relative or a friend to sell the agricultural chemicals. In addition, there are limited numbers of authorised chemical inspectors appointed by TPHPA, and hence, the Ministry of Agriculture allows agricultural extension officers trained by Sokoine University of Agriculture and other agricultural bodies to conduct pesticides inspection. Unfortunately, there was also often a conflict of interest, as some of the agricultural extension officers also own agrovet stores.

Illegal importation of substandard pesticides into the market and lack of facilities for adequate disposing of pesticides were also identified as additional challenges. This results in improper use of pesticides and an increase in the risk of environmental contamination.

> "*(…) otherwise we will be flooded with chemicals. But there are few that still come in, because we still have porous boundaries, from Rwanda, Kenya, and Burundi. You find that the regulatory procedures in Kenya are more lenient than ours so other people have found a way to bring in the product through other countries, so you might find product coming in from Rwanda, Burundi, and even from the south coming from Malawi and Zambia coming in as contraband*" (senior staff at public sector, age not known).

However, the issue of sub-standard pesticides could be controlled by using a bar code system.

> "*That should be the case for all regulated products, especially those we import. There should also be a system for those that we produce in the country, a system of controlling quality and we should find a way to identify that this product is from Tanzania agrochemical producers or suppliers. There should be a code and to create something which is unique and difficult for people to develop counterfeit. An electronic system for instance if you are in Mpanda district in west Tanzania, you see a product, able to scan, get all the details like QR and others. I should be able to scan using my phone and identify if a product is fake and be able to isolate it. The product information should also include where it comes from*" (director of public sector, age not disclosed).

Suggested improvements for pesticides management. The use of a self-surveillance system was suggested as a potential approach to improve management of pesticide usage. There was one example of a successful pilot done in northern Tanzania [310], which established self-surveillance programmes to empower farmers with knowledge and skills to report the pesticide products and quantities and improve decisions on use and dosage.

Mixing of different pesticides was already widely employed by farmers. However, during the programme, the farmers also learned how to select the appropriate pesticides. Farmers became actively involved in observing problems at the farms and reported adverse events of pesticides on their health and even ecosystems.

> "*What we have been able to pilot is this one tool "self-surveillance system" which was used in Asia and Asia Pacific. It had been used a lot by the Asian people and they were able to help in the banning of pesticides such as Paraquat (paraquat dichloride) and Endosulfan (organochlorine) because they used to record their effects and reported them. After activists picked it up, they blew it into a national issue and the government*

had to listen and make a decision" (female senior lecturer at public sector, age not disclosed).

There was training the trainers who could be ambassadors in the local communities to improve pesticide use. In this study, farmers were able to record, discuss as a team on any adverse events associated with the pesticides they have been using, and later decide the way forward to manage the problem in consultation with an agricultural officer.

"*(…) and we tried to train trainers, training a few farmers and letting them go teach others. We taught them how to analyze those forms, conduct calculations, see their effects, and then sit as a group to discuss. They could see that after using large amounts of pesticides many people in their groups would get headaches or become dizzy so a specific pesticide wasn't right, they would decide on whether to change it or leave it all together*" (female senior lecturer at public sector, age not disclosed).

Another suggested approach was recycling empty pesticide containers instead of burying, throwing away, or burning. The stakeholders suggested that all leftovers, expired products, or invalid pesticides should be taken to a centralized station following national guidelines, to be collected and disposed by appropriate authorities. Lastly, barcode systems could help regulate the quality of pesticides coming in the country and control substandard pesticide products.

Need for multi-sector collaborations and community empowerment. The key informants acknowledged the need for a holistic approach for integrating relevant sectors in the management of pesticide usage in both public health and agricultural practices. While the main challenge has been implementation costs, and lack of commitment among sectors, possible collaborations could involve pesticides regulatory bodies and the relevant ministries as well as the malaria control programme.

"*But it's very important to have people from different sectors on issues regarding pesticides because pesticides are used everywhere for different purposes and sometimes are misused not only in the farms but also in the community, some people just use them to kill mosquitoes in their houses, for example, these times, there are fumigation companies everywhere. These companies most likely use the same pesticides approved for agricultural purposes*" (male senior lecturer at public sector, age not disclosed).

In addition, empowerment could start by raising awareness and participatory community involvement on pesticide management and alternative pest control. Open dialogues could be one of the platforms for the stakeholders to meet and hold discussions on the agricultural pesticides usage and its association with public health.

> "*Most farmers apply pesticides based on their experience, not sure if they are aware of correct amount and when to spray, so I think, health sector and the agriculture sector need to be interacting at a certain level either through programme interventions or through meetings whereby the open dialogues on the pesticides products and usage practices which seems to be cutting across the two sectors are discussed*" (male director of public sector, age not disclosed).

However, they emphasized that the partnership needs a policy framework that will guide the collaborative approach.

> "*In my opinion, integrating only agricultural and public health sectors in pesticides management at the local community level may not be enough. This is because most of these programmes are governed by policy and regulations. Effective implementation programme would require policymakers being part of the game changer*" (director of public sector, age not disclosed).

6.5 Discussion

The use of agricultural pesticides for crop protection is rapidly increasing in sub-Saharan Africa, including Tanzania. However, intensive use of agricultural pesticides may influence insecticide resistance in crop pests, cause pest resurgence, and has been linked to other issues, such as pesticide self-poisoning by farmers [311,312] and pesticide residue in foods. These chemical residues also accumulate in the aquatic mosquito breeding sites where most of the farming practices are taking place, resulting in a selection pressure on mosquito's larvae, thus driving the development of insecticide-resistant mosquitoes [121,122,124,126,313-317].

The current study investigated the knowledge, views, and practices among Tanzanian farmers in two districts about the issue of insecticide resistance in malaria mosquitoes associated to their long-term usage of agricultural pesticides. Additionally, opportunities to engage the farming sector in management of insecticide resistance in malaria vectors and crop pests were explored by direct participation. Most farmers reported pests as a serious challenge to effective crop production, and synthetic pesticides were heavily relied on. The fear of losing crops, subsidies for agrochemical input, and the ready availability of pesticides influenced farmers' decisions to use pesticides over non-chemical options. While all farmers described pest and disease descriptively, most were not knowledgeable on pest biology, thus pesticides were sprayed haphazardly. Considerable knowledge gaps in pest and diseases identification among farmers have been reported previously [318,319]. Most of the agricultural insecticides utilized had a broad spectrum, were non-selective, and were indiscriminately sprayed based on farmer experience and informal knowledge obtained from the sellers [293]. These findings are consistent with results from previous studies in Tanzania, which reported a lack of knowledge and poor pesticide usage and disposal practices among farmers and pesticide sellers [150,310]. A recent performance audit report by the Controller and Auditor General of the National Audit of Tanzania highlighted similar issues and recommended actions to be taken to improve knowledge on pesticide handling and agricultural practices among users [291]. It was concluded that there is inadequate implementation of pesticide laws and regulations governing pesticides management in Tanzania [143,291].

While most farmers were generally knowledgeable on the link between mosquitoes and malaria, they were less acquainted with the biology and ecology of mosquitoes and their breeding sites. A study by Afrane and colleagues in Ghana found that all farmers who participated in their study had not seen mosquito larvae before and were not aware that water used for agricultural practices support mosquito breeding habitats [320]. In the current study, most farmers were unaware of insecticide resistance in mosquitoes and they could not associate with the selection pressure from pesticides usage. Some farmers claimed an increase in mosquito population density in their localities. *Culex* and *Mansonia* were the predominant mosquito genera in the six study villages [321-325] that cause biting nuisances and might be perceived as malaria vectors. In the southern part of Côte d'Ivoire, farmers also heavily used agricultural pesticides, while they were unaware of the threat to develop insecticide resistance in mosquito vectors [326]. The lack of knowledge of mosquito ecology and biology and the concept of insecticide resistance in mosquito among farmers could have negative implications when designing and implementing vector control programmes. The study recommends regular educational programmes, including community engagement sessions and active involvement in research activities and malaria vector control programmes, in line with previous experiences and recommendations [327-329].

Over 96% of farmers indicated that they had never heard about the concept of integrated pest management (IPM), although a third had previously implemented some non-chemical pest control practices, which are among components of IPM programmes. The alternative traditional pest control methods to synthetic pesticides include the use of wood ash and manual weeding [330]. However, farmers did not routinely use non-chemical pest control methods, as these methods were considered less effective compared to pesticides, and could not be deployed at large scale because of the fear of reduced crop yields due high incidence of pest infestations. Intercropping farming practice (e.g., maize intercropped with beans, or maize intercropped with sesame) was routinely implemented by 42 farmers. This strategy was primarily considered as a means of maximizing the use of land in order to increase crop yields rather than IPM. Similarly, bean farmers in Tanzania were not aware of other benefits of intercropping, in addition to enhancing the productivity of the farmland [331]. In the present study, 75% of farmers were not familiar with biological/natural pest enemies. Previous studies have shown that these cultural practices such as intercropping could encourage predator biodiversity and reduce the incidence of crop pests, while minimizing the need for using synthetic pesticides [331].

There are several successful approaches for engaging communities in the fight against malaria [332]. This study explored possible ways to engage and empower farmers through participatory workshops and practical sessions with the farmers. The study provided a forum for the health researchers and agricultural experts to discuss, interact, actively engage, and empower farming communities with basic knowledge and skills on malaria issues, crop pests, pesticide management, and general good agricultural practices. Improved agricultural practices, including improved management of agricultural pesticides, may contribute in preventing/delaying insecticide resistance in both mosquito vectors and pest crops. This approach was based on knowledge sharing and learning practices by researchers who promote awareness among farmers on the linkage between malaria and agriculture, insecticide resistance in malaria vectors and pest crops, and collective resistance management strategies through directly empowering farmers, enhanced with agricultural experts. Indeed, farmers were offered an opportunity to interact with agricultural experts and researchers. The study focused on empowering farmers with basic knowledge and skills on good agricultural practices, including agrochemicals management, which in turn could indirectly minimize the odds of insecticide resistance development in malaria vectors.

Participatory workshops and actively involving the community in mosquito control has been reported previously [333,334], and could improve uptake of research outcomes into the communities [335], enhance their knowledge on agro-ecosystem practices linked with malaria, while empowering them to make sound agricultural decisions. Feedback sessions with the community and other relevant stakeholders encourage sharing of research findings that could initiate policy changes. As a logical next step, an engagement study with the farmers, researchers, public health, and agricultural experts through workshops and practical field sessions is indicated. Stories should be shared on morphological recognition of different mosquito species, including *Anopheles* and *Culex*, insecticide resistance in mosquitoes, and potential associations with the overwhelming use of agricultural pesticides. Discussions with the farmers on resistance management include proper usage, storage, disposal of agricultural pesticides, and alternative crop pests control other than using agricultural pesticides to prevent or delay the development of insecticide resistance crop pests and malaria mosquitoes.

The majority of the stakeholders acknowledged that agricultural practices have significant implications in malaria vector control. They are aware that pesticide usage practices could be the root cause of insecticide resistance in vectors. They did, however, warn that pesticide usage for crop protection cannot be fully avoided, but must be minimized, used sparingly, or integrated with non-chemical methods. They noted that there is a gap in linking agriculture and public health, probably due to limited resources, such as a shared budget for implementation and lack of commitment across the sectors. While an integrated vector management (IVM) concept for malaria control is encouraged and has been promoted in other East African countries, it is not optimally implemented due to shortage of financial resources and poor implementation approaches [336]. The majority of stakeholders advocated for public forum and field-based farmer field school learning programmes to raise awareness and active participation of communities, policy, and decision-makers. Existing national malaria control strategies could adopt and customize the WHO integrated vector and pest management (IVPM) policy framework in collaboration with key stakeholders from other sectors for a successful malaria vector control programme.

To sustain the effectiveness and efficacy of vector control interventions, WHO recommends IVM strategies that encourage collaborative efforts within the health sector and across other sectors [300,301]. Besides, the Food and Agriculture Organization (FAO) of the United Nations promotes combined pest management approaches to reduce pesticide application, through IPM farmer field schools programmes [337]. The integrated strategy has the potential to bridge the gap between agriculture and health; nevertheless, it is underutilized in low- and middle-income countries [338]. In Tanzania, the concept of IPM is adopted in the pesticides regulatory policy [309,339], but its implementation in the communities is limited due to lack of awareness, a top-down delivery, and the widely available and heavy use of subsides as agrochemical inputs. IPVM approaches engage and empower farmers in controlling crop pests, mosquito densities, and malaria prevalence [16].

Due to financial and time constraints, the current study only covered four discussion workshops and three learning-by-doing sessions in the field unlikely a typical farmer field school. With the limited budget, the study did not monitor and evaluate the effect of community-based participatory workshops and fields training on improved agro-ecosystem practices linked to mosquito and malaria among participants. Hence, future studies should also monitor the impact of participatory workshops on improved knowledge and skills among farmers and other key stakeholders.

6.6 Conclusions

Farmers had some general knowledge about the presence of mosquitoes in their surroundings and that mosquitoes might be associated with malaria transmission. However, farmers could not distinguish malaria from non-malaria vectors, and they failed to make a link between agricultural pesticide use and insecticide resistance in malaria vectors. Both pyrethroids and organophosphate were used either for public health applications or by farmers. The creation of awareness among the farming community about malaria vectors, the use of agricultural pesticides, and the likelihood of influencing insecticide resistance in malaria vectors is critical in integrated insecticide resistance management strategies in malaria mosquitoes and agricultural pests. For successful pesticide resistance control in mosquito and crop pests, community participation, advocacy, and integrating programmes across researchers, public health, and agricultural sectors are required.

Abbreviations

FAO: Food and Agriculture Organization
IPM: Integrated pest management
IRS: Indoor residual spraying
IVM: Integrated vector management
IVPM: Integrated vector and pest management
LLINs: Long-lasting insecticidal nets
NIMR: National Institute for Medical Research
s.s.: *sensu stricto*
TFDA: Tanzania Food and Drugs Authority
TPHPA: Tanzania Plant Health and Pesticides Authority
TPRI: Tropical Pesticides Research Institutes
WHO: World Health Organization

Acknowledgements

We are grateful for the support and experiences provided by all participants during the interviews, survey questionnaire, participatory learning workshops, and field activities. Noelia Pama and Tumpe Mwandiyla are acknowledged for assisting researchers during engagement sessions with the community, data collection, and transcriptions of the interviews. We would like to thank the Editor and anonymous reviewers for providing useful comments and suggestions that helped improving our manuscript.

Author contributions

NSM and FOO conceptualized the study. NSM designed the study protocol, interview guides, and learning sessions guide; MT, BAT, MF, YPM, JU, and FOO contributed to study design and revised the study materials prior to the actual study. NSM performed the interviews, coordinated the participatory workshops and practical sessions with the support from the trained researchers. NSM supported data translation and analyzed the data. NSM drafted the original manuscript. All authors provided significant contributions to the initial draft of the manuscript and approved the final draft before submission.

Funding

This project was funded by Wellcome Trust Intermediate Fellowship in Public Health and Tropical Medicine awarded to FOO (grant no.: WT102350/Z/13/Z). NSM obtained a PhD fellowship financially supported by the Swiss Government Excellence Scholarship via the Federal Commission for Scholarships for Foreign Students FCS (ESKAS; grant identification no. 2017.0786). The funders had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

6.7 Availability of data and materials

The raw datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

6.8 Ethics approval and consent to participate

This project was approved by the institutional review board of the Ifakara Health Institute (reference no. IHI/IRB/NO: 11-2015) and the Medical Research Coordinating Committee at NIMR in Tanzania (reference no. NIMR/HQ/R.8a/Vol.IX/2031). All participants were informed about the study objectives and procedures, and that the discussions were being recorded. Written informed consent was obtained prior to study enrollment. Participation was voluntary, and hence, participants could withdraw from the study at any time without further obligations. To maintain confidentiality, participants were given identification numbers instead of using their names.

Consent for publication

Not applicable

7 Chapter 7: General Discussion

7.1 Summary, research questions and key findings of the study

With the support from the Global funds and the President's Malaria Initiatives (PMI), Long Lasting Insecticidal Impregnated nets (LLINs), and Indoor Residual Spraying (IRS) have been widely distributed and used for controlling malaria and other vector-borne diseases in sub-Saharan Africa. Between 2000 and 2015, 663 million clinical malaria cases were averted (68% reduction) in Africa due to malaria vector control mainly LLINs [29]. The Tanzania's malaria control strategic plan for 2014–2020 aimed at reducing malaria prevalence from 10% in 2014 to 5% in 2016 and less than 1% in 2020. However, there has been an increase in malaria prevalence, with an average prevalence from 9.5% in 2012 to 14.8% in 2016 [8,9]. The current TMIS of 2017 showed an overall malaria prevalence is still as high as 7% among children, and the malaria transmissions is highly heterogeneous across the regions [10]. One of the possible setbacks for malaria control, like other malaria-endemic countries, could be the mosquito vector populations have developed resistance to the core insecticidal interventions after prolonged exposures. Evidence showed that the use of agricultural pesticides for crop protection could also enhance selection pressure and increase resistance to public health pesticides.

In Tanzania, there is still a knowledge gab on the correlations between agricultural practices, including pesticides usage and selection of insecticide resistance in mosquitoes. Most of the farming activities are not incorporated in malaria control programs. Yet, land-use patterns for agricultural activities can influence mosquito population density and pesticide applications on the crop can gradually drive resistance in mosquito larvae and likely to cause resistance in adult mosquitoes to public health insecticides. The WHO through GPIRM developed various approaches for the management of insecticide resistance in malaria vectors aims to prevent or slow down resistance development or retain sustainability of vectors to the current interventions so to sustains the success made in the fight against vector-borne disease. However, the current GPRM does not address the drawbacks from pesticide usage in agriculture and how it could be integrated into resistance monitoring programmes. This study assessed seasonal and spatial changes in insecticide resistance and resistance mechanisms in *Anopheles arabiensis* and *Culex pipiens* complex, in areas where LLINs have been the primary malaria intervention strategy and agricultural pesticide is widely used in rural southern

Tanzania. The specific questions, key findings, study limitations, recommendations for future research and conclusion are discussed below;

Research questions one: *Is there a seasonal and spatial variations of insecticide resistance in An. arabiensis, one of the primary malaria vectors in Ulanga district, southern Tanzania?*

There were substantial fluctuations in phenotypic resistance over time and space in *An. arabiensis* to pyrethroids, DDT and bendiocarb. The differences in insecticide resistance in *An. arabiensis* were observed at fine scales even in villages that were nearby to each other, 5kms apart. Even though resistance was widely distributed across the study ward, mosquito populations from Minepa ward showed the highest resistance frequency, with lowest mosquito mortality and most mosquito populations were more resistant to insecticides in dry seasons than in wet seasons. Across the study wards, insecticides were also found highly used against pests in crops in dry seasons. In Minepa rice production were highly practised throughout the year, and insecticides were more regularly applied for crops protection compared to other study wards. These resistance variations could be due to differences in the biology and genetics of the vector populations, presence of contaminants in particular ecological settings, as reported in a previous study by Verhaeghen *et al.* [198]. DDT and bendiocarb have not used by the public health for malaria vector control in the study areas suggest the observed resistance in the mosquito populations could be due to presence of cross-resistance between classes or selection pressures from agricultural pesticides usage. The impact of agricultural pesticides in the selection of resistant mosquitoes has already been reported extensively [85,86,120-126].

Absence of knock-down resistance genes and partly/complete restoration of pyrethroids and bendiocarb susceptibility after pre-exposures to synergists suggests the involvement of detoxification enzymes P450 monooxygenases and esterases mediating the resistant phenotypes. These findings are of great significance, for the improvement of the current insecticide resistance management strategies taking into considerations the substantial fluctuations in phenotypic resistance profiles in vectors across seasons and locations for better allocation of effective vector control tools. New innovative vector control tools including the new generations PBO-treated LLINs such as Olyset® Plus that contain synergists or bi-treated LLINs with different mode of actions such as Olyset Duo® and Interceptor® G2 could be a possible opportunity in mitigating the spread of pyrethroids resistance, prolong the effectiveness and efficacy of the pyrethroid-based interventions thus sustain and further the gains made against malaria [340].

Research questions two: *What is the insecticide susceptibility status of the "house mosquito" Culex pipiens complex and resistance mechanisms in rural south-eastern Tanzania*

The findings showed that *Culex* species are resistant to most classes of public health insecticides. Insecticide resistance and resistance mechanisms in *Cx.* species varied at a fine geographical scale, between adjacent wards, and seasons. Resistance phenotypes were partly mediated by metabolic mechanisms, but require further evaluation through biochemical and molecular techniques that detect the gene overexpression/activity of detoxification enzymes. The spatial and seasonal fluctuations of resistance profiles and its mechanisms in adults *Cx*. species might be due to different ecological systems with varying exposures and enhanced metabolic detoxification enzymes on mosquito larvae from agricultural pesticides sprayed in different seasons as reported previously [109,128,341].

The most abundant mosquito in the area was *Cx*. species. About 79% of the indoors biting densities were due to *Cx*. species mainly (94%) *Cx. pipiens* complex, of which 81% were classified by PCR as *Cx. quinquefasciatus*. The increased biting densities of *Cx*. mosquitoes combined with lack of knowledge on the difference between malaria and non-malaria vectors among the communities are likely to be perceived that the LLINs or any interventions in place is/are not effective against malaria vectors even if they interventions work. This might reduce community adherence and overall sustainability of the malaria vector control interventions. Indeed, the current study found that most farming communities perceived insecticide resistance as an increased in any mosquito abundance in their environment. Recent study showed that despite high IRS coverage in Bioko Island (Equatorial Guinea), *Culex* biting rates were high both indoors and outdoors [342]. In this study *An. gambiae* s.l were found susceptible to cabamates and organophospahtes, in contrast *Cx.* species were resistant to all four IRS insecticides recommended by the WHO, likely to be perceived by the community as intervention failure since only 3.2% knew malaria was transmitted by Anopheles different from the nuisance mosquitoes [342]. Other related studies have previously reported one of the factors impeding community acceptability and use of malaria vector control tools is perceived increased mosquito density [343]. In these studies, community motivation for accepting/using the IRS intervention was based whether the intervention is beneficial, primarily in reducing mosquito abundance and bites other than prevention of malaria transmission [342-344].

Research questions three: *What are the types and classes of pesticides sold and used in agriculture and farmer's knowledge and practices on pesticides in southern Tanzania.*

The findings revealed that that pesticide were readily available and widely used in agriculture against pests and diseases in the study areas. Various types of pesticides with different chemical active ingredients were sold at the agrovet stores and used by rice, vegetables and other farmers. Most of insecticides and fungicides have similar chemical compositions and share target sites as those used in public health against diseases and vectors. Pyrethroids mostly lambda-cyhalothrin, cypermethrin and Imidacloprid (neonicotinoids) were the most common agricultural insecticides sold to farmers. The herbicide glyphosate (amino-phosphonates) (59.0%), and the fungicides dithiocarbamate and acylalanine (54.5%), and organochlorine (27.3%) were also readily available in the agrovet shops and widely used by farmers. Although both retailers and farmers had at least primary-level education and recognized pesticides by their trade names, they lacked knowledge on pest control or proper usage of these pesticides. Most of the farmers (54.4%, n = 316) relied on instructions from untrained pesticides dealers. Most of pesticide mixing practices were performed alongside water bodies. Overall, 93.7% (400) farmers practised pesticides mixing in their farms, often in close proximity to water sources. One-third of the farmers disposed of their pesticide leftovers (30.0%, n = 128) and most farmers disposed of empty pesticide containers into rivers or nearby bushes (55.7%, n = 238). These improper pesticide use practices have negative implications for malaria vector control tools

Research questions four: *What are the farmers' opinions on insecticide resistance in mosquitoes, alternative pests control and IPM approaches? Can a participatory field-based learning approach bridge the gap between farmers, researchers, and agricultural experts from both agriculture and public health sectors in rural southern Tanzania?*

The study findings indicated that most farmers (94%) considered pesticide use as the first and effective method for pest control and improve crop productivity. A small proportion of farmers had ever used non-chemical strategies but found them ineffective. Slightly less than half of the farmers who participated in the study (n=198, 46.4%) reported being able to identify and name pests on the farms. However, most used local names to identify and describe crop pest and diseases, which is not enough information when selecting pesticide before applications.

Knowledge of the association between agricultural practices and mosquito ecology was poor among farmers. Only (7%) of farmers considered their farms such as irrigated field as potential sources of aquatic mosquito breeding sites. The lack of awareness on the ecology of malaria vector among farmers in the study area is likely to increase the mosquito productivity by creating more breeding habitats. Most farmers could not link any effects that agricultural pesticides may have on mosquitoes, and three quarters had never heard of insecticide resistance in malaria mosquitoes.

While the key informants from public health, environment, and agriculture sectors acknowledged that agricultural practices might have necessary implications in malaria vector control by driving insecticide resistance in mosquitoes, however, they noted that pesticide-based crop protection could not be ignored entirely and instead called for minimized, judicious use and integration with non-chemical methods.

Participatory workshops and field-based practical sessions served as a platform for the farmers to meet with the scientists, agricultural and veterinary experts. All farmers learned to distinguish between larvae or adults of malaria vectors and non-vectors, crop pests and diseases, appropriate pesticides selection and use, and basic knowledge and procedures for detecting insecticide resistance in malaria vectors. The resistance tests conducted together with the farmers showed that *Anopheles arabiensis* mosquitoes were still resistant to the three main insecticides with 24-hour mortality rates of 46-62%.

7.2 Policy implications of the research findings for malaria control

The national malaria control programme (NMCP) of Tanzania has been implementing two main strategies as part of resistance management strategies in line with the GPIRM. These include rotations/mixtures of insecticide with different mode of action and target sites. For example, between 2007 and 2011 IRS programmes in all PMI-supported districts of Lake Zone with the highest malaria burden, were performed using pyrethroid lambda-cyhalothrin (ICON 10CS, Syngenta, Basel, Switzerland). In 2011 malaria vectors developed resistance to pyrethroids therefore the IRS policy switched to bendiocarb (Ficam 80% wettable powder, Bayer) in 2012 and later replaced by pirimphos-methyl in 2013 after detection of bendiocarb resistance. A mixtures approach has also been practiced in some regions including the use of Olyset® Plus in Muleba, Kagera region in North-West Tanzania, where metabolic resistance was confirmed. Among the oldest yet successful malaria vector control interventions include larval source management or larviciding with biolarvicides (*Bti)* and (*Bs)*, however these control approaches are less promoted and implemented by the NMCP programmes and moreover mosquito vectors are likely to develop resistant to these microbial agents especially in agro-ecological settings. It is clear that there is a lack of intersectoral collaboration for pesticide management used for public health and agriculture that contribute in the selection of resistant vectors. The findings of this thesis have policy implications for malaria, other vector-borne disease control programmes, agriculture and environmental sectors as described below;

8) Revise the current NMCP insecticide resistance monitoring plan by expanding the sentinel sites within the districts/wards other than extrapolating resistance data from few representative sentinel sites that are far from each other, with varying selection pressures, for country-level decisions. Continued monitoring is crucial to ensure optimal resistance management and effectiveness of the malaria vector control.

9) The national IRS programmes should operate in line with agricultural pesticide spraying programmes, when selecting class of insecticide and spraying period. This will consider the observed spatial (correlation in space) and temporal variations (correlation in time) in resistance frequency in mosquitoes and ensure targeted and effective interventions are implemented.

- Review and strengthen the current national regulations and policies guiding pesticides manufactures, registration, quality and standards, trading, handling, supply/selling, use, disposal and overall management. Considering a limited options of insecticides are available for public health use, insecticides usage should be harmonized across public and agriculture sectors. Currently agriculture has a wide range of insecticides with more than 600 classes/active ingredients and different formulations unlike public health. One of possible approaches could be to allocate pyrethroids (permethrin, deltamethrin), organophosphates (pirimiphos-methyl, Actellic 300SC)) and carbamates (bendiocarb) solely for public health purposes (LLINs and IRS) and keep other classes of insecticides for agricultural pest control. Management and disposal of obsolete pesticides is centralized and carried out by the NEMC but remaining/unused pesticides and empty pesticide containers are still indiscriminately discarded into the environment. One possible solution could be creating a centralized controlled system at the district/regional level where all pesticide leftover from farms can be collected, later returned back to the supplier and get recycled, thus suppress pesticide waste and accumulation in the environment. To minimize repacking, manipulation and wastage of pesticide, manufactures/distributers could pack and seal small quantities based on local needs. Establish a comprehensive pesticide monitoring and control system in the country by using additional bar-code label or quick response (QR) code on the pesticide products that can be scanned instantly using a mobile smartphone to authenticate their registration status, formulations, validity, dosage, and specific crop before use. In addition, pesticide policy could impose restrictions of pesticides-buying practices that require a customer to seek prescription and an approval certificate from the agricultural/veterinary extension officers.

- In coordination with the ministry of agriculture, increase the number of trained agricultural extension officers at village level to support farmers through empowering them with proper agricultural knowledge for an improved farming practices in line with the national agricultural policy of 2007. Strengthen mechanism in place to ensure pesticides dealers are adequately trained on overall pesticide management before initiation of pesticides business. Increase human resources and finance support for regular pesticide inspection and spot checking at the market ensuring the adherence to the pesticide handling guidelines.

- The current NMCP insecticide resistance and malaria control strategies should consider integrating with agricultural pesticide application practices and general pest management programmes. Possible opportunities could be creating a networks of subsistence farmers, agricultural experts, public health officials, and researchers and engage them through participatory workshops and field learning activities. This will improve community knowledge on mosquito biology linked with agricultural practices, awareness among the farming community on the proper usage and disposal of agricultural pesticide, and enhance community ownership of malaria control program and uptake of research findings. Trained farmers could support implementation of other supplementary vector control strategies such as larva source management or larviciding using biolarvicides (*Bti)* and (*Bs)* in few, detectable and permanent aquatic mosquito breeding sites possibly related to agricultural practices but with minimal pesticide use. Possible opportunity could be combining/apply biolarvicides with agricultural input such as fertilizers to save cost and improve productivity.

- Incorporating *Culex* species into malaria vector management plans to ensure community acceptability and sustainability of the current and future mosquito control programmes.

- Alternatively, integrated pest and vector management (IPVM) methods which involves biological, cultural vector and pest control, the use of pest resistant crop, thus minimal or no using of pesticides to managing insecticide resistance.

7.3 Strength of the study

The strength of this research is that it used a range of multi-disciplinary approach during data collection and reporting. These including observational study at the pesticide stores and pesticide applications in the farms. It also involved the entomological collection of mosquito larvae in the field, laboratory measurements of insecticide resistance and additional synergist bioassays in adult mosquito, and molecularly genotyping of knock-down resistance mutation mechanisms in mosquito vectors. Besides, the study used social skills, through mixed-method research to assess knowledge, broader perspectives, and practises among farmers, pesticide dealers, and key informant/policy advisory, on agricultural pesticides, use and management practices. An integrative weaving approach was used in analysing and interpretation of qualitative and quantitative data. Additionally, participatory engagements and learning activities were carried out with the communities through workshops and field visits.

7.4 Study limitations and recommendations for future research

The following are study limitations and recommendations for future research

1. In the current study, insecticide resistance measurement in the wild mosquito vectors was based on the WHO insecticide susceptibility bioassays that assess phenotypic/frequency of resistant mosquitoes. Future studies are recommended to determine the intensity/levels of resistance based on a dose-response relationship and/ increasing the time post-exposure for more informative data.

2. Other than the use of synergist test to detect metabolic resistance, this study could not quantify the levels of non-specific enzymes such as esterases, monooxygenase, and glutathione S-transferases (GST) that mediating metabolic resistance in the field-collected mosquito vectors. Future studies should also consider screening slight large number specimens to detect low level of kdr mutation frequencies that could have been missed out at the initial phase of resistance development.

3. This study did not evaluate the epidemiological implications of the insecticide resistance evolution on increased malaria transmissions, and reduced efficacy of existing malaria interventions; thus, additional studies are recommended to be carried out in the future.

4. This research did not analyse pesticide residues/level of pesticide contamination in the water/soil in the farm sites where mosquito larvae were sampled, for adult's exposure to insecticide using WHO susceptibility bioassays. Future studies should consider chromatography analysis of water and soil from breeding sites to quantify possible pesticide residues.

5. Due to financial and time constraints, the study did not monitor and evaluate the effect of community-based participatory workshops and fields training on the improved agroecosystem practices linked to mosquito and malaria among participants. Hence, future studies should also monitor the impact of participatory workshops on enhanced knowledge and skills among farmers and other key stakeholders. During the participatory research, a few participants dropped out in the preceding workshops and field visits due to lack of financial incentives. Future community-based mosquito and

malaria interventional trials could consider incorporating incentives, either monetary or non-monetary, to motivate and improve community participants' uptake, participation, and sustainability.

7.5 Conclusions

Major conclusions drawn from this thesis are described below:

1. Insecticides resistance in *An. arabiensis* vectors and *Culex pipiens* complex varies substantially in time and over a small geographical space. The spatial and temporal heterogeneity in insecticide resistance poses challenges for vector control and resistance management programmes and suggests that the current vector control and resistance management programmes should be tailored to meet these local differences.

2. The highest indoor biting nuisance and resistance in *Culex* species could negatively influence the adherence and coverage of vector control interventions. Additional studies are necessary to investigate the epidemiological importance of the species in transmission filariasis and arboviruses in the study area. Malaria vector control programmes should also consider monitoring the resistance profile of culicines as part of the routine resistance management plan.

3. The absence of knock-down resistance mutations in the field *An. arabiensis* populations and substantial suppression of pyrethroids and DDT resistance in *Culex* species after synergist exposures indicated that a metabolic-based mechanism might be present in these species. The use of dual insecticide LLINs with different classes of insecticide or pyrethroid-PBO LLINs could delay resistance development or restore susceptibility in local vectors.

4. Despite the presence of insecticide resistance, communities should keep using the LLINs to minimize the risk of malaria transmission. Since the nets provide only physical barriers, complementary tools such the LLINs contain dual insecticides with different modes of action or incorporated with synergist may enhance the killing efficacy against vectors, and sustain/further the gains made against malaria.

5. Insecticide susceptibility of male mosquitoes could be useful when designing control methods targeting males, such as sterile insect technique (SIT), spraying of male swarms with insecticides and use of attractive toxic sugar baits.

6. Pesticides are the key component in the agriculture toolbox and are readily available, some are counterfeit, sold on the black market, applied haphazardly, and disposed of incorrectly by farmers. Farmers commonly practised pesticide tank mixtures primarily to save time and increase the pesticide performance against a range of crop pests rather than management of resistance in pest.

7. Pesticides are applied intensively for crop protection against pests in Ulanga and Kilombero district where malaria transmissions persist. The unrestricted use of pyrethroids or any approved public health insecticides, for agricultural purposes in the farm settings, implies possible contributions of the ongoing insecticide resistance in wild mosquitos, thus compromising the effectiveness of the current and future malaria vector control interventions.

8. Farmers are not knowledgeable on the agro-ecology linkage and effect of pesticides applications on driving selection for resistance in mosquitoes.

9. Even though it might be challenging to change the agricultural land use and pesticide applications patterns as they are primarily driven by economic and livelihoods necessity. Thus this study recommends awareness improvements among subsistence farmers on the ecology of mosquito vectors, the use of non-chemical pest control tools such as crop rotations and biological, pest-resistant crops. Besides, integrated vector and pest management methods could reduce mosquito populations destiny, risk of malaria transmission and minimize pesticide use/ensure insecticide are judiciously used among both sectors.

10. This report emphasises the need to reform current insecticide resistance management strategies in mosquito vectors in the view of agricultural pesticide practices. It calls for the collaboration and coordination among public health, agriculture and environment sectors to minimize/prevent double insecticide exposure pressures from both sectors thus delay insecticide resistance development. Surveillance of insecticide resistance in mosquito vectors should be a central component of the planning and evaluation of both agriculture pest and vector control programs.

11. Participatory approach and field visits could improve understanding of farmers on agro-ecology system and risks of malaria infections, empower farmers with hand on knowledge on how to manage crop pests and improve collaborations of different

stakeholders (e.g. farmers, researchers, public health officers, and agricultural experts) for more effective resistance management.

8 The data collection tools including the questionnaire and interview guide used in the study

8.1 Appendix 1a: Survey questionnaire to assess farmer's pesticide knowledge, usage practices and perceived effects of pesticide in mosquito vectors

Interview Information

Date:

Name of the investigators

General data/Demographic Characteristics

Participant name/ID:

Sex

i) Male
ii) Female

What is your marital status?

a) Singe
b) Marriage
c) I don't prefer to disclose

What is your age in years?

i) 18-30
ii) 31-40
iii) 41-50
iv) 51-60
v) >60

What is your position in the community?

i) Normal citizen
ii) Hamlet leader
iii) Ward leader
iv) Agricultural officer
v) Veterinary officer
vi) Others

Where do you live?

- Name of Hamlet
- Name of Ward
- Name of District

What is your educational attainment?

i) Primary school

ii) Secondary school

iii) College/university

iv) Professional training

v) No formal training

What is/are your main economic activities?

i) Small-scale subsistence farming activities

ii) Large-scale farming for food and business

iii) Livestock keeping

iv) Small-scale business

v) Large-scale business

vi) Private employment

i) Others

Farming practices

1) Do you own a farm for farming activities?
   a) Yes
   b) No
2) Have you ever rented a farm for crop production?

a) Yes

b) No

c) I don’t remember

3) Where is the location of the farm?
   a) Minepa
   b) Mavimba
   c) Lupiro
   d) Mbasa
   e) Katindiuka
   f) Other farmlands
4) What is the size of the farmland used for crop production in hectares?
   a) <1 hectare

b) 1-5 hectare
c) 5-10 hectares
d) >10 hectares

5) What is the type of crops you cultivated? Circle all applicable
   a) Cereals such as rice, maize
   b) Legumes such as beans
   c) Fruits and vegetables
   d) Others crops, please mention all

6) Among these, which are the rice cultivation methods you have been practising?
   a) Irrigation system i.e. Ngapa
   b) Non-irrigation on the hills
   c) Both irrigation and non-irrigation
   d) Others

Various agrochemicals and usage practices among the farmers

7) Do you know about agricultural pesticides?
   a) Yes
   b) No

8) Have you ever used pesticides in your farming activities? (if No end here)
   a) Yes
   b) No

9) How long have you been using agricultural pesticides?
   a) < 3 years
   b) 3 years' now
   c) 3-10 years
   d) More than 10 years
   e) Others, please specify years

10) Which group of pesticides among these do you use? (multiple selections)
   a) Herbicides
   b) Insecticides
   c) Fungicides
   d) Rodenticides
   e) Others (specify)

11) Which pesticides among these do you use? (refer to the checklist with all the pesticides mentioned during the in-depth interview and check out all listed/specified by the farmer)
12) Are you aware some pesticides are banned or restricted for use in farming activities?
    a) Yes
    b) No
13) Among the pesticides you have been using, are there some that have been banned or restricted for use?
    c) Yes
    d) No

14) Where do you normally get your agricultural pesticides?
    a) I purchase from the agrovet stores in my village
    b) I purchase from the agrovet stores in the city such as Ifakara town or Dar e salaam
    c) I purchase from people selling in the streets
    d) I purchase from the agricultural officer
    e) Others (please specify)

Pesticide mixing, knowledge and frequencies of application

15) Where do you conduct your pesticide mixing/dilution with water before applying?
    a) At home outdoor
    b) In the farm nearby water source
    c) In the farm far from the sources of water
    d) Others (specify)
16) How do you measure the amount of pesticides before mixing with water/ what equipment do you use to measure pesticide?
    a) I use an empty bottle of cola soda to measure pesticide
    b) I estimate the amount of pesticide by eyes
    c) I use syringe pipe to measure insecticide
    e) I use an empty pesticide bottle to measure herbicide
    f) I use the measuring equipment that is brought alongside with the pesticides
    g) I use the cover of the pesticide bottle to measure pesticide
17) Which method of application of agrochemicals do you use?
    a) Spraying after convectional mixed two or more different chemicals and water in a spray tank
    b) Spraying individual chemical mixed with water in a spray tank

c) Sometimes I do either a or b
d) Others, please specify

18) Do you mix and use more than one pesticide (pesticide cocktail) in a one spray tank?
a) Yes
b) No

19) Why do you practice mixing of more than one pesticide in one spray tank?
a) Spraying pesticides in combination save time
b) Spraying pesticides in combination simplify work
c) Combined pesticides are more effective than individual pesticide
d) I don't know

20) What are the common pesticides/products you have ever mixed in one spray tank?
a) Two herbicides
b) Two insecticides
c) One fertilizer and one insecticide
d) One fertilizer and one herbicide
e) One insecticide and one fungicide
f) One insecticide and one herbicide
g) Other mixtures (specify)

21) When do you normally spray various agricultural pesticides?
a) I spray pesticides after rainfall
b) I spray herbicides between November to January before planting rice or any other crops
c) I spray herbicides any time of the year when I see pests in the farm
d) I spray insecticide mostly in dry seasons
e) I spray insecticide only when there are pests any time of the year
f) I spray fungicide in the dry season
g) I spray fungicide in the wet season
h) I spray fungicide any time of the year

22) How often do you spray various pesticides during cropping period?
a) Once every two weeks
b) Twice every week
c) 2-4 times per growing season
d) I spray until I finish all the pesticide I purchased
e) Any time I find pests in the farm
f) Depend on the recommendation on the pesticide product label
g) I don't remember

Knowledge, source of knowledge, storage practises of pesticides among farmers

23) Have you ever participated in any training on proper pesticide usage and management practices?
    a) Yes
    b) No
24) Do you read pesticide product label before applying?
    a) Yes
    b) No
25) Do you know the ingredients/contents of the agricultural pesticides you have been using?
    a) Yes
    b) No
26) Do you know that various pesticide has different formulations?
    a) Yes
    b) No
27) Where do you get instructions on pesticide selection, usage and storage of various agrochemicals? Please select all applicable. If the participant didn't choose **a** and **d**, please skip the next two questions.
    a) From the sellers of the agrovet stores
    b) I rely on my personal experience after using the chemicals for a long time
    c) I participated in professional training on how to use various agrochemicals
    d) From the agricultural officers
    e) I read the pesticide label information before applying pesticide, only if written in Swahili
    f) Others, please mention

28) What is the type of information or instructions gained from either the sellers of agrochemicals or agricultural officers? Please select all applicable
    a) Amount of pesticides to be sprayed
    b) Amount of water to be used for mixing pesticides
    c) Consideration of wind direction during spraying
    d) The use of personal protective equipment during mixing and spraying pesticides
    e) Considering the size of weeds when measuring the dosage of pesticides
    f) Considering the proportion of pest infestations when measuring pesticides
    g) I don't remember
    h) Others, please specify
29) How much do you get satisfied with the instructions or information provided by the sellers/ and agricultural officers?
    a) Highly satisfied

b) Satisfied
c) Low satisfied
d) Not satisfied at all

30) In your opinion what do you know about pesticide recommended dosage?
a) Any pesticide amount that kills all pest in the farm
b) Any quantity of pesticide that is suggested by the pesticide and requires dilution with 15-20 litres of water in a spray tank/solo
c) Half of the pesticide found in the bottle mixed with 15-20 litres of water in a solo
d) All quantity of pesticide purchased that require dilution with 15-20 litres of water
e) Amount/ rate of pesticide found on the label for a particular crop
f) Any amount of pesticide mixed with water enough to spray the farm
g) I don't know

31) Do you think it is necessary to apply the correct pesticide recommended dosage as specified on the label information?
a) Yes
b) No
c) I don't know

32) Which group of pesticides are highly used in vegetable cultivation?
a) Herbicides
b) Insecticides
c) Fungicides
d) All of the above
e) None of the above

33) Which type of pesticides are highly used in fruits cultivation?
a) Herbicides
b) Insecticides
c) Fungicides
d) All of the above
e) None of the above

34) Which type of pesticides are highly used in rice cultivation?
a) Herbicides
b) Insecticides
c) Fungicides
d) All of the above
e) None of the above

35) At which stage/period of the plant developments do you spray insecticides?
   a) At nursery stage
   b) Flowering period
   c) Transplanting
   d) Before harvesting
   e) All of the above
   f) Others (specify)

36) At which stage/period of the plant developments do you spray herbicides?
   a) At nursery stage
   b) During farms preparation
   c) Flowering period
   d) Transplanting
   e) Before harvesting
   f) All of the above
   g) Others (specify)

37) How much herbicides do you use per hectare? (example if you target weeds in rice farm)
   a) Less than 10mls
   b) 10-40mls
   c) 50-150mls
   d) 150-250mls
   e) 200-500mls
   f) More than 500mls
   g) I don't know

38) How much insecticide do you use per hectare? (example in tomatoes farm)
   a) Less than 10mls
   b) 10-40mls
   c) 40-150mls
   d) 150-250mls
   e) 200-500mls
   f) More than 500mls
   g) I don't know

39) What do you do with the remaining/extra (both mixed/diluted or undiluted) pesticides?
   a) Discard them in running water or bushes in the farms
   b) Throw them in the pit latrine
   c) Spray in my pit toilet to kill mosquitoes and other insects

d) Spray in my house to kill insects such as mosquitoes and cockroaches
e) Store them in the house until next farming season
f) Giveaway to my neighbor farmer to use in his/her farm
g) Bury them in the farm until next farming season
h) I had never had any remain pesticides
i) Others, please specify

40) What do you do with the empty containers after finishing the pesticides??
a) Throw them away in the running water/ bushes in the farms
b) Throw them in the bushes
c) Burn them in the farms
d) Bury them in the farms
e) Throw them in the pit latrine
f) Re-use for storage agrochemicals
g) Re-use for other household purposes
h) Others (specify)

41) Do you think there is a need to have a central station to collect leftover, old or expired agricultural pesticides?
a) Yes
b) No
c) I don't know

42) What are the challenges you have been facing when using agricultural pesticides?
a) Health effects such as skin irritation, skin burning, headache, and difficulty in breathing when mixing and after spraying pesticides
b) Pesticides are costly
c) Pesticides have a short shelf life, and they don't last long so we can keep for the next farming season
d) Some pesticides are too much diluted from the suppliers/dealers as they do not perform as expected
e) Some pesticides have lost their killing efficacy, not as previously
f) Some pesticides are counter fake
g) Pest rebound even after spraying pesticides
h) Others
i) I have never experienced any challenge since I started using pesticides

43) Do you know mosquito larvae?
a) Yes

b) No

44) Have you ever seen mosquito larvae before?
   a) Yes
   b) No

45) Where do you think malaria mosquito breed (multiple responses)?
   a) Rivers
   b) Swamps and streams
   c) Stagnant water pools
   d) Rice fields
   e) unused concrete holes
   f) Pit latrines
   g) Open discarded containers with stagnant water
   h) Others
   i) I don’t know

46) Do you think water sources from agroecosystem can create breeding sites for malaria mosquitoes?
   a) Yes
   b) No
   c) I don’t know

47) Why do you use pesticides?
   a) To reduce pest’s infestations & increase agriculture production
   b) An easy and effective method to control pest and diseases in crop
   c) Subsidy and availability of pesticides "kilimo kwanza"
   d) Due to an increased pest’s incidence

48) Can you identify pests type in the farm?
   a) Yes
   b) No

49) What are the criteria 's do you use when selecting and using pesticides (multiple selection?
   a) estimate the size of the farmland
   b) rely on how big the insect pests have infested in the farm
   c) identify the type of weed spray Roundup for hard weeds and 2,4D for soft weeds
   d) spray any pesticide as long as it was effective previously
   e) others (specify)

50) Are you aware of any of the non-chemical pest management methods?
   a) Yes
   b) No

51) Have you ever use of any non-chemical pest management methods?
   a) Yes
   b) No

52) Was the non-chemical pest management method effective?
   a) Yes
   b) No
   c) I don't remember

53) Which of the following cultural pest management practices you have ever used (multiple selection)?
   a) Intercropping (planting more than one different crop in one farm land)
   b) Crop rotation (growing of two or more different types of crop in the same area at different cropping season)
   c) Mulching (covering the soil with materials to retain moisture and minimize weed growth)
   d) I cannot remember
   e) I have never practice any of the above
   f) I don't know

54) have you ever heard of natural enemies for pests control?
   a) category
   b) Yes
   c) No
   d) I don't know

55) Have you heard of integrated pest management?
   a) Yes
   b) No

56) In your opinion what are the effects of agricultural pesticides in malaria mosquitoes?
   a) I don't know
   b) Kill mosquitoes
   c) do not have any effect in malaria mosquitoes
   d) Chase away the mosquitoes
   e) Increase mosquito population density

57) If you look in the past 5 years compare to now, what is the malaria transmission trend in your village settings?
   a) Malaria cases have reduced compare to the past 5 years
   b) Malaria cases are increasing compared to the past 5 years
   c) Malaria transmission remains stable
   d) Others

58) What is/are methods do use in your family against mosquitoes and malaria transmission?
   a) Bed nets treated with insecticides from the factory
   b) Cleanliness of the environment, such as drainage of small pools and clearing bushes
   c) I spray aerosols such as Rungu
   d) Bed nets treated manually
   e) Mosquito coils
   f) Topical repellents
   g) Wearing long-sleeve clothes

h) Others

59) What are the challenges you have been facing in using mosquito and malaria control methods?
a) I don't have any challenge
b) We use malaria control methods but still get bitten by mosquitoes and get malaria
c) They have lost their repellent effect
d) They have lost their killing effectiveness
e) They do not offer enough protection
f) Bed nets create hot environment during sleeping

60) Have you ever heard about insecticide resistance in malaria mosquitoes?
a) Yes
b) No

61) In your opinion what is insecticide resistance in malaria mosquitoes?
a) I don't know
b) Mosquitoes cannot be killed or repelled by the insecticides
c) Increase in mosquito population density
d) Mosquitoes cause more malaria in our village settings
e) Mosquitoes are no longer responsive to the insecticidal interventions
f) Increase in malaria transmission trends in our village settings
g) Others

62) Do you think insecticide resistance in malaria vectors exists in your village setting?
a) Yes
b) No

63) In your opinion, what are the methods for controlling insecticide resistance in malaria mosquitoes?
a) Alternative use of biological, cultural and environmental methods for controlling pests and mosquitoes
b) Establish integrative pest and vector management program that will engage both farmers, researchers and other officials in resistance management
c) Minimize/judiciously use of agricultural chemicals
d) Minimize the use of public health pesticides against mosquitoes
e) Others (specify)

8.2 Appendix 1b: Guide for conducting in-depth interviews, to assess awareness, practices and perceptions of farming communities regarding pesticide use and consequences on mosquito vectors

Date of interview:

Names/initials of investigators:

Participant name/No:

Age:

Gender:

Education attainment of the participant:

Marital status:

Occupation:

Village/Ward/ Sub-village:

District:

Information about the farmers

1) How much land (in terms of acres) do you farm?
2) Which village or sub-village do you do your farming activities?
3) Which crop(s) do you farm?
4) Which seasons of the year do you do your farming activities? (Probe type of crops, probe on reasons)
5) Do you ever engage in the irrigation crop cultivation system? (Probe on type of crops, seasons, source of water)
6) When do you harvest your crops? (Probe on different types of crops, seasonality and farming methods)

Knowledge on different types/class of agricultural pesticides used

7) What are the agricultural pesticides do you know? (Probe on which chemicals they use, the purpose of using, effectiveness, seasonality)
8) Can you please mention all the pesticides you have been using in your farming activities?
9) Why do you use these pesticides?
10) Among the insecticides which ones do you use mostly in i) vegetable ii) rice, iii) maize iv) storage of cereals
11) At which stage/period of the crop development do you spray insecticides? Why?
12) At which stage/period of the crop development do you spray herbicides? Why?
13) At which stage/period of the crop developments do you spray fungicides?
14) Which season do you use insecticides? Why in the seasons mentioned above?
15) Which season do you use herbicides? Why in the seasons mentioned above?
16) Which season do you use fungicides? Why in the seasons mentioned above?
17) Do you know the content or ingredients of the pesticides you have been using? (probe if they read labels)

Information on purchasing of chemicals

18) Where do you normally get or purchase your agricultural supplies, including pesticides? (Probe on types and use for chemicals, preference, seasonality, amount, pesticides, herbicides, mixing)

Pesticide knowledge and usage practices

19) How do you know how to use the different agricultural pesticides you normally purchase? (Probe on the sources of information, knowledge on different types of chemicals, different types of crops, on different seasons, amount, mixing, frequency of use, considerations, herbicides, pesticides)
20) Who are your primary sources of information on how to use pesticides? (Probe on exact information given, type of chemicals, type of crops, frequency of getting information restrictions and considerations, usefulness and satisfaction of information)
21) Where do you prepare and mix pesticides before applying?
22) How do you know the correct pesticide application dosage? (probe if they read the pesticide labels)
23) How do you know pesticide formulations and, frequency of application?
24) How often do you spray insecticides and in vegetables?
25) How often do you apply herbicides in rice?
26) How often do you spray insecticide in rice?
27) Have you ever mix more than one pesticides in one spray tank before applying? If yes, why?
28) How do you know the pesticide has worked effectively?
29) After mixing and applying pesticides, how and where do you wash your pesticide equipment?
30) What challenges do you normally face when using pesticides? (probe on challenges on health, knowledge, financial, and probe on how they overcome challenges)
31) How do you usually store or dispose of the leftover diluted, and extra undiluted pesticides? (Probe on storage, disposal)
32) How do you store or dispose of empty pesticide container?

Do you have any questions/ comments regarding anything we have discussed?

Thank you very much for your time and knowledge. Just in case you have questions/concern regarding this study, please do not hesitate to contact the investigators below.

8.3 Appendix 1c: In-depth interviews guide for assessing pesticide sold in the agrovet stores, knowledge and practices on agricultural pesticide among pesticide dealers

Date:

Name/initials of the investigator's

Shop number (to be given by the investigator):

Name of the participant:

Location of the agrovet store, village/town:

District:

Demographic profile of the participant

1) Sex
2) How old are you (years)?
3) What is your position in this agrovet store?
4) What is your educational attainment?
5) Please specify any other income generating activities?
6) Can you explain if you have ever participating in any seminar, workshop or training on proper use and management of pesticides as a pesticide dealer? (probe if he/she has received any relevant professional training)

Type of agricultural products sold at the shop

1) What are the agricultural supplies do you sell?
2) Do you do wholesale or retail pesticide selling? (probe and observe if they decant pesticide into small containers)
3) A. What are the types of agricultural pesticides do you sell at your shop?

   B. Can I please see and photograph the pesticides that you are selling at your shop? (record the pesticides type, active ingredient in the checklist, and take a photograph for further analysis)

3. How long have you been selling pesticides?
4. Where do you purchase most of your pesticides?

Pesticide awareness and management practices

1) Who are your most frequent customers? *(**probe on different types of customers**)*

2) What type of pesticides do your different customers buy? ***(probe on the different customers and what they buy)***

3) Where do most of your customers come from?

4) On average. How frequently do your customers come to purchase herbicides? (***answers in terms on times per day/week/month***)

5) On average. How frequently do your customers come to purchase insecticides? (***answers in terms on times per day/week/month***)

6) On average, how much pesticides do you sell? (***answers in terms on the amount per day/week/month***)

7) Which pesticides are the most preferred/purchased and why?

8) Which pesticides group are highly demanded by rice farmers and why? (probe if there is specific pesticide type and reasons of preference)

9) Which pesticides group are highly demanded by vegetable farmers and why? (probe if there is specific pesticide type and reasons of preference)
10) Which insecticides are highly preferred by rice farmers and why?
11) Which insecticides are highly preferred by vegetable farmers and why?
12) A. Which seasons of the year do you have the highest pesticide-sales? (probe why)
B. What pesticides are most popular in the dry season? (probe type/group of pesticides and if corresponding to the season when farmers cultivate a certain type of crop)

C. What pesticides are most popular in the wet season? (probe type/group of pesticides and if corresponding to the season when farmers cultivate a certain type of crop)

D. What seasons do you have the least sales?
13) What pesticides are the least popular?

14) How much on average, do people purchase different chemicals? ***(probe on the amount per person)***
15) A. Do your customers know the dosage of pesticides to use (probe if the customers ask for advice on how to use pesticides, including dosage)?

B. If they ask you on dosage, how much do you recommend them to use? What do you consider when advising them on pesticide mixing and rates of application?

C. Do farmers understand the pesticides information on the label? (probe if they advise them to read pesticide labels)

14) How do you dispose of the old pesticide stocks?

15) What do you do with the expired pesticides? (probe how they store/dispose them)

15) How do you store and dispose of empty pesticide containers?

Do you have any questions/ comments regarding anything we have discussed?

Thank you very much for your time and knowledge. Just in case you have questions/concern regarding this study, please do not hesitate to contact the investigators below.

9 References

1. WHO (2019) World Malaria Report: Geneva:World Health Organization.
2. Organization WH (2018) High burden to high impact: a targeted malaria response. World Health Organization.
3. Feachem RG, Chen I, Akbari O, Bertozzi-Villa A, Bhatt S, et al. (2019) Malaria eradication within a generation: ambitious, achievable, and necessary. The Lancet 394: 1056-1112.
4. WHO (2016) The World Health Organization's Global Technical Strategy for Malaria 2016 - 2030. Geneva, World Health Organization.
5. Organization WH (2016) World Malaria Report 2016 (World Health Organization, Geneva.
6. WHO (2017) World Malaria Report Geneva, World Health Organisation.
7. PMI (2019) Presidents Malaria Initiative, Malaria Operational Plan: Tanzania FY 2019. USAID.
8. MoHCDGEC MoH, National Bureau of Statistics , Office of Chief Government Statistician , ICF International (2016) Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS) 2015–2016. MoHSW, MoH, NBS, OCGS, and ICF International Dar es Salaam, Tanzania and ….
9. TACAIDS Z, NBS OCGS I (2013) Tanzania HIV/AIDS and Malaria Indicator Survey 2011–12. Dar es Salaam, Tanzania Dar es Salaam, Tanzania: Tanzania Commission for AIDS (TACAIDS), Zanzibar AIDS Commission (ZAC), National Bureau of Statistics (NBS), Office of the Chief Government Statistician (OCGS), and ICF International.
10. Ministry of Health CD, Gender, Elderly, Children MoH, National Bureau of Statistics , Office of the Chief Government Statistician , ICF (2017) Tanzania Malaria Indicator Survey 2017. MoHCDGEC, MoH, NBS, OCGS, and ICF Dar es Salaam, Tanzania, and Rockville ….
11. Lee H, Halverson S, Ezinwa N (2018) Mosquito-borne diseases. Primary Care: Clinics in Office Practice 45: 393-407.
12. Pates H, Lines J, Keto A, Miller J (2002) Personal protection against mosquitoes in Dar es Salaam, Tanzania, by using a kerosene oil lamp to vaporize transfluthrin. Medical and veterinary entomology 16: 277-284.
13. Chavasse D, Lines J, Ichimori K, Marijani J (1995) Mosquito control in Dar es Salaam. I. Assessment of *Culex quinquefasciatus* breeding sites prior to intervention. Medical and veterinary entomology 9: 141-146.
14. Subra R (1981) Biology and control of *Culex pipiens quinquefasciatus* Say, 1823 (Diptera, Culicidae) with special reference to Africa. International Journal of Tropical Insect Science 1: 319-338.

15. Matowo NS, Abbasi S, Munhenga G, Tanner M, Mapua SA, et al. (2019) Fine-scale spatial and temporal variations in insecticide resistance in *Culex pipiens* complex mosquitoes in rural south-eastern Tanzania. Parasites & vectors 12: 1-13.
16. CDC (2015) Fast facts on Neglected Tropical Diseases (NTDs). Atlanta, Centers for Disease Control and Prevention.
17. WHO (2010) First WHO report on neglected tropical diseases: working to overcome the global impact of neglected tropical diseases. Geneva, World Health Organization.
18. Jones C, Ngasala B, Derua YA, Tarimo D, Reimer L, et al. (2018) Lymphatic filariasis transmission in Rufiji District, southeastern Tanzania: infection status of the human population and mosquito vectors after twelve rounds of mass drug administration. Parasites & vectors 11: 588.
19. WHO (2010) Progress report 2000-2009 and strategic plan 2010-2020 of the global programme to eliminate lymphatic filariasis: halfway towards eliminating lymphatic filariasis: World Health Organization.
20. Ottesen EA (2000) The global programme to eliminate lymphatic filariasis. Tropical Medicine & International Health 5: 591-594.
21. Malecela MN, Lazarus W, Mwingira U, Mwakitalu E, Makene C, et al. (2009) Eliminating LF: a progress report from Tanzania. J Lymphoedema 4: 10-12.
22. McMahon J, Magayuka S, Kolstrup N, Mosha F, Bushrod FM, et al. (1981) Studies on the transmission and prevalence of bancroftian filariasis in four coastal villages of Tanzania. Annals of Tropical Medicine & Parasitology 75: 415-431.
23. Sang R, Kioko E, Lutomiah J, Warigia M, Ochieng C, et al. (2010) Rift Valley fever virus epidemic in Kenya, 2006/2007: the entomologic investigations. The American journal of tropical medicine and hygiene 83: 28-37.
24. Mweya CN, Kimera SI, Kija JB, Mboera LE (2013) Predicting distribution of *Aedes aegypti* and *Culex pipiens* complex, potential vectors of Rift Valley fever virus in relation to disease epidemics in East Africa. Infection ecology & epidemiology 3: 21748.
25. Heinrich N, Saathoff E, Weller N, Clowes P, Kroidl I, et al. (2012) High seroprevalence of Rift Valley fever and evidence for endemic circulation in Mbeya region, Tanzania, in a cross-sectional study. PLoS neglected tropical diseases 6: e1557.
26. Chipwaza B, Mugasa JP, Selemani M, Amuri M, Mosha F, et al. (2014) Dengue and Chikungunya fever among viral diseases in outpatient febrile children in Kilosa district hospital, Tanzania. PLoS neglected tropical diseases 8: e3335.
27. Hertz JT, Munishi OM, Ooi EE, Howe S, Lim WY, et al. (2012) Chikungunya and dengue fever among hospitalized febrile patients in northern Tanzania. The American journal of tropical medicine and hygiene 86: 171-177.

28. Weissenböck H, Hubálek Z, Bakonyi T, Nowotny N (2010) Zoonotic mosquito-borne flaviviruses: worldwide presence of agents with proven pathogenicity and potential candidates of future emerging diseases. Veterinary microbiology 140: 271-280.

29. Bhatt S, Weiss D, Cameron E, Bisanzio D, Mappin B, et al. (2015) The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature 526: 207-211.

30. Organization WH (2019) World malaria report 2019.

31. Malaria RB (2008) The global malaria action plan. Roll Back Malaria partnership.

32. WHOPES (2015) World Health Organization Pesticide Evaluation Scheme. Geneva, World Health Organisation.

33. Manga L (2002) Vector-control synergies, between'roll back malaria'and the Global Programme to Eliminate Lymphatic Filariasis, in the African region. Annals of tropical medicine and parasitology 96: S129-132.

34. WHO (2017) Global vector control response 2017–2030: A strategic approach to tackle vector-borne diseases. World Health Organization.

35. WHO (2014) WHO recommended insecticide products for treatment of mosquito nets for malaria vector control. Geneva, World Health Organisation.

36. WHO (2015) WHO recommended insecticides for indoor residual spraying against malaria vectors. Geneva, World Health Organisation.

37. Renggli S, Mandike R, Kramer K, Patrick F, Brown NJ, et al. (2013) Design, implementation and evaluation of a national campaign to deliver 18 million free long-lasting insecticidal nets to uncovered sleeping spaces in Tanzania. Malar J 12: 85.

38. The United Rebublic of Tanzania MoHaSW, National Malaria Control Programme, (2014) National Malaria Strategic Plan 2014 – 2020.

39. Bonner K, Mwita A, McElroy PD, Omari S, Mzava A, et al. (2011) Design, implementation and evaluation of a national campaign to distribute nine million free LLINs to children under five years of age in Tanzania. Malaria Journal 10: 73.

40. Hanson K, Marchant T, Nathan R, Mponda H, Jones C, et al. (2009) Household ownership and use of insecticide treated nets among target groups after implementation of a national voucher programme in the United Republic of Tanzania: plausibility study using three annual cross sectional household surveys. Bmj 339.

41. Lalji S, Ngondi JM, Thawer NG, Tembo A, Mandike R, et al. (2016) School distribution as keep-up strategy to maintain universal coverage of long-lasting insecticidal nets: implementation and results of a program in Southern Tanzania. Global Health: Science and Practice 4: 251-263.

42. Protopopoff N, Matowo J, Malima R, Kavishe R, Kaaya R, et al. (2013) High level of resistance in the mosquito *Anopheles gambiae* to pyrethroid insecticides and reduced susceptibility to bendiocarb in north-western Tanzania. Malaria Journal 12: 149.

43. Soper FL, Wilson DB (1943) *Anopheles gambiae* in Brazil 1930 to 1940. *Anopheles gambiae* in Brazil 1930 to 1940.

44. Shousha AT (1948) Species-eradication: The Eradication of *Anopheles gambiae* from Upper Egypt, 1942-1945. Bulletin of the World Health Organization 1: 309.

45. Watson M (1921) The prevention of malaria in the Federated Malay States: a record of twenty years' progress: EP Dutton & Company.

46. Kitron U, Spielman A (1989) Suppression of transmission of malaria through source reduction: antianopheline measures applied in Israel, the United States, and Italy. Reviews of infectious diseases 11: 391-406.

47. Killeen GF, Fillinger U, Knols BG (2002) Advantages of larval control for African malaria vectors: low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. Malaria Journal 1: 8.

48. Keiser J, Singer BH, Utzinger J (2005) Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. The Lancet infectious diseases 5: 695-708.

49. Tusting LS, Thwing J, Sinclair D, Fillinger U, Gimnig J, et al. (2013) Mosquito larval source management for controlling malaria. Cochrane Database of Systematic Reviews.

50. Walker K, Lynch M (2007) Contributions of Anopheles larval control to malaria suppression in tropical Africa: review of achievements and potential. Medical and veterinary entomology 21: 2-21.

51. Jacups S, Kurucz N, Whitters R, Whelan P (2011) Habitat modification for mosquito control in the Ilparpa Swamp, Northern Territory, Australia. Journal of Vector Ecology 36: 292-299.

52. Imbahale SS, Mweresa CK, Takken W, Mukabana WR (2011) Development of environmental tools for anopheline larval control. Parasites & Vectors 4: 130.

53. Imbahale SS, Githeko A, Mukabana WR, Takken W (2012) Integrated mosquito larval source management reduces larval numbers in two highland villages in western Kenya. BMC Public Health 12: 362.

54. Fillinger U, Lindsay SW (2006) Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. Tropical Medicine & International Health 11: 1629-1642.

55. Yapabandara A, Curtis C, Wickramasinghe M, Fernando W (2001) Control of malaria vectors with the insect growth regulator pyriproxyfen in a gem-mining area in Sri Lanka. Acta tropica 80: 265-276.

56. Howard AF, Zhou G, Omlin FX (2007) Malaria mosquito control using edible fish in western Kenya: preliminary findings of a controlled study. BMC public health 7: 199.

57. Fillinger U, Kannady K, William G, Vanek MJ, Dongus S, et al. (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: 20.

58. el Gaddal AA (1985) The Blue Nile Health Project: a comprehensive approach to the prevention and control of water-associated diseases in irrigated schemes of the Sudan. The Journal of tropical medicine and hygiene 88: 47.

59. Organization WH, UNICEF (2017) Global vector control response 2017-2030.

60. WHO (2004) Global strategic framework for integrated vector management. World Health Organization.

61. van den Berg H, Kelly-Hope LA, Lindsay SW (2013) Malaria and lymphatic filariasis: the case for integrated vector management. The Lancet infectious diseases 13: 89-94.

62. Muturi EJ, Mbogo CM (2006) Relationship between malaria and filariasis transmission indices in an endemic area along the Kenyan Coast. Journal of vector borne diseases 43: 77.

63. Mbogo C, Baya N, Ofulla A, Githure J, Snow R (1996) The impact of permethrin-impregnated bednets on malaria vectors of the Kenyan coast. Medical and veterinary entomology 10: 251-259.

64. Bøgh C, Pedersen EM, Mukoko DA, Ouma JH (1998) Permethrin-impregnated bednet effects on resting and feeding behaviour of lymphatic filariasis vector mosquitoes in Kenya. Medical and Veterinary Entomology 12: 52-59.

65. Ogoma SB, Lweitoijera DW, Ngonyani H, Furer B, Russell TL, et al. (2010) Screening mosquito house entry points as a potential method for integrated control of endophagic filariasis, arbovirus and malaria vectors. *PLoS Neglected Tropical Diseases* 4: e773.

66. Chanda E, Masaninga F, Coleman M, Sikaala C, Katebe C, et al. (2008) Integrated vector management: the Zambian experience. Malaria Journal 7: 164.

67. Van den Berg H, Das P, von Hildebrand A, Ragunathan V (2006) Evaluation of the integrated pest and vector management (IPVM) project in Sri Lanka: mission report, July 2006.WHO Regional Office for South-East Asia.

68. WHO (2012) Global Plan for Insecticide Resistance Management in Malaria Vectors. Geneva, World Health Organisation.

69. Hargreaves K, Koekemoer L, Brooke B, Hunt R, Mthembu J, et al. (2000) *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. Medical and veterinary entomology 14: 181-189.

70. Churcher TS, Lissenden N, Griffin JT, Worrall E, Ranson H (2016) The impact of pyrethroid resistance on the efficacy and effectiveness of bednets for malaria control in Africa. Elife 5: e16090.

71. Maharaj R, Mthembu D, Sharp B (2005) Impact of DDT re-introduction on malaria transmission in KwaZulu-Natal. South African Medical Journal 95: 871-874.

72. Cohen JM, Smith DL, Cotter C, Ward A, Yamey G, et al. (2012) Malaria resurgence: a systematic review and assessment of its causes. Malar J 11: 122.

73. Alout H, Labbé P, Chandre F, Cohuet A (2017) Malaria vector control still matters despite insecticide resistance. Trends in parasitology 33: 610-618.

74. Thomas MB, Read AF (2016) The threat (or not) of insecticide resistance for malaria control. Proceedings of the National Academy of Sciences 113: 8900-8902.

75. Kleinschmidt I, Bradley J, Knox TB, Mnzava AP, Kafy HT, et al. (2018) Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: a WHO-coordinated, prospective, international, observational cohort study. The Lancet infectious diseases 18: 640-649.

76. Livadas GA, Georgopoulos G (1953) Development of resistance to DDT by *Anopheles sacharov*i in Greece. Bulletin of the World Health Organization 8: 497.

77. de Zulueta J (1959) Insecticide resistance in Anopheles sacharovi. Bulletin of the World Health Organization 20: 797.

78. Metcalf RL (1989) Insect resistance to insecticides. Pesticide science 26: 333-358.

79. Hamon J, Subra R, Sales S, Coz J, Organization WH (1968) Présence dans le Sud-Ouest de la Haute-Volta d'une population d'*Anopheles gambiae*" A" résistante au DDT. Genève: Organisation mondiale de la Santé.

80. Chandre F, Darriet F, Manguin S, Brengues C, Carnevale P, et al. (1999) Pyrethroid cross resistance spectrum among populations of Anopheles gambiae ss from Cote d'Ivoire. Journal of the American Mosquito Control Association 15: 53.

81. Knox TB, Juma EO, Ochomo EO, Jamet HP, Ndungo L, et al. (2014) An online tool for mapping insecticide resistance in major Anopheles vectors of human malaria parasites and review of resistance status for the Afrotropical region. Parasites & vectors 7: 76.

82. Matiya DJ, Philbert AB, Kidima W, Matowo JJ (2019) Dynamics and monitoring of insecticide resistance in malaria vectors across mainland Tanzania from 1997 to 2017: a systematic review. Malaria Journal 18: 102.

83. Kisinza WN, Nkya TE, Kabula B, Overgaard HJ, Massue DJ, et al. (2017) Multiple insecticide resistance in *Anopheles gambiae* from Tanzania: a major concern for malaria vector control. Malar J 16: 439.

84. Matowo NS, Munhenga G, Tanner M, Coetzee M, Feringa WF, et al. (2017) Fine-scale spatial and temporal heterogeneities in insecticide resistance profiles of the malaria

vector, Anopheles arabiensis in rural south-eastern Tanzania. Wellcome Open Research 2.

85. Nkya TE, Akhouayri I, Kisinza W, David J-P (2013) Impact of environment on mosquito response to pyrethroid insecticides: facts, evidences and prospects. Insect biochemistry and molecular biology 43: 407-416.
86. Nkya TE, Mosha FW, Magesa SM, Kisinza WN (2014) Increased tolerance of *Anopheles gambiae* ss to chemical insecticides after exposure to agrochemical mixture. Tanzania Journal of Health Research 16.
87. Matowo NS, Tanner M, Munhenga G, Mapua SA, Finda M, et al. (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: 257.
88. Ranson H, Jensen B, Vulule J, Wang X, Hemingway J, et al. (2000) Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. Insect molecular biology 9: 491-497.
89. Martinez-Torres D, Chandre F, Williamson M, Darriet F, Berge JB, et al. (1998) Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* ss. Insect molecular biology 7: 179-184.
90. Mouhamadou CS, N'Dri PB, Behi Kouadio Fodjo CGS, Affoue F-PK, Koudou BG (2019) Rapid spread of double East-and West-African kdr mutations in wild *Anopheles coluzzi* from Côte d'Ivoire. Wellcome open research 4.
91. Kabula B, Kisinza W, Tungu P, Ndege C, Batengana B, et al. (2014) Co-occurrence and distribution of East (L1014S) and West (L1014F) A frican knock-down resistance in *A nopheles gambiae* sensu lato population of T anzania. Tropical Medicine & International Health 19: 331-341.
92. Corbel V, N'Guessan R (2013) Distribution, mechanisms, impact and management of insecticide resistance in malaria vectors: a pragmatic review. Anopheles mosquitoes-New insights into malaria vectors: IntechOpen.
93. Hemingway J, Hawkes NJ, McCarroll L, Ranson H (2004) The molecular basis of insecticide resistance in mosquitoes. Insect biochemistry and molecular biology 34: 653-665.
94. David J-P, Ismail HM, Chandor-Proust A, Paine MJI (2013) Role of cytochrome P450s in insecticide resistance: impact on the control of mosquito-borne diseases and use of insecticides on Earth. Philosophical Transactions of the Royal Society B: Biological Sciences 368: 20120429.

95. Wondji CS, Irving H, Morgan J, Lobo NF, Collins FH, et al. (2009) Two duplicated P450 genes are associated with pyrethroid resistance in *Anopheles funestus*, a major malaria vector. Genome research 19: 452-459.

96. Nkya TE, Akhouayri I, Poupardin R, Batengana B, Mosha F, et al. (2014) Insecticide resistance mechanisms associated with different environments in the malaria vector *Anopheles gambiae*: a case study in Tanzania. Malar J 13: 10.1186.

97. VAUGHAN A, HAWKES N, HEMINGWAY J (1997) Co-amplification explains linkage disequilibrium of two mosquito esterase genes in insecticide-resistant *Culex quinquefasciatus*. Biochemical Journal 325: 359-365.

98. Vontas J, Blass C, Koutsos A, David JP, Kafatos F, et al. (2005) Gene expression in insecticide resistant and susceptible *Anopheles gambiae* strains constitutively or after insecticide exposure. Insect molecular biology 14: 509-521.

99. RANSON H, ROSSITER L, ORTELLI F, JENSEN B, WANG X, et al. (2001) Identification of a novel class of insect glutathione S-transferases involved in resistance to DDT in the malaria vector *Anopheles gambia*e. Biochemical Journal 359: 295-304.

100. Vanlalhruaia K (2016) Characterization and expression profiles of Glutathione-S-transferase (GSTs) gene in Anopheles mosquito vector complex.

101. Sokhna C, Ndiath M, Rogier C (2013) The changes in mosquito vector behaviour and the emerging resistance to insecticides will challenge the decline of malaria. Clinical Microbiology and Infection 19: 902-907.

102. Musiime AK, Smith DL, Kilama M, Rek J, Arinaitwe E, et al. (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: 1-9.

103. Reddy MR, Overgaard HJ, Abaga S, Reddy VP, Caccone A, et al. (2011) Outdoor host seeking behaviour of *Anopheles gambiae* mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. Malaria Journal 10: 184.

104. Moiroux N, Gomez MB, Pennetier C, Elanga E, Djènontin A, et al. (2012) Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in Benin. The Journal of infectious diseases 206: 1622-1629.

105. Corbel V, Akogbeto M, Damien GB, Djenontin A, Chandre F, et al. (2012) Combination of malaria vector control interventions in pyrethroid resistance area in Benin: a cluster randomised controlled trial. The Lancet infectious diseases 12: 617-626.

106. Kitau J, Oxborough RM, Tungu PK, Matowo J, Malima RC, et al. (2012) Species shifts in the Anopheles gambiae complex: do LLINs successfully control Anopheles arabiensis? PloS one 7: e31481.

107. Sougoufara S, Ottih EC, Tripet F (2020) The need for new vector control approaches targeting outdoor biting Anopheline malaria vector communities. Parasites & Vectors 13: 1-15.

108. Russell TL, Govella NJ, Azizi S, Drakeley CJ, Kachur SP, et al. (2011) Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. Malaria journal 10: 80.

109. Reid MC, McKenzie FE (2016) The contribution of agricultural insecticide use to increasing insecticide resistance in African malaria vectors. Malaria journal 15: 107.

110. Paul P, Kangalawe RY, Mboera LE (2018) Land-use patterns and their implication on malaria transmission in Kilosa District, Tanzania. Tropical diseases, travel medicine and vaccines 4: 6.

111. Swai JK, Finda MF, Madumla EP, Lingamba GF, Moshi IR, et al. (2016) Studies on mosquito biting risk among migratory rice farmers in rural south-eastern Tanzania and development of a portable mosquito-proof hut. Malaria journal 15: 564.

112. Hetzel MW, Alba S, Fankhauser M, Mayumana I, Lengeler C, et al. (2008) Malaria risk and access to prevention and treatment in the paddies of the Kilombero Valley, Tanzania. Malaria journal 7: 7.

113. Matthys B, Vounatsou P, Raso G, Tschannen AB, Becket EG, et al. (2006) Urban farming and malaria risk factors in a medium-sized town in Cote d'Ivoire. The American journal of tropical medicine and hygiene 75: 1223-1231.

114. Klinkenberg E, McCall P, Wilson MD, Amerasinghe FP, Donnelly MJ (2008) Impact of urban agriculture on malaria vectors in Accra, Ghana. Malaria Journal 7: 151.

115. Klinkenberg E, McCall P, Hastings I, Wilson M, Amerasinghe F, et al. (2005) High malaria prevalence and urban agriculture in Accra, Ghana. Emerg Infect Dis 11: 1290-1293.

116. Padonou GG, Sezonlin M, Ossé R, Aizoun N, Oké-Agbo F, et al. (2012) Impact of three years of large scale Indoor Residual Spraying (IRS) and Insecticide Treated Nets (ITNs) interventions on insecticide resistance in *Anopheles gambiae* sl. in Benin. Parasites & vectors 5: 72.

117. Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C, et al. (2016) Averting a malaria disaster: will insecticide resistance derail malaria control? The Lancet 387: 1785-1788.

118. Trape J-F, Tall A, Diagne N, Ndiath O, Ly AB, et al. (2011) Malaria morbidity and pyrethroid resistance after the introduction of insecticide-treated bednets and artemisinin-based combination therapies: a longitudinal study. The Lancet infectious diseases 11: 925-932.

119. Chapin G, Wasserstrom R (1981) Agricultural production and malaria resurgence in Central America and India. Nature 293: 181-185.

120. Diabate A, Baldet T, Chandre F, Akoobeto M, Guiguemde TR, et al. (2002) The role of agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae* sl in Burkina Faso. The American journal of tropical medicine and hygiene 67: 617-622.

121. Nkya T, Poupardin R, Laporte F, Akhouayri I, Mosha F, et al. (2014) Impact of agriculture on the selection of insecticide resistance in the malaria vector *Anopheles gambiae*: a multigenerational study in controlled conditions. Parasit Vectors 7: 480.

122. Elissa N, Mouchet J, Rivière F, Meunier J-Y, Yao K. Resistance of *Anopheles gambiae* ss to pyrethroids in Côte d'Ivoire; 1993. Institute of Tropical Medicine. pp. 291-291.

123. Akogbeto M, Djouaka R, Noukpo H (2005) Use of agricultural insecticides in Benin. Bulletin de la Societe de pathologie exotique (1990) 98: 400-405.

124. Yadouleton A, Martin T, Padonou G, Chandre F, Asidi A, et al. (2011) Cotton pest management practices and the selection of pyrethroid resistance in *Anopheles gambiae* population in Northern Benin. Parasit Vectors 4: 1-11.

125. Yadouleton AW, Asidi A, Djouaka RF, Braïma J, Agossou CD, et al. (2009) Development of vegetable farming: a cause of the emergence of insecticide resistance in populations of Anopheles gambiae in urban areas of Benin. Malaria Journal 8: 103.

126. Georghiou GP (1990) The effect of agrochemicals on vector populations. Pesticide resistance in arthropods: Springer. pp. 183-202.

127. Riaz MA, Poupardin R, Reynaud S, Strode C, Ranson H, et al. (2009) Impact of glyphosate and benzo [a] pyrene on the tolerance of mosquito larvae to chemical insecticides. Role of detoxification genes in response to xenobiotics. Aquatic Toxicology 93: 61-69.

128. Poupardin R, Reynaud S, Strode C, Ranson H, Vontas J, et al. (2008) Cross-induction of detoxification genes by environmental xenobiotics and insecticides in the mosquito Aedes aegypti: Impact on larval tolerance to chemical insecticides. Insect biochemistry and molecular biology 38: 540-551.

129. David J-P, Coissac E, Melodelima C, Poupardin R, Riaz MA, et al. (2010) Transcriptome response to pollutants and insecticides in the dengue vector Aedes aegypti using next-generation sequencing technology. BMC genomics 11: 216.

130. Dabire RK, Namountougou M, Diabaté A, Soma DD, Bado J, et al. (2014) Distribution and frequency of kdr mutations within *Anopheles gambiae* sl populations and first report of the ace. 1 G119S mutation in *Anopheles arabiensis* from Burkina Faso (West Africa). PLoS One 9: e101484.

131. Thomas MB, Godfray HCJ, Read AF, van den Berg H, Tabashnik BE, et al. (2012) Lessons from agriculture for the sustainable management of malaria vectors. PLoS Med 9: e1001262.

132. Sternberg ED, Thomas MB (2018) Insights from agriculture for the management of insecticide resistance in disease vectors. Evolutionary applications 11: 404-414.

133. Koffi AA, Alou LPA, Kabran J-PK, N'Guessan R, Pennetier C (2013) Re-visiting insecticide resistance status in *Anopheles gambiae* from Cote d'Ivoire: a nation-wide informative survey. PLoS One 8: e82387.

134. Chouaïbou M, Etang J, Brevault T, Nwane P, Hinzoumbé CK, et al. (2008) Dynamics of insecticide resistance in the malaria vector *Anopheles gambiae* sl from an area of extensive cotton cultivation in Northern Cameroon. Tropical Medicine & International Health 13: 476-486.

135. Abuelmaali SA, Elaagip AH, Basheer MA, Frah EA, Ahmed FT, et al. (2013) Impacts of agricultural practices on insecticide resistance in the malaria vector *Anopheles arabiensis* in Khartoum State, Sudan. PLoS One 8: e80549.

136. Mzilahowa T, Ball A, Bass C, Morgan J, Nyoni B, et al. (2008) Reduced susceptibility to DDT in field populations of *Anopheles quadriannulatus* and *Anopheles arabiensis* in Malawi: evidence for larval selection. Medical and Veterinary Entomology 22: 258-263.

137. Talom AD, Essoung MA, Gbankoto A, Tchigossou G, Akoton R, et al. (2020) A preliminary analysis on the effect of copper on *Anopheles coluzzi*i insecticide resistance in vegetable farms in Benin. Scientific reports 10: 1-10.

138. Hien AS, Soma DD, Hema O, Bayili B, Namountougou M, et al. (2017) Evidence that agricultural use of pesticides selects pyrethroid resistance within *Anopheles gambiae* sl populations from cotton growing areas in Burkina Faso, West Africa. PLoS One 12: e0173098.

139. Mouhamadou CS, de Souza SS, Fodjo BK, Zoh MG, Bli NK, et al. (2019) Evidence of insecticide resistance selection in wild Anopheles coluzzii mosquitoes due to agricultural pesticide use. Infectious diseases of poverty 8: 64.

140. TPRI (2018) Registered plant protection substances for use in the United Republic of Tanzania. Registrar of pesticides, Tropical Pesticides Research Institute.

141. Dar es Salaam: Ministry of Agriculture FSaC (1999) United Republic of Tanzania: Plant Protection Regulations.

142. AGENDA A (2006) Case Study on Trade and Utilization of Pesticides in Tanzania: Implication to Stockpiling, 2006.

143. Stadlinger N, Mmochi AJ, Kumblad L (2013) Weak governmental institutions impair the management of pesticide import and sales in Zanzibar. Ambio 42: 72-82.

144. Lekei EE, Mkalanga H, Mununa F (2014) Characterization and potential health risks of pesticides registered and used in Tanzania. Afr Newsl Occup Health Safety 24: 56-59.

145. Lekei EE, Ngowi AV, London L (2014) Farmers' knowledge, practices and injuries associated with pesticide exposure in rural farming villages in Tanzania. BMC public health 14: 389.

146. Rajabu J, Tarimo M, Hangali T (2017) Health effects, trends and knowledge on pesticide use in Tanzania. International Journal of Scientific Research and Innovative Technology 4: 100-122.

147. Ngowi A, Mbise T, Ijani A, London L, Ajayi O (2007) Pesticides use by smallholder farmers in vegetable production in Northern Tanzania. Crop Protection (Guildford, Surrey) 26: 1617.

148. Philbert A, Lyantagaye SL, Nkwengulila G (2019) Farmers' pesticide usage practices in the malaria endemic region of North-Western Tanzania: implications to the control of malaria vectors. BMC public health 19: 1456.

149. Chouaïbou MS, Fodjo BK, Fokou G, Allassane OF, Koudou BG, et al. (2016) Influence of the agrochemicals used for rice and vegetable cultivation on insecticide resistance in malaria vectors in southern Côte d'Ivoire. Malaria Journal 15: 426.

150. Lekei EE, Ngowi AV, London L (2014) Pesticide retailers' knowledge and handling practices in selected towns of Tanzania. Environmental health 13: 79.

151. Afrane YA, Lawson BW, Brenya R, Kruppa T, Yan G (2012) The ecology of mosquitoes in an irrigated vegetable farm in Kumasi, Ghana: abundance, productivity and survivorship. Parasites & vectors 5: 1-7.

152. N'Dri BP, Heitz-Tokpa K, Chouaïbou M, Raso G, Koffi AJ, et al. (2020) Use of Insecticides in Agriculture and the Prevention of Vector-Borne Diseases: Population Knowledge, Attitudes, Practices and Beliefs in Elibou, South Côte d'Ivoire. Tropical medicine and infectious disease 5: 36.

153. Sparks TC, Nauen R (2015) IRAC: Mode of action classification and insecticide resistance management. Pesticide biochemistry and physiology 121: 122-128.

154. WHO (2016) Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva, World Health Organisation.

155. Brogdon WG, Chan A (2012) Guideline for evaluating insecticide resistance in vectors using the CDC bottle bioassay. Altlanta, USA: Centers for Disease Control and Prevention.

156. Kabula B, Tungu P, Malima R, Rowland M, Minja J, et al. (2014) Distribution and spread of pyrethroid and DDT resistance among the Anopheles gambiae complex in Tanzania. Medical and veterinary entomology 28: 244-252.

157. Ranson H, Abdallah H, Badolo A, Guelbeogo WM, Kerah-Hinzoumbé C, et al. (2009) Insecticide resistance in *Anopheles gambiae*: data from the first year of a multi-country study highlight the extent of the problem. Malar J 8: 299.

158. Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, et al. (2011) Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends Parasitol* 27: 91-98.

159. Ranson H, Lissenden N (2016) Insecticide resistance in African Anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. Trends in parasitology 32: 187-196.

160. Kisinza W KB, Tungu P, Sindato C, Mweya C, Massue D, Emidi B, Kitau J, Chacha M, Batengana B, , Matowo J MS, Malima R, and Magesa S, (2011) Detection and Monitoring of Insecticide Resistance in Malaria

Vectors in Tanzania Mainland. Technical Report of the National Institute for Medical Research, Tanzania: 1-45.

161. Bousema T, Drakeley C, Gesase S, Hashim R, Magesa S, et al. (2011) Identification of hot spots of malaria transmission for targeted malaria control. Journal of Infectious Diseases 201: 1764-1774.

162. Bejon P, Williams TN, Liljander A, Noor AM, Wambua J, et al. (2010) Stable and unstable malaria hotspots in longitudinal cohort studies in Kenya. PLoS medicine 7: 915.

163. Cotter C, Sturrock HJ, Hsiang MS, Liu J, Phillips AA, et al. (2013) The changing epidemiology of malaria elimination: new strategies for new challenges. The Lancet 382: 900-911.

164. National Malaria Control Programme (Tanzania), WHO (Tanzania), Ifakara Health Institute (Tanzania), KEMRI-Wellcome Trust (Kenya) (2013) An epidemiological profile of malaria and its control in mainland Tanzania. Report funded by Roll Back Malaria and Department for International Development-UK July 2013.

165. Bass C, Nikou D, Donnelly MJ, Williamson MS, Ranson H, et al. (2007) Detection of knockdown resistance (kdr) mutations in *Anopheles gambiae*: a comparison of two new high-throughput assays with existing methods. Malaria Journal 6: 1-14.

166. Organization WH (1998) Techniques to detect insecticide resistance mechanisms (field and laboratory manual). World Health Organization.

167. Brogdon WG (1989) Biochemical resistance detection: an alternative to bioassay. Parasitology Today 5: 56-60.

168. Chouaïbou M, Zivanovic GB, Knox TB, Jamet HP, Bonfoh B (2013) Synergist bioassays: A simple method for initial metabolic resistance investigation of field Anopheles gambiae sl populations. Acta tropica 130: 108-111.

169. Nwane P, Etang J, Chouaïbou M, Toto JC, Koffi A, et al. (2013) Multiple insecticide resistance mechanisms in *Anopheles gambiae* sl populations from Cameroon, Central Africa. Parasites & vectors 6: 41.

170. Khatib RA, Killeen GF, Abdulla SM, Kahigwa E, McElroy PD, et al. (2008) Markets, voucher subsidies and free nets combine to achieve high bed net coverage in rural Tanzania. Malaria Journal 7: 98.

171. Schellenberg JA, Abdulla S, Minja H, Nathan R, Mukasa O, et al. (1999) KINET: a social marketing programme of treated nets and net treatment for malaria control in Tanzania, with evaluation of child health and long-term survival. Transactions of the Royal Society of Tropical Medicine and Hygiene 93: 225-231.

172. West PA, Protopopoff N, Rowland M, Cumming E, Rand A, et al. (2013) Malaria risk factors in North West Tanzania: the effect of spraying, nets and wealth.

173. West PA, Protopopoff N, Wright A, Kivaju Z, Tigererwa R, et al. (2014) Indoor Residual Spraying in Combination with Insecticide-Treated Nets Compared to Insecticide-Treated Nets Alone for Protection against Malaria: A Cluster Randomised Trial in Tanzania. PLoS medicine 11: e1001630.

174. Organization WH (2016) World malaria report 2015: World Health Organization.

175. Ministry of Health CD, Gender, Elderly and Children (MoHCDGEC) [Tanzania, Mainland] MoHMZ, National Bureau of Statistics (NBS), Office of the Chief, Government Statistician (OCGS) aI (2016) Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS) 2015-16.

176. The Tanzania National Malaria Control Programme (2014-2020) The National Malaria Control Programme Strategic Plan, "Invest in the future defeat Malaria".

177. PMI (2015) Presidents Malaria Initiative, Malaria Operational Plan: Tanzania FY2015. USAID.

178. Protopopoff N, Matowo J, Malima R, Kavishe R, Kaaya R, et al. (2013) High level of resistance in the mosquito *Anopheles gambiae* to pyrethroid insecticides and reduced susceptibility to bendiocarb in north-western Tanzania. Malar J 12: 149.

179. Djègbè I, Boussari O, Sidick A, Martin T, Ranson H, et al. (2011) Dynamics of insecticide resistance in malaria vectors in Benin: first evidence of the presence of L1014S kdr mutation in *Anopheles gambiae* from West Africa. Malaria journal 10: 261.

180. Ochieng'Opondo K, Weetman D, Jawara M, Diatta M, Fofana A, et al. (2016) Does insecticide resistance contribute to heterogeneities in malaria transmission in The Gambia? Malaria journal 15: 1.

181. Saavedra-Rodriguez K, Beaty M, Lozano-Fuentes S, Denham S, Garcia-Rejon J, et al. (2015) Local evolution of pyrethroid resistance offsets gene flow among Aedes aegypti collections in Yucatan State, Mexico. The American journal of tropical medicine and hygiene 92: 201-209.

182. Matowo NS, Moore J, Mapua S, Madumla EP, Moshi IR, et al. (2013) Using a new odour-baited device to explore options for luring and killing outdoor-biting malaria vectors: a

report on design and field evaluation of the Mosquito Landing Box. Parasites & Vectors 6: 137.

183. Okumu FO, Moore J, Mbeyela E, Sherlock M, Sangusangu R, et al. (2012) A Modified Experimental Hut Design for Studying Responses of Disease-Transmitting Mosquitoes to Indoor Interventions: The Ifakara Experimental Huts. *PLoS One* 7: e30967.
184. Mayagaya VS, Nkwengulila G, Lyimo IN, Kihonda J, Mtambala H, et al. (2015) The impact of livestock on the abundance, resting behaviour and sporozoite rate of malaria vectors in southern Tanzania. Malaria Journal 14: 17.
185. Kaindoa EW, Matowo NS, Ngowo HS, Mkandawile G, Mmbando A, et al. (2017) Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in south-eastern Tanzania. PLoS One 12: e0177807.
186. Kaindoa EW, Mkandawile G, Ligamba G, Kelly-Hope LA, Okumu FO (2016) Correlations between household occupancy and malaria vector biting risk in rural Tanzanian villages: implications for high-resolution spatial targeting of control interventions. Malaria journal 15: 199.
187. Ferguson HM, Ng'habi KR, Walder T, Kadungula D, Moore SJ, et al. (2008) Establishment of a large semi-field system for experimental study of African malaria vector ecology and control in Tanzania. Malaria Journal 7: 158.
188. Ogoma SB, Lorenz LM, Ngonyani H, Sangusangu R, Kitumbukile M, et al. (2014) An experimental hut study to quantify the effect of DDT and airborne pyrethroids on entomological parameters of malaria transmission. Malaria Journal 13: 131.
189. Ogoma SB, Ngonyani H, Simfukwe ET, Mseka A, Moore J, et al. (2014) The mode of action of spatial repellents and their impact on vectorial capacity of *Anopheles gambiae* sensu stricto. PLoS One 9: e110433.
190. Hunt R, Brooke B, Pillay C, Koekemoer L, Coetzee M (2005) Laboratory selection for and characteristics of pyrethroid resistance in the malaria vector *Anopheles funestus*. Medical and Veterinary Entomology 19: 271-275.
191. Nardini L, Christian RN, Coetzer N, Ranson H, Coetzee M, et al. (2012) Detoxification enzymes associated with insecticide resistance in laboratory strains of *Anopheles arabiensis* of different geographic origin. Parasites & vectors 5: 113.
192. Oliver SV, Brooke BD (2013) The effect of larval nutritional deprivation on the life history and DDT resistance phenotype in laboratory strains of the malaria vector *Anopheles arabiensis*. Malaria Journal 12: 44.
193. R Core Team (2012) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, 2012. ISBN 3-900051-07-0.
194. Vincent K (2008) Probit analysis. San Francisco: San Francisco State University.

195. WHO (2014) Management of Insecticides Resistance in Vectors of Public Health Importance. Geneva, World Health Organisation.

196. Foster GM, Coleman M, Thomsen E, Ranson H, Yangalbé-Kalnone E, et al. (2016) Spatial and temporal trends in insecticide resistance among malaria vectors in chad highlight the importance of continual monitoring. PloS one 11: e0155746.

197. Deming R, Manrique-Saide P, Barreiro AM, Cardeña EUK, Che-Mendoza A, et al. (2016) Spatial variation of insecticide resistance in the dengue vector *Aedes aegypti* presents unique vector control challenges. Parasites & vectors 9: 67.

198. Verhaeghen K, Van Bortel W, Roelants P, Okello PE, Talisuna A, et al. (2010) Spatio-temporal patterns in kdr frequency in permethrin and DDT resistant *Anopheles gambiae* ss from Uganda. The American journal of tropical medicine and hygiene 82: 566-573.

199. Matambo T, Abdalla H, Brooke B, Koekemoer L, Mnzava A, et al. (2007) Insecticide resistance in the malarial mosquito *Anopheles arabiensis* and association with the kdr mutation. Medical and veterinary entomology 21: 97-102.

200. Berticat C, Boquien G, Raymond M, Chevillon C (2002) Insecticide resistance genes induce a mating competition cost in *Culex pipiens* mosquitoes. Genetical research 79: 41-47.

201. Platt N, Kwiatkowska R, Irving H, Diabaté A, Dabire R, et al. (2015) Target-site resistance mutations (kdr and RDL), but not metabolic resistance, negatively impact male mating competiveness in the malaria vector *Anopheles gambiae*. Heredity.

202. Rowland M (1991) Activity and mating competitiveness of γHCH/dieldrin resistant and susceptible male and virgin female *Anopheles gambiae* and *An. stephensi* mosquitoes, with assessment of an insecticide-rotation strategy. Medical and veterinary entomology 5: 207-222.

203. Bellini R, Calvitti M, Medici A, Carrieri M, Celli G, et al. (2007) Use of the sterile insect technique against *Aedes albopictus* in Italy: First results of a pilot trial. Area-wide control of insect pests: Springer. pp. 505-515.

204. Harris AF, McKemey AR, Nimmo D, Curtis Z, Black I, et al. (2012) Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. Nature biotechnology 30: 828-830.

205. Diabate A, Tripet F (2015) Targeting male mosquito mating behaviour for malaria control. Parasites & vectors 8: 1-13.

206. Müller GC, Beier JC, Traore SF, Toure MB, Traore MM, et al. (2010) Successful field trial of attractive toxic sugar bait (ATSB) plant-spraying methods against malaria vectors in the *Anopheles gambiae* complex in Mali, West Africa. Malaria Journal 9: 210.

207. Stewart ZP, Oxborough RM, Tungu PK, Kirby MJ, Rowland MW, et al. (2013) Indoor application of attractive toxic sugar bait (ATSB) in combination with mosquito nets for control of pyrethroid-resistant mosquitoes.

208. WHOPES (2013) WHOPES-recommended compounds and formulations for control of mosquito larvae. Geneva, World Health Organization Pesticide Evaluation Scheme.

209. Jao LT, Casida JE (1974) Insect pyrethroid-hydrolyzing esterases. Pesticide Biochemistry and Physiology 4: 465-472.

210. Farnham AW (1998) The mode of action of piperonyl butoxide with reference to studying pesticide resistance. Piperonyl butoxide, the insecticide synergist Academic, London: 199-213.

211. Darriet F, Chandre F (2011) Combining piperonyl butoxide and dinotefuran restores the efficacy of deltamethrin mosquito nets against resistant *Anopheles gambiae* (Diptera: Culicidae). Journal of medical entomology 48: 952-955.

212. Bingham G, Strode C, Tran L, Khoa PT, Jamet HP (2011) Can piperonyl butoxide enhance the efficacy of pyrethroids against pyrethroid-resistant Aedes aegypti? Tropical Medicine & International Health 16: 492-500.

213. MR F, Makkapati A (2007) Efficacy of piperonyl butoxide (PBO) as a synergist with deltamethrin on five species of mosquitoes. J Commun Dis 39: 159-163.

214. Okumu FO, Chipwaza B, Madumla EP, Mbeyela E, Lingamba G, et al. (2012) Implications of bio-efficacy and persistence of insecticides when indoor residual spraying and long-lasting insecticide nets are combined for malaria prevention. Malaria Journal 11: 378.

215. Kabula B, Kisinza W, Tungu P, Ndege C, Batengana B, et al. (2014) Co-occurrence and distribution of East (L1014S) and West (L1014F) African knock-down resistance in *Anopheles gambiae* sensu lato population of Tanzania. Tropical Medicine & International Health 19: 331-341.

216. Hamon J, Burnett G, Adam J-P, Rickenbach A, Grjébine A (1967) *Culex pipiens* fatigans Wiedemann, Wuchereria bancrofti Cobbold and the economic development of tropical Africa. Bulletin of the World Health Organization 37: 217.

217. Mweya CN, Kimera SI, Mellau LS, Mboera LE (2015) Inter-epidemic abundance and distribution of potential mosquito vectors for Rift Valley fever virus in Ngorongoro district, Tanzania. Global health action 8.

218. Bhattacharya S, Basu P, Sajal Bhattacharya C (2016) The southern house mosquito, Culex quinquefasciatus: profile of a smart vector. J Entomol Zool Stud 4: 73-81.

219. Turell MJ, Linthicum KJ, Patrican LA, Davies FG, Kairo A, et al. (2008) Vector competence of selected African mosquito (Diptera: Culicidae) species for Rift Valley fever virus. Journal of Medical Entomology 45: 102-108.

220. Huff CG (1965) Susceptibility of mosquitoes to avian malaria. Experimental parasitology 16: 107-132.

221. Farajollahi A, Fonseca DM, Kramer LD, Kilpatrick AM (2011) “Bird biting” mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. Infection, genetics and evolution 11: 1577-1585.

222. Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, et al. (2005) Epidemiology and transmission dynamics of West Nile virus disease. Emerging infectious diseases 11: 1167.

223. Brugman V, Hernández-Triana L, Medlock J, Fooks A, Carpenter S, et al. (2018) The role of culex pipiens l.(diptera: culicidae) in virus transmission in Europe. International journal of environmental research and public health 15: 389.

224. Pfeffer M, Dobler G (2010) Emergence of zoonotic arboviruses by animal trade and migration. Parasites & Vectors 3: 35.

225. Farajollahi A, Fonseca DM, Kramer LD, Kilpatrick AMJI, genetics, evolution (2011) “Bird biting” mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. 11: 1577-1585.

226. WHO (2012) Global plan for insecticide resistance management in malaria vectors. World Health Organization.

227. Kelly-Hope L, Ranson H, Hemingway J (2008) Lessons from the past: managing insecticide resistance in malaria control and eradication programmes. The Lancet infectious diseases 8: 387-389.

228. Curtis C, Pasteur N (1981) Organophosphate resistance in vector populations of the complex of Culex pipiens L.(Diptera: Culicidae). Bulletin of Entomological Research 71: 153-161.

229. Jones CM, Machin C, Mohammed K, Majambere S, Ali AS, et al. (2012) Insecticide resistance in Culex quinquefasciatus from Zanzibar: implications for vector control programmes. Parasit Vectors 5: 78.

230. Orshan L, Kelbert M, Pener H (2005) Patterns of insecticide resistance in larval Culex pipiens populations in Israel: dynamics and trends. Journal of vector ecology 30: 289.

231. Silvestrini F, Severini C, di Pardo V, Romi R, de Matthaeis E, et al. (1998) Population structure and dynamics of insecticide resistance genes in *Culex pipiens* populations from Italy. Heredity 81: 342.

232. Kato F (2007) Development of a major rice cultivation area in the Kilombero Valley, Tanzania.

233. Kaindoa EW, Matowo NS, Ngowo HS, Mkandawile G, Mmbando A, et al. (2017) Interventions that effectively target *Anopheles funestus* mosquitoes could significantly

improve control of persistent malaria transmission in south–eastern Tanzania. PLoS ONE 12: e0177807.

234. Okumu FO, Chipwaza B, Madumla EP, Mbeyela E, Lingamba G, et al. (2012) Implications of bio-efficacy and persistence of insecticides when indoor residual spraying and longlasting insecticide nets are combined for malaria prevention. Malaria J 11: 378.

235. Sattler MA, Mtasiwa D, Kiama M, Premji Z, Tanner M, et al. (2005) Habitat characterization and spatial distribution of Anopheles sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. Malaria Journal 4: 1.

236. Kaindoa EW, Mkandawile GB, Lingamba GF, Killeen GF, Okumu FO (2013) Longitudinal surveillance of disease-transmitting mosquitoes in rural Tanzania: creating an entomological framework for evaluation. The Lancet 381: S70.

237. Lines J, Curtis C, Wilkes T, Njunwa K (1991) Monitoring human-biting mosquitoes (Diptera: Culicidae) in Tanzania with light-traps hung beside mosquito nets. Bulletin of Entomological Research 81: 77-84.

238. Edwards FW (1941) Mosquitoes of the Ethiopian Region. III.-Culicine adults and pupae. London British Museum (Natural History): 1-515.

239. Smith JL, Fonseca DM (2004) Rapid assays for identification of members of the Culex (Culex) pipiens complex, their hybrids, and other sibling species (Diptera: Culicidae). The American journal of tropical medicine and hygiene 70: 339-345.

240. Abbott W (1925) A method of computing the effectiveness of an insecticide. J econ Entomol 18: 265-267.

241. Niang EHA, Konaté L, Diallo M, Faye O, Dia I (2016) Patterns of insecticide resistance and knock down resistance (kdr) in malaria vectors *An. arabiensis*, *An. coluzzii* and *An. gambiae* from sympatric areas in Senegal. Parasites & vectors 9: 71.

242. Nwane P, Etang J, Chouaïbou M, Toto JC, Koffi A, et al. (2013) Multiple insecticide resistance mechanisms in *Anopheles gambiae* sl populations from Cameroon, Central Africa. Parasites & vectors 6: 1.

243. Organization WH (2018) Global report on insecticide resistance in malaria vectors: 2010–2016.

244. Killeen GF, Govella NJ, Lwetoijera DW, Okumu FO (2016) Most outdoor malaria transmission by behaviourally-resistant *Anopheles arabiensis* is mediated by mosquitoes that have previously been inside houses. Malaria journal 15: 225.

245. Abdullahi AI, Yusuf Y (2015) Response of *Anopheles gambiae* detoxification enzymes to levels of physico-chemical environmental factors from northwest Nigeria. Bayero Journal of Pure and Applied Sciences 7: 93-104.

246. Diao X, Hazell PB, Resnick D, Thurlow J (2007) The role of agriculture in development: Implications for Sub-Saharan Africa: Intl Food Policy Res Inst.

247. Staatz JM, Dembele NN (2008) Agriculture for development in sub-Saharan Africa.

248. Kishimba M, Henry L, Mwevura H, Mmochi A, Mihale M, et al. (2004) The status of pesticide pollution in Tanzania. Talanta 64: 48-53.

249. Swai JK, Finda MF, Madumla EP, Lingamba GF, Moshi IR, et al. (2016) Studies on mosquito biting risk among migratory rice farmers in rural south-eastern Tanzania and development of a portable mosquito-proof hut. Malaria J 15: 564.

250. Tanner M, De Savigny D, Mayombana C, Hatz C, Burnier E, et al. (1991) Morbidity and mortality at Kilombero Tanzania 1982-88.

251. Schellenberg D, Menendez C, Kahigwa E, Font F, Galindo C, et al. (1999) African children with malaria in an area of intense *Plasmodium falciparum* transmission: features on admission to the hospital and risk factors for death. The American journal of tropical medicine and hygiene 61: 431-438.

252. Hartung C, Lerer A, Anokwa Y, Tseng C, Brunette W, et al. Open data kit: tools to build information services for developing regions; 2010. pp. 1-12.

253. Kuckartz U (2007) MAXQDA: Qualitative data analysis. Berlin: VERBI software.

254. Gale NK, Heath G, Cameron E, Rashid S, Redwood S (2013) Using the framework method for the analysis of qualitative data in multi-disciplinary health research. BMC medical research methodology 13: 117.

255. Fetters MD, Curry LA, Creswell JW (2013) Achieving integration in mixed methods designs—principles and practices. Health services research 48: 2134-2156.

256. Zidan N, El-Naggar JB, Aref SA, El-Dewy ME (2012) Field evaluation of different pesticides against cotton bollworms and sucking insects and their side effects. Journal of American Science 8: 128-136.

257. Mutagahywa J, Ijumba JN, Pratap HB, Molteni F, Mugarula FE, et al. (2015) The impact of different sprayable surfaces on the effectiveness of indoor residual spraying using a micro encapsulated formulation of lambda-cyhalothrin against Anopheles gambiae ss. Parasites & vectors 8: 203.

258. Tungu PK, Malima R, Mosha FW, Lyimo I, Maxwell C, et al. (2015) Evaluation of ICON Maxx, a long-lasting treatment kit for mosquito nets: experimental hut trials against anopheline mosquitoes in Tanzania. Malaria journal 14: 225.

259. Mulamba C, Riveron JM, Ibrahim SS, Irving H, Barnes KG, et al. (2014) Widespread pyrethroid and DDT resistance in the major malaria vector Anopheles funestus in East Africa is driven by metabolic resistance mechanisms. PloS one 9.

260. Wood O, Hanrahan S, Coetzee M, Koekemoer L, Brooke B (2010) Cuticle thickening associated with pyrethroid resistance in the major malaria vector *Anopheles funestus*. Parasites & vectors 3: 67.

261. Fan S, Zhang F, Deng K, Yu C, Liu S, et al. (2013) Spinach or amaranth contains highest residue of metalaxyl, fluazifop-p-butyl, chlorpyrifos, and lambda-cyhalothrin on six leaf vegetables upon open field application. Journal of agricultural and food chemistry 61: 2039-2044.

262. N'Guessan R, Boko P, Odjo A, Chabi J, Akogbeto M, et al. (2010) Control of pyrethroid and DDT-resistant *Anopheles gambiae* by application of indoor residual spraying or mosquito nets treated with a long-lasting organophosphate insecticide, chlorpyrifos-methyl. Malar J 9: 44.

263. Chandre F, Darriet F, Doannio JM, RiviÈre F, Pasteur N, et al. (1997) Distribution of organophosphate and carbamate resistance in *Culex pipiens* quinquefasciatus (Diptera: Culicidae) in West Africa. Journal of medical entomology 34: 664-671.

264. Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the status and global strategy for neonicotinoids. Journal of agricultural and food chemistry 59: 2897-2908.

265. WHO (2020) Prequalification Vector Control: Prequalified lists of vector control products. Geneva, World Health Organisation.

266. Agossa FR, Padonou GG, Koukpo CZ, Zola-Sahossi J, Azondekon R, et al. (2018) Efficacy of a novel mode of action of an indoor residual spraying product, SumiShield® 50WG against susceptible and resistant populations of *Anopheles gambiae* (sl) in Benin, West Africa. Parasites & vectors 11: 293.

267. Crossthwaite AJ, Rendine S, Stenta M, Slater R (2014) Target-site resistance to neonicotinoids. Journal of chemical biology 7: 125-128.

268. Protopopoff N, Wright A, West PA, Tigererwa R, Mosha FW, et al. (2015) Combination of insecticide treated nets and indoor residual spraying in northern Tanzania provides additional reduction in vector population density and malaria transmission rates compared to insecticide treated nets alone: a randomised control trial. PLoS One 10.

269. Corbel V, Hougard J-M, Guessan RN, Chandre F (2003) Evidence for selection of insecticide resistance due to insensitive acetylcholinesterase by carbamate-treated nets in *Anopheles gambiae* ss (Diptera: Culicidae) from Côte d'Ivoire. Journal of medical entomology 40: 985-988.

270. FAO (2012) International code of conduct on the distribution and use of pesticides: Guidelines on Prevention and Management of Pesticide Resistance. Rome,Food and Agriculture Organization of the United Nations

271. Organization WH (2007) Report of the 10th WHOPES Working Group meeting Review of Spinosad 0.5% GR and 12% SC, Lambda-Cyhalothrin 10% CS, KO TAB 1-2-3 Interceptor. 11-14 December 2006. WHO/CDS/NTD/WHOPES.

272. Matowo J, Kulkarni MA, Mosha FW, Oxborough RM, Kitau JA, et al. (2010) Biochemical basis of permethrin resistance in Anopheles arabiensis from Lower Moshi, north-eastern Tanzania. Malar J 9: 10.1186.

273. Djouaka RF, Bakare AA, Coulibaly ON, Akogbeto MC, Ranson H, et al. (2008) Expression of the cytochrome P450s, CYP6P3 and CYP6M2 are significantly elevated in multiple pyrethroid resistant populations of *Anopheles gambiae* ss from Southern Benin and Nigeria. BMC genomics 9: 538.

274. Shao D, Edward S (2014) Combating fake agro-inputs products in Tanzania using mobile phones. International Journal of Computer Applications 97.

275. January B, Rwegasira M, Tefera T (2018) Lepidopteran stem borer species abundance and associated damages on irrigated Kilombero low land rice ecosystem in Tanzania. Journal of Entomology 15:28-35.

276. Mengistie BT, Mol AP, Oosterveer P (2017) Pesticide use practices among smallholder vegetable farmers in Ethiopian Central Rift Valley. Environment, Development and Sustainability 19: 301-324.

277. Finda MF, Moshi IR, Monroe A, Limwagu AJ, Nyoni AP, et al. (2019) Linking human behaviours and malaria vector biting risk in south-eastern Tanzania. PloS one 14: e0217414.

278. WHO (2018) World Malaria Report: Geneva:World Health Organization.

279. Ministry of Health CD, Gender, Elderly, Children MoH, National Bureau of Statistics , Office of the Chief Government Statistician , ICF (2018) Tanzania Malaria Indicator Survey 2018. MoHCDGEC, MoH, NBS, OCGS, and ICF Dar es Salaam, Tanzania, and Rockville ….

280. Chacky F, Runge M, Rumisha SF, Machafuko P, Chaki P, et al. (2018) Nationwide school malaria parasitaemia survey in public primary schools, the United Republic of Tanzania. Malar J 17: 452.

281. Thawer SG, Chacky F, Runge M, Reaves E, Mandike R, et al. (2020) Sub-national stratification of malaria risk in mainland Tanzania: a simplified assembly of survey and routine data. Malar J 19: 1-12.

282. Organization WH (2020) The potential impact of health service disruptions on the burden of malaria: a modelling analysis for countries in sub-Saharan Africa.

283. Sherrard-Smith E, Hogan AB, Hamlet A, Watson OJ, Whittaker C, et al. (2020) The potential public health consequences of COVID-19 on malaria in Africa. Nat Med 26: 1411-1416.

284. Ranson H, Lissenden N (2016) Insecticide resistance in African Anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. Trends Parasitol 32: 187-196.

285. Ranson H, N'guessan R, Lines J, Moiroux N, Nkuni Z, et al. (2011) Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol 27: 91-98.

286. Hancock PA, Hendriks CJ, Tangena J-A, Gibson H, Hemingway J, et al. (2020) Mapping trends in insecticide resistance phenotypes in African malaria vectors. PLoS Biol 18: e3000633.

287. Maharaj R, Mthembu D, Sharp B (2005) Impact of DDT re-introduction on malaria transmission in KwaZulu-Natal. S Afr Med J 95: 871-874.

288. Hargreaves K, Koekemoer L, Brooke B, Hunt R, Mthembu J, et al. (2000) *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. Med Vet Entomol 14: 181-189.

289. Matiya DJ, Philbert AB, Kidima W, Matowo JJ (2019) Dynamics and monitoring of insecticide resistance in malaria vectors across mainland Tanzania from 1997 to 2017: a systematic review. Malar J 18: 102.

290. van den Berg H, Gu B, Grenier B, Kohlschmid E, Al-Eryani S, et al. (2020) Pesticide lifecycle management in agriculture and public health: Where are the gaps? Sci Total Environ: 140598.

291. National Audit Office URoT (2018) Performance audit report on the management of pesticides in agriculture.

292. Reid MC, McKenzie FE (2016) The contribution of agricultural insecticide use to increasing insecticide resistance in African malaria vectors. Malar J 15: 107.

293. Matowo NS, Tanner M, Munhenga G, Mapua SA, Finda M, et al. (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. Malar J 19: 257.

294. Mboera LE, Senkoro KP, Mayala BK, Rumisha SF, Rwegoshora RT, et al. (2010) Spatio-temporal variation in malaria transmission intensity in five agro-ecosystems in Mvomero district, Tanzania.

295. Ijumba J, Lindsay S (2001) Impact of irrigation on malaria in Africa: paddies paradox. Med Vet Entomol 15: 1-11.

296. Klinkenberg E, McCall P, Hastings IM, Wilson MD, Amerasinghe FP, et al. (2005) Malaria and irrigated crops, Accra, Ghana. Emerg Infect Dis 11: 1290.

297. Mutero CM, Kabutha C, Kimani V, Kabuage L, Gitau G, et al. (2004) A transdisciplinary perspective on the links between malaria and agroecosystems in Kenya. Acta tropica 89: 171-186.

298. Swai JK, Finda MF, Madumla EP, Lingamba GF, Moshi IR, et al. (2016) Studies on mosquito biting risk among migratory rice farmers in rural south-eastern Tanzania and development of a portable mosquito-proof hut. Malar J 15: 564.

299. Dunn CE, Le Mare A, Makungu C (2011) Malaria risk behaviours, socio-cultural practices and rural livelihoods in southern Tanzania: implications for bednet usage. Soc Sci Med 72: 408-417.

300. Berg Hvd, Mutero CM, Ichimori K, Organization WH (2012) Guidance on policy-making for integrated vector management.

301. Organization WH (2012) Handbook for integrated vector management: World Health Organization.

302. Matowo NS, Abbasi S, Munhenga G, Tanner M, Mapua SA, et al. (2019) Fine-scale spatial and temporal variations in insecticide resistance in *Culex pipiens* complex mosquitoes in rural south-eastern Tanzania. Parasit Vectors 12: 1-13.

303. Matowo NS, Munhenga G, Tanner M, Coetzee M, Feringa WF, et al. (2017) Fine-scale spatial and temporal heterogeneities in insecticide resistance profiles of the malaria vector, *Anopheles arabiensis* in rural south-eastern Tanzania. Wellcome Open Res 2.

304. Ngufor C, Fagbohoun J, Critchley J, N'Guessan R, Todjinou D, et al. (2017) Which intervention is better for malaria vector control: insecticide mixture long-lasting insecticidal nets or standard pyrethroid nets combined with indoor residual spraying? Malar J 16: 340.

305. Gale NK, Heath G, Cameron E, Rashid S, Redwood S (2013) Using the framework method for the analysis of qualitative data in multi-disciplinary health research. BMC Med Res Methodol 13: 117.

306. Verbi S (1989) MAXQDA, software for qualitative data analysis. Sozialforschung GmbH: 1989-2010.

307. Kuckartz U (2001) MAXQDA, software for qualitative data analysis. Berlin: VERBI Software. Consult. Sozialforschung. GmbH.

308. Fetters MD, Curry LA, Creswell JW (2013) Achieving integration in mixed methods designs—principles and practices. Health Serv Res 48: 2134-2156.

309. United Republic of Tanzania: Plant Protection Act (1997) Acts supplement on the Gazette of the United Republic of Tanzania 78 (27): 252-281.

310. Ngowi AV, Semali I (2011) Controlling pesticide poisoning at community level in lake Eyasi Basin, Karatu District, Tanzania. Eur J Cancer 16: 139-148.

311. Gunnell D, Knipe D, Chang S-S, Pearson M, Konradsen F, et al. (2017) Prevention of suicide with regulations aimed at restricting access to highly hazardous pesticides: a systematic review of the international evidence. Lancet Glob Health 5: e1026-e1037.

312. Knipe DW, Gunnell D, Eddleston M (2017) Preventing deaths from pesticide self-poisoning—learning from Sri Lanka's success. Lancet Glob Health 5: e651-e652.

313. Diabate A, Baldet T, Chandre F, Akoobeto M, Guiguemde TR, et al. (2002) The role of agricultural use of insecticides in resistance to pyrethroids in Anopheles gambiae sl in Burkina Faso. Am J Trop Med Hyg 67: 617-622.

314. Nkya TE, Mosha FW, Magesa SM, Kisinza WN (2014) Increased tolerance of *Anopheles gambiae* ss to chemical insecticides after exposure to agrochemical mixture. Tanzan J Health Res 16.

315. Akogbeto M, Djouaka R, Noukpo H (2005) Use of agricultural insecticides in Benin. Bull Soc Pathol Exot 98: 400-405.

316. Yadouleton AW, Asidi A, Djouaka RF, Braïma J, Agossou CD, et al. (2009) Development of vegetable farming: a cause of the emergence of insecticide resistance in populations of Anopheles gambiae in urban areas of Benin. Malar J 8: 103.

317. Nkya TE, Akhouayri I, Kisinza W, David J-P (2013) Impact of environment on mosquito response to pyrethroid insecticides: facts, evidences and prospects. Insect Biochem Mol Biol 43: 407-416.

318. Khan M, Damalas CA (2015) Farmers' knowledge about common pests and pesticide safety in conventional cotton production in Pakistan. Crop Protection 77: 45-51.

319. Chipeta MM, Shanahan P, Melis R, Sibiya J, Benesi IR (2016) Farmers' knowledge of cassava brown streak disease and its management in Malawi. Int J Pest Manag 62: 175-184.

320. Afrane YA, Lawson BW, Brenya R, Kruppa T, Yan G (2012) The ecology of mosquitoes in an irrigated vegetable farm in Kumasi, Ghana: abundance, productivity and survivorship. Parasit Vectors 5: 1-7.

321. Kaindoa EW, Mkandawile G, Ligamba G, Kelly-Hope LA, Okumu FO (2016) Correlations between household occupancy and malaria vector biting risk in rural Tanzanian villages: implications for high-resolution spatial targeting of control interventions. Malar J 15: 199.

322. Matowo NS, Moore J, Mapua S, Madumla EP, Moshi IR, et al. (2013) Using a new odour-baited device to explore options for luring and killing outdoor-biting malaria vectors: a report on design and field evaluation of the Mosquito Landing Box. Parasit Vectors 6: 137.

323. Matowo NS, Koekemoer LL, Moore SJ, Mmbando AS, Mapua SA, et al. (2016) Combining synthetic human odours and low-cost electrocuting grids to attract and kill outdoor-biting mosquitoes: Field and semi-field evaluation of an improved mosquito landing box. PLoS One 11: e0145653.

324. Ogoma SB, Mmando AS, Swai JK, Horstmann S, Malone D, et al. (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 Negl Trop Dis 11: e0005455.

325. Ogoma SB, Lweitoijera DW, Ngonyani H, Furer B, Russell TL, et al. (2010) Screening mosquito house entry points as a potential method for integrated control of endophagic filariasis, arbovirus and malaria vectors. PLoS Negl Trop Dis 4: e773.

326. N'Dri BP, Heitz-Tokpa K, Chouaïbou M, Raso G, Koffi AJ, et al. (2020) Use of Insecticides in Agriculture and the Prevention of Vector-Borne Diseases: Population Knowledge, Attitudes, Practices and Beliefs in Elibou, South Côte d'Ivoire. Trop Med Int Health 5: 36.

327. Mazigo HD, Obasy E, Mauka W, Manyiri P, Zinga M, et al. (2010) Knowledge, attitudes, and practices about malaria and its control in rural northwest Tanzania. Malar Res Treat 2010.

328. Moshi IR, Ngowo H, Dillip A, Msellemu D, Madumla EP, et al. (2017) Community perceptions on outdoor malaria transmission in Kilombero Valley, Southern Tanzania. Malar J 16: 274.

329. Ukpong I, Opara K, Usip L, Ekpu F (2010) Community perceptions about malaria, mosquito and insecticide treated nets in a rural community of the Niger Delta Nigeria: implications for control. J Parasitol (Faisalabad) 5: 248-257.

330. Nampeera EL, Nonnecke GR, Blodgett SL, Tusiime SM, Masinde DM, et al. (2019) Farmers' Knowledge and Practices in the Management of Insect Pests of Leafy Amaranth in Kenya. J Integr Pest Manag 10: 31.

331. Laizer HC, Chacha MN, Ndakidemi PA (2019) Farmers' knowledge, perceptions and practices in managing weeds and insect pests of common bean in Northern Tanzania. Sustainability 11: 4076.

332. Van den Berg H, Jiggins J (2007) Investing in farmers—the impacts of farmer field schools in relation to integrated pest management. World Development 35: 663-686.

333. Parker C, Garcia F, Menocal O, Jeer D, Alto B (2019) A Mosquito Workshop and Community Intervention: A Pilot Education Campaign to Identify Risk Factors Associated with Container Mosquitoes in San Pedro Sula, Honduras. Int J Environ Res Public Health 16: 2399.

334. Castro MC, Tsuruta A, Kanamori S, Kannady K, Mkude S (2009) Community-based environmental management for malaria control: evidence from a small-scale intervention in Dar es Salaam, Tanzania. Malar J 8: 57.

335. Van den Berg H, van Vugt M, Kabaghe AN, Nkalapa M, Kaotcha R, et al. (2018) Community-based malaria control in southern Malawi: a description of experimental

interventions of community workshops, house improvement and larval source management. Malar J 17: 266.

336. Mutero CM, Schlodder D, Kabatereine N, Kramer R (2012) Integrated vector management for malaria control in Uganda: knowledge, perceptions and policy development. Malar J 11: 1-10.

337. Pontius J, Dilts R, Bartlett A (2002) Ten years of IPM training in Asia-From farmer field school to Community IPM. Bangkok: Food and Agricultural Organization.

338. Parsa S, Morse S, Bonifacio A, Chancellor TC, Condori B, et al. (2014) Obstacles to integrated pest management adoption in developing countries. Proc Natl Acad Sci USA 111: 3889-3894.

339. Nyambo T, Varela AM, Seguni Z, Kirenga G (2003) Integrated pest management in Tanzania. Integrated pest management in the global arena: 145-152.

340. Protopopoff N, Mosha JF, Lukole E, Charlwood JD, Wright A, et al. (2018) Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two factorial design trial. The Lancet 391: 1577-1588.

341. Strode C, Steen K, Ortelli F, Ranson H (2006) Differential expression of the detoxification genes in the different life stages of the malaria vector *Anopheles gambiae*. Insect molecular biology 15: 523-530.

342. Fuseini G, Nguema RN, Phiri WP, Donfack OT, Cortes C, et al. (2019) Increased biting rate of insecticide-resistant *culex* mosquitoes and community adherence to irs for malaria control in urban malabo, bioko island, equatorial guinea. Journal of medical entomology 56: 1071-1077.

343. Ingabire CM, Rulisa A, Van Kempen L, Muvunyi C, Koenraadt CJ, et al. (2015) Factors impeding the acceptability and use of malaria preventive measures: implications for malaria elimination in eastern Rwanda. Malaria journal 14: 136.

344. Munguambe K, Pool R, Montgomery C, Bavo C, Nhacolo A, et al. (2011) What drives community adherence to indoor residual spraying (IRS) against malaria in Manhiça district, rural Mozambique: a qualitative study. Malaria journal 10: 344.

10 Curriculum Vitae

***Nancy Stephen Matowo**, BSc, MSc, PhD*

***Current organization**: London School of Hygiene and Tropical Medicine*

***Current position**: Research Fellow*

***Email address**: nstephen20@gmail.com*

PROFILE

I am a Medical Entomologist and vector control specialist with over 9 year-experience in public health/vector control research. I hold a Research Fellow position with the Department of Infectious Disease Epidemiology at the London School of Hygiene and Tropical Medicine (LSHTM). I joined LSHTM in 2018 and I am currently based at National Institute for Medical Research, Mwanza centre in Tanzania. My research interests focus on mosquito biology, design, and field evaluation and implementation of innovative vector control interventions. I have established and extensively investigated spatial and temporal variations of insecticide resistance and associated resistance mechanisms in malaria vectors in rural Tanzania. I have broad experience coordinating research teams in large field-based projects. Currently, I manage an entomological component of a large cluster randomized controlled trial assessing the effectiveness of novel dual active ingredients next-generation long-lasting insecticide nets (LLINs) against malaria transmitted by pyrethroid-resistant mosquito populations in rural Tanzania. I have published extensively in peer-reviewed journals and presented research outputs at both national and international forums. I provide technical assistance to Vector Control Technical Working Group (VCTWG) of the National Malaria Control Programme.

EDUCATION BACKGROUND/ PROFESSIONAL QUALIFICATIONS

1) **PhD in Epidemiology/Public Health.** The University of Basel, Faculty of Science; and Swiss Tropical and Public Health Institute, Department of Environmental and Public Health, Basel, Switzerland. *completed December 2020*

2) **Master of Science in Medicine (Biology and Control of African Disease Vectors).** The University of the Witwatersrand, School of Pathology, Faculty of Health Sciences, Johannesburg, South Africa. *completed July 2015*

3) **Bachelor of Science in Environmental Health Sciences**. Muhimbili University of Health and Allied Sciences (MUHAS), School of Public Health and Social Sciences, Department of Environmental and Occupational Health Sciences, Dar-es-Salaam, Tanzania. *completed December 2009*

SELECTED RESEARCH PROJECTS AND CONSULTANCIES	
Name of project:	Efficacy of different types of bi-treated long lasting insecticidal nets and deployment strategy for control of malaria transmitted by pyrethroid resistant vectors, Tanzania
Client / Funder:	DFID/MRC UK/NIHR/Wellcome, Joint Global Health trial

SELECTED RESEARCH PROJECTS AND CONSULTANCIES					
Year:	2018-2021	**Location:**	Tanzania and Benin	**Funding:**	3,794,760 USD
Role:	Lead Medical Entomologist of a large-scale cluster-randomized net evaluation trial. I involved in the preparation of SOPs and protocols, implementation of field activities, training and supervision of the team involved in the vector surveillance, coordination of insecticide resistance monitoring, molecular analysis, statistical data analysis and manuscripts write up.				
Name of project:	Videographic analysis and experimental evaluation of female mosquito host-seeking responses to optimize a new odour-baited device for monitoring outdoor-biting malaria vectors,				
Client / Funder:	The Wellcome Trust				
Year:	2014-2017	**Location:**	Tanzania	**Funding:**	164,759 USD
Role:	Principal Investigator, coordinated the entomological field and semi-field activities on videographic analysis and quantification of mosquito host-seeking behaviour towards odour-baited mosquito control traps and human volunteers using infrared cameras. Mentored one research assistant and 4 field technicians.				
Name of project:	Using molasses for agricultural productivity and mosquito vectors control				
Client / Funder:	Rising Stars in Global Health Award Phase I Grant from Grand Challenges Canada™				
Year:	2014-2016	**Location:**	Tanzania	**Funding:**	88,974 USD
Role:	Co-Principal Investigator supported in the study design, data collection, analysis and manuscript writing, review and editing.				
Name of project:	Outdoor Mosquito Control as a Complimentary Strategy to Accelerate Malaria Elimination in Africa				
Client / Funder:	Bill and Melinda Gates Foundation (BMGF) and Grand Challenges Canada (GCC)				
Year:	2011-2014	**Location:**	Tanzania	**Funding:**	615,670 USD
Role:	Research officer/Entomologist; • Developed, and field testing community driven odour-baited mosquito landing box for controlling malaria outdoors • Established and conducted the WHO insecticides susceptibility bioassays profile in urban and rural areas of the study sites • Ensured that all relevant quality control procedures and ethical guidelines are adhered to the study protocols • Wrote and published high quality scientific papers on your research work in peer reviewed and internationally recognized journals				
Name of project:	Auto dissemination of Insecticides for Malaria Vector- Control				
Client / Funder:	Bill and Melinda Gates Foundation				
Year:	2011-2016	**Location:**	Tanzania	**Funding:**	2.2 million USD
Role:	Research Officer/Entomologist; • Conducted field surveillance of mosquito larvae, characterised and geo-located breeding sites for malaria vectors in the rural, southern of Tanzania • Performed semi-field tests demonstrating the auto-dissemination of Pyriproxyfen, to their breeding sites • Performed CDC bottle bioassays on sterility effects of PPF on mosquitoes				

RELEVANT MEDIA INTERVIEWS

Media coverage

I participated in various media interviews regarding malaria control using our novel prototype (The Mosquito Landing Box). Below is a sample of relevant media coverage events.

1) December 2016: Interview with the Deutsche Welle (DW) featured at World in Progress: Staying one step ahead of Malaria: Available at: http://dw.com/p/2TWMQ.

2) January 2016: Interview with the Newsweek: A new mosquito trap mimics human odours and carbon dioxide to catch the insects: Available at http://www.newsweek.com/2016/02/12/mosquito-trap-uses-human-scent-bait-421829.html.

3) 15th April 2015: Interview with Mwananchi. CO.TZ: New technology for controlling mosquitoes and malaria transmission (The odour-baited mosquito landing box equipped with electrocuting grids).

4) February 2014: Interview and video shooting with Al Jazeera, for a program named Lifelines: The Quest For Global Health: EndGame: Full Video: http://www.youtube.com/watch?v=kmrvDrGaUo, which was first broadcasted on Al Jazeera English on 24 April 2014 at 20:00 GMT.

RELEVANT PEER-REVIEWED PUBLICATIONS

First - author publications

1) **Matowo NS**, Tanner M, Temba BA, Finda M, Mlacha YP, Utzinger J, Okumu FO. (2022). Participatory approaches to raise awareness among subsistence farmers in Tanzania about the spread of insecticide resistance in malaria vectors and the possible link to improper agricultural pesticide use. In Press, Malaria Journal

2) **Matowo NS**, Martin J, Kulkarni MA, Mosha JF, Lukole E, Isaya G, Shirima B, Kaaya R, Moyes C, Hancock PA, Rowland M, Manjurano A, Mosha FW, Protopopoff N, Messenger LA (2021). "An increasing role of pyrethroid-resistant *Anopheles funestus* in malaria transmission in the Lake Zone, Tanzania". Scientific Reports 11(1): 13457

3) **Matowo NS**, Tanner M, Munhenga G, Mapua SA, Finda M, Utzinger J, Ngowi V, Okumu FO. (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): 257.

4) **Matowo NS**, Abbasi S, Munhenga G, Tanner M, Mapua SA, Oullo D, Koekemoer LL, Kaindoa E, Ngowo HS, Coetzee M, Jürg U, Okumu FO (2019). Fine-scale spatial and temporal variations in insecticide resistance in *Culex pipiens* complex mosquitoes in rural southeastern Tanzania. Parasites & vectors 12: 1-13.

5) **Matowo NS**, Munhenga G, Tanner M, Coetzee M, Feringa WF, Ngowo HS, Koekemoer LL, Okumu FO. Fine-scale spatial and temporal heterogeneities in insecticide resistance profiles of the malaria vector, *Anopheles arabiensis* in rural southeastern Tanzania. [version 1; referees: 1 approved]. Wellcome Open Res 2017, 2:96 (doi:10.12688/welcome open res.12617.1)

6) **Matowo NS**, Koekemoer LL, Moore SJ, Mmbando AS, Mapua SA, Coetzee M, Okumu FO. Combining synthetic human odours and low-cost electrocuting grids to attract and kill outdoor-biting mosquitoes: field and semi-field evaluation of an improved mosquito landing box. PLoS ONE 2016 Jan 20; 11(1):e0145653: Available at: http://dx.doi.org/10.1371/journal.pone.0145653.

7) **Matowo NS,** Moore J, Mapua S, Madumla EP, Moshi IR, Kaindoa EW, Mwangungulu SP, Kavishe DR, Sumaye RD, Lwetoijera DW, and Okumu FO: Using a new odour-baited device to explore options for luring and killing outdoor-biting malaria vectors: a report on design and field evaluation of the Mosquito Landing Box. Parasites and Vectors. 2013, 6:137: Available at http://www.parasitesandvectors.com/content/6/1/137.

Middle-author publications

1) Mosha JF, Kulkarni MA, Lukole E, **Matowo NS**, Pitt C, Messenger LA, Mallya E, Jummanne M, Aziz T, Kaaya R, Shirima BA, Isaya B, Taljaard M, Martin J, Hashim R, Thickstun C, Manjurano A, Kleinschmidt I, Mosha FW, Rowland M, Protopopoff N. “Effectiveness and cost-effectiveness of three types of dual active ingredient treated nets compared to standard pyrethroid long-lasting nets to prevent malaria transmitted by pyrethroid-resistant mosquitoes in Misungwi, Tanzania: A four arm, single-blind, cluster-randomized trial” The Lancet 399 (10331): 1227-1241.

2) Mponzi WP, Swai JK, Kaindoa EW, Kifungo K, Eiras AE, Batista EP, **Matowo NS**, Sangoro PO, Finda MF, Mmbando AS, Gavana T, Ngowo HS. Okumu FO (2022). "Observing the distribution of mosquito bites on humans to inform personal protection measures against malaria and dengue vectors." PloS one 17(7): e0271833.

3) Mosha JF, Kulkarni MA, Messenger LA, Rowland M, **Matowo N**, Pitt C, Lukole E, Taljaard M, Thickstun C, Manjurano A, Mosha FW. “Protocol for a four parallel-arm, single-blind, cluster-randomised trial to assess the effectiveness of three types of dual active ingredient treated nets compared to pyrethroid-only long-lasting insecticidal nets to prevent malaria transmitted by pyrethroid insecticide-resistant vector mosquitoes in Tanzania”. BMJ Open. 2021 Mar 1;11(3): e046664.

4) Opiyo MA, Ngowo HS, Mapua SA, Mpingwa M, Nchimbi N, **Matowo NS**, Majambere S, Okumu FO. “Sub-lethal aquatic doses of pyriproxyfen may increase pyrethroid resistance in malaria mosquitoes”. PloS One. 2021 Mar 18;16(3): e0248538

5) Mmbando AS, Kaindoa EW, Ngowo HS, Swai JK, **Matowo NS**, Kilalangongono M, Lingamba GP, Mgando JP, Namango IH, Okumu FO, Nelli L. “Fine-scale distribution of malaria mosquitoes biting or resting outside human dwellings in three low-altitude Tanzanian villages”. PloS one. 2021 Jan 28;16(1): e0245750

6) Finda MF, Limwagu AJ, Ngowo HS, **Matowo NS**, Swai JK, Kaindoa E, Okumu FO. (2018). Dramatic decreases of malaria transmission intensities in Ifakara, southeastern Tanzania since the early 2000s. Malaria Journal 17(1): 362.

7) Batista EP, Mapua SA, Ngowo H, **Matowo NS**, Melo EF, Paixão KS, Eiras AE, Okumu FO (2019). Videographic analysis of flight behaviours of host-seeking *Anopheles arabiensis* towards BG-Malaria trap. PloS One 14: e0220563.

8) Mmbando AS, Ngowo HS, Kilalangongono M, Abbas S, **Matowo NS**, Moore SJ, Okumu FO. Small-scale field evaluation of push-pull system against early- and outdoor-biting malaria mosquitoes in an area of high pyrethroid resistance in Tanzania [version 1; referees: awaiting peer review]. Wellcome Open Res 2017, 2:112 (doi: 10.12688/welcome open res.13006.1).

9) Kaindoa EW, **Matowo NS**, Ngowo HS, Mkandawile G, Mmbando A, Finda M, Okumu FO. Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in southeastern Tanzania. PLoS ONE 2017 May 18;12(5): e0177807.

10) Mmbando AS, Okumu FO, Mgando JP, Sumaye RD, **Matowo NS**, Madumla E, Kaindoa E, Kiware SS, Lwetoijera DW. 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. 2015 Dec 9; 14(1):494: Available at: http://www.malariajournal.com/content/14/1/494.

11) Okumu FO, Sumaye RD, **Matowo NS**, Mmbando SA, Mwangungulu SP, Moshi IR, Madumla EP, Mapua SA, Mkandawile GB , Mgando J, Mtali S, Ligamba G, Kaindoa EW, Swai JK, Hamis M, Mteteleka S, Ngowo HS, Limwagu A, Minja E, Lwetoijera DW. Scent Imitation: Innovations for tackling residual malaria mosquitoes in Africa, International Innovation, 2014. Issue 142: Available on pages 94-96 at http://www.researcheurope.com/magazine/ISSUE/142/index.html?utm_campaign=120614+ADD8+issue+142++referred+contacts+email&utm_source=emailCampaign&utm_medium=email&utm_content.

12) Harris C, Lwetoijera DW, Dongus S, **Matowo NS**, Lorenz LM, Devine GJ and Majambere S: Sterilizing effects of Pyriproxyfen on *Anopheles arabiensis* and its potential use in malaria control. Parasites and Vectors.2013, 6:144: Available at http://www.parasitesandvectors.com/content/pdf/1756-3305-6-144.pdf.

13) Okumu FO, Sumaye RD, **Matowo NS**, Mwangungulu SP, Kaindoa EW, Moshi IR, Madumla EP and Lwetoijera DW: Outdoor mosquito control using odour-baited devices: development and evaluation of a potential new strategy to complement indoor malaria prevention methods (2013): Malaria World Journal, 2013.4:(6) Available at http://www.malariaworld.org/mwj/2013/gce-special-outdoor-mosquito-control-using-odour-baited-devices-development-and-evaluation.

RELEVANT WORKSHOPS & TRAINING COURSES PARTICIPATED

1) **5th -7th March 2018:** Participated in training and received a certificate of completion in a course on Writing a Journal Article and Getting it Published, organized by Institute of Social and Preventive Medicine (ISPM), University of Bern, Switzerland.

2) **19th – 30th June 2017:** Successfully participated and received a certificate of completion in a course on "Using Geographic Information Systems (GIS) in disease control programmes" jointly organized by the Royal Tropical Institute, ITC/University of Twente and KIT, The Netherlands.

3) **14th - 16th February 2016:** Participated in basic statistical R-course organized by Ifakara Health Institute, Tanzania.

4) **18th – 29th January 2016:** Acquired training and practical experiences on the computer system and EthoVision XT software for analysis of mosquito behaviours using infrared cameras at Noldus Information Technology, bv, Wageningen, The Netherlands.

SELECTED SCIENTIFIC CONFERENCES ATTENDED WITH PRESENTATIONS

1) **1st - 5th July 2018**: Attended and presented at the 1st Malaria World Congress 2018 held in Melbourne Convention and Exhibition Centre, Melbourne, Victoria, Australia, Title of presentation: Fine-scale spatial and temporal heterogeneities in insecticide resistance profiles of the malaria vector, *Anopheles arabiensis* in rural south-eastern Tanzania.
2) **September 6th – 10th 2015:** Participated and presented at the 9th European Congress on Tropical Medicine and International Health (ECTMIH) held in Basel, Switzerland. Title of the presentation: Attracting and instantly killing outdoor-biting malaria vectors using odour-baited mosquito landing boxes (MLB) equipped with low-cost electrocuting grids.
3) **July 2015**: Presented at the Malaria (GRS) Gordon Research Conference "Translating Malaria Research to the Field", held in Melia Golf Vichy Catalan Business and Convention Center, Girona, Spain. Title of the presentation: Using a New Odour-Baited Device to Explore Options for Luring and Killing Outdoor-Biting Malaria Vectors: Design and Field Evaluation of the Mosquito Landing Box
4) **February 2nd - 7th 2014:** Participated and gave a poster presentation on targeting the residual malaria transmission using an odour-baited device, at the Keystone Symposium (The Science of Malaria Eradication), held at the Fiesta Americana, Mérida, Yucatán, Mexico.