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Investigating effects of agricultural pesticides on susceptibility and fitness parameters of Malaria vectors in rural south eastern, Tanzania

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INVESTIGATING EFFECTS OF AGRICULTURAL PESTICIDES ON SUSCEPTIBILITY AND FITNESS PARAMETERS OF MALARIA VECTORS IN RURAL SOUTH EASTERN, TANZANIA

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A Dissertation Submitted in Partial Fulfilment of the Requirements of the Degree of Master of Science in Public Health Research of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

The aim of this study was to investigate effects of agrochemicals on susceptibility and fitness of the malaria vectors, *An. gambiae s.l* across farming areas in Tanzania. An exploratory mixed-methods study was conducted to explore awareness on pesticide use among community members in four villages (V1-V4) in south-eastern Tanzania. *An. arabiensis* larvae were collected from agricultural fields in the same villages and their emergent adults examined for insecticide susceptibility, egg-laying, and wing lengths (as proxy for body size). These tests were repeated using two groups of laboratory-reared *An. arabiensis*, one of which was pre-exposed for 48hrs to sub-lethal aquatic doses of agricultural pesticides found in the villages. Results revealed that farmers lacked awareness of the linkages between public health and agriculture sectors but were interested in being more informed. Agrochemical usage was reported as extensive in V1, V2 & V3 but minimal in V4. Similarly, mosquitoes from V1-V3 but not V4 were resistant to pyrethroids, and either pirimiphos-methyl, bendiocarb or both. Adding the synergist, piperonyl butoxide, restored potency of the pyrethroids. Pre-exposure of laboratory-reared mosquitoes to pesticides during aquatic stages did not affect insecticide susceptibility in emergent adults of the same filial generation. There was also no effect on fecundity, except after pre-exposure to organophosphates, which were associated with fewer eggs and smaller mosquitoes. In this study, susceptibility of mosquitoes to public health insecticides was lower in villages reporting frequent use of pesticides compared to villages with little or no pesticide use. In conclusion, safeguarding the potential of insecticide-based interventions requires improved understanding of how agricultural pesticides influence important life-cycle processes and transmission potential of mosquito vectors.

DECLARATION

I, Naomi Urio do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation titled "Investigating the effect of agricultural pesticides on susceptibility and fitness parameters of malaria vectors in south-eastern, Tanzania" is my original work and has never been or intending to be submitted for a degree award in any other university.

________________	03.08.2022
Naomi Urio	Date

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CERTIFICATION

This is to certify that I have read and approved the dissertation titled, “Investigating the effects of agricultural pesticides on the susceptibility and fitness parameters of malaria vectors in south eastern, Tanzania” submitted by Naomi Urio (M040/T19) in partial fulfilment of the requirements for the award of Master of Science in Public Health Research of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

I would like to dedicate this work to my parents, Mr. Humphrey Urio and Mrs. Doris Urio.

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
CI	Confidence Interval
CSP	Chemosensory Protein
DDT	Dichlorodiphenyltrichloroethane
FGD	Focus Group Discussion
GST	Gluthatione – S -transferase
IHI	Ifakara Health Institute
IRS	Indoor Residual Spray
ITNs	Insecticide Treated Nets
KDT_{50}	Time taken for 50% of mosquitoes to knock down
LLINs	Long-lasting Insecticide Treated Nets
NIMR	National Institute of Medical Research
PMI	President’s Malaria Initiative
RR	Resistance ratio
SAP	Sensory Appendage Proteins
UDP	Uridine diphosphate glucuronosyltransferase

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Vector control has played a key role in the control of malaria globally (Bhatt *et al.*, 2015; Killeen, 2014; Kiszewski *et al*., 2004; Moyes *et al*., 2020; Wilson *et al*., 2020). The primary interventions, namely insecticide-treated nets (ITNs) and indoor residual spraying (IRS) are estimated to have contributed nearly 70% of all the gains accrued against malaria starting 2000 (Bhatt *et al.*, 2015). Unfortunately, the disease remains a major public health concern in sub-Saharan Africa, where over 95% of the cases and deaths now occur (Bhatt *et al.*, 2015; WHO, 2021). Despite the gradual declines that started around 2000, the incidence and mortality rates of malaria now appear to be increasing, especially in the high-burden countries (WHO, 2021). The success of vector control interventions is now threatened by multiple challenges, including but not limited to, certain shifts in malaria vector species populations (Mwangangi *et al*., 2013; Sougoufara *et al*., 2016), changes in mosquito biting patterns (Cooke *et al*., 2015; Moiroux *et al*., 2014), and the widespread insecticide resistance in mosquito populations (Hemingway *et al*., 2004). Insecticide resistance is particularly challenging because the chemicals are widely used in both public health and agriculture, yet the stewardship of these products is not integrated between the two sectors.

The broad linkages between agricultural practices and transmission of insect-borne diseases are widely appreciated in Africa and elsewhere (Hawkes & Ruel, 2006;Ijumba *et al*., 2002; Janko *et al*., 2018; Matowo *et al*., 2020; Mutero *et al*., 2006; Philbert *et al*., 2019). On one hand, crop farming and livestock-keeping also form an important basis of livelihoods for millions of people in malaria-endemic communities (Paul *et al*., 2018; Rulisa & Kempen, 2022). On the other hand, vector-borne and zoonotic diseases can disrupt the same livelihoods by lowering productivity and draining household incomes (Hawkes & Ruel, 2006; Lipton*et al.*,1988). While the resulting impact of specific farming practices are varied (Ijumba & Lindsay, 2001), evidence suggests multiple associations between the ecology of malaria vectors and cultivation of crops such as rice and sugarcane (Demissew *et al*., 2020; Ijumba & Lindsay, 2001; Ijumba *et al*., 2002; Mwangangi *et al*., 2010; Oguoma & Ikpeze, 2009). Suitable ecological conditions, such as the shallow and slow-moving fresh water systems found in rice fields, may contribute to higher densities of malaria vectors as compared to non-rice growing areas (Bradley, 1988; Chan *et al*., 2022; Lacey & Lacey, 1990). Large populations of vector species such as *Anopheles arabiensis* may proliferate in rice growing areas and can increase malaria transmission (Chan *et al*., 2022; Mukiama & Mwangi, 1989; Mwangangi *et al*., 2006). Besides crop-farming, other forms of agriculture, i.e. livestock keeping also influences malaria transmission. For example, mosquito blood-feeding is influenced by availability and densities of non-human vertebrates and livestock (Killeen *et al*., 2001; Takken & Verhulst, 2013).

The use of agricultural pesticides is perhaps the most adversely linked to malaria transmission. Resistance in malaria vectors is predominantly attributed to selection pressures when mosquitoes are exposed to public health insecticides mainly IRS and ITNs (Kisinza *et al*., 2011; N'Guessan *et al*., 2007). However, agricultural pesticides potentially also contribute to resistance through the selection pressures imparted during the aquatic stages (Matowo *et al*., 2010; Nkya *et al*., 2013; Ranson *et al*., 2009); as mosquitoes emerging from areas of intense pesticide use show decreased susceptibility to insecticides (Matowo *et al*., 2010; Overgaard, 2006;Ranson *et al*., 2009; Yadouleton *et al*., 2011). For example, in Côte d'Ivoire, extensive use of pesticides was associated with significant loss of susceptibility in key malaria vector species (Fodjo *et al*., 2018). This association between agriculture and malaria control is strongly attributed to similarities in chemicals used, shared modes of action, simultaneous application of the chemicals, and their extensive use in agriculture (Matowo *et al*., 2020; Vanek *et al*., 2006).

Since most investigations in this subject have been aimed at safeguarding the performance of insecticide-based vector control tools, notably ITNs and IRS, there is limited understanding of how agrochemicals influence malaria vector populations, their fitness as well as their survival. Similarly, whereas it is generally agreed that insecticide resistant mosquitoes incur significant fitness costs compared to susceptible mosquitoes, it remains unclear how such factors influence the overall pathogen transmission potential. A recent review concluded that while resistance-associated fitness costs are common, the available evidence is difficult to summarise due to the variations in resistance mechanisms, the different insecticides tested and the inconsistencies in experimental designs (Freeman *et al*., 2021).

Insecticide resistance is broadly an energy-intensive process, which leads to changes in mosquito physiology and behaviours and might affect the vector competence (Chen *et al*., 2021), usually in the context of other biological factors that influence vectorial capacity either favourably or negatively (Beerntsen *et al*., 2000; Tabachnick, 1994; William *et al*., 2002).

These biological characteristics often include larval development, survival, and fecundity, all of which can be altered in insecticide-resistant vector populations (Brito *et al.*, 2013). In one study from Cameroon, researchers established a colony of field-collected *Anopheles* mosquitoes strongly resistant to both DDT and pyrethroids, then assessed different life traits including fecundity, larval development, emergence rates, and longevity of surviving adults. Compared to mosquitoes from a susceptible laboratory colony, they observed that the resistant *Anopheles* had fewer eggs, delayed larval development, reduced emergence rates and reduced survival of the emergent adults (Nkondjio *et al.*, 2020). Another study in Kenya showed that pyrethroid-resistant *Anopheles gambiae* mosquitoes exhibited delays in development during aquatic stages and also had reduced survivorship (Osoro *et al.*, 2021). Furthermore, malaria infection rates in West Africa were higher in resistant mosquitoes than in susceptible ones, even though the latter had lower proportions of parous females (Collins *et al.*, 2019). Studies on *Aedes* have demonstrated similar fitness costs, sometimes extending to reduced mating success (Smith *et al.*, 2021), though in some cases these costs were marginal (David *et al.*, 2018).

Taken together, these findings suggest that the associations between resistance and the fitness of vectors are complex and should be investigated for different localities to examine how exposures to specific pesticides may influence overall fitness and the resulting malaria transmission.

1.2 Statement of the problem

In rural south-eastern Tanzania, insecticides used in agriculture and vector control share similar active ingredients, yet many farmers in the area have poor pesticide management practices and awareness (Matowo *et al.*, 2020). In these areas, the malaria vector species, *An. arabiensis* is known to breed in the same fields where pesticides are used. These vector populations are therefore endlessly exposed to insecticides either in the aquatic stage on the farms, or when the adults come into contact with insecticide-impregnated bed nets inside homes, thus increasing resistance pressures. Entomological surveys have demonstrated that the insecticide-resistance in the vectors here varies with season and location (Matowo *et al.*, 2017; Pinda *et al.*, 2020), and is much stronger in *An. funestus* than in *An. arabiensis* (Pinda *et al.*, 2020). It plausible that the resistance in the area may be at least partly linked to agricultural pesticide use and is contributing to the persistent transmission by the residual malaria vectors. However, there is insufficient knowledge on how pesticide use may be directly or indirectly influencing the fitness and transmission activity of malaria vectors in the area

1.3 Rationale of the study

The main malaria control interventions in Tanzania are LLINs and IRS, which equally depend on the utilization of chemical insecticides (NMCP, 2014). The effectiveness and efficacy of these interventions rely on, among many factors, the susceptibility and fitness of local malaria vectors. Determining the effect of agricultural pesticides on the local malaria vector will help in the strategic deployment and implementation of these malaria control tools.

1.4 Objectives

1.4.1 General Objective

To investigate the effects of agricultural pesticides on the susceptibility and fitness parameters of malaria vectors in south eastern, Tanzania

1.4.2 Specific Objectives

The study intended to achieve the following objectives:

(i) To identify common pesticides and farming practices amongst farmers in the area

(ii) To determine the susceptibility of *An. arabiensis* after exposure to commonly pesticides (wild and colony strains)

(iii) To compare fitness traits (fecundity and wing lengths) between exposed *An. arabiensis* and unexposed *An. arabiensis*

1.5 Research Questions

(i) What are the common agricultural pesticides used and farming practices in Ulanga and Kilombero districts?

(ii) What are the effects of agricultural pesticides on *An. arabiensis* susceptibility to insecticides and resistance mechanisms after exposure to agricultural pesticides in the field and laboratory?

(iii) How does the exposure to agricultural pesticides affect fecundity and wing length in *An. arabiensis?*

1.6 Significance of the study

This study will provide baseline information of possible associations between resistance status of *Anopheles arabiensis* in villages and varying agricultural pesticides use. These results may assist in insecticide management strategies between the agricultural and public health sector; and also improve future efforts to control the vectors in the area.

1.7 Delineation of the study

Past studies in the area, have either independently focused on farming practices across the area, or insecticide susceptibility status of malaria vectors. The effects of agricultural pesticides on malaria vectors that breed in farms is poorly understood and thus the available information is limited. However, results from this study could be used as a baseline to give an indication of the current situation in the area.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria burden

In the beginning of the twentieth century, malaria transmission occurred in vast areas of the world including South America, Europe, the Middle East and northern Australia areas which are now free of transmission (Guinovart *et al.*, 2006). For many years, *Plasmodium falciparum* has been known to mostly inhabit the tropic and sub-tropic regions, with most of the burden being observed in Africa and south Asia. The geographical extent of malaria has declined significantly as malaria was eliminated from many countries. However, a larger population is now exposed to malaria transmission due to population growth. The WHO African Region, which now bears more than 95% of the malaria burden, has seen its population increase from roughly 665 million in 2000 to 1.2 billion in 2020; and with this, the overall malaria burden has stagnated (WHO, 2021). Malaria interventions have curtailed a large portion of the disease cases and deaths over the past two decades (Bhatt *et al.*, 2015), and prevented 1.7 billion cases and 10.6 million deaths since 2000 (WHO, 2021). However these efforts are now under threat due to a number of reasons, insecticide resistance being one of them (Hemingway *et al.*, 2004).

2.2 Insecticide resistance

2.2.1 Evolution of insecticide resistance

Insecticides comprise a wide range of chemical and biochemical components that either collectively kills an insect or prevent it from taking part in destructive behaviours (Ware, 2004). Insecticides are primarily used to manage vectors like ticks and mosquitoes that are greatly responsible for the spread of public health diseases such as malaria and Lyme disease (Gupta *et al.*, 2019). Before 1946, only about 12 cases of insecticide resistance were reported (Georghiou, 1986). During that time insecticides used were inorganic and were equally a threat to both insects and humans (Mallet, 1989). Furthermore, these inorganic chemicals had multiple modes of action hence delaying the process of resistance (Georghiou, 1986). After the Second World War, the chemical components of insecticides changed to organic compounds (i.e. synthetic pyrethroids, organophosphates, organochlorines, and carbamates). Unlike inorganic compounds such as arsenicals, these compounds have a specific mode of action, are much safer for humans and the environment, and can target specific insects. Despite the major advantages of organic insecticides, their use has made the evolution of insecticide resistance a much faster process (Mallet, 1989).

Majority of the interventions in vector control are insecticide based. Primary interventions such as ITNs and IRS predominantly rely on the use of pyrethroids, organophosphates, and carbamates (WHO, 2012). The extensive use of these chemicals in vector control as well as unregulated use of agricultural pesticides by farmers and retailers has resulted to be the biggest driver of insecticide resistance in malaria vectors (Matowo *et al*., 2020). Mainly due to the high selection pressure these mosquitoes are subjected to either on the farms or through interventions such as ITNs and IRS (WHO, 2012).

2.2.2 Mechanisms of insecticide resistance

Resistance can be explained as an organism's ability to withstand doses of an insecticide that would normally be lethal to other organisms from a population from the same species (Coleman & Hemingway, 2007). Globally, resistance has occurred in more than 500 species of mosquitoes, 50 of them being malaria vectors (Hemmingway& Ranson, 2000). Resistance mechanisms in malaria vectors can be broadly grouped into: metabolic, target site, behaviour avoidance, and cuticle resistance (WHO, 2012).

2.2.3 Metabolic resistance

The most common type of resistance in insects is metabolic resistance and is mostly detected using bioassays with live mosquitoes or biochemical and molecular assays with dead mosquitoes (WHO, 2022). This type of resistance is based on the over expression or increased catalytic activity of insecticide detoxifying enzymes, resulting in alterations in enzyme affinity for the insecticides and thus resistance (Gullemaud *et al*., 1997). This detoxification of insecticide molecules by enzymes happens before it can reach the target site (Brooke & Koekemoer, 2010).

There are three main groups of enzymes associated with metabolic resistance in malaria vectors: esterases, glutathione-S-transferases (GSTs) and monooxygenases (P450s). However, other enzyme families may also take part in this mechanism such as UDP glucosyl-transferases (UGTs) (Hemingway *et al*., 2004; Li *et al*., 2007). Over and under expression of these enzymes has been linked to resistance to specific insecticide groups:

monooxygenases to pyrethroid resistance (Miller, 1988). Glutathione-S-transferases to dichlorodiphenyltrichloroethane (DDT) resistance (H. Ranson *et al*., 2001) and esterases to organophosphate and pyrethroids (Brogdon *et al*., 1999).

2.2.4 Target site mutation

Target site resistance arises from a change in the gene structure coding target proteins which interact with the insecticides. This mutation alters the protein receptor within the mosquito that the insecticide is meant to attach itself to, thus blocking/reducing the harmful effect of the insecticide (Davies &Williamson, 2009). The two common types of target-site resistance: are knockdown resistance (kdr) and altered acetylcholinesterase (altered AChE). These mutations may act in a single manner or together in an organism (Chanda *et al*., 2016). In kdr single or multiple mutations occur in the genes encoding for the sodium voltage channel (Coleman & Hemingway, 2007). Under normal circumstances, pyrethroids and organochlorines bind to sodium gated channels as their mode of action, however in kdr mutated organisms, insecticides cannot bind to these channels and thus becomes less effective (Vais *et al*., 2001). The altered form of the acetylcholinesterase enzyme prevents organophosphates and carbamates from binding to AChE on the postsynaptic neuron thus, allowing for the synapse to function normally rendering the insecticide ineffective (Hemmingway& Ranson, 2000).

2.2.5 Behavioural resistance

Behavioural avoidance develops through prolonged exposure of a mosquito to an insecticide. Mosquitoes change their host-seeking behaviour rather than their biochemical/physiological components (Brown, 1986). These behavioural changes include avoidance of insecticide-treated surfaces, changes in resting and feeding patterns in relation to the use of insecticides (Chareonviriyaphap *et al*., 2013). An example of behavioural change was seen in the *An. funestus* species in Tanzania, where this vector began to dominantly bite outdoors instead of indoors due to the increased use of nets treated with pyrethroids (Riveron*et al.,*2018).

2.2.6 Cuticular resistance

Slow or prevented absorption of insecticide on the cuticle and digestive linings of an insect is known as reduced penetration. It is known to be a minor resistance mechanism due to an increase in cuticle thickness and is quite rare (Riveron*et al.,*2018; Fine *et al*., 1963). In a study done by Lilly *et al.* (2016), the thickness of the mosquito cuticle was positively associated with the time to knockdown effect.

2.2.7 Sensory Appendage Proteins

Sensory appendage proteins are part of the chemosensory protein (CSP) family. Their main function is the transportation of hydrophobic compounds through the sensillum lymph (Ingham *et al.*, 2020). One recent study showed that SAP2 is found in the legs of malaria-carrying mosquitoes and is said to confer pyrethroid resistance to the *Anopheles* mosquitoes whereas before it showed no function in insecticide resistance (Ingham *et al.*, 2020). SAP2 expression is found to be at its peak amongst populations that are already resistant to insecticides and is exacerbated by contact with appendage proteins and pyrethroid molecules. When the sensory appendage protein 2 is silenced, it wholly restores mosquito susceptibility to the insecticide (Ingham *et al.*, 2020).

2.3 Insecticide resistance monitoring

In order to effectively tackle insecticide resistance amongst malaria vectors, frequent monitoring is required. However, this poses to be a challenge in many malaria endemic African countries (Ranson, 2017). In Tanzania, a routine entomologic monitoring of resistance supported by PMI was established and consists of three main areas: (a) to conduct yearly countrywide monitoring of resistance to insecticides used in vector control, (b) to perform monthly bioassay monitoring of residual insecticidal activity of the IRS scheme, and (c) to monitor vector species abundance and distribution. Currently, a great number of sites in the country conduct insecticide resistance monitoring annually and do routine tests for select resistance mechanisms (PMI, 2022). The allocation of more resources such as funding, equipment, and skills would enable quicker and more routine monitoring of resistance amongst malaria vectors (WHO, 2009).

2.4 Current insecticide resistance management strategies

In hopes to provide direction and guidance to countries experiencing high rates of insecticide resistance WHO published a Global Plan for Insecticide Resistance Management (WHO, 2012). This plan aims to preserve and sustain the sensitivity of malaria vectors to chemicals used in vector control interventions. Programmes that reduce resistance in vector populations, e.g. by rotating insecticides before resistance is detectable, are an important component of vector control (Curtis *et al.*, 1993). Using a single class of insecticide in interventions is less effective than combining classes of insecticides, using mosaic designs, and rotating the use of insecticides (Guillet *et al.*, 2001; Mosha *et al.*, 2008). It can however be challenging to find the perfect combination which kills the vector regardless of resistance to another insecticide (Corbel *et al.*, 2012). As of now, research institutions such as NIMR and IHI work with the Tanzanian government to carry out annual countrywide insecticide resistance tests (PMI, 2022). Future prospects need to divert and focus insecticide resistance management strategies on non-insecticide vector control methods in relation to integrated vector management (WHO, 2009; WHO, 2012).

2.5 The link between agricultural practices and public health

Over the past decades, populations across Africa have increased exponentially. The need for an increase in food production in African countries resulted in the up scaling of various strategies, such as better irrigation systems and greater use of agrochemicals (pesticides/herbicides) to obtain a greater yield of produce (ADB, 2016). However, these strategies could possibly aggravate health risks and enhance conditions favouring transmission of infectious diseases such as malaria. Rice is one of Africa's fastest growing crops, with a 600 percent rise in harvested areas since the 1960s (FAO, 2022). Evidence suggests multiple associations between the ecology of malaria vectors and cultivation of crops such as rice and sugarcane (Demissew *et al*., 2020; Ijumba & Lindsay, 2001; Ijumba *et al*., 2002; Mwangangi *et al*., 2010; Oguoma & Ikpeze, 2009). Suitable ecological conditions, such as the shallow and slow-moving fresh waters systems found in rice fields, may contribute to higher densities of malaria vectors as compared to non-rice growing areas (Bradley, 1988; Chan *et al*., 2022; Lacey & Lacey, 1990). Furthermore, malaria burden in rice-growing areas is believed to be around twice as much as in non-rice-growing areas, implying that rice farming is linked with more intense transmission for unprotected populations (Chan *et al*., 2022).

After the second world war, development of affordable, effective, and safe insecticides revolutionized crop production (Ware, 2004). These advancements in agriculture occurred parallel to malaria control campaigns. Vector control interventions; ITNs and IRS were implemented in the mid 2000s using a relatively limited arsenal of insecticide initially intended for agriculture (Ranson *et al*., 2011; Yewhalaw *et al*., 2011). The similarity in chemical classes between the two sectors is suggested to be the core reason behind the increasing resistance in malaria vectors to common public health insecticides (pyrethroids, organophosphates, carbamates, and organochlorines) (Reid & McKenzie, 2016). Malaria vector species which naturally breed in rice farms, are proposed to be continuously exposed to insecticides in agrochemicals during their aquatic stages (Matowo *et al.*, 2010; Ranson *et al.*, 2009). Prolonged exposures across subsequent generations along with selection pressures from ITNs and IRS could possibly induce resistance and thus threaten the efficacy of these insecticides in both sectors.

2.6 Community awareness or involvements in vector control

The design and implementation of vector management systems can involve both governments and local communities. To develop comprehensive, community-wide malaria control programs, it is essential to understand what the government and communities are doing presently to protect people from exposure and how they are delivering malaria control interventions (Asale *et al.*, 2019). Community involvement, can accelerate the development of long-lasting solutions by focusing on the causes of the issue and mobilizing all spheres of society (Kibe *et al.*, 2006). An in-depth understanding of community attitudes, knowledge, and behaviour can help reshape the control program's strategy and serve as the foundation for effective health education messages (Govere *et al.*, 2000). Various communities in Kenya actively carried out vector control activities such as; spraying houses with permethrin, treating water bodies with used engine oil, sewing, dipping and selling of ITNs and community clean up days. Their active participation contributed to the reduction in malaria incidences and mortalities in the area. However, multiple challenges were faced such as lack of funds and participation of community leaders, chiefs and elders, thus reducing community mobilization (Kibe *et al.*, 2006).

Community members such as farmers are a core group in society when it comes to the implementation of vector control strategies. Awareness and proper knowledge on the use of pesticides between retailers and farming community is mandatory, and can be done through regular educational engagement activities and workshops (Matowo *et al.*, 2020). Government officials must be dedicated to this collaboration and put policies in place to encourage community participation in vector management in order for community ownership and sustainability are to be attained (Matthews, 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The field study was conducted in agricultural districts of Ulanga and Kilombero in south-eastern Tanzania, where the main malaria vectors include *Anopheles funestus* and *Anopheles arabiensis* (Kaindoa *et al*., 2017; Matowo *et al*., 2017). Four villages were covered, namely Minepa (V1) 8.23°S, 36.75°E; altitude 268m) and Lupiro (V2) (8.41°S, 36.81°E; altitude = 389m) in Ulanga district and_Kisawasawa (V3) (7.91°S, 36.82°E; altitude = 728m) and Njage (V4) (8.3°S, 36.14°E; altitude = 519m) in Kilombero district. The area experiences 15-35ºC daily temperatures and 1300 - 3600mm annual precipitation (World Weather Online, 2021). Economic activities include crop-farming, fishing, and livestock keeping (Kato, 2007;Matowo *et al*., 2020). The main malaria vector control method in the area is ITNs (Renggli *et al*., 2013).

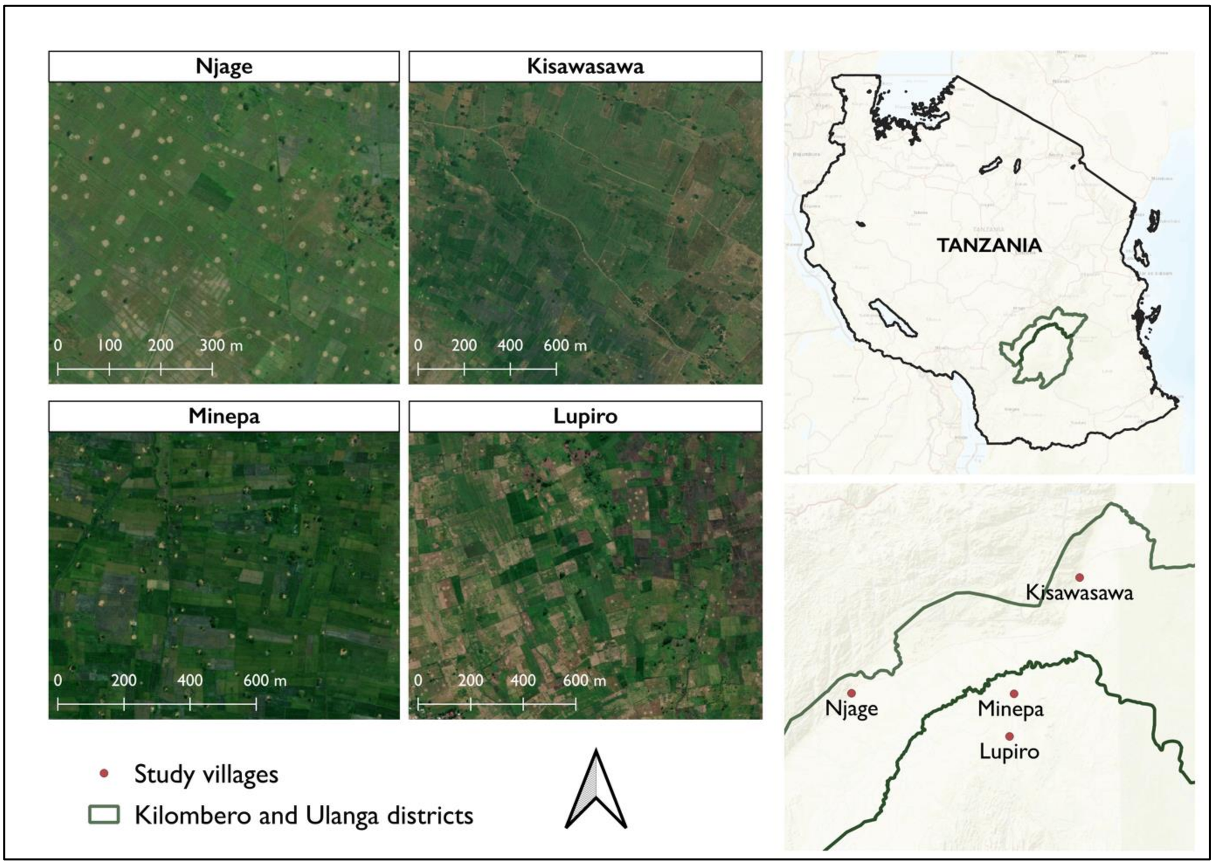

Figure 1: Map of Kilombero and Ulanga districts showing the four study villages

3.2 Qualitative assessment of the use of agricultural pesticides

A qualitative assessment was conducted in the four study villages, by carrying out focus group discussions (FGDs) to identify the common agricultural pesticides used in each village, followed by direct observations of pesticide use in the farms.

A discussion guide was prepared for the FGDs to capture the following key areas of discussion: (a) farming practices, (b) types of chemicals used in the farm, (c) farming seasons and crop types, (d) sources of agricultural pesticides and (e) management and disposal of the pesticides. An FGD was conducted in each village, bringing together eight local community representatives recruited upon signing the informed consent. These representatives were community leaders, practicing subsistence farming, and included both males and female adults. The discussions were facilitated by three researchers from IHI, who were all knowledgeable on agricultural practices and malaria control interventions in the region and had experience conducting qualitative research in rural communities. Before starting the discussions, facilitators presented a brief overview of the study topic to the participants; and explained the reason for conducting the FGDs. Each session lasted about 60-75 minutes and followed the discussion guide.

In addition, direct observations were made in the farms in the study villages to record signs of pesticide use, disposal, and methods of application. Where possible, pictorial evidence was gathered to complement the direct observations.

3.3 Mosquito collections and rearing of larvae

Field mosquitoes were collected in their larval stages during the dry season months of July and September 2021. The study villages were inspected for presence of aquatic habitats known to contain *An. gambiae s.l* before the actual mosquito collections were done. The standard 350mL dippers were used to collect the larvae; and the collections were transported to the VectorSphere Mosquito Laboratory facility at IHI for further experiments. Larvae were identified using the dichotomous key of Gillies and Coetzee(Gillies & Coetzee, 1987). In the insectary, the larvae were kept in 5L rearing dishes, under controlled conditions of 25-27°C and 80% relative humidity. The larvae were fed on Tetramin® fish food and the emergent adults were supplied with a 10% sugar solution. As described below, *An. arabiensis* females from a laboratory colony maintained under the same conditions since 2009, were also used in some comparative tests.

3.4 Assessment of insecticide susceptibility of the field-collected mosquitoes

Emergent adult female mosquitoes aged 3–5 days old and not previously blood-fed were used according to the WHO insecticide susceptibility test procedures. A total of 120 mosquitoes per test were used over four replicates, each requiring 20–25 mosquitoes. The bioassays were done at 25°C ± 2 °C and 80% ± 10% relative humidity.

The candidate insecticides and doses included: 0.75% permethrin (pyrethroids class I), 0.05% lambda-cyhalothrin (pyrethroid class II), 0.25% pirimiphos-methyl (organophosphate), 4% DDT (organochloride) and 0.1% bendiocarb (carbamate). The mosquitoes were exposed for one hour, and knockdown recorded at intervals of 10, 15, 20, 30, 40, 50, 60 minutes. The mosquitoes were then transferred to clean holding tubes, supplied with 10% sugar solution, and observed to assess mortality after 24 hours. For comparison, similar tests were conducted using the laboratory-reared mosquitoes.

The synergist, 4% Piperonyl Butoxide (PBO), was used to assess possible involvement of mixed-function oxidases in metabolic resistance. In these tests, the mosquitoes were pre-exposed to PBO or control and then to the candidate insecticides. Four groups of 20-25 mosquitoes each were used, and the groups were exposed for one hour to either PBO alone, PBO followed by respective candidate insecticide for one hour, the candidate insecticide alone, or the control papers. The mosquitoes were monitored for 24-hour mortality and the tests replicated three times.

3.5 Assessment of fitness parameters of field-collected mosquitoes

Field collected larvae from the four study villages (V1-V4) that were brought to the insectary and kept till they pupated, after which they were collected in cups, and placed in standard 15 x 15 x 15cm cages. Upon adult emergence, the mosquitoes were fed on 10% glucose solution via soaked cotton pads. Mosquitoes were provided their first blood meal on the third day after emergence, to give them time to mate. Fully fed mosquitoes were moved into individual cups with a damp filter paper at the base of the cup to stimulate oviposition conditions. Eggs laid by each mosquito were counted under a stereomicroscope as a measure of fecundity.

Wing sizes were also measured and used as a proxy for body size of the mosquitoes. To do this, the adult female mosquitoes emerging from the field larvae collections, were anaesthetized at -10°C for 7 minutes and a single wing was removed from either the left or right side of the mosquito. Distilled water was used to fix the wings onto slides and the length from the apical notch to the auxiliary margins was measured using a micrometer ruler under a stereo microscope.

3.6 Exposure of mosquitoes to sub-lethal doses of agricultural pesticides

These tests were done to simulate field exposures to common agrochemicals and assess effects on emergent adults. Groups of 3rd instar *An. arabiensis* larvae from the laboratory colonies which were susceptible to a number of candidate insecticides were exposed to a range of pesticide concentrations (Table 1), to observe the effect of the active treatments and determine the doses below which there were still substantial emergent adults for further experimentation. For this experiment, three insecticides from three pesticide classes (pyrethroids, organophosphates and carbamates) were selected, so as to be representative of the commonest pesticide classes used by communities in the study villages (as determined by the FGDs and the field observations).

In each test, 25 larvae were introduced into 1.2L basins containing a 1L of water, into which the candidate pesticides were introduced. The pesticide concentrations ranged from 1×10^{-4} to 1×10^{-8} g/L (for lambda cyhalothrin and pirimiphos methyl) and 5×10^{-4} to 3.5×10^{-3} g/L (bendiocarb). Three replicates were completed for each concentration (treatment) and control, all under the same conditions. All larvae exposed and unexposed were fed on Tetramin ® fish food and monitored every 12 hours for mortality until 120 hours.

A lethal concentration (LC) of 15% mortality was selected for subsequent experiments, since at this level there was sufficient adult emergence despite significant pesticide exposure. Fresh batches of fourth-instar larvae were therefore exposed to the LC_{15} pesticides concentrations in the 1.2L trays for 48 hours, during which the larvae were fed Tetramin® fish food. Pupae from these trays were transferred to cups containing only water and placed in small cages (15 × 15 × 15cm) for emergence.

Table 1: Selected pesticides used for experiments of sub-lethal exposures of Anopheles mosquitoes

Trade name	Insecticide class	Active ingredient	Concentration of active ingredient	Description and Recommended use
Ninja 5EC	Pyrethroid	Lambda-cyhalothrin	50g/L	Fast-acting and broad-spectrum; For use on the cashew nuts, vegetables & fruits;Targets sucking insect pests
Twigaphos 48EC	Organophosphate	Chlorpyrifos	480g/L	For the control of insects in cotton, coffee, cashew, and vegetables.
Akheri Powder	Carbamate	Carbaryl	5% w/w	For domestic use against crawling insects such as fleas, ants, cockroaches etc.

3.7 Assessment of insecticide susceptibility and fitness parameters of laboratory mosquitoes

Once the sub-lethal dose was determined, the 3rd to 4th instar larvae were introduced and maintained until pupation. The pupae collected from each treated dish (exposed mosquitoes) and from concurrent controls (unexposed mosquitoes) were counted and placed in cages for emergence. Three to five days old female adult mosquitoes were then used to carry out insecticide susceptibility bioassays as described above for field-collected mosquitoes. Fecundity and wing lengths were assessed in the same way as described above for field-collected mosquitoes.

3.8 Data analysis

The quantitative data was analysed using the open-source software, R programming version 4.0.5 (RCoreTeam, 2021). In tests using mosquitoes pre-exposed to sub-lethal aquatic doses of pesticides, Probit analysis was done using the '*ecotox*' library (Wheeler *et al.*, 2006) to determine appropriate sub-lethal concentrations to be used in subsequent experiments. To assess the insecticide resistance profiles, data analysis was done according to the WHO susceptibility test guidelines. The resistance or susceptibility status was defined based on the WHO criteria, where 98–100% mortality indicates susceptibility; 90–97% mortality requires further confirmation of possible resistance, and less than 90% mortality indicates resistance (WHO, 2016). The resistance/susceptibility graphs were plotted using *ggplot2* package (Chang, 2013). Knockdown times were calculated using the PoLo Plus software (Software, 2006) using log-probit analysis. For fecundity and wing length, the *dabestr* package in R was used to generate a two-group estimation (Gardner-Altman) plots (Ho *et al.*, 2019). The estimation plots were used to display distributions of residual mean differences in the number of eggs laid and wing lengths in the different experiments. The results were presented in summary graphs or tables.

For the qualitative assessment, audio recordings of the focus group discussions and key informant interviews were transcribed and translated from Swahili to English. Any notes written during the discussion were added to the written transcripts. The written transcripts were reviewed and analysed on Microsoft word. Analyses were conducted separately for different villages. The FGD guide and objective of the study were used to develop deductive codes. Inductive codes were derived from detailed studying of the written transcripts. Once the coding was completed, codes were grouped, and emerging patterns were used to identify themes.

3.9 Ethical considerations

Approval to conduct this research study was granted by IHI’s review board (REF: IHI/IRB/No: 30-2021). Participants involved in the focused group discussions were not subjected to harm in anyway whatsoever. Their dignity and respect were a priority throughout, and full consent was obtained from the participants prior to the discussion. Lastly, the protection of the privacy of the participants was ensured through anonymity.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Qualitative findings

4.1.1 Farming practices in south-eastern Tanzania

Rice was the commonest crop across the four villages and its cultivation was either rain-fed or irrigation dependent. Most villages reported cultivating rice during both seasons. During the wet season they depended on rain as a source of water and during the dry season they rely mostly on irrigation systems with farms near rivers to access the water. Their planting methods were broadcasting seeds, planting single seeds, or transplanting seeds. A common challenge was the need to spray agrochemicals on the farms at the same time to avoid re-invasion of pests from neighbouring farms. The participants noted that in cases where this was not done, they often had to re-spray because of such re-invasions:

> *Spraying every two weeks is not fixed, you can spray pesticides every week. For example, when having two adjacent plots, the owner of one has been attacked by pests because they cannot afford to purchase pesticides. If the other plot owner decides to spray pesticides on his own land, pests will die but after a few days, the pesticide has worn out and pests from the adjacent plot will attack again. So, you find yourself spraying pesticides multiple times every week. Unless you decide to buy and spray your neighbour's plot with pesticides if you can afford to (Participant 4, village 2).*

4.1.2 Common herbicides and pesticides used

The use of herbicides was common across villages. Farmers reported to mostly using bispyribac-sodium and glyphosate containing herbicides to control weeds on their farms. Participants from three out of four of the villages (i.e. Villages V1, V2 and V3) reported relying on pesticides to deal with pests affecting their crops. The most common pesticides reported were of pyrethroids and organophosphate classes (Table 2). Herbicides and pesticideswere used multiple times, due to the frequent occurrence of weeds and pests on their crops. It was also mentioned that application of these chemicals was greater during the dry seasons compared to wet seasons due to the abundance of pests and weeds in that period. Most farmers in these villages poorly disposed of their pesticide containers, by either leaving them in their fields or nearby rivers. However, farmers in one village (V4) reported barely using pesticides on their farms, noting that they did not find it necessary:

> *When we talk about pests affecting our crops, I can say that about 97% of farmers in our village aren't really affected by pests, as it is not a big problem. To say that we look for alternative methods to look for pesticides that work is rare, we take it as a normal thing, which does not have major effects on us as farmers (Participant 7, village 4).*

4.1.3 Awareness of the linkages between agricultural pesticides use insecticide resistance and mosquito control

Many of the community leaders across all the farming villages were unaware of the association between the chemicals used on their farms and malaria vector control methods such as ITNs and IRS. There was no evidence of the farmers associating agricultural pesticide use with insecticide resistance in mosquitoes. However, there was an association of pesticide use with mosquito mortality. In one village (V2), the farmers reported that they knew that the mosquito larvae died immediately after they had sprayed chemicals on their farms. However, they had never tried to use the same chemicals to kill mosquitoes at home or surrounding areas:

> *"We know when we have killed mosquitoes and the scientists don't get anything when they come and collect mosquitoes on the rice farms" (Participant 2, village 2).*

Farmers in all four villages mentioned that they wanted to know more about the association between agrochemical use on their farms and malaria control. They were willing to undergo any form of training to improve their knowledge on the subject at hand. They suggested that experts in the respective sectors should advice farmers on which chemicals to use in order not to harm malaria control efforts, but still maintain their farm produce.

> *"We can sit together with scientists and listen to advise given to us on the types of chemicals to useso that we do not affect malaria control. We can make plans that can help get rid of some barriers in malaria control efforts" (Participant 3, village 3).*

Table 2: Common agricultural pesticides used in respective villages, their chemical class, frequency of use, and resistance status

Village	Pesticide/Herbicides	Trade name	Chemical class	Active ingredient	Frequency of use	Resistance status
V1	Both pesticides & herbicide (Widespread use)	Karate 5EC	Pyrethroid	Lambda-cyhalothrin	High	CR
		KungFu 5EC	Pyrethroid	Lambda-cyhalothrin	High	CR
		Rapid Attack 344SE	Pyrethroid+ Neonicotinoids	Cypermethrin+ Imidacloprid	Moderate	CR -
V2	Both pesticides & herbicide (Widespread use)	KungFu 5EC	Pyrethroid	Lambda-cyhalothrin	High	CR
		Profecron 720EC	Organophosphate	Profenofos	Moderate	PR
		Dasba 40EC	Organophosphate	Chlorpyrifos	Moderate	PR
V3	Both pesticides & herbicide (Widespread use)	Actellic 50EC	Organophosphate	Pirimiphos-methyl	High	PR
		Karate 5EC	Pyrethroid	Lambda-cyhalothrin	High	CR
		KungFu 5EC	Pyrethroid	Lambda-cyhalothrin	High	CR
		Profecron 720EC	Organophosphate	Profenofos	High	CR
V4	Only Herbicides (Marginal use)	2, 4 D Amine	AryloxyacidesII	2, 4 D- dimethyl amine salt	Moderate	-

CR: Confirmed resistance, PR: Possible resistance

4.2 Susceptibility status field-collected and laboratory reared mosquitoes

Insecticide susceptibility in field mosquitoes: The susceptibility test results are summarised in Fig. 2 and Table 3. *An. arabiensis* from all four villages were susceptible to DDT. Resistance to the two candidate pyrethroids, lambda cyhalothrin and permethrin, was observed in three of the four villages (V1, V2 and V3), while resistance to the carbamate, bendiocarb was observed in two villages (V1 and V3). Similarly for the organophosphate, pyrimiphos methyl, there was resistance in mosquitoes from V1 and V3, as well as signs of possible resistance in V2. On the contrary, mosquitoes from V4 were susceptible to all the candidate insecticides tested (mortality range: 98 -100%) (Fig. 2). Comparative tests done using laboratory reared mosquitoes of the same species revealed full susceptibility to all the candidate insecticides (Fig. 2). The knockdown times (including time taken to 50% knock-down) were recorded for the two pyrethroids (lambda cyhalothrin and permethrin) and the organochloride (DDT), and are summarised in Table 3.

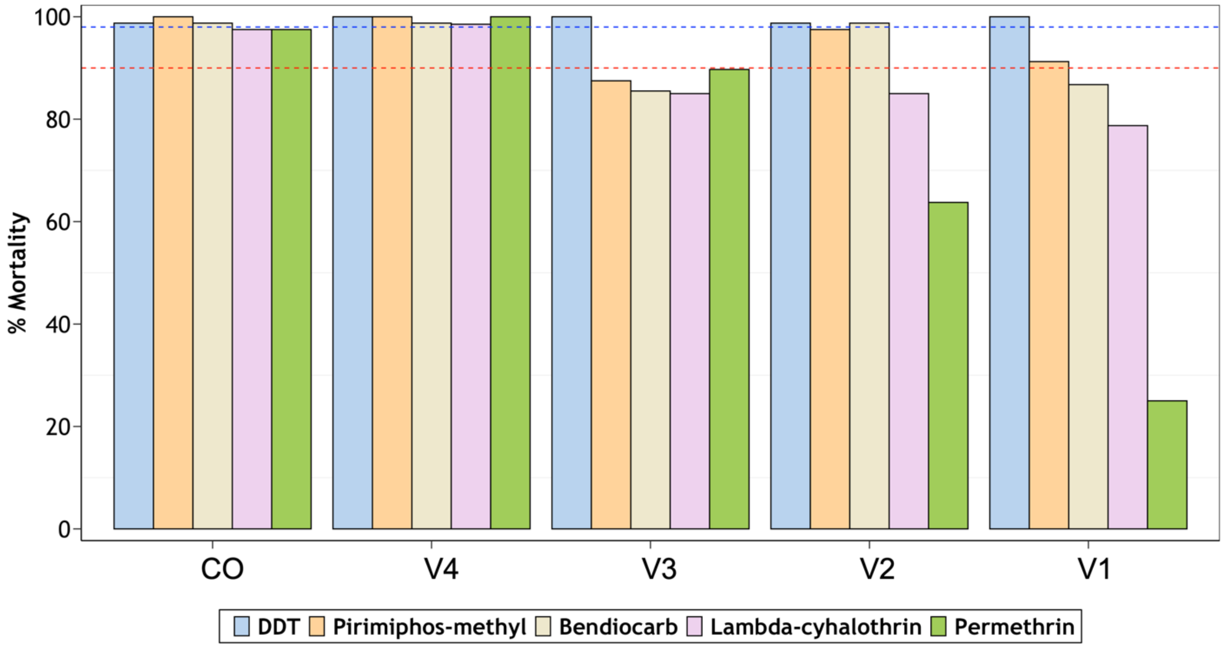

Figure 2: Percentage mortality in field collected An.arabiensis exposed to standard concentrations of five insecticides

Table 3: The knockdown time (KDT50) of *An. arabiensis* mosquitoes to three insecticide classes

	Organochloride			**Pyrethroids**					
	DDT			**Permethrin**			**Lambda-cyhalothrin**		
	KDT50	**% Mortality**	**RR (95% CI)**	**KDT_{50}**	**% Mortality**	**RR (95% CI)**	**KDT50**	**% Mortality**	**RR (95% CI)**
Lab-reared	31.5 (30.0 - 32.9)	98.75	1	17.2 (16.0 - 18.5)	97.5	1	9.0 (6.4 - 11.0)	97.5	1
V1	43.9 (41.7 - 46.0)	100	1.4 (1.4 - 1.4)	37.1 (34.6 - 39.6)	25	1.9 (1.8 - 1.9)	38.0 (33.6 - 43.6)	78.75	4.2(4.0 - 5.2)
V2	35.5 (33.3 - 37.5)	98.75	1.13 (1.1 - 1.1)	28.5 (26.1 - 30.9)	63.75	1.6 (1.6 - 1.6)	17.3 (14.1 - 20.4)	85	1.9 (1.9 - 2.2)
V3	28.6 (26.4 - 30.9)	100	0.9 (0.9 - 0.9)	18.3 (16.8 - 19.8)	89.71	1.5 (1.4 - 1.5)	18.4 (15.2 - 21.4)	85	2.04 (1.9 - 2.4)
V4	26.5 (24.4 – 28.7)	100	0.8 (0.8 – 0.9)	21.5 (20.0 – 23.1)	100	1.3 (1.2 -1.4)	19.4 (18.5 - 20.3)	98.5	2.2 (1.8 – 2.9)

Effects of the synergist, piperonyl butoxide (PBO) and possible involvement of metabolic resistance: In the three villages where there was pyrethroid resistance (V1-V3), the potency of the two pyrethroids was restored when mosquitoes were first exposed to the synergist, PBO, prior to the insecticide exposure. The resulting mortalities exceeded 98% in all cases across the three villages (Fig. 3), thus suggesting that the resistance observed here was primarily due to elevated expression of monooxygenases.

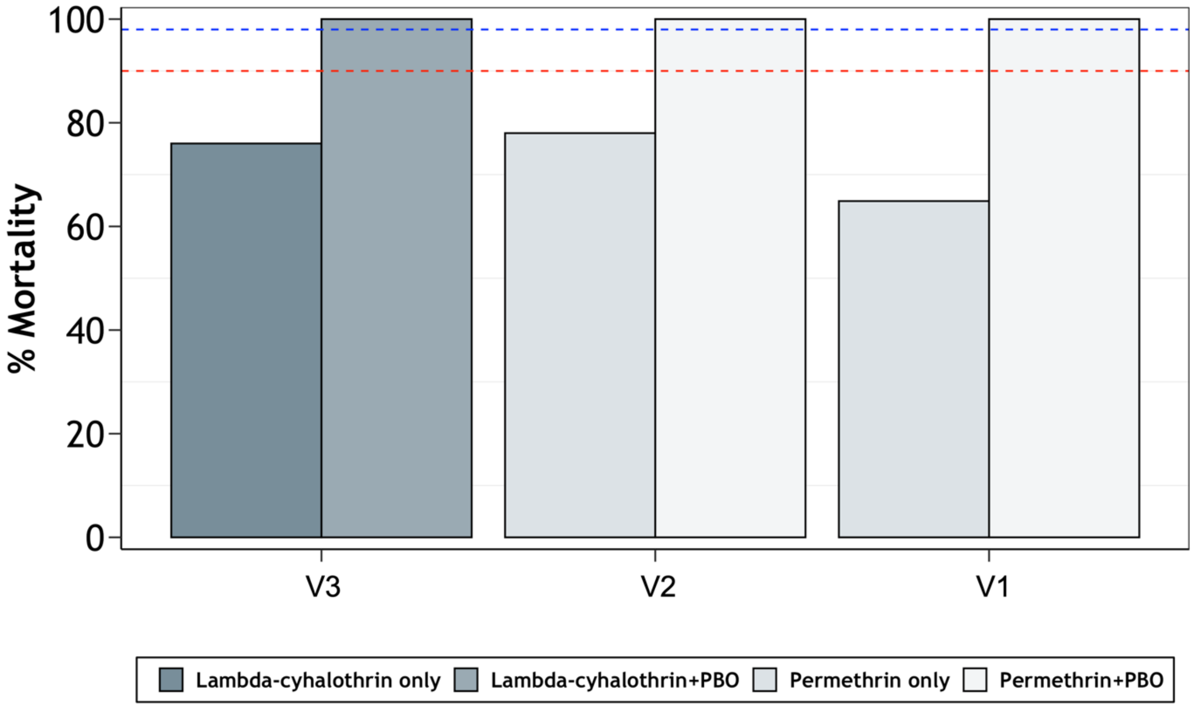

Figure 3: Percentage mortality of field collected *An. arabiensis* mosquitoes exposed to pyrethroids and PBO

Effects of sub-lethal pesticide exposures on insecticide susceptibility on laboratory-reared mosquitoes: Following the initial tests, a lethal concentration (LC) of 15% mortality was chosen for subsequent experiments because it enabled sub-lethal exposure while also ensuring sufficient adult emergence (Table 4). The effects of the sub-lethal exposures on the levels of insecticide susceptibility, fecundity and wing lengths of emergent adults are summarised below.

Table 4: Specific lethal concentrations used in subsequent laboratory experiments

Treatment	**Active ingredient**	**Concentration**	**Dose (g/L)**	**95% CI (g/L)**
Pyrethroid (Ninja 5EC)	Lambda-cyhalothrin	LC_{15}	2.3×10^{-7}	3.35×10^{-6} - 2.33×10^{-7}
Organophosphate (Twigaphos)	Pirimiphos-methyl	LC_{15}	1.0×10^{-7}	9.42×10^{-13} – 6.36×10^{-7}
Carbamate (Akheri)	Carbaryl	LC_{15}	2.1×10^{-4}	4.86×10^{-3} – 1.1×10^{-4}

CI: Confidence interval

There were no clear differences in insecticide susceptibility between mosquitoes emerging from exposed and non-exposed larvae. However, when the larvae were pre-exposed to the pyrethroids and DDT, the emergent adults appeared slightly less susceptible against these same insecticides (Fig.5).

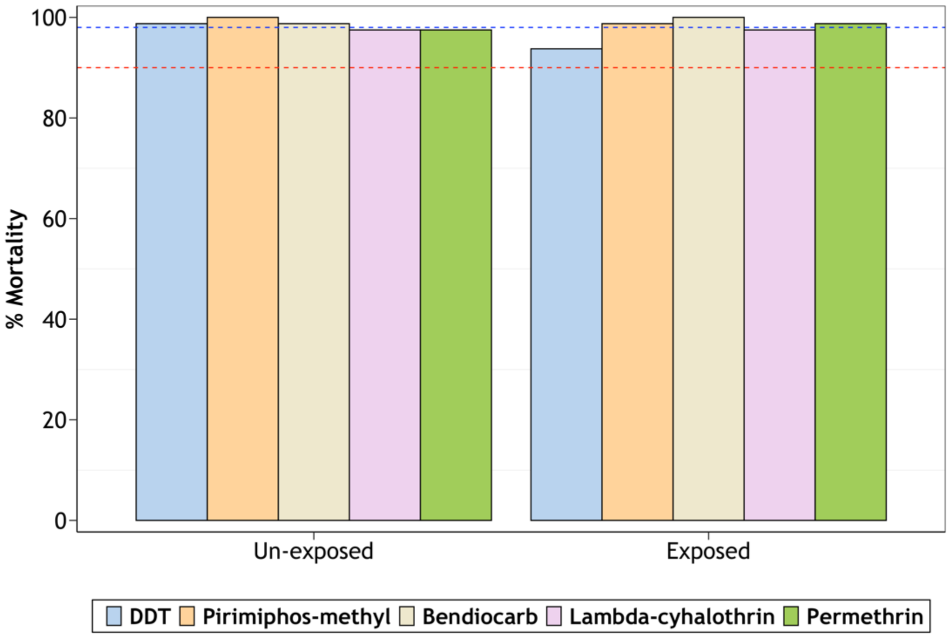

Figure 4: Percentage mortality in mosquitoes emerging from larvae

4.3 Effects of pesticide exposure on fitness parameters in field and laboratory reared mosquitoes

4.3.1 Field mosquitoes

Fecundity: Mosquitoes collected from the field had clutch sizes with eggs ranging from 8-190 (V1), 6-150 (V2), 12-178 (V3), 9-121 (V4) as estimated by direct counts under a dissecting microscope (Fig. 4a). The highest number of eggs laid was in V3, where the mean number of eggs was 73.23 (95% CL: 55.7 – 88.7). The analysis of the mean residual differences showed that overall, the fecundity was similar across the villages. There were also no significant differences in numbers of eggs laid by the field-collected relative to the laboratory-reared female mosquitoes (Fig. 4a).

Wing length: For field mosquitoes, the wing length ranged between 2.4 and 3.2 mm, with variations between the villages (Fig.4b). V3 mosquitoes had the largest wings (mean wing length = 3.1mm (95% CL: 3.06 – 3.14)) whereas V1 mosquitoes had the smallest wings (mean wing length = 2.9 mm (95% CL: 2.82 – 2.98)). In all study villages, the field-collected females were all smaller than females from the reference laboratory-reared colonies, which had wing lengths ranging from 2.6 mm to 3.6 mm (Fig.4b).

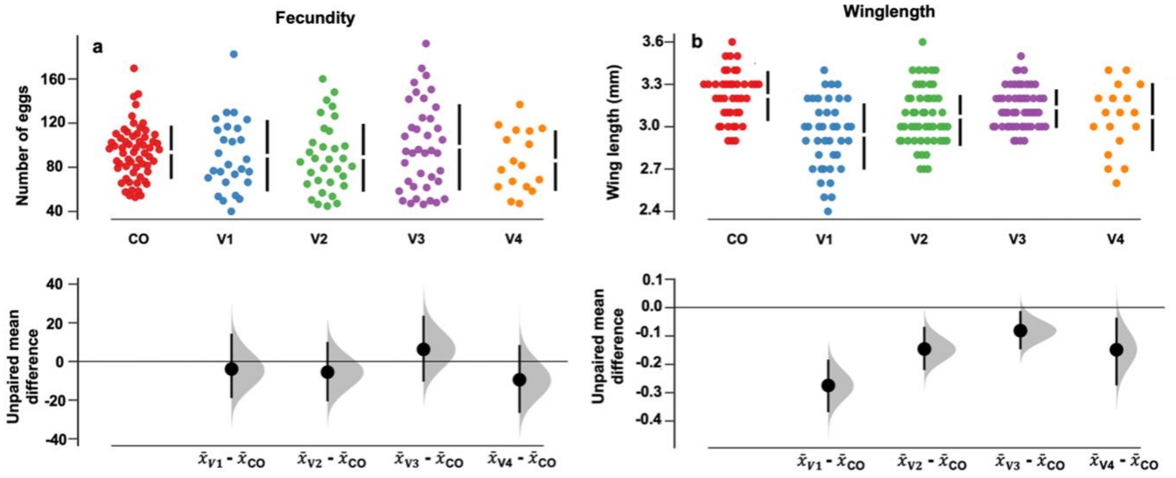

Figure 5: Estimated mean number of (a) eggs laid (b) wing lengths in field collected *An. arabiensis*

4.3.2 Laboratory reared mosquitoes

Fecundity: There were only marginal reductions in fecundity of laboratory-reared mosquitoes emerging from larvae exposed to pesticides, except in the case of organophosphate exposure which significantly reduced fecundity. The un-exposed mosquitoes had egg clutches with a range of 16-162 eggs/female. In comparison, there were 19-92 eggs in carbamates-exposed mosquitoes, 10-127 eggs in pyrethroid-exposed mosquitoes and 9-90 eggs in organophosphate-exposed mosquitoes (Fig.6a). The unexposed mosquitoes had the highest number of eggs (mean = 67 (95% CL: 59.4 – 74.6)) while those exposed to organophosphates had the lowest fecundity (mean = 52 (95% CL: 43.1 – 60.1)).

Wing lengths: In the mosquitoes from pesticide-exposed larvae, wing length ranged between 2.6 mm to 3.6 mm, with the organophosphate group showing the lowest mean wing length. The unexposed group had the largest wings (Fig.6b). Significant reduction of wing sizes was observed after exposure to pyrethroids and organophosphates. On the contrary, exposure to carbamates did not influence the wing lengths

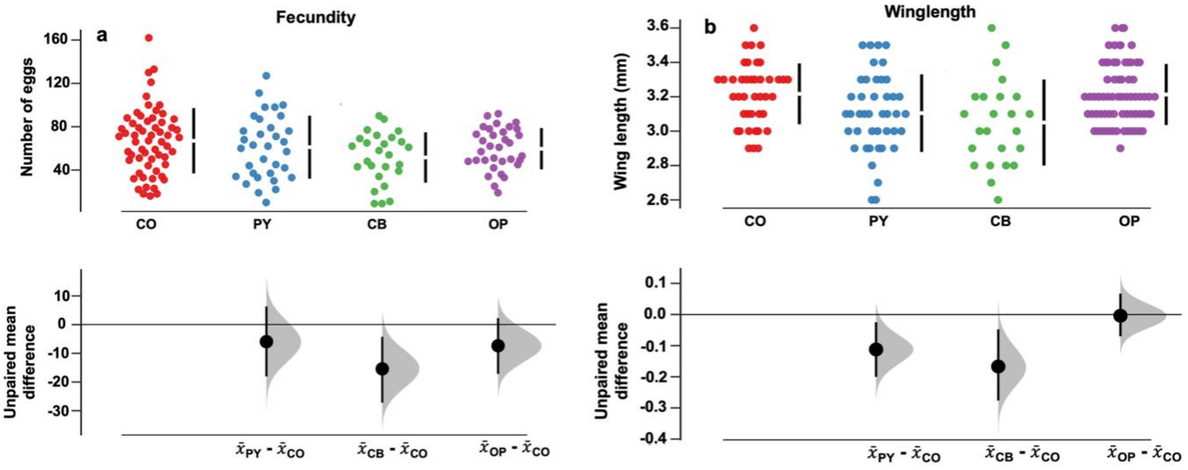

Figure 6: Estimated mean number of eggs laid (a) and (b) wing lengths by *An. arabiensis* mosquitoes emerging from larvae exposed to different pesticides

4.4 Discussion

The interactions between public health and agricultural sectors remain poorly understood, yet both have significant impacts on human livelihoods. The development and spread of insecticide resistance in disease vectors and crop pests, and the measures developed to address the challenges provide specific channels to explore these interactions.

Insecticide resistance in malaria vectors might not be solely due to public health use in interventions, such as ITNs and IRS. For more consolidated insecticide resistance management, the agricultural sector must therefore be an integral part of malaria control and elimination programs. The WHO Resistance management plan already borrows heavily from agricultural lessons but additional integration is necessary at the level of implementation (WHO, 2012). To support this, research is needed to assess how disease vectors are affected in communities where pesticide exposures arise from the two sectors variedly. This study investigated potential impacts of agrochemicals on the fitness and susceptibility of malaria vectors to commonly used public health insecticides. The study relied on a combination of methods including focus group discussions and direct observations to explore agricultural practices in four villages, followed by direct experimental assessments of resistance, fecundity and wing lengths. Additional tests were conducted, in which where laboratory-reared mosquitoes were exposed to sub-lethal concentrations of pesticides and the same fitness parameters assessed, in comparison to controls without the pre-exposures.

Previous studies postulated that large-scale agrochemical use may increase resistance levels in malaria vector populations and therefore compromise the performance of public health tools such as ITNs and IRS (Matowo *et al.*, 2010; Overgaard, 2006; Ranson *et al.*, 2009; Yadouleton *et al.*, 2011). Separately, it has also been demonstrated that resistant mosquitoes incur certain fitness costs and may therefore not be as efficient vectors as susceptible ones (Collins *et al.*, 2019; Nkondjio *et al.*, 2020; Osoro *et al.*, 2021; Smith *et al.*, 2021). However, a wider analysis of these factors suggest variations that make it difficult to draw a conclusion on the overall direction of the impacts (Freeman *et al.*, 2021). It is therefore important that local studies are conducted to investigate the inter-linkages between agricultural practices and the responsiveness of malaria vectors to control by insecticidal interventions.

This study demonstrated that *An. arabiensis* mosquitoes in the study villages in south-eastern Tanzania were generally resistant to insecticides used in public health and agriculture, except in villages that reported minimal pesticide use. Though this work does not itself fully elucidate the role of agrochemicals in the development of insecticide resistance, the observations are indicative of a pathway of association, possibly even causal. The study village that had the highest levels of susceptibility was also the one with the lowest pesticide use as reported during the qualitative studies. Villages reporting frequent pesticide use (e.g. multiple times in a space of a week) could potentially create a highly insecticide concentrated environments, resulting in heightened selection pressures. It remains unclear how much pesticide use is necessary to generate the negatively impactful selection pressures, thus more research is required in this area. Since the chemical classes are common in public health and agriculture, with agriculture uses far exceeding public health, it is often assumed that resistance can arise in vector populations from either source. Studies have shown resistance to certain chemicals soon after introduction of or even without prior public health use (Ranson, 2017).

The qualitative findings of this study revealed a broad use of agrochemicals by farmers, often without appropriate guidance on dosage and waste disposal. This corroborates with earlier studies conducted in the same area by Matowo *et al.* (2020). Additionally, a lack of awareness by farmers on the negative effects of agrochemicals on malaria control was noted and further emphasises the need to provide targeted training and sensitization to improve pesticide stewardship in crop-farming communities. Fortunately, this study also revealed that the farmers were eager and willing to undergo formal training to better understand the link between the two sectors. It is necessary to urgently integrate practices in both sectors, to ensure insecticide resistant management strategies are effective.

Insecticide potency in pyrethroids (lambda-cyhalothrin and permethrin) was fully restored (to 100%) by first exposing resistant field mosquitoes to the synergist, PBO. This restoration could be a sign of metabolic resistance as the mixed-function oxidase enzymes within these mosquitoes may have been suppressed (WHO, 2016). This indicates that even if field mosquitoes are resistant to pyrethroids, bed nets impregnated with pyrethroids and PBO could be considered as a supplementary tool to the conventional LLINs (Asidi *et al.*, 2012). Additionally, newer interventions such as non-pyrethroid actives, along with non-pyrethroid IRS may result in greater community protection against malaria (Protopopoff *et al.*, 2018).

This study also showed that the fitness parameters such as fecundity and wing lengths only marginally reflected the differences in agricultural pesticide use. However, KDT_{50} for DDT and lambda-cyhalothrin increased profoundly in colony exposed mosquitoes. This data further confirms the observations that even low concentrations of insecticide exposure could alter the tolerance of mosquitoes to insecticides, and hence repetitive exposure across several generations might induce insecticide resistance (Nkya *et al.*, 2014). Since these experiments were done with laboratory colonies, and observations made on emergent adults, it is likely that the effects of sub-lethal pesticide exposure requires multiple generations to manifest on the mosquito fitness. There were however, clear signs that in some cases, especially when the pesticide exposure was from organophosphate and pyrethroids, that the mosquitoes had significantly reduced fecundity and wing lengths in the emergent adults within the same generation. This could give an indication that induced resistance in mosquitoes could indirectly affect fecundity through reduction of body size. Possibly suggesting that mosquito behavioural and biological responses may be pesticide-specific as observed in Kibuthu *et al.*(2016). A similar observation was made with *An. funestus* in Tanzania whereby mosquitoes with small wing lengths, laid fewer eggs as compared to their larger counterparts (Nambunga *et al* unpublished data). This is therefore an area of research requiring additional investigations to fully understand the causal pathways and the important factors involved.

Findings from this study showed that field mosquitoes from all four villages were significantly smaller compared to the colony unexposed group. This was an expected finding as various studies have shown that mosquitoes from the wild are much smaller than those reared in the laboratory (Briegel *et al.*, 2001; Mogi *et al.*, 1996; Yeap *et al.*, 2013). This difference may be linked to the type of environment and resources available for the insect to thrive. Laboratory mosquitoes tend to have a more conducive environment, including nutritious food, minimal overcrowding, frequent blood meals, no insecticide selection pressure, and optimum temperature and humidity (Jirakanjanakit *et al.*, 2005; Leemingsawat & Dujardin, 2008; Yeap *et al.*, 2013).

Even though the main objectives of this study were achieved, there were various limitations. Firstly, field mosquito collections were done during the dry season, thus the findings might be different during the wet season. Secondly, only one lethal concentration was used for sub-lethal exposure due to a limited number of colony mosquitoes.Thirdly, no molecular analysis was done to identify the morphologically indistinguishable members of the *An. gambiae* complex since recent studies from the same area have indicated the complex now comprises nearly exclusively of *An. arabiensis* (Pinda *et al.*, 2020). Lastly, sub-lethal pesticide exposures were done only over a single generation and therefore did not allow for multi-generational observations of potential effects. It is recommended for future studies perform susceptibility bioassays during both wet and dry seasons as well as use a range of lethal concentrations for sub-lethal dose experiments across multiple generations. Such studies should also consider other forms of pesticide use, such as on livestock, which may also influence insecticide susceptibility and fitness of the mosquitoes, especially where the vector species have zoophilic tendencies.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

For more consolidated insecticide resistance management plans, the agricultural sector must be an integral in malaria control and elimination programs. Moreover, safeguarding the potential of insecticide-based interventions requires improved understanding of how agricultural pesticides influence important life-cycle processes and transmission potential of mosquito vectors. In this study, susceptibility of mosquitoes to public health insecticides was lower in villages reporting frequent use of pesticides compared to villages with little or no pesticide use. Variations in the fitness parameters, fecundity and wing length, marginally reflected the differences in exposure to agricultural pesticides, and should be investigated further. Pesticide use may impart additional life-cycle constraints on mosquito vectors, but this is likely to occur over multiple-generational exposures.

5.2 Recommendations

From the findings from this study, I strongly propose the following:

(i) More rigorous and expanded research on the effects of pesticides on mosquito fitness parameters in mosquitoes from more villages with varying levels of pesticide use.

(ii) Farmers and agrochemical retailers should undergo training on insecticide resistance training by experts in order to curb negative impacts on malaria vector control

(iii) Further studies should carry out pesticide exposure on laboratory mosquitoes over multi-generations, in order to determine how many generations it takes to induce resistance, and hopefully predict if the same is happening in field populations

(iv) Integration of the malaria and agricultural sector should be greatly encouraged to establish effective policies suitable to both sectors

(v) Greater engagement of stakeholders from multiple sectors, including agriculture, environment and public health to participate in disease control and livelihood planning.

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APPENDICES

Appendix 1: Insecticide susceptibility form for tested *An. arabiensis*

Village code ☐☐☐ Test number ☐☐☐☐ Date (dd-mm-yy) ☐☐/☐☐/ ☐☐

Investigator name:

Areainformation

Country:.. Province: ....

District: ....Commune: ....Village:..............................

Sample information

Species tested: ....Species control:...

Sex: .... Age (days): ……...

Collection method

Human landing indoor ☐ Resting night indoor☐ Resting morning indoor☐ Cattle collect☐ Human landing outdoor ☐ Resting night outdoor☐ Other: specify Larval collection☐ Progeny F1☐ Colony☐ Name of colony strain:.......................................

Physiological stage

Non-blood fed ☐ Blood fed ☐ Semi-gravid ☐Gravid ☐

Test insecticide information

Insecticide tested: Date of expiry: ☐☐/☐☐/☐☐

Impregnated papers prepared by: Date box first open: ☐☐/☐☐/☐☐

Concentration (1x/5x/10x): ....

Number of times this paper is used: ☐☐

Storage conditions: Room temperature ☐ Refrigerated ☐

Test conditions

Temperature °C □□.□ □□.□

Relative humidity(%) □□ □□

No. exposed	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Control 1		Control 2	
Number of knocked down (KD) mosquitoes after exposure for min												
	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Control 1		Control 2	
	Time	No. KD	Time	No. KD	Time	No. KD	Time	No. KD	Time	No. KD	Time	No. KD
START												
10 min												
15 min												
20 min												
30 min												
40 min												
50 min												
60 min												

b. Number of dead/ alive mosquitoes at the end of holding period

	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Control 1	Control 2
No. dead						
No. alive						

Appendix 2: Form for recording fecundity

Species: _____________Field/Lab : ___________ Temperature: ____________

Humidity: _____________Date started: ____________ Date ended: _____________

Investigator: ___________Village/Group: ______________

Mosquito ID	Number of eggs laid

Appendix 3: Form for recording wing length

Species: _____________Field/Lab : ___________ Temperature: ____________

Humidity: _____________ Date started: ____________Date ended: _____________

Investigator: ___________Village/Group: ______________

Mosquito ID	Wing length(mm)

RESEARCH OUTPUTS

Output 1: Paper publishedin the*Parasites & Vectors* journal

Urio, N., Pinda, P., Ngonzi, A., Muyaga, L., Msugupakulya, B., Finda, M., ... & Okumu, F. O. (2022). Effects of agricultural pesticides on the susceptibility and fitness of malaria vectors in rural south-eastern Tanzania.

Output 2: Co-author in paper published in Pan African Medical Journal

Amos Justinian Ngonzi*et al*. Susceptibility status of major malaria vectors to novaluron, an insect growth regulator South-Eastern Tanzania. Pan African Medical Journal. 2022;41:273. doi: 10.11604/pamj.2022.41.273.33793

Output 3: Poster