EVALUATION OF POTENTIAL VECTOR CONTROL METHODS THAT TARGET OUTDOOR FEEDING *ANOPHELES* MOSQUITOES

MARY MATHEW MATHANIA

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY FOR SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA

2017

ABSTRACT

Insecticide-Treated Nets (ITNs/Long Lasting Insecticide-Treated Nets (LLINs) and Indoor Residual Spraying (IRS) have contributed to halving global malaria incidence and mortality rates. Achieving zero malaria transmission using these methods which target indoor-feeding mosquitoes is hampered by residual malaria transmission attributable to outdoor-feeding mosquitoes. This study evaluated potential malaria vector-control methods that target outdoor-feeding *Anopheles* mosquitoes to contribute to efforts to eliminate residual malaria transmission. Specifically, it set out: (i) To assess community knowledge and awareness on malaria and its vectors, (ii) To determine spatial distribution of *Anopheles* adults and larvae, (iii) To determine effects of combined use of chemo-attractants and non excito-repellant insecticide (Chlorfenapyr) applied to contaminating devices in attracting and killing outdoor *Anopheles* mosquitoes and (iv) To evaluate method of attracting gravid *Anopheles gambiae s.s* to oviposit in artificially-created ovicidal breeding sites. To meet specific objective (i), a cross-sectional questionnaire study which explored knowledge and awareness on malaria was carried out in Dodoma and Morogoro on 400 respondents. The questionnaire study revealed that a vast majority (78.8%) were not aware that early outdoor biting of mosquito was a risk factor for malaria transmission. The main *Anopheles* breeding sites were found to be rice paddies (25.2%), ditches (23.3%) and septic tanks/pits (18.8%). Adult *Anopheles* mosquitoes were collected mainly from ceiling (42.3%) and stored/piled junks (16.3%) in the hot-wet season and from under beds (32.1%), undisturbed curtains (25.3%) and store rooms (23.7%) in cold-dry seasons. On testing a novel Umbrella-topped Mosquito Contaminating Device (UtMCD) developed in the present studies as a tool for attracting and killing outdoor *Anopheles* mosquitoes (specific objective iii) it was revealed that, adjusting for season and study location, the number of *Anopheles* mosquitoes caught were significantly associated with type of UtMCD set-up used. From among the UtMCD

set-ups ***A-D*** and device **E** tested (***A:*** Device alone, ***B:*** Device with attractants alone, ***C:*** Device with insecticide alone, ***D:*** Device with insecticide and attractants and **E***:* Device (Okomu), UtMCD set-up *D* significantly caught more *Anopheles* mosquitoes than UtMCD set-up ***A*** (AMR=2.96,p<0.0001). Mortality analysis showed that UtMCD set-ups ***D***, ***C*** and device ***E*** had higher percentage mortality: ***D*** (87.7%); ***C*** (89.4%) and ***E*** (84.9%) than UtMCD set-up ***A*** (19.2%) and UtMCD set-up ***B*** (17.4%). In the studies on artificially-created breeding sites it was found that the odds of not hatching for eggs deposited in hay infusion +vinegar sites was significantly greater than that of eggs deposited in water alone (AOR=80.6, p<0.001). In conclusion, the studies revealed there is a need for increased awareness on malaria transmission by outdoor early-feeding *Anopheles* and furthermore, the novel UtMCD (with chemo-attractants and insecticide) as well as artificial breeding sites with attractants and an ovicidal agent (vinegar) have potential for use in integrated vector and larval source management to eliminate residual malaria transmission.

DECLARATION

I, Mary Mathew Mathania, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted to any other institution.

_____ _____

Mary Mathew Mathania Date

(PhD Candidate)

The above declaration confirmed

_____ _____

Prof. Richard S. Silayo Date

(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENT

Firstly I thank God Almighty for His Love and continual blessing. He is a well-spring of guidance and strength to me My sincerest gratitude goes to my supervisor, Professor Richard S. Silayo for tireless efforts in guidance, contributions and encouragement towards completion of this thesis. He was always inspirational and a wonderful role model being not only an awesome supervisor but also like a father and a friend to me. I really enjoyed his supervision. My gratitude also goes to Sokoine University of Agriculture. I appreciate all I have learnt from Sokoine University of Agriculture and the support I got from all staff. I am also deeply grateful to Prof. Sharadhuli I. Kimera for his detailed constructive criticism and invaluable contribution to this work. Additionally, I am deeply indebted to Prof. Elikira Kimbita the former and Prof Gerald Misinzo the current Head of Department of Veterinary Microbiology and Parasitology (currently Department of Microbiology, Parasitology and Biotechnology of College of Veterinary Medicine and and BioMedical Sciences) as they sacrificed much to see this work become successful. My sincere appreciation goes to my employer St John's University of Tanzania for granting me study leave and financial support to pursue my PhD program. I also extend my sincere gratitude to Prof Dominic M. Kambarage and Prof Esron Karimuribo for the excellent financial support I received from the INTRA_ACP mobility project allowing me to carry out a part of the studies in Zambia. My heartfelt gratitude goes to Dr. Jennifer Stevenson for her tireless mentorship in Entomology and molecular assay during the entire period I visited Malaria Research Trust (MRT) at Macha-Choma Zambia. She was very instrumental in growth of my knowledge in Malaria Entomology. I extend my thankfulness to all MRT staff for their superb support in the laboratory. Special thanks go to Limonty Simubali, Ben Katowa, Jailos Lubinda, Natasha Laban and field and insectary team led by Bruice Malembeka, Alpha Simudoombe and Joe

Mwaanga for their wonderful cooperation. My grateful thanks also go to Mr. Faraji Abilahi, Mr. Abbas K. Saidi, Mr. Francis A. Tumbo and other staff at Ifakara Health Institute (IHI) for their support in mosquito molecular and serological work done at their institute. Special gratitude goes to my colleagues Dr. Barnabas Mngumi, Dr. Joseph Mdachi, Mr Abdallah Husein and to the field team led by Mr. Hamisi Nyoni for giving me support in data collection and analysis. I am also thankful to all staff at SUA Pest Management Centre (SPMC) for excellent supervision when I was keeping mosquitoes in their insectary. All this could not be successful without Prof.Bukheti Kilonzo, Dr. Ladslaus L. Mnyone and Ms. Resta R. Maganga. Without commitments from the Administrative authorities of Morogoro and Dodoma municipalities and willingness of all study respondents, all activities related to the execution of this PhD programme would have been futile. I am deeply indebted to them for their excellent cooperation and willingness to participate and they were the main stakeholders for turning my PhD into reality. To my lovely friends Dr. Sabina P.Sheppa, Dr. Franscis Mdoe, Dr. David.Z Munisi, Dr.Tumbwene Mwansisya, Dr. Richard Mongi, Elisa Mwega, and Leonard Kamanga, I thank them all for keeping the boat afloat despite the massive turbulences in the rough seas. Last but not the least, I wish to express my sincere appreciation and heartfelt gratitude to my lovely husband Gibson Frank Mawalla and my sons Joshua, Jovin, Salema and my nieces Emiliana, Given, my sweet maids Fortunata and Diana for their moral support. I know they missed me when I was away but they always believed that *"my success was also theirs"*. Special thanks should also go to my lovely parents (Mr and Mrs Mathew Mathania), my beloved brothers and sisters as well as in-laws for their prayers and always giving me excellent moral support. "*Thank you very much. May Almighty God bless you all.*"

DEDICATION

This thesis is dedicated to my lovely husband, Gibson F. Mawalla, my adorable sons Joshua, Jovin and Salema, my niece Given and to my dear parents Mr. and Mrs. Mathew Mathania and beloved brothers and sisters. They have always offered encouragement and support in my endeavours.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ACT	Artemisinin combination therapies
BP	Base Pair
DDT	dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Tri_Phosphate
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme-linked immune-sorbent assay
GPS	Geographic Positioning System
HMIS	Health Management Information System
IRS	Indoor residual spray
ITN	Insecticides treated nets
IVM	Integrated vector management
LLINs	Long-lasting Insecticide-treated Nets
LSM	Larval source management
M	Molar
MoHSW	Ministry of Health and Social welfare
PCR	Polymerase chain reaction
PMIs	The President's Malaria Initiatives
QA	*Anopheles quadriannulatus*
RBM	Roll Back Malaria
rDNA	ribosomal deoxyribonucleic acid
S.l	Sensu lato
S.S.	Sensu stricto

SDS	Sodium Dodecyl Sulfate
Taq	Thermus aquaticus xiii
TDHS-MIS	Tanzania Demographic and Health Survey and Malaria Indicator Survey
THMIS	Tanzania HIV/AIDS and Malaria Indicator Survey
UN	Universal
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

For many years, controlling human – vector interaction has been an overarching goal in the efforts of combating malaria by protecting people from potentially infectious mosquito bites through the use of core indoor interventions such as insecticide-treated nets / long lasting insecticide-treated nets (ITNs/LLINs) and indoor residual spraying (IRS) (Steketee *et al.,* 2010; Murray *et al.,* 2012; WHO, 2015). However in areas where transmission is focal and breeding habitats are accessible and fixed, these core interventions can be supplemented by other methods such as larval source reduction (Maheu-Giroux and Castro, 2013). Moreover, the applications of these measures either singly or in combination reduce vector abundance, human-vector contact and vector infectivity ultimately lowering malaria transmission (Eisele *et al.,* 2010; Steketee *et al.,* 2010).

In malaria endemic areas particularly in Sub-Saharan Africa where indoor interventions have been intensively used, malaria incidence and mortality rate have been reduced by 79% and 55% respectively between 2000 and 2015 (WHO, 2015). This success has been attributed to the improved and increased coverage of malaria vector control interventions mainly scaling-up of ITNs and IRS (Teklehaimanot *et al.,* 2007; WHO, 2015).

ITNs/LLINs and IRS which are the conventional indoor intervention play a role of reducing vectorial capacity of local vector populations below the critical threshold required for malaria reproduction rate (R_o, the expected number of human cases in the population) to be less than 1 (Enayati and Hemingway, 2010). Consequently the

ITNs/LLINs and IRS reduce vector daily survival rates, thus *Plasmodium* in the malaria vector will not complete its life cycle and subsequently local malaria transmission will be lowered (Kelly-Hope and McKenzie, 2009; Shaukat *et al.,* 2010).

However, despite scaling up of ITNs and IRS through high coverage and utilization of these interventions, there are ongoing lower but significant malaria transmission reported in different parts of the world which has been described as residual malaria transmission (Mboera *et al*., 2013; Russell *et al*., 2013; Killeen, 2014; WHO, 2015). The residual transmission has been attributed to outdoor feeding behaviour adopted by most malaria vectors hence making these intradomiciliary tools to have little impact on these vector species (Reddy *et al.*, 2011; Govella *et al.,* 2013; Russell *et al.,* 2013; Smith *et al.,* 2013; Durnez and Coosemans, 2014; Killeen, 2014).

Historically, malaria vectors exhibit exophilic/exophagic or endophilic/endophagic behaviour whereby the former rest and feed outdoor and the later rest and feed indoor (Gillies, 1954; Mnzava, 1984). A number of studies have shown that wide-scale use of ITNs/LLINs and IRS as indoor malaria control interventions contributed to adaptation behavior (as a result of excito-repellance of chemicals used to treat the nets), whereby indoor *Anopheles* mosquitoes are now biting outdoors (Okumu *et al.,* 2010a; Govella *et al.,* 2013). These mosquitoes escape indoor-based interventions through avoidance behaviour (reduced house entry, outdoor early feeding, avoid contact with indoor treated surface or nets, and obtaining blood meals from non-human hosts) (Killeen, 2014).

In Tanzania and elsewhere in Africa, recent data reported a great shift of most anthropophilic vectors (e.g. *An gambiae s.s* and *An. funestus*) from being endophilic/endophagic to being exophilic/exophagic vectors (Govella *et al.,* 2013;

Matowo *et al.,* 2013; Protopopoff *et al.,* 2013; Renggli *et al.,* 2013). Available evidence shows that *An. gambiae s.s* and *An. funestus* (endophilic/endophagic) and *An. arabiensis* (exophilic/exophagic) often occurred together outside human dwellings (Russell *et al.,* 2010; Russell *et al.,* 2011; Kitau *et al.,* 2012). Furthermore in places previously dominated by *An. gambiae* s.s and *An. funestus* such as Tanga, Pwani, these vector species have largely been overtaken by *An. arabiensis* which are now the predominant malaria vectors (Russell *et al.,* 2010; Derua *et al.,* 2012; Kabula *et al.,* 2011). Additionally in areas where *An. gambiae s.s* still dominates, this species exhibits outdoor early biting presumably as an adaptation mechanism to avoid indoor control intervention tools (Geissbühler *et al.,* 2007; Reddy *et al.,* 2011; Russell *et al.,* 2011; Kitau *et al.,* 2012; Bayoh *et al.,* 2014; Durnez and Coosemans, 2014).

Behaviour change of endophilic and endophagic malaria vectors such as *An. gambiae s.s* and *An. funestus* to feed outdoor early in the evening before people have retired to bed, has caused the available indoor tools (ITNs/IRS) not confer full protection of humans against bites by these species (Mng'ong'o *et al.,* 2011; Kabula *et al.,* 2011; Derua *et al.,* 2012; Kitua *et al.,* 2012; Russell *et al.,* 2012; Protopopoff *et al.,* 2013; Majambere *et al.,* 2013). Similarly, following the decline in *An. gambiae s.s* population and a rise in proportional abundance of *An. arabiensis,* in some places in Tanzania (Kabula *et al.,* 2011; Kitua *et al.,* 2012; Russell *et al.,* 2012) the current frontline vector control tools alone are insufficient to attain the ultimate goal of World Health Organization (WHO) to zero down malaria transmission (Okumu *et al.,* 2010a; Majambere *et al.,* 2013; Takken and Verhulst, 2013; Protopopoff *et al.,* 2013). Thus there are increasing calls for development of additional vector control tools which will supplement the existing malaria control interventions and move beyond control to malaria elimination (Ferguson *et al.* 2010; Mboera *et al.,* 2013; Hemingway, 2014; Killeen 2014; WHO, 2015).

1.2 Problem Statement

Effective malaria vector control depends on mosquito behaviours (exophilic/exophagic or endophilic/ endophagic) (Pates and Curtis, 2000). The vectors with low behaviour vulnerability to existing vector controls such as ITNs and IRS have mediated the enormous transmission across the endemic region of tropical countries. For many years ITNs and IRS have been used as the mainstream tool to fight against malaria. Despite the fact that the malaria morbidity and mortality in some localities has been reduced to half the previous number of cases due to utilization of ITNs/LLINs, IRS and artemisinin-based combination therapy (ACT), malaria still ranks as number one among top ten diseases reported in Tanzania health facilities (MoHSW, 2015; MTUHA, 2015a and b) (MTUHA is short form for the Kiswahili "Mfumo wa Taarifa za Uendeshaji wa Huduma za Afya" meaning "Health Management Information System (HMIS)". Besides, just recently Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS, 2016) reported that malaria prevalence has increased from 9.5% to 14.1% causing enormous social, economic and health burden to many Tanzanians. Over 93% of the 45 million people in mainland Tanzania are at risk of contracting malaria (MoHSW, 2015; Mboera *et al*., 2013). It has been reported that malaria contributes to about 42% and 49% of all outpatients ≤5 years of age and those aged ≥5 years respectively. In terms of inpatient admissions, malaria accounts for 39.4% of children under the age of 5 years and 47.5% of children above 5 years (MOHSW, 2015). At the beginning of this study (November 2012) malaria prevalence in Morogoro and Dodoma regions in Tanzania was reported to be 13.0% and 2.5% respectively (THMIS, 2012). Very recently, TDHS-MIS (2016) reported increased malaria prevalence in some regions of the country and Morogoro was among the regions recorded to have high (22.5%) malaria prevalence in 2016. Although use of ITNs/LLINs was the main method of personal protection against mosquito bites (household caverage 66.8%), malaria prevalence was observed to have

increased (TDHS-MIS, 2016). Recent studies involving entomological monitoring throughout the country indicated that malaria vectors increasingly bite outdoors in early evening when most of the people are not protected by ITNs/LLINs and may be the cause of residual malaria transmission (malaria transmission that persist after full achievement of universal coverage with effective LLINs and or IRS interventions) (Kelly-Hope and McKenzie 2009; Okumu *et al.,* 2010a; Govella *et al.,* 2013; Russell *et al.,* 2013). To eliminate this residual malaria transmission, novel control methods which will target the responsible vectors (i.e. outdoor early biting *Anopheles* mosquitoes) are required.

1.3 Rationale and Significance of the Study

Vector control remains the most important component of malaria control in Tanzania and elsewhere. Use of ITNs/LLINs and IRS have been going on for past decades with great achievements. However, the constant use of ITNs/LLINs and IRS has caused mosquitoes to develop insecticide resistance or develop behaviours to avoid the excito-repellent insecticide applied on the bed nets (Russell *et al*., 2013). It has been observed that malaria vectors which were biting indoor at midnight are now biting outdoor early in the evening (Geissbühler *et al*., 2007; Russell *et al.,* 2011). The use of ITNs/LLINs which were meant to kill mosquitoes attempting to feed on people who are sleeping under treated mosquito nets, and IRS which kills mosquitoes that land on the insecticide-treated surface (Killen, 2014) are no longer sufficient to combat all transmission because they do not give complete personal protection against outdoor biting mosquitoes (Pates and Curtis, 2005; Geissbühler *et al.*, 2007; Russell *et al*., 2013). The present study aimed to provide information about vector control methods that would contribute to tackling the outdoor feeding *Anopheles* mosquitoes. The results of this study will add new knowledge that will contribute to improvement of intergrated malaria control.

1.4 Objectives

1.4.1 Overall objective

The overall objective of the research was to contribute to control of residual malaria transmission attributed to outdoor feeding *Anopheles* mosquitoes.

1.4.2 Specific objectives

The specific objective were

i. To determine community knowledge and awareness of malaria, *Anopheles* and its biting behaviour in the study area.

ii. To determine seasonal spatial and temporal distribution of adults and larvae of *Anopheles* mosquitoes in the study area.

iii. To determine the effects of combined use of chemo-attractants and non excito-repellant insecticide (Chlorfenapyr) applied to contaminating device on attracting and killing outdoor *Anopheles* mosquitoes.

iv. To evaluate the effect of attracting gravid *Anopheles gambiae s.s* to oviposit in artificially created ovicidal breeding sites.

1.5 Null Hypotheses

1. There is no difference between study participants and general population in terms of knowledge and awareness on malaria, *Anopheles* and its biting behavior

2. Spatial and temporal distribution of adults and larvae of *Anopheles* mosquitoes in study area does not differ between seasons.

3. The effects of combined use of chemo-attractants and non excito-repellant insecticide applied on contaminating device for attracting and killing *Anopheles* mosquitoes are not different across different set-ups of contaminating and other devices.

4. There is no difference in responses of gravid *Anopheles gambiae* towards attractants with or without vinegar and no effects on hatchabilityof deposited eggs in all experiments

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Malaria

Malaria in humans is caused by five species of parasites belonging to the genus *Plasmodium* (WHO, 2015). Four of these- *Plasmodium falciparum, P. malariae P. ovale (P. ovale curtisi and P. ovale wallikeri)* and*P. vivax*, are human species transmitted from one person to another through the bite of infected female mosquitoes in the genus *Anopheles*. In recent years human cases of malaria due to a fifth species, *P. knowlesi* have been reported in South-East Asia (Cox-Sing and Singh, 2008; White *et al*., 2014). This species (*P. knowlesi*) normally causing malaria in monkeys, can accidentally infect humans when *Anopheles* mosquito infected from feeding on the monkeys, bites a human (zoonotic transmission) (Cox-Sing and Singh, 2008).

Malaria infection begins when infected female *Anopheles* mosquito inoculates malaria parasites into human body during a blood meal. About 10-100 sporozoites are injected into human skin and migrate to the liver (Doolan *et al*., 2009; Nkhoma *et al*., 2012). Once parasites are fully matured in the liver, they rupture the hepatic cells and are released into blood circulation as merozoites. The merozoites immediately invade red blood cells (RBCs) and form a ring stage called trophozoite. When trophozoites mature they divide mitotically to form multinucleated schizonts which contain an average of 10 merozoites. The matured schizonts rupture the RBCs and release merozoites into the blood circulation. It is during this stage that the characteristic fever, chills and malaise are felt (Greenwood *et al*., 2008; Doolan *et al*., 2009). The intraerythrocytic cycle may be repeated a number of times but eventually some of merozoites which infect new RBCs undergo sexual development and become gametocytes (Jones and Good, 2006;

Greenwood *et al*., 2008). These gametocytes will be ingested by female *Anopheles* mosquito during blood meal and then undergo another life cycle (sporogony cycle) in the invertebrate host (female *Anopheles* mosquito) or will die if not taken by the female *Anopheles* mosquito (Greenwood *et al*., 2008).

Within the invertebrate host (mosquito) midgut, the macrogametocyte (female gametes) undergoes maturation process which involves morphological changes, in which in the case for *P. falciparum* they round up and become spherical. Microgamete (male gametes) undergoes rapid nuclear division to produce eight flagellated microgametes. The flagellated microgamete fertilizes female macrogamete to produce zygote (diploid cell). The zygote elongates into a motile ookinete which burrows through the epithelium of the mosquito stomach to develop into immotile oocyst on the outer surface of the gut. Subsequently it undergoes sporogony to produce thousands of uninucleate sporozoites which will be released throughout the mosquito haemocoel. Most of these sporozoites penetrate the salivary glands and remain there until the mosquito inject them into the vertebrate host with the saliva during blood meal (Smith *et al.*, 2000; Greenwood *et al.*, 2008; Gerald *et al.*, 2011).

2.2 Malaria vectors

Human malaria vectors belong to the genus *Anopheles.* This genus is estimated to comprise about 400 species spatially distributed all over the world. Of these, only 40 are associated with transmission of human malaria (WHO, 2015). It has been reported that mosquitoes can transmit malaria parasites to humans once they meet the following conditions i) **Mosquito abundance**: *Anopheles* species have to exist in high number to ensure they encounter an infectious human and pick up malaria parasite. ii) **Longevity**: Individual mosquito needs to survive long enough to allow parasite to develop within it.

iii) **Capacity:** Mosquito need to be able to carry enough parasites in salivary gland to ensure parasites are transmitted to the next human. iv) **Mosquito –human interaction:** The species prefer to feed on humans and breed near human dwelling so that it can be easy to find people to feed upon.

The Genus *Anopheles* is distributed throughout the world but in the Afro-tropical regions the major malaria vectors are *Anopheles gambiae* complex and *Anopheles funestus* group (Gillies, 1954; Cano *et al.,* 2006; Lehmann *et al.,* 2014). In Tanzania predominant species is *Anopheles gambiae* complex of which *An. gambiae sensu stricto* (*An. gambiae s.s*) and *An. arabiensis* are most important malaria vectors (Mnzava, 1984; Mnzava and Kilima, 1986). Three other species in the *A, gambiae* complex namely An. *merus*, *A. melas* and *An bwambae* are not (with the exception of *An. merus*) commonly found in Tanzania. Another species *A. quadriannulatus* which occurs in two forms A and B and was generally regarded as not an important vector has sporadically been reported to be positive for malaria parasites as a result of occasional feeding on people (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987).

The *An. funestus* group consists of nine indistinguishable subspecies namely; *An. funestus sensu stricto, An. funestus vaneedeni, An. funestus parensis, An. funestus aremi, An. funestus fuscivenosus, An. funestus confuses, An. funestus leesoni, An. funestus rivolorum and An. funestus brucei*. However *An. funestus s.s.* is the predominant one, both in numbers and geographical distribution, and also the most anthropophilic vector in this group (Gillies and Coetzee, 1987).

2.2.1 Life cycle of malaria vectors

Anopheles mosquito depends on two environments for completion of its life cycle which goes through four stages namely egg, larvae, pupa and adult (Figure 1). In this cycle water is essential component for juvenile stages (egg, larva and pupa) whereas aerial and terrestrial environment is essential for adult stage (Foster and Walker, 2002; Wirth 2002).

Before oviposition, female mosquito begins to search for a suitable breeding site to lay its eggs when it is fully gravid. Once it finds appropriate breeding site for oviposition, it lays about 100-150 eggs on water surface while hovering above the water (Foster and Walker, 2002). The singly-laid eggs have lateral floats which enable them to float on water. If not disturbed (by temperature or pH, dessication UV, chemicals) eggs can hatch within 2-3 days (Clement, 2000). Female mosquitoes lay multiple batches of eggs throughout their life time and they require a blood meal for every batch of eggs they lay. The gravid mosquito can lay eggs in permanent and temporary breeding sites such as shallow pools along edges of rivers and lakes, water in tins, coconut husks and other containers, foot prints, rice paddies, swamps, puddles formed by tire tracks and ditches (Sattler *et al*., 2005; Vanek *et al.,* 2006).

The larva is extremely active and feeds on nutrients such as algae, bacteria and other microorganisms found in water. It shuttles between the subsurface where it feeds and the surface where, due to lack of a siphon, it positions itself parallel to the water surface to obtain oxygen (Foster and Walker, 2002; Wirth 2002*).* Larvae develop through four stages (instars), the time required for each stage being dependent on various factors including temperature and water pH (Clement, 2000). At room temperature larvae take 7 to 10 days to develop from first to fourth instar.

Larvae of *Anopheles* mosquito are most commonly found in unpolluted, sunlit pools of water (Vanek *et al.,* 2006). Recent studies have reported that *Anopheles gambiae* complex can breed in polluted water and urbanization ecosystem was strongly associated with this new adaptation (Keating *et al.,* 2004; Sattler *et al.,* 2005; Awolola *et al.,* 2007; Castro *et al.,* 2010). The comma-shaped pupa is very active like the larval stage but does not feed.It lasts two to three days (at ambient temperature) then sheds out its skin to become adult mosquito (Foster and Walker, 2002; Wirth 2002; Vanek *et al.,* 2006;).

The adult mosquito which emerges from pupa stays on water surface for few minutes to dry up then fly away. In most cases male *Anopheles* mosquitoes emerge first and they form large swarms around dusk (Vanek *et al.,* 2006). Mating usually occurs within one to two days after emerging. Female *Anopheles* mosquitoes will fly into swarms of male *Anopheles* mosquitoes for mating. The female mosquito mates once in its life time and after insemination the spermatozoa are stored in spermathecae within the female mosquito. In its life time, the female mosquito continues to draw sperm from spermatheca after every egg lay to fertilize the released ova (Foster and Walker, 2002). Male *Anopheles* lives only for about a week while female *Anopheles* can live for 3-4 weeks or longer (more than a month in captivity or during dry/cold weather) (Vanek *et al.,* 2006). Male *Anopheles* mosquitoes do not feed on blood on account of their mouth parts not being adapted for insertion under the skin to obtain blood. They feed on nectar.to get energy. Female *Anopheles* mosquitoes also feed on nectar as a source of energy but have to feed on blood for egg development (Vanek *et al.,* 2006; Foster and Walker, 2002).

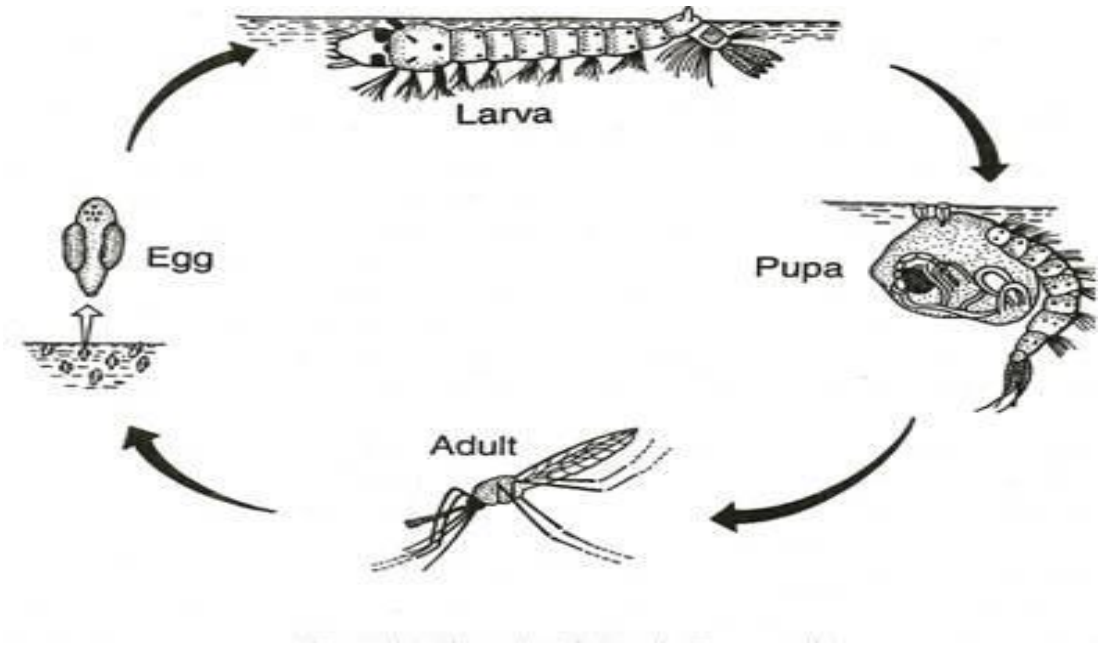


Figure 1: Life cycle of *Anopheles* mosquito (Source: Wirth 2002)

2.2.2 Spatial and temporal distribution of malaria vectors

The distribution of *Anopheles* mosquitoes is determined by macro (climate and topography) and micro (availability of aquatic ecological habitats) factors. Climate and topography are major determinants of macro spatial and temporal distribution of *Anopheles* mosquitoes within the district, region, country or continent (Ernst *et al.*, 2006; Gage *et al*., 2008; Kulkarni *et al*., 2010; Texier *et al*., 2013). The micro-spatial distribution of vectors within the community depends on the availability of aquatic habitats proximal to human dwellings (Gu *et al*., 2008; Geissbühler *et al*., 2009). The distribution of immature stage of mosquitoes is mainly determined by the availablility of aquatic habitats spatialy distributed in a given geographical area (Gouagna *et al.*, 2012). These habitats must have potentials for supporting the development/survival of the malaria vector larvae, since not all aquatic habitats are suitable breeding habitat for *Anopheles* mosquitoes (Anderson *et al.,* 2001). There are several environmental factors which determine the occurrence and abundance of mosquito larvae. These factors include climate (e.g. temperature, rainfall), physical and chemical conditions of the aquatic

habitats, and biological characteristics of breeding habitats (Bayoh and Lindsay, 2003; Minakawa *et al.,* 2005; Mwangangi *et al.,* 2007a; Paaijmans *et al.,* 2008).

Rainfall is one among the major determinants of spatial distribution of mosquito larvae because it provides enormous breeding sites for female mosquitoes to oviposit. However when there is high rainfall, breeding sites can be flushed out impacting larval distribution (Cohuet *et al.,* 2004). Furthermore water temperature, pH, salinity, turbidity, vegetation cover and presence of predators and competitors are other important factors determining the abundance and distribution of anopheline larvae at species level (Mereta *et al.,* 2013). For instance, larvae of *An. gambiae* complex and *An. funestus* are often found in shallow water that contains vegetation, whereas *An. merus* breeds in saline waters (Minakawa *et al.,* 2005; Mwangangi *et al.,* 2007a). Additionally, previous studies on *An. gambiae s.l* larvae showed that the distribution of *Anopheles* larvae does not depend only on large/huge permanent habitats but rather in small human-made aquatic habitats such as empty containers, ditches, small holes and foot prints (Mwangangi *et al.,* 2007a; Mereta *et al.,* 2013).

Oviposition and larval aquatic habitat preference which determine the spatial distribution of larvae often varies among mosquito species. Before oviposition, a gravid mosquito is guided by two distinct behaviours to search for suitable aquatic habitat for oviposition. These behaviours are preoviposition (orientation of aquatic habitats) and oviposition (the actual deposition of eggs) (Rinker *et al.,* 2013; Herrera-Varela *et al.,* 2014).

During pre-oviposition, gravid mosquitoes use long range visual cues to search and locate aquatic habitat (Okal *et al.,* 2013). It has been reported that water vapour is a key attractant in pre-oviposition process which guides gravid mosquito to locate breeding sites

and searching for aquatic habitats usually occurs during twilight or in the morning (Huang *et al.,* 2005; Okal *et al.,* 2013). Once the mosquito locates the habitat, selections of suitable habitat for oviposition become very important. In nature there are many aquatic sites but some remain uncolonized, suggesting that some habitats are more attractive than others (Fillinger *et al.,* 2009; Gouagna *et al.,* 2012). Before oviposition gravid mosquitoes use short range cues (olfactory) to sense volatile chemical compound emitted by the aquatic habitat (Fillinger *et al.,* 2009; Gouagna *et al.,* 2012; Rinker *et al.,* 2013). For example oviposition pheromone of egg, larval and pupa origin has been reported to be potent attractants which attract gravid mosquito towards breeding sites to oviposit (Blackwell and Johnson, 2000; Fillinger *et al.,* 2009). Moreover, presence of certain vegetation in aquatic habitat and semio-chemicals mainly of microbial origin stimulate mosquito oviposition (Fillinger *et al.,* 2009; Gouagna *et al.,* 2012). Vegetation such as rice grass has been reported to contain oviposition stimuli for *Anopheles* mosquitoes (Gouagna *et al.,* 2012). The compounds isolated from hay infusion such as 3-methyl indole and 4-ethylphenol were shown to induce positive oviposition response in *An. gambiae* complex and *Ae. albopictus* (Allan and Kline, 1995). Another study reported by Kweka *et al.,* (2011) showed that cow urine contains chemical substances such as ammonia which influence gravid mosquitoes such as *An. arabiensis* to oviposit.

Distributions of adult mosquitoes are usually seasonal and normally follow rainfall patterns which cause the population densities to differ both across and within the countries (Oesterholt *et al.,* 2006). For example, during and after rainy season, density of some mosquito species such as *Anopheles gambiae* complex peak up due to availability of breeding sites while during dry -cold seasons mosquitoes density decline (Parham *et al.,* 2012). In cold/dry season, mosquitoes may disappear from the vicinity because temperature and humidy do not favour them and thus they may become dormant/ inactive

or hibernate in warm places in or outside human dwellings (Kirby *et al*., 2009; Parham *et al.,* 2012).

In tropical areas, the cold-dry season survival mechanism of mosquitoes species is one of the most important strategies which help the mosquito to survive because many mosquitoes die off when the temperature drops (Minakawa *et al.,* 2001; Lehmann *et al.,* 2010). Favorable ambient temperature for *Anopheles* species is 15 - 35 ºC (Bayoh *et al,* 2001; Beck-Johnson *et al.,* 2013). In cold weather mosquitoes become inactive and reduce their metabolic activity for survival (Robich *et al.,* 2005; Huestis *et al.,* 2012; Lehmann *et al.,* 2014). During dry/ cold seasons *Anopheles* mosquitoes seek hibernating shelter in warmer places (for example in caves, stables, earth burrows, cellars of barns) and take the hibernation squat position by bending their legs and tucking their body close to the surface they sat on and leave those places in warm season when the temperatures increase (Yaro *et al.,* 2012; Beck-Johnson *et al.,* 2013; Lehmann *et al.,* 2014).

2.2.3 Human vector interaction

Female mosquitoes are attracted to their hosts by physical and chemical cues emanating from them. These cues allow the mosquito to differentiate between favorable and unfavorable host to feed upon (Pitts *et al*., 2011). When female mosquitoes are host-searching for blood meal, they use odorant receptors in their olfactory organ to locate the host (Pask *et al.,* 2011). There are two main behaviours which *Anopheles* mosquitoes use in host-seeking, namely i) host recognition/preference and ii) time of feeding (Pask *et al*., 2011; Pitts *et al*., 2011).

In host recognition/ preference, mosquito can be anthropophilic (prefer to feed on human e.g. *An. gambiase s.s*); facultative feeders (they can feed on human or animal e.g. *An.*

arabiensis) or zoophilic (prefer to feed on animal e.g. *An. leucosphyrus*) (Qiu *et al.,* 2004; Cox-Singh and Singh, 2008; Pitts *et al*., 2011). Host odour plays a major role in enabling female mosquito to locate a host. For example many *An. gambiae* complex recognize specific alcohols and heterocyclics secreted within human sweat and by skin microbiota (Carey *et al.,* 2010; Verhulst *et al.,* 2011; Rinker *et al.,* 2013; Takken, and Verhulst, 2013). Compounds such as ammonia have been identified as some of the main components of sweat detected by *Anopheles* mosquitoes in the course of identifying the orientation of human hosts (Smallegange *et al.,* 2005). Other components from sweat are indole, diethyl-ether, 1-dodecanol, 6-methyl-5-hepaten-2-one and acetone and carboxylic acids emitted from human foot attract female *Anopheles* mosquitoes towards a host (Verhulst *et al.,* 2011; Smallegange *et al*., 2010a). Carbon dioxide emitted from human breath is another powerful chemo-attractant (McMeniman *et al.,* 2014; Van Loon *et al.,* 2015; Webster *et al*., 2015). Gillies and De Meillon, (1968) reported that carbon dioxide attracts female mosquitoes from a distance of 18-36 m.

To date many of these odorants can be synthesized *in vitro* to mimic real human and can be used to lure mosquitoes (Logan and Birkett, 2007; Okumu *et al.,* 2010b). Advancement such as production of CO_2 from fermentation of sugars using yeast has been used in experimental sampling of *Anopheles* mosquitoes (Saitoh *et al.,* 2004; Smallegange *et al.,* 2010b). Additionally, carboxylic acids which are emitted from unwashed nylon stockings/socks have been reported to attract female mosquito to baited traps used in catching mosquitoes (Mboera *et al.,* 2000; Busula *et al.,* 2015). It has been shown in many studies that combinations of these synthetic odours have great synergistic effect in attracting female *Anopheles* mosquitoes toward traps (Smallegange *et al*., 2005; Logan and Birkett, 2007; Pask *et al.,* 2011). It is therefore foreseen that by understanding the role of odour stimuli in human vector interaction, one can design a contaminating

device (with insecticide and chemo-attractants) to lure and kill female *Anopheles* mosquitoes and thus protect the human host from mosquito bite (Okumu *et al.,* 2010a; Homan *et al.,* 2016).

Another behaviour used by mosquito in blood feeding is time orientation, which is highly modulated by Zeitgeber time cycle (Githeko *et al*., 1996). Zeitgeber cycle explains how external environmental cue coordinates mosquito's biological rhythms towards 24-hour light/dark cycle (Sharma, 2003). Other environmental oscillation such as humidity, temperature and food availability have been reported to modify Zeitgeber rhythms (Githeko *et al*., 1996; Sharma, 2003). In blood meal foraging, female *Anopheles* species exhibit different biting behaviours. Some *Anopheles* mosquitoes are crepuscular (active during the dawn and dusk) while others are nocturnal (active at night) (Pates and Curtis, 2005). In sunny habitats, biting hours occur around dusk, dawn and at night because the air is more humid and light intensity is low (Githeko *et al*., 1996). For instance, Afro tropical malaria vectors such as *An. gambiae* s.s have a tendency to bite at midnight (Geissbühler *et al.,* 2007; Kabbale *et al.,* 2013). However recent studies have shown changed biting and resting behaviour of these species due to selective pressure brought by insecicides applied on nets and walls which cause mosquitoes to avoid the existing vector control tools such as ITNs and IRS(Pates and Curtis, 2005; Geissbühler *et al.,* 2007; Reddy *et al*., 2011; Russell *et al.,* 2011; Cooke *et al.,* 2015).

2.3 Existing Vector Control Tools

Vector control is an essential component of malaria prevention and control as recommended by WHO global malaria control programmes. The principal goal of malaria vector control is to reduce the vectorial capacity within the community by providing cost effective interventions which will confer individual physical barrier against infective

mosquito bites (License *et al.,* 2011; Kim *et al.,* 2012; WHO, 2015). There are three main control strategies which are used in malaria vector interventions namely i) Reducing human-vector contact, ii) Reducing adult mosquito abundance and iii) Larval control (Betson *et al.,* 2009). In the first two control strategies LLINs / ITNs and IRS are commonly applied to reduce vector daily survival rates (WHO, 2015) whereas larval control is used to supplement the major interventions. Key considerations for selection of these strategies in a given epidemiologic setting are focused on affordability, accessibility and acceptability to local community (Enayati and Hemingway, 2010; WHO, 2015).

Insecticide-treated bed nets (ITNs), wearing protective clothing, and use of mosquito repellents have been used to reduce human-vector contact by preventing the mosquito from feeding on human (Bayoh *et al.,* 2010; Russell *et al.,* 2010; Mutuku *et al.,* 2011). There are two important categories of insecticide -treated bed nets namely conventional insecticide-treated bed nets (ITNs) and long lasting insecticide-treated nets (LLINs) (WHO, 2014). The former is the mosquito net that has been impregnated by dipping the nets into the insecticide. The effectiveness of these nets is attained when the net is re-treated after two to three time washes in a year. The latter, (LLINs) are factory-treated mosquito bed nets that have insecticide incorporated within the fibers. These nets retain effectiveness for at least three years, so they do not need re-treatments (WHO, 2015). It is only recently that WHO has recommended the use of long lasting insecticide-treated nets (LLINs) rather than conventional insecticide- treated nets (ITNs) because LLINs eliminate the need of re-treatment, thus reducing insecticide use in the community and minimize the environmental hazards occurring during re-treatments of conventional ITNs (WHO, 2014).

LLINs/ITNs have shown greater success in combating malaria and have been associated with the decrease of malaria prevalence in the countries where LLINs/ITNs coverage is high (WHO, 2015). This is achieved firstly, by providing personal protection to individuals who sleep under treated bed nets (individual level protection) and secondly, by conferring extended effect to an entire area (community or mass effect) by killing mosquitoes that contact the net thus affecting the vector population and lowering transmission intensity in the community regardless of whether or not people are using LLINs/ITNs (WHO, 2015). The community wide use of ITNs/LLINs shortens the life span of female mosquitoes and therefore the mosquitoes will not survive long enough to transmit parasites (Kulkarni *et al.,* 2007; License *et al.,* 2011).

In sub-Saharan regions of Africa, The President's Malaria Initiatives (PMIs)-ITNs through RBM has scaled up the use of LLINs by increasing accessibility to the most effective and affordable protective measures against mosquitoes i.e. use of ITNs and LLINs (WHO, 2015). These have been achieved through distribution of LLINs in all malaria endemic regions, ensuring that everyone in the community is protected by these nets. Where the supplies are constrained, the nets should be given to the most vulnerable group such as under-five-year old children and pregnant women (RBM, 2008). It is estimated that worldwide a cumulative 1.2 billion fewer malaria cases and 6.2 million fewer malaria deaths have occurred from 2001 to 2015 due to utilization of malaria control intervention. Of these 69% were averted due to use of LLINs/ITNs, 21% due to artemisinin-based combination therapy (ACT) and 10% due to IRS (WHO, 2015).

In Tanzania free distribution of LLINs to risk population began in late 2008 through Tanzania National Voucher Scheme (TNVS) or "*Hati Punguzo"* programme (THMIS, 2008). Currently LLINs are distributed free of charge to all households by specific health

campaign such as universal coverage campaign (UCC) which was last carried out in 2015. These nets were distributed to households by Ward Executive Officers (WEO) (MoHSW, 2016 unpublished data). It has been reported that across Tanzania 91.5% of households have received one or more LLINs for free as part of a campaign while 8.5% of household purchased bednets from retail shops, vendor or market (Acheson *et al.*, 2015; MoHSW, 2015). Although there was scaling up of ITNs/LLINs, their use confer full personal protection to individuals who sleep under treated mosquito nets and exclude those who are active outdoors or do not use nets at all (Reddy *et al*., 2011; Russell *et al*., 2013; Killeen, 2014).

Indoor residual spraying (IRS) is another mosquito control intervention whereby insecticides are sprayed on the interior surface of human dwellings where mosquitoes tend to rest after taking blood meal (Lahondère and Lazzari, 2012). The main effect of IRS is to kill mosquitoes which are entering the house and resting on the sprayed surface long enough to pick up the fatal dose of insecticide. When IRS is implemented properly, it will provide protection to community through rapid mass effect on mosquito population by reducing mosquito density and vectorial capacity to transmit malaria parasites (Lahondère and Lazzari, 2012). The impact of IRS in reducing incidence of malaria in a targeted area can be seen when at least 85% of households are sprayed with insecticide and in addition repeated spraying is made mandatory to maintain effectiveness against mosquitoes (WHO, 2008). The frequency of spraying is determined by insecticide used. For instance Lambda cyhalothrin needs to be repeated after every six months (WHO, 2008).

In Tanzania the application of IRS has been adopted to complement the scaling up of LLINs/ITNs in epidemic prone districts of mainland Tanzania while in Zanzibar almost

the whole island were covered with IRS (THMIS, 2012). In mainland Tanzania, IRS was applied Muleba whereby about 92% of houses were sprayed with IRS. In 2010 the programme was extended to other regions in Lake Zone e.g Geita. The percentage of households covered by IRS was 40% in Mwanza 50% in Geita and 61% in Mara. Other parts of mainland Tanzania, except Eastern zone which received 10% of IRS coverage, received only 1% IRS intervention (THMIS, 2012).

Decline in malaria prevalence has been reported in places where IRS and LLINs were used in combination. For instance in 2008 Kagera Region had malaria prevalence of 41.1% (THMIS, 2008) but by 2012 prevalence had dropped to 8.3. Furthermore the Ministry of Health and Social Wellfare reported the mortality rate among under-five-year old children in Tanzania had dropped by nearly 60% as a result of IRS and ITNs use (MHoSW, 2012). Although this intervention is highly effective in reducing malaria transmission in area where these interventions were implemented properly, there has been reported failure due to substantial insecticide avoidance by malaria vector by creating a new behaviour of feeding and resting outdoors (Mboera *et al.,* 2013). IRS which is an intradomiciary tool was developed to reduce malaria parasite transmission in areas where the primary vectors feed and/or rest indoors is not effective against outdoor *Anopheles* mosquitoes (WHO, 2008; Kleinschmidt *et al.,* 2009; Pluess *et al*., 2010).

Space spraying is another technique used to control adult mosquitoes. The insecticide application is in form of fog (referred to as an aerosol). This fog which is in aerosol/liquid form is dispersed into the air by using fogging machine or mist blower (Conlon, 2011). The insecticide destroys flying mosquitoes when they come into contact with insecticide in the air. This method is used when there is high mosquito density during epidemics/outbreaks (Barber *et al.,* 2007; Lothrop *et al.,* 2007). The main advantages of

space spraying are immediate effect on the adult mosquitoes, less labour required, large area can be treated fairly quickly, less insecticide used, and it kills exophagic/exophilc mosquitoes (Esu *et al.,* 2010). This technique has been used in epidemics if there is sufficient evidence that the main causal factor is abnormal density of exophagic and exophilic malaria vectors (Conlon, 2011). The spray is designed for rapid knock down, rapid mortality and rapid control of epidemics and emergence situation e.g. in refugees camps. The insecticides such as Malathion and Fenthion are usually sprayed in and around houses, in the open space where outdoor vectors can be found (Barber *et al.,* 2007). Similarly, in the households aerosol spray (can spray) has been used to repel and kill mosquitoes which entered the house (Fradin and Day, 2002). Unlike fog spraying, aerosol can-spray is confined to sprayed house with limited ventilation and its effect would either paralyse and kill (knock down effect) and/or deter mosquitoes from entering a room/house (excite-repellent effect) (Barnard and Xue, 2004). Commonly used insecticides in aerosol spray-cans are synthetic pyrethroids such as Sumithrin, Transfluthrin, and S-bioalletrin (WHOPES, 2013). However, even natural pyrethroids are reported to be used as household aerosol insecticide. In poor resource countries use of aerosol can-spray has been reported to be more expensive therefore only few families can afford to use this method therefore most the time they use mosquito coils (Fradin and Day, 2002).

Mosquito coils confer protection against mosquito bite through the spatial action of emanated pyrethroids vapor particles (Ogama *et al.,* 2012). This control tool inhibits the action of olfactory receptor of mosquitoes thus interferes the host seeking behavior as a result mosquitoes are repelled and failed to feed the host (Smith *et al.,* 1972; Moore *et al.,* 2007; Bohbot *et al.,* 2011). Although mosquito coils are relative cheap as compared to

other emanators such as vaporizer mats, smoke has been reported as undesirable experience among users (Hudson *et al.,* 1971; Liu *et al.,* 2003).

Mosquito larval control is an effective tool in integrated vector management (IVM) strategies for reducing malaria transmission. (Utzinger *et al.,* 2001; Keiser *et al.,* 2005) Larval source management (LSM) is one of most efficient tools in integrated vector management (IVM) which is used to prevent vector-borne diseases such as malaria through management of breeding sites that are few, fixed and findable (Barat, 2006). Unlike LLINs and IRS which target the adult mosquito, LSM targets the immature aquatic stages of the mosquito (the eggs, larvae and pupae), thereby reducing the abundance of adult vectors (Killeen *et al.,* 2002). This approach attempts to complement the existing malaria vector control (ITNs & IRS) which has been traditionally the mainstay vector control method for many decades (Killeen *et al.,* 2002; Barat, 2006; WHO, 2015). There are two types of larval source management which have been used to prevent completion of immature stage of mosquito. These types are: i) larval source reduction by environmental management (modification and manipulation) ii) larviciding (Walker and Lynch, 2007).

Environmental management for larval control refers to the planning, organization and monitoring of activities which modify environmental factors related to vector breeding habitats (Walker and Lynch, 2007). These breeding sites can be natural or manmade and can range from shallow open sunlit pools to water bodies such as drains, brick pits, hoof prints, pools, banks of rivers and lakes (Service, 2000; Edillo *et al.,* 2006). The aim of environmental management is to reduce the numbers of mosquito larvae and pupae and ultimately decrease the population of both indoor and outdoor malaria vectors (Walker and Lynch, 2007).

Environmental modification occurs when there are permanent changes in the land, water or vegetation. For instance, filling of breeding sites with soil, stones and proper water drainage by construction of open waterways with tidal gates, land reclamation, landscaping are approaches used in environmental modification (Walker and Lynch, 2007). Environmental manipulation is temporary method which needs to be repeated over and over. For example increasing the flow of streams, ditches, covering up empty containers and cans, flushing, drain clearance to eliminate pools and removal of vegetation and shading are important ways used to prevent mosquito aquatic habitats (Walker and Lynch, 2007). Female mosquitoes do not always need big swamps, ponds or other big water bodies to lay their eggs; even small amounts of water collected eg, cans, hoof prints and tyres can serve as breeding sites.

In Tanzania, environmental management has been implemented in urban settings e.g in Dar es Salaam, where construction waterways gates and manipulation of breeding sites in human settlements was carried out throughout the eradication era (1970s to 1980s), although it was eventually neglected due to economic crisis (Bang *et al.*. 1977; Castro *et al*. 2004). However recent studies have suggested that environmental management should gain more attention and should be incorporated in existing malaria vector control tools (Gu *et al.,* 2006).

Larviciding is another tool in larval source management which uses chemicals, biological agents or toxins to kill mosquito larvae (Castro *et al.*, 2010). For water collections that cannot be dealt with by environmental manipulation, larviciding is the best alternative of controlling immature stages of mosquitoes in fixed and findable breeding habitats (Awolola *et al.,* 2007; Castro *et al.,* 2010). A successful larviciding depends on spatial distribution and identification of mosquito breeding sites in the given geographical area

and requires repeated spraying of the chemicals into those aquatic habitats. Moreover, larviciding can be effective when the area is densely populated with fixed and findable breeding sites e.g. in towns and cities (Lynch, 2007; Maheu-Girouxand Castro, 2014).

Larviciding entails the killing of mosquito larvae at their breeding sites before they emerge into adult mosquitoes. It utilizes chemicals, toxins or biological agents including those which prevent mosquito metamorphosis (Awolola *et al.*, 2007; Castro *et al.,* 2010) Several compounds have been used to kill mosquito larvae and pupae. These include crude oil, kerosene, temephos, chlorpyriphos, and pirimiphos-methyl (Barber *et al.,* 2007). Moreover biological control such as use of *Gambusia* and the spore-forming bacteria *Bacillus thuringiensis* var. *israelensis(Bti)*and *B. sphaericus (Bs)* have been shown in many parts of the world to kill mosquito larvae with great success (Fillinger and Lindsay 2006; Majambere *et al.,* 2007; Shililu *et al.,* 2007). One study done in lowland and highland Kenya showed that microbial larvicides reduced *Anopheles* larvae density by 95% with concomitant reduction of mosquito bite by 90% (Fillinger and Lindsay, 2006).

Currently in Tanzania, larviciding has been practiced in urban setting such as in Dar es Salaam where a large scale community-based larviciding is carried out by the Urban Malaria Control Programme (UMCP) (Vanek *et al.,* 2006; Fillinger *et al.,* 2008; Chaki *et al.,* 2009; Maheu-Giroux and Castro *et al.,* 2014; Chaki *et al.,* 2014). The UMCP uses microbial larvicides of spore-forming bacteria origin, (*Bacillus thuringiensis* var. *israelensis (Bti), B. sphaericus (Bs)* which are sprayed on the entire water surfaces found in the communities (Chaki *et al.,* 2014; Maheu-Giroux and Castro *et al.,* 2014). Chaki *et al.,* (2014) reported great contribution of microbial larviciding in reducing malaria

transmission in Dar es Salaam and rural areas of Tanzania (Fillinger *et al.,* 2008; Magesa *et al.,* 2009; Kramer *et al.,* 2014; Mboera *et al.,* 2014; Rahman *et al.,* 2016).

Oviciding is a preventive method which is used to interrupt the development of eggs to larvae and as a result, development of other stages i.e pupae and adults is inhibited (Mullai and Jabanesan, 2006; Panneerselvam and Murugan, 2013; Govindarajan and Sivakumar, 2014). For many years plant extracts, essential oils, and bioactive compounds from several plants species have been proven to release toxicants which halt the growth of immature stages of mosquito vectors (Sukumar *et al.,* 1991; Pushpalatha, 2001; Muthukrishnan and Pushpalatha, 2001; Rajkumar and Jebanesan, 2007). These plant extracts kill mosquito eggs (ovicidal) and/or larvae (larvicidial) ultimately reducing mosquito population density (Govindarajan *et al.,* 2008; Khandagle, 2011).

In recent years many researches in vector control have focused more on interrupting immature stages (e.g. prevent hatchability) using plant derivatives which are environmentally safe (Mullai and Jabanesan, 2006; Rajkumar and Jebanesan, 2007; Rahuman *et al.,* 2008; Elimam *et al.,* 2009). Studies conducted in Middle East have shown that plant derivatives such as marine sponge (*Cliona celata*), *Ipomoea cairica L, Areca catechu, Nicotiana tabacum* have ovicidal effects on *C. quinquefasciatus, A. aegypti and An. stephensi* which cause zero hatchability to eggs exposed to the mentioned plant extracts (Mullai and Jabanesan, 2006; Pushpanathan *et al.,* 2006; Govindarajan *et al.,* 2011; Govindarajan and Karuppannan, 2011; Panneerselvam and Murugan, 2013; Ahbirami *et al.,* 2014; Govindarajan and Sivakumar, 2014; Reegan *et al.,* 2015).

On the other hand, application of acid (e.g Vinegar which consist of 5–20% acetic acid - CH_3COOH) compound in aquatic environment (breeding site) for mosquito vectors alters

the pH of water, following which, the altered fluid ions limit the normal mosquito metamorphosis since for *Anopheles* mosquitoes this takes place within the pH ≤ than 4 (Sutcliffe and Hildrew, 1989; Vangenechten *et al.,* 1989; Clark *et al.,* 2004). One study conducted by Clement (2000) reported that when the pH of aquatic habitat for mosquitoes is kept low (pH< 4) or high (pH > 11) immature mosquitoes will not develop due to inactivation of metabolic enzymes (such as choriolytic enzyme). Similar findings were reported by Truchot (1987); Clark *et al.* (2004) and Thamer and Abdulsamad (2005).

Additionally, Thamer and Abdulsamad (2005) reported the hatchability of mosquito eggs and larval survival (with exception of e.g *An. merus* which is salt-tolerant) is not only affected by low or high pH but also sodium chloride concentration (NaCl). These compounds (low pH, high concentration of NaCl) deactivate metalloenzyme e.g. carbonic anhydrase, the enzyme that play a fundamental physiological role in regulation of ions in breeding habitats. Therefore when the ambient pH for aquatic habitat is disturbed by being made more acidic (low pH), its effects will prevent hatchability process thus mosquito eggs will not develop to larva, pupa and adult, ultimately reducing the mosquito population density (Patrick *et al.,* 2002). For this reason, acid (vinegar) can be used as ovicide and prevent egg hatching consequently hindering completion of aquatic life cycle of *Anopheles* mosquito.

Vinegar sour testing liquid containing acetic acid (CH_3COOH) produced from fermented ethanol which has a pH range from 2-4 (Natera *et al.,* 2003). Vinegar is a popular ancient chemical compound which has been used as medicine (cleaning wounds) (Rund, 1996; Jung *et al.*, 2002), disinfectant and antiseptic (Rutala *et al*., 2000), and in food preservative (Natera *et al.,* 2003, and culinary (Sengun *et al.,* 2005; Sugiyama *et al.,* 2003). At concentration of ≥ 0.0025% of acetic acid solution is effective at inhibiting the

growth microorganism of *Escherichia coli*, group D *Enterococcus, Bacteroides fragilis, Staphylococcus aureus*and *Pseudomonas aeruginosa* (Dohar *et al.,* 2003). Similarly at concentration of 2% acetic acid solution at pH 2, vinegar inhibits egg hatching of *Anopheles* and *Aedes*, mosquitoes (Clark *et al.,* 2004)

Application of vinegar (2% acetic acid (-CH_3COOH) compound in aquatic environment (breeding site) alters the pH of water, following which, the altered fluid ions limit the normal mosquito metamorphosis since for *Anopheles* mosquitoes this takes place within the pH ≤ than 4 (Sutcliffe and Hildrew, 1989; Vangenechten *et al.,* 1989; Clark *et al.,* 2004). One study conducted by Clement (2000) reported that when the pH of aquatic habitat for mosquitoes is kept low (pH< 4) immature mosquitoes do not develop to adult mosquitoes due to inactivation of metabolic enzymes (such as choriolytic enzyme), similar findings were reported by Truchot (1987); Clark *et al.* (2004) and Thamer and Abdulsamad (2005). Nonetheless, vinegar (acetic acid compound) deactivates metalloenzyme e.g. carbonic anhydrase, the enzyme that play a fundamental physiological role in regulation of ions in breeding habitats. Therefore when the ambient pH for aquatic habitat is disturbed by being made more acidic (low pH), its effects will prevent hatchability process thus mosquito eggs will not develop to larva, pupa and adult, ultimately reducing the mosquito population density (Patrick *et al.,* 2002).

2.4 Insecticides and Insecticide Resistance

WHO has recommended four chemical classes of insecticides namely; carbamates (e.g Bendiocarb), organophosphates (e.g OP pirimiphos methyl), organochlorine (e.g. DDT, dieldrin) and pyrethroids (e.g. Deltamethrin, Lambda-cyhalothrin, Alpha cypermethrin) to be used in malaria vector controls (IRAC, 2010; Rivero *et al.,* 2010). There are many insecticides which belong to these chemical classes but only twelve insecticides (which

belong to all four chemical classes) have been approved by WHO to be used for IRS application, while only six insecticides (belonging to pyrethroid class alone) have been approved for LLINs/ITNs use (WHOPES, 2014).

Furthermore all these insecticides work by targeting receptors or enzymes in the nervous system of *Anopheles* vectors i.e. acetylcholinesterase (AChE), the voltage-dependent sodium channel (NaV), and gamma-Aminobutyric acid (γ-aminobutyric acid (GABA) (Fukuto, 1990; Rivero *et al.,* 2010). Toxicity of carbamates and organophosphates is caused by their ability to inactivate acetylcholinesterase (AChE) an enzyme responsible for hydrolysis of neurotransmitting agent acetylcholine (Ach) to choline and acetic acid at the cholinergic nerve synapse of the insect. When AChE is inactivated by organophosphates or carbamate ester, concentration of acetylcholine in the nervous junction will remain high. Continuous stimulation of the muscle or nerve fiber due to accumulation of acetylcholine cause nerve paralysis and eventually knock down of the insect (Fukuto, 1990; Rivero *et al.,* 2010). Pyrethroids and DDT on the other hand act on the voltage-gated sodium channel protein found in insect nerve membranes. DDT acts on peripheral nervous system while Pyrethroids act on both peripheral and central nervous systems. When the insecticide comes in contact with the mosquito, bind delays the closing of the sodium channel as a consequence of which neurotransmitters are released continuously into neuromuscular junction leading to prolonged action potential causing repetitive neuron firing, twitching, paralysis and subsequent death of the insect (knockdown) (Davies *et al.,* 2007; WHOPES, 2014;).

To withstand the effect of insecticides,*Anopheles* mosquitoes have developed different types of resistance including knockdown resistance (*kdr*) which enable them to survive even when they are exposed to insecticides (Hemingway *et al.,* 2002; Czeher *et al.,* 2008;

Russell *et al.,* 2011). By definition, insecticide resistance is the ability of an insect population to withstand the effects of an insecticide by becoming resilient to its toxic effects by means of natural selection and mutations as a result of which, the insecticide fails to provide the intended level of control (IRAC, 2011). The resistant strains have mutation at the main binding targets (AChE, CNaVdp andγ-aminobutyric acid) resulting in cross resistance to all insecticide acting on the same target. Once the main targets are altered by mutation, insecticide will not be able to interact with binding site and thus the insecticide will be ineffective (Hemingway *et al.,* 2002; Czeher *et al.,* 2008; Wondji *et al.,* 2009; Gatton *et al.,* 2013).

There are three major mechanisms which are responsible for mosquito insecticide resistance: i) resistance by modifying the target site of insecticides: this occurs when mosquitoes possess *kdr* mutation genes which change target site (NaV), consequently reduce the ability of insecticide to bind on the sodium voltage gated channels hence endure prolonged exposure to insecticide without being knocked down (Davies *et al.,* 2007; Ndjemai *et al.,* 2009; IRAC, 2011) ii) Metabolic resistance which occurs when there is an increased activity of one or more enzymes (e.g cytochrome P450) as a result the insecticide is detoxified before it reaches the target site thereby impairing toxicity effect of the insecticide (Ranson *et al.,* 2002; Feyereisen *et al.,* 2005) and iii) Behaviour resistance which refers to any modification/adaptation to mosquito behaviour that facilitates the avoidance of contact with the insecticides as a result of which the mosquito will not pick the required lethal dose (Geissbühler *et al.,* 2007; Czeher *et al.,* 2008; Russell *et al.,* 2011; Reddy *et al.,* 2011; Russell *et al.,* 2013; Killeen, 2014).

Many studies have reported that use of insecticides in agriculture practice and constant use of insecticide /over reliance on a single class of insecticide, the pyrethroids have

resulted in insecticide resistance (Zaim *et al.,* 2000; Oduola *et al.,* 2012; Aïzoun *et al.,* 2013). The synthetic pyrethroids which have killing and excito repellent properties kill or deter mosquitoes from entering inside the house and consequently influence the mosquitoes to change their resting and feeding behavior (Bayoh *et al.,* 2010; Reddy *et al.,* 2011; Russell *et al.,* 2011). In many places throughout Africa a reduced indoor biting by *Anopheles* mosquitoes has been reported to be due to constant use of ITNs/ LLINs and IRS (Killeen, 2014). Malaria vectors such as *An. gambiae s.s* and *An. funestus* have shown substantial change in some areas by feeding early in the evening before people have retired to bed where they could be protected by insecticide- treated bed nets (Mathenge *et al.,* 2001; Reddy *et al.,* 2011).

Recent studies have reported pyrethroids resistant populations of *An. gambiae* complex and *An. funestus* in West Africa (e.g. Burkina Faso, Nigeria, Ghana) (Yawson *et al.,* 2004; Corbel *et al.,* 2007), East Africa (Tanzania, Kenya, Uganda) (Kulkarni *et al.,* 2007; Verhaeghen *et al.,* 2010; Ranson and Lissenden, 2016), Central Africa (Etang *et al.,* 2007), Mozambique (Coleman *et al.,* 2008), Sudan (Abdalla *et al.,* 2008) Ethiopia (Balkew *et al.,* 2010) and South Africa, Zambia and Zimbabwe (Choi*et al.,* 2014; Hargreaves *et al.,* 2000; Munhenga *et al.,* 2008). Similarly, carbamate and organophosphate resistant populations of *An. gambiae* have been reported in West Africa (Corbel *et al.,* 2007; Namountougou *et al.,* 2012; Aïkpon *et al.,* 2013).Therefore due application of the insecticides, malaria vectors have adopted the mechanisms (physiological and behavioural resistance) to evade insecticide applied on LLINs/ITNs and IRS

2.5 Residual Malaria Transmission

Residual malaria transmission refers to all forms of malaria transmission that persists despite high coverage of good quality LLINs and IRS. The main cause of residual malaria transmission is reported to be due to outdoor feeding behaviour of malaria vectors which could be brought by insecticidal pressure observed in malaria vector interventions (Martin-Campos *et al*., 2012; Chareonviriyaphap *et al*., 2013; Russell *et al*., 2013; Killeen, 2014). Most of malaria vectors have adopted survival avoidance behavior, which permit the vector either avoid or enter but safely leave the house protected with LLINs and or IRS and allow maximizing their feeding probability of continued searching until they find unprotected host indoor or outdoor (Russell *et al*., 2013; Killeen and Chitnis, 2014). Once *Anopheles* mosquitoes encounter unprotected victim in the house sprayed with IRS they bite them and rapidly exit the house soon after the blood meal and rest outdoor (Reddy *et al.,* 2011; Kitau *et al.,* 2012; Russell *et al*., 2013). In some areas where malaria is endemic and outdoor early biting *Anopheles* mosquitoes are prevalent, the risk of contracting malaria is high because *Plasmodium falciparum* annual entomological inoculation rate (aPfEIR) among individuals is also high (Kitau *et al.,* 2012). Thus residual malaria transmission has been a challenge to malaria control programmes by rendering malaria elimination to be extremely difficult in the absence of new vector control strategies (Campos *et al.,* 2012; Russell *et al*., 2013; Chareonviriyaphap*et al.,* 2013; Killeen, 2014). Figure: 2 show the conceptual framework of residual malaria transmission caused by changed feeding behaviour of *Anopheles* mosquitoes.

Traps and contaminating devices for malaria vector control

Over the years, there have been some considerations of mosquito control using techniques which attract and kill outdoor host-seeking vectors (Okumu *et al.,* 2010a; Okumu *et al.,* 2013; Mmbando *et al.,* 2015). Most recently, luring and killing using odour-baited traps

against outdoor host-seeking mosquitoes to complement LLINs were reported to be successfully used in Kenya (Rapley *et al.,* 2009; Ritchie *et al.,* 2009; Hiscox *et al.,* 2012; Okal *et al.,* 2013). Similar methods of luring and killing other blood sucking insects such as tsetse flies (Knols *et al.,* 1993), horse flies (Day and Sjogren, 1994) and crop pests (Copping and Menn, 2000) have been done and shown great achievements of reducing population density of these insects

Studies done in Ifakara, Tanzania by Matowo *et al.,* 2013 and Okumu *et al.,* 2010a reported catching outdoor mosquitoes by using odour baited traps (odour-baited Mosquito Landing Box (MLB) and odour baited station (OBS) .The studies used the same techniques of luring and killing outdoor feeding *Anopheles* mosquitoes by setting traps outside the human dwelling to protect human from mosquito bite while they are outside the houses. According to the study reports, the traps were able to catch different species of mosquitoes and with regards to number of *Anopheles* collected, the traps caught a large number of *An. arabiensis* followed by *An. funestus* whereas other mosquitoes caught were *Culex* and *Mansonia* species (Okumu *et al.,* 2010a; Matowo *et al.,* 2013). Although the traps had potential of catching outdoor mosquitoes in the communities, the devices and reagents were a bit expensive prompting the authors to suggest the use of cost effective devices with affordable attractants and killing agents which are environmentally safe, long lasting and have different mode of action other than pyrethroids which are used on LLINs/ITNs to reduce the risk of cross resistance (Matowo *et al.,* 2013).

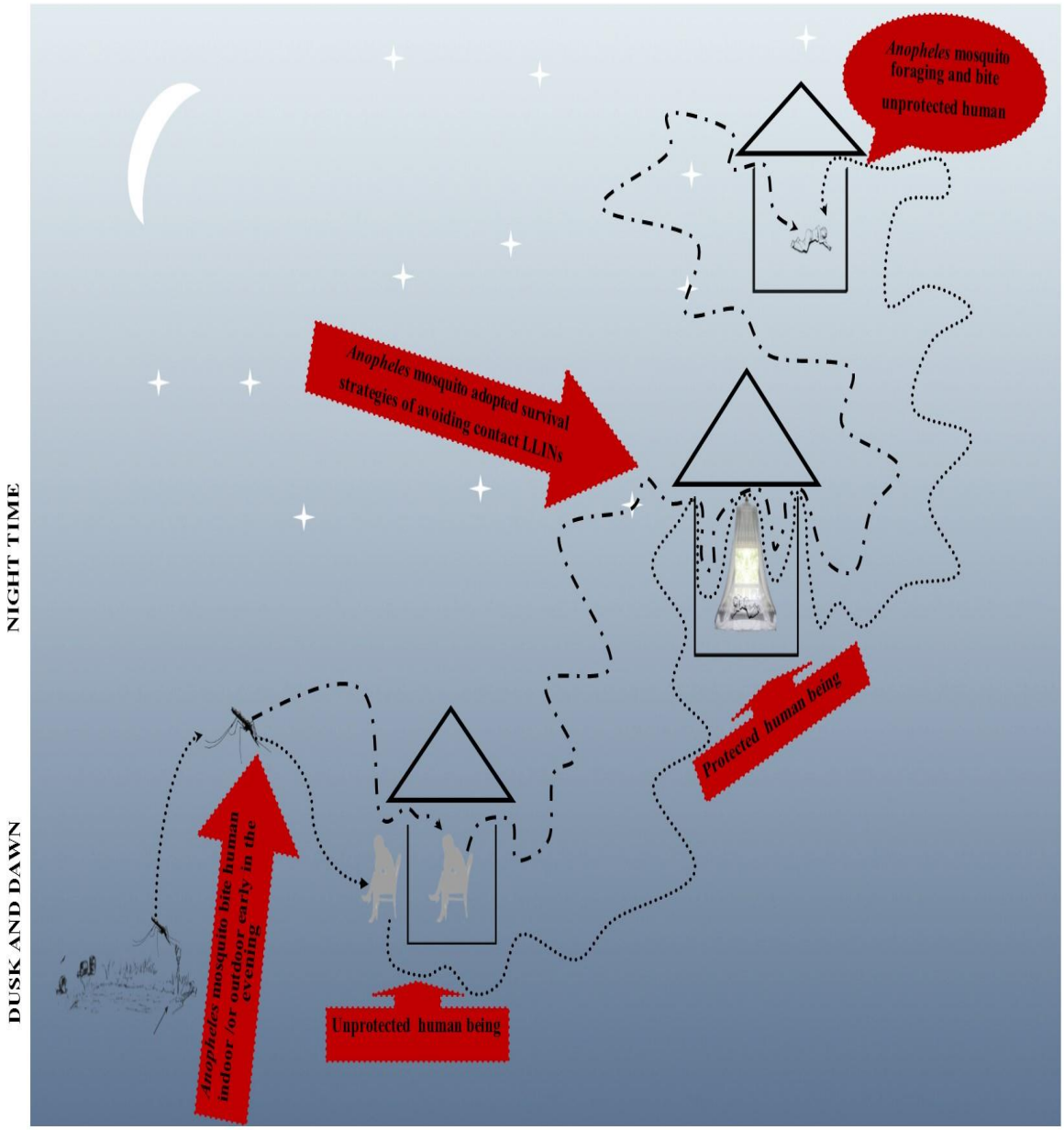


Figure 2: Modified conceptual framework of residual malaria transmission caused by changed feeding behaviour of *Anopheles* mosquitoes (Source: Killeen, 2014).

2.6 Potential Insecticide Resistance Management Strategies

Several strategies have been used in Insecticide Resistance Management (IRM) to delay the spread of insecticide resistance in malaria vectors. These strategies are basically based on IRS intervention since the strategies for LLINs are still limited (WHO, 2015). There are four main IRM strategies for IRS intervention namely; rotations of insecticides, combination of interventions, mosaic spraying and use of mixtures (WHO, 2009).

In rotation of insecticides two or more insecticide with different mode of action are rotated from one year to the next while in combination of interventions, two or more insecticide-based interventions (with different classes or mode of action) are used in the same house. For mosaic spraying, one insecticide is used in one geographical area and other insecticide with different classes or mode of action will be used in neighbouring area while use of mixtures, is operated by mixing of two or more insecticides with different classes to make up a single product or formulation resulting in the mosquito coming into contact with two insecticides at once (IRAC, 2011). The above-mentioned IRM strategies are for IRS intervention but for LLINs intervention the only available IRM option is combination of interventions (i.e. LLINs and IRS) (N'Guessan *et al.,* 2010; Raghavendra *et al.,* 2010).

Strategies of using chemical synergist in insecticide resistance management have proven to be successful against resistant mosquitoes. However the cost of adopting and implementating IRM has been a biggest challenge (Thomas *et al.,* 2012; N'Guessan *et al.,* 2010; Brown*et al.,* 2013). Additionally, cross resistance between insecticides and multiple resistance strains of mosquitoes have been reported, which threaten the effectiveness of resistance management strategies (Nauen, 2007).

The use of late acting insecticides such as entomopathgenic fungi e.g *Metarhizium anisopliae* and *Beauveria bassiana* has been suggested to be used in malaria vector control intervention as mosquitoes resistant to insecticdes are vulnerable to fungal infection (Read *et al.,* 2009; Lynch *et al.,* 2012). Furthermore insecticide with different mode of action of the recommended four chemical classes of insecticides such as chlorfenapyr can be used in malaria vector control to delay evolution of insecticide resistance (Mosha *et al.,* 2008; Reddy *et al.,* 2011).

Chlorfenapyr is an insecticide belonging to a pyrrole class which has been used to control termites and other insects in different parts of the world (Lovell *et al.,* 1990; Pimprale *et al.,* 1997; Sheppard *et al.,* 1998). It acts by targeting the oxidative pathway in the mitochondria of the insect resulting in disruption of ATP production and as a consequence the insect will lose energy leading to cell dysfunction then death (WHOPES, 2013). This insecticide has low mammalian toxicity and is classified by WHO criterion as slightly hazardous insecticide (Tomlin, 2000; WHOPES, 2013). Due to different mode of action Chlorfenapyr cannot cause cross resistance as is the case with other neurotoxic insecticides such as pyrethroids and DDT (Pridgeon *et al.,* 2008; Oliver *et al.,* 2010).

Several studies have been carried out in experimental huts (in Benin, Tanzania, Gambia, India) to test the efficacy and toxicity of Chlorfenapyr against pyrethroid resistant strains of *Anopheles* mosquitoes. These studies showed that Chlorfenapyr causes high mosquito mortality rates compared to synthetic pyrethroids (Mosha *et al.,* 2008; Pridgeon *et al.,* 2008; Oliver *et al.,* 2010; Oxborough *et al.,* 2010; Ngufor *et al.,* 2011). Large scale experiments were carried out in some villages of India, Gambia, and Benin to evaluate and compare the residual effects, toxicity and efficacy of Chlorfenapyr on sprayed walls. The results showed that *An. gambiae* complex were susceptible to Chlorofenapyr with 72

hour mortality rate of more than 85% (Raghavendra *et al.,* 2011). Therefore WHOPES (2013) has recommended Chlorfenapyr as less toxic insecticide able to kill pyrethroid and DDT resistant mosquito populations. The insecticide has been registered in 19 countries for the control of various arthropods including bedbugs, mite pests on cotton, ornamentals and a number of vegetable crops (Rand, 2004). Chlorfenapyr is also suggested to be good insecticide for malaria vector control in areas with pyrethroid-resistance (Mosha *et al*., 2008; Raghavendra *et al.,* 2011).

2.7 Community Knowledge on Malaria and its Vectors

Families within the communities are the primary context within which most health problems and illnesses occur and they have a powerful influence to make informed health choices in issues concerning health e.g. malaria (Steketee and Campbell, 2010). Community knowledge, perceptions and attitudes about malaria transmission risk, causation, symptom identification, treatment, and prevention influence efforts to address malaria infection (Deressa *et al.,* 2003). When the community lack enough knowledge on malaria and its vectors the impact will be seen in low utilization of available malaria control interventions (e.g ITN/IRS)(RBM, 2008). For instance, studies conducted in Ethiopia on knowledge, attitude and practice of the community towards malaria revealed that individuals who had low malaria knowledge did not participate fully in malaria control programs and consequently, malaria interventions were not sustainable in those communities (Dagne and Deressa, 2008; Baume *et al.,* 2009; Jima *et al.,* 2010; Abate *et al.,* 2013). On the other hand, high level of community knowledge on malaria has been reported to contribute towards the utilization of malaria control intervention and the overall impact in reduction of malaria burden (Adongo*et al.,* 2005; Mazigo *et al.,* 2010).

Knowledge on malaria and its vectors varies among communities and also among individuals within the households (Dagne and Deressa, 2008). In rural community (e.g Ethiopia, and Tanzania) knowledge and perception on mode of transmission and breeding sites has been reported to be low compared to urban settings (Jima *et al.,* 2010; Mazigo *et al.*, 2010; Abate *et al.,* 2013).

Research on knowledge, perception, attitude and practice on malaria has extensively been carried out but requires to be continuously carried out on account of continued changes on the malaria situation. For instance, there are reported changed biting patterns of malaria vectors but there is scant data on community knowledge and awareness regarding this behavior change in malaria vectors. Gauging of community knowledge on behavioral feeding patterns of *Anopheles* mosquitoes will inform measures to be taken to increase knowledge essential for new approaches to control such as personal protection against outdoor early biting mosquitoes.

Concluding remarks;

Most of malaria control programmes in the past decades have relied mainly on controlling the vectors. These programmes, in particular ITNs and IRS, have succeeded to avert 68% of malaria cases and reduce malaria morbidity and mortality in malaria endemic regions (Bhatt *et al.,* 2015) but due to complexity, bio diversity and behaviour change of vector *Anopheles* mosquitoes, reducing malaria deaths to near zero by 2015 has not been achieved (WHO, 2015). This implies that no single tool can be sufficient to control the disease successfully. Therefore there is a need to develop other tools which can complement the existing malaria control interventions (WHO, 2015).

This study therefore aimed to contribute to elimination of residual malaria transmission attributed to outdoor feeding *Anopheles* mosquitoes by development and evaluation of vector control methods that target immature stage of mosquitoes and those that lure and kill adult mosquitoes outdoors. The findings of the studies will add knowledge on malaria vector control tools which will be used against outdoor malaria vectors and ultimately lead to reduced malaria transmission.

CHAPTER THREE

3.0 MATERIALS AND METHODS

This chapter highlights the materials and methods used in this study. It focuses on the study sites where data were collected, and also explains in detail, sampling designs and data collection techniques for each objective. Finally the chapter clarifies how data was managed and analyzed.

3.1 Study Area

This study was conducted in two regions of Tanzania: Dodoma and Morogoro and at Macha Research Trust- MRT in Choma district Zambia. For the sites in Tanzania, Morogoro and Dodoma regions were conveniently selected to represent high and low malaria prevalence areas with permanent and seasonal mosquito breeding sites. Morogoro is among regions with high prevalence of malaria (13.0%) whereas Dodoma falls into regions with low malaria prevalence (2.5%) (THMIS, 2012). Thus studies to meet specific objectives one, two and three were conducted in Dodoma and Morogoro, Tanzania while some molecular analyses of samples (adult *Anopheles* mosquitoes) and activities for objective four and some molecular analysis were carried out at Macha Research Trust- MRT in Choma Zambia. The remained work on molecular identification of adult mosquitoes collected from Morogoro and Dodoma was carried out at Ifakara Health Institute (IHI) located in Morogoro Region Tanzania.

Morogoro municipality which has a population of 350,000 (NBS, 2012) is found in the eastern part of Tanzania, 169 kilometres west of Dar es Salaam the country's largest city and commercial centre and 223 kilometres east of Dodoma the country's capital city. Morogoro town (6°49’S and 37°40’E) lies at the foot of the Uluguru Mountains and an

average altitude of 522 m above mean sea level. It experiences short rains during December and January and long rains from March to June. Total average annual rainfall is 783.5 mm, mean relative humidity 72%, minimum temperature 22 °C, and maximum temperature 33 °C during wet seasons (December–May). During cold season (June-September) minimum and maximum temperatures are 15°C and 19°C maximum respectively.

Dodoma municipality located at 6°25'S and 35°75'E is 486 kilometres west of Dar es Salaam and has a population of 410,956 (NBS, 2012). It covers an area of 2,669 square kilometers of which 625 square kilometers are urbanized. It is semi-arid, with total annual average rainfall 478.4 mm, mean relative humidity 67%. During the wet season minimum and maximum temperatures are 22 °C, and 31°C respectively while in the cold season (June –September) the minimum and maximum temperature are to 13 °C 18°C. The town receives short rains in December and long rains from February to March.

Choma (16°49'S and 26°59'E), Zambia is the capital of Southern Province of Zambia connecting Lusaka and Livingstone. The city has a population of about 40,000 people (ZABS, 2010) and it serves as the commercial hub for central region of the Southern Province. The study was conducted in Macha (16°26'S and 26°47'E), 70 Kilometers from Choma district at Macha Research Trust (MRT). Since 2003 the Macha mission center established a strong malaria research center in collaboration with Johns Hopkins Bloomberg School of Public Health. This Malaria Research Trust has well-equipped entomological departments and laboratory for molecular assays.

In Macha, temperatures vary between 14°C and 28°C, the highest temperature occurring between the beginning of October and the end of December and the lowest temperature

being recorded in June and July while mean relative humidity is 77%. Average rains start in mid October and continue to the beginning of April. The mean rainfall is 800 mm per annum.

3.2 Methods

3.2.1 Assessing knowledge and awareness on malaria, malaria vector and community understanding on biting behavior change of *Anopheles* mosquitoes

3.2.1.1 Study design

The study was cross sectional survey whereby the sampling unit was a household. . A typical household was defined as a residential house owned or rented and occupied by one or more than one family.

3.2.1.2 Sample size estimation

The sample size was estimated using the following formula.

Formulae and assumptions

$$n = \frac{z^2 p(100 - p)}{\varepsilon^2}$$

Where:

n=Minimum sample size

Z= 1.96, that corresponds to 95% CI

ε= marginal error (0.5)

p= Prevalence of population with moderate knowledge on malaria and its vector was taken to be 50%

With this formula a total of 384 respondents were to be interviewed. However, to cover for non-responses it was planned to sample 400 respondents

To obtain the 400 prospective respondents, a total of 200 households would have been involved in the study but in the course of the study some households that had been targeted did not have a senior pupil to be interviewed. Therefore to attain a total of 400 interviewees, a total of 218 households were included in the study. In total 250 adults and 150 pupils were involved in the study as shown in Table 1.

Table 1: Study participants from each of study districts (Dodoma and Morogoro)

Study site	Number of households	Number of participants (n)	
Dodoma	106	Adults	136
		Pupils	64
Morogoro	112	Adults	114
		Pupils	86
Total	218		400

Sampling techniques

A systematic random sampling was adopted whereby the first household was chosen randomly and the subsequent units were obtained by calculating a sampling interval. The sampling frame was drawn from a list of households based on the last population census of 2012 as provided by the ward executive office. The sampling started by selecting one household from the list at random and then every k^{th} household (which was calculated from the formula-k=N/n) in the frame was selected where *k* was sampling interval, *N* was population size and *n* was the sample size. Household head or second in-charge and a senior primary school child who agreed to participate were involved in the study. In this study second in-charge was an adult who represented the household head when he or she was absent, and senior primary school child was an eldest pupil aged 10- 15 years.

3.2.1.3 Inclusion and Exclusion criteria

Inclusion criteria

Head or adult second in-charge of the house and senior primary school pupil who were available at the day of data collection and agreed to participate were involved in the study

Exclusion criteria

Head or adult second in-charge of household and senior primary school pupil who did not consent to participate in study were excluded from the study.

3.2.1.4 Data collection

A structured, pre-tested Kiswahili (as translated from English version) questionnaire consisting of open-ended and closed questions was used as data collection tool (Apendix 1 and 2). It was designed to be completed in the presence of an enumerator and collected after completion. For illiterate respondents, the enumerator carried out interviews using the same questionnaire. For each household, two persons namely the head or second-in-charge and the most senior primary school child who were available during the study were selected to fill the questionnaire. In order to gauge knowledge and awareness on malaria and mosquitoes, eight questions were asked. These questions were: what causes malaria, risk of malaria transmission, what is active mosquito biting time, potential breeding site, vector control, malaria symptoms, what to do when encountering those symptoms and identification of mosquito pictures. Questions on awareness of changed biting behaviour of *Anopheles* were focused on outdoor early mosquito bites in relation to malaria transmission and malaria vector controls. Primary school children had an additional question (available at the end of questionnaire) which sought to find out what health information (between HIV and malaria) they get more frequently at school. In a

given street, whole exercise of data collection was completed within one day to preclude, as much as possible, information sharing between respondents.

3.2.1.5 Data processing and analysis

Data collected were coded and entered into Excel then plugged into Epi Info 7 ready for data analysis. All questions of knowledge on mosquitoes and malaria and awareness of changed biting behavior of *Anopheles* mosquito were scored with one mark for correct answer, so as to categorize participants with 'good knowledge'. Zero was given for incorrect answers and those participants giving incorrect answers were categorized as having 'poor knowledge'. For awareness, participants who got correct answers were categorized as being 'aware' and those participants who got incorrect answers were categorized to be "not aware". Categorization of participants on knowledge and awareness were based on the cut-off point of average score which was 4 out of 8 for knowledge question and 3 out of 6 for awareness questions.

All data were summarized into proportions and cross tabulations were done to look for association between independent and dependent variables. Chi-square test (χ^2) was used to test for statistical significance and the association was considered statistically significant if p value was <0.05. Logistic regression analysis was performed to test the association between binary response variables and their corresponding explanatory variables.

3.2.2 Determining spatial and temporal distribution of adults and larvae of *Anopheles* mosquitoes in study areas

3.2.2.1 Study area

This survey was conducted in two regions, Dodoma and Morogoro as described in study area section. The data were collected in two seasons, cold-dry season from June to September 2014 and in hot-wet seasons from January to February 2015. In all two seasons larvae and adult mosquitoes were collected in same locations. Larvae were sampled from all encountered potential breeding sites whereas adult mosquitoes were collected inside and outside human dwellings during the study period.

3.2.2.2 Study design

The study was an ecological study of repeated cross-sectional type.

3.2.2.3 Sampling techniques

Purposively, two wards were selected in Dodoma and Morogoro urban based on presence of seasonal and temporary breeding sites (presence of artificial breeding sites, natural swamps and rice paddies) with local high incidence of malaria within the wards (MTUHA (a and b), 2014 unpublished data).

3.3.2.4 Data collection for larvae

Breeding site identification

Searching for water bodies in the areas under study was done by going around all streets of the wards. Ten-Cell leaders of these streets in the wards lead the research team into different places within the wards for identification and location of water bodies which may contain *Anopheles* mosquitoes. Once the water bodies were found to have *Anopheles* larvae, researchers recorded that water body as potential breeding site. Each potential

breeding site encountered in the study area was given a unique identification number and its position was recorded using hand-held Global Positioning System (GPS) device. Thereafter, classification of potential breeding sites in different habitat characteristics was done using visual observation and tape measure. The parameters such as water depth, habitat size, distance of water body to houses, water type, water current and surrounding land cover was recorded for every accessible potential aquatic mosquito habitat during study survey. Water depth was classified as shallow when the water level was <0.5m (e.g hoof prints, burrow pits, tyre tracks) and deep when it was >0.5m (e.g. ditches, holes). Habitats that had diameter of one meter or less were categorized as small breeding sites (e.g. water in containers, rain pools) whereas for those with diameter greater than one meter were categorized as large breeding habitats (e.g . rice paddies) (Sattler *et al.*, 2005)

Distance of the potential habitat to the human dwelling was measured by tape measure and three categories were recorded, <10m, 10-100m and >100m. Water types (clean or polluted), light intensity (full sunlight, partial sunlight and shaded) and water current (stagnant or slow flow) were recorded using visual perceptions. All visual classifications were done by the same person to maintain consistency. Lastly, the surrounding land cover around each potential aquatic habitat was recorded as long grasses, short grasses and type of vegetation e.g. rice. All these parameters were recorded on larval collection form (Appendix 4).

Larval collection

Sampling of *Anopheles* larvae were conducted in the morning around 0700 hrs to 1100 hrs during the study periods (June to September 2014 and January to February 2015). Larvae sampling was done using the standard dipping method with a 350ml mosquito scoop as described by Service (1993). Visual identification of presence of larvae and its

abundance in each dipping were used to describe the larvae density. When larvae were seen without dipping or when nearly every dipping contained *Anopheles* species that habitat was recorded as having high *Anopheles* larvae density and if dipping contained few *Anopheles* larvae the habitat was categorized as having low *Anopheles* larvae density. After collection, mosquito larvae were taken to the laboratory for *Anopheles* species identification**.** Positioning of the larvae parallel to the water surface and absence of siphon were used to identify *Anopheles* larvae. Pupae were excluded from the study as they could not be visually identified from the field. Thereafter larvae were taken to the insectary (with temperature of 28±5 ºC, relative humidity of 70-78%) and reared until they emerged to adult mosquitoes so that they could be identified by amplification of ribosomal DNA using polymerase chain reaction (PCR) (Scott *et al.*, 1993).

Data processing and analysis

Coordinates of potential breeding sites were entered into Geographical information system (ArcGIS-ArcInfo Version 9.1) software to plot the maps. These maps were used to localize and visualize all surveyed aquatic habitats. Data on different habitat characteristics, presence and density of mosquito larvae were entered into SPSS V.16. Descriptive statistics were arrived at by calculating different proportions for the variables. The impact of different water body characteristics on the density of mosquito larvae was explored individually. Comparisons between proportions were made using Chi-square test. All variables were incorporated in a mathematical model and their overall impact on the density of *Anopheles* larvae tested using multiple logistic regression. Variables with p-value less than 0.05 in multiple logistic regression models were retained for interpretation. Emerged female *Anopheles* mosquitoes were identified to species level using PCR.

3.2.2.5 Data collection on adult mosquito

Collections of adult mosquitoes were conducted during cold-dry season from June to September 2014 and in hot-wet season from January to February 2015. Ten houses (in Morogoro) and ten houses (in Dodoma) were randomly selected from each street within the sampled wards. Households that were included in the mosquito sampling were mapped using a hand held global positioning system (GPS) device. Collections of mosquitoes were done outside and inside human dwellings in places such as veranda, in unfinished buildings (inside and outside), fences, in stored junks and in tree holes as well as behind curtains,under beds, under couches using vacuum backpack aspirator while knock down catches using pyrethrum aerosol spray (from Rungu®- aerosol spray cans) in places like store rooms.. The catching was performed in the morning from 0700 to 1000 hrs and in the evening from 1700-1900. All information pertaining to mosquitoes were filled in respective data collection forms (Appendix 5).

During mosquito collection, a vacuum aspiration which contained a collection cup at the back of the aspirator was used to collect mosquitoes caught in the field. The mosquitoes in the collecting cup were emptied into transporting plastic cups (with street labels) which were carried to the field and then taken to the insectary for counting and sorting. Female mosquitoes were identified morphologically and thereafter analyzed to species siblings by PCR.

Identification of sheltering places of *Anopheles mosquitoes* during cold-dry season (June-September)

Identification of hiding places for *Anopheles* mosquitoes were conducted in June to September 2015 at randomly selected houses in each street (Ten houses in Morogoro and ten houses in Dodoma) by using spray sheet collection method with knock down catches

using pyrethrum aerosol spray (from Rungu®- aerosol spray cans) in places like store rooms while in undisturbed places like under the bed, store rooms, behind the curtains, shelves, ceilings, aspirator was used to collect mosquitoes. Thereafter all mosquitoes caught from aspirator cups and knock down catches (fallen on the white sheet) were temporarily stored in the transporting plastic cups (which carry labels identifying street and sheltering places) and then taken to the laboratory to be counted, sorted by sex and morphologically identified. After morphological identification, each female *Anopheles* mosquito was preserved individually in an eppendorf filled with silica gel. The silica gel was used to store mosquitoes in dry condition while waiting to be transferred to the laboratory for molecular (PCR).assays

3.2.2.6 Data processing and analysis

Data on the number of *Anopheles* mosquitoes collected in different places were modeled using Poisson regression model so as to determine the impact of the study site characteristics on the distribution of *Anopheles*. For morphological and molecular assays, collected mosquitoes were taken to the laboratory where they were examined morphologically. Identified *Anopheles* mosquitoes were sampled and subjected to PCR for species identification as described in General Laboratory Analysis.

3.2.3 Determining the effects of combined use of chemo-attractants and non excito-repellant insecticide applied on contaminating device

3.2.3.1 Study area

This study was done in selected two wards in Dodoma and Morogoro urban. The area has been described in general study area section at the beginning of Chapter 3. This study was conducted in hot-wet season from January to February 2015 and in cold- dry season from June to September 2015. Again the study was repeated in wet and dry season from

December to June 2016 for assessing residual effect of the insecticide (Chlorfenapyr) on contaminating device.

3.2.3.2 Study design

Field experimental study.

3.2.3.3 Optimization of prototype mosquito contaminating device

The specific objective of the study was to determine the efficiency of the contaminating device with combined application of chemo-attractants and Chlorfenapyr, a non excito-repellant insecticide. Since there were different combinations of chemo-attractants used, it was pertinent to optimize/validate the tools prior to experiments so that they could yield good results. The experiment involved use of contaminating devices with chemo-attractants (CO_2, carboxylic acid, ammonia) and Chlorfenapyr.

Designing of a prototype mosquito contaminating device

In design of the mosquito contaminating device, different materials such as cotton sheet, wood, and nails were used. Pilot study modification of contaminating device in terms of shape, size, and colour of cloths used to cover the mosquito contaminating device was done to improve its ability to catch mosquitoes and also to suit people's perception and acceptability. Some initial models were not further developed because of superstitious views (witchcrafts) on the shape and materials used. (One such model not further developed due to superstitious views on it, was fabricated with wooden box covered with coconut leaves to create a thatched roof). The finally accepted device (which cost Tsh. 37,000/=; equivalent to 16 $) for evaluation was named Umbrella-topped Mosquito Contaminating Device (UtMCD).The (UtMCD) was made of wooden frame measuring 1m x 0.5m x 0.3m. Three quarters of all four sides of the frame was covered with a black

cotton cloth and remaining portion was left open to form an eave space for letting mosquitoes enter inside the device. In addition, a round wire (mimicking an umbrella with a radius of 0.25m) covered with black cloth was fixed on top of the frame. The umbrella-like top was meant to increase surface area for mosquito to land or hide underneath. The frame was mounted on the wooden basement which has four pedestals. These pedestals hold firmly the UtMCD when it is placed on the ground. To prevent insects such as ants from climbing into the contaminating device, the pedestals were put inside small bowls containing water.To make the device easy to carry, the frame was designed in such a way that it can be dismantled for transportation and reassembled on site. The cloths which cover the frame and umbrella-like top were sprayed with Chlorfenapyr 240g Al/L SC insecticide which has non-excito repellent properties.

Preparation of mosquito chemo-attractants

Three chemo-attractants (which are Carbon dioxide [CO_2], odour from unwashed nylon socks and ammonia) were used to bait the UtMCD. In this study CO_2 was locally made from sugar-yeast culture. Yeast and sugar in 25 g and 250 g amounts respectively were added into 3L of warm water contained in a 5L container wrapped with aluminum foil to conserve the temperature. The container with this mixture had a small opening with a diameter of 1 mm punched on the lid to allow CO_2 to be released slowly. The container was placed inside the UtMCD at the wooden basement. From 1-2 hours after mixture preparation, a continuous plume of CO_2 was released from the container (the CO_2 plume released from the container was confirmed by extinguishing a match-stick flame placed close to the outlet). Ammonia was another chemo-attractant used to lure female mosquitoes toward the UtMCD. Five grams of ammonia pallets were poured in the petri dish and placed at the wooden basement inside the contaminating device. A pair of unwashed nylon socks was also used as human foot odour (carboxylic acid) bait on the

UtMCD. These socks were hung under the umbrella-like cover of the UtMCD. These socks were worn for two consecutive days before experiment (wearer put on the socks at 0600 hrs and off at 2200 hrs) by five research assistants. Each research assistant was assigned to provide a pair of unwashed socks for fixed one UtMCD until the experiments were over, thus a pair of new socks was given to wearers for the subsequent experimental rotation. Each experiment needed new unwashed socks, new sugar-yeast culture and 5 g ammonia pallet and new cloth sprayed with Chlorfenapyr.

Chemo-attractants were also optimized. For instance, since CO_2 was prepared from sugar –yeast cultures it was imperative to get suitable composition ratio which will yield enough CO_2 for experiments. Hence from different combination ratios of the sugar-yeast culture which were used to release CO_2, a ratio of 25 g of yeast to 250 g sugar in 3L of warm water in 5L container (Kweka *et al.*, 2013).

Therefore the tool of choice for experiment was contaminating device covered with black cloth, CO_2 from the sugar-yeast culture with a ratio of 25 g of yeast to, 250 g sugar in 3L of warm water, a pair of worn unwashed socks, 5 g ammonia pallet and Chlorfenapyr 240g Al/L SC (non excito- repellant insecticide).

3.2.3.4 Data collection

Organization of Umbrella-topped Contaminating Device (UtMCD) for mosquito collection

Five UtMCDs were used in the mosquito collections. These were fitted with different substances (contents). The first UtMCD did not contain any odour or insecticide (UtMCD alone) it was labeled **A**. The second UtMCD labeled **B** had chemo-attractants while the third UtMCD labeled **C** had insecticide (Chlorfenapyr) alone, the fourth UtMCD, labeled

D, had both chemo-attractants and insecticide (Chlorfenapyr) while the fifth device labeled **E**, was comparable contaminating device without topped umbrella but contained both chemo-attractants and insecticide (Chlorfenapyr). The experiments were designed to be rotated in five randomly selected households in two streets within the wards (Mnalani and Swaswa in Dodoma and Mahita and Misufini in Morogoro). The positions of contaminating devices were interchanged (5x5 Latin square design) after every one day so that at the end of the study experiments (after completing five rotations), each contaminating device had been in each location once.

These UtMCDs were set in the evening (around 1600 hrs) outside human dwelling 10 m away from the house, because the UtMCD were developed purposely to lure and kill outdoor feeding mosquitoes. The UtMCDs were mounted on the ground inside the toned netting cage made on iron frame measuring 4m x 2m x 4m (Plate 1). This netting cage was used to prevent dead mosquitoes from being blown away by wind. The netting cage had a zip which could be unzipped to allow a researcher to enter during mosquito collections. After one day (at 1600 hrs) researcher entered inside the netting cage to collect mosquitoes which were found inside the netting cage and also inside and outside of the UtMCD. The live mosquitoes were aspirated by vacuum backpack aspirator and mouth aspirator while the dead mosquitoes were collected by tweezers. The dead mosquitoes were put into plastic cups while live mosquitoes were transferred into holding mosquito cages carrying the same label of the contaminating devices. The live mosquitoes were supplied with 10% glucose to enhance mosquito survival so that delayed mortality and survival of mosquitoes would be assessed. The delayed mortality was recorded at 24 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs (WHOPES, 2013). Mosquitoes which survived five days (120 hrs) were counted for survival analysis. In another experiment, residual effect of Chlorfenapyr was assessed using a contaminating device which contained

Chlorfenapyr and chemo-attractant i.e. UtMCD labelled D, for 9 months with mosquito mortality rate for caught mosquitoes being assessed at three months, six months and nine months.

In the insectary, all collected dead mosquitoes were counted and examined under stereomicroscope to sort out female and male mosquitoes (sex sorting). Female mosquitoes were counted then identified morphologically into different genera and also the abdomen of these mosquitoes was examined to categorize them into unfed and fed mosquitoes. Individual mosquitoes were stored in eppendorf tubes containing silica gel until used for PCR identification of sibling species, ELISA for CSP sporozoite infection rate and blood meal detection.

Plate 1: UtMCD dimension, set-up, and mosquito collection. UtMCD with its dimensions (A and B); UtMCD inside the cage C; Mosquitoes collection D

3.2.3.5 Laboratory activities

Genus and species identification

All collected mosquitoes were examined under stereo microscope to sort out female and male mosquitoes after which female mosquitoes were counted and then identified morphologically to genus and then species level as described under "general laboratory analysis'

Mosquito infection rate and blood source identification

In identification of blood meal source, abdomens of female *Anopheles* mosquitoes were examined to categorize fed and unfed mosquitoes. The bloodfed mosquitoes were subjected to PCR for identification of blood meal preference. For sporozoites infection rate of *Anopheles* mosquitoes, head and thorax of each mosquito were subjected to ELISA-CSP test (Burkot *et al.,* 1984). In ELISA results, samples were considered positive for sporozoites if absorbance value exceeded the 2X average absorbance of the four values of negative controls shown in first and second columns of left hand side (NB: Negative control were uninfected *Anopheles* mosquitoes from colony mosquitoes)

Mortality rates:

Mortality rates of female *Anopheles* mosquitoes exposed to UtMCDs with or without insecticide (Chlorfenapyr) were determined. The immediate (after 24-48 hours) and delayed mortality (after 72 hours) was observed and recorded. The mortality rates in five contaminating devices were compared.

3.2.3.6 Data processing and analysis

Statistical analysis was performed using SPSS V.16.The outcome variable was presented as means and standard errors. The Poisson regression model was employed to determine the predictors of number of *Anopheles* caught. Percentage mortality of female *Anopheles* mosquitoes which came into contact with insecticide were calculated and compared with UtMCDs without insecticide. Cox's proportional hazards model was chosen to investigate the effect of UtMCDs on survival of the *Anopheles* mosquitoes**.** ELISA plate reader and PCR electrophoresis were used to read the results for sporozoite infection rate and blood meal source of *Anopheles* mosquitoes respectively (described in details in general laboratory analysis section).

3.2.4 Assessing attractiveness and ovicidal effects of artificially created breeding sites ovipositing gravid *Anopheles* gambiae

3.2.4.1 Study area

All the experiments for this specific objective were conducted in insectary and mosquito sphere (mosquito house that simulates a natural mosquito ecosystem) (Knols *et al.,* 2002) at Macha Research Trust (MRT), Choma, Zambia.The area has been described in general study area section at the beginning of Chapter 3. The experiments were conducted from April to June 2015.

3.2.4.2 Study design

The study was laboratory small scale semi-field study.

3.2.4.3 Rearing and mosquito colony maintenance

*Anopheles gambiae s.s (*Kisumu colonies) were obtained from the insectary at Macha Research Trust (MRT) in Choma, Zambia. These mosquitoes were reared in the insectary cages at 28±2ºC with relative humidity of 70-80%. The mosquitoes were given 10% glucose as a source of energy. Anaesthetized mice placed in mosquito-rearing cages were used as source of blood meal for the female mosquitoes to lay eggs (for optimization of tool) and adults for experiments. Two days after the mosquitoes were offered a blood meal, an egg pot containing water, with wet filter paper in it, was placed in the mosquito cage for oviposition by the blood-fed female *Anopheles* mosquitoes. A day after mosquitoes had oviposited, some eggs were taken from filter paper for ovicidal bioassay (optimization of tool) and the remaining eggs were transferred into the trays and reared so that they can emerge as adults. These adult mosquitoes were used in the experiments to assess the attractiveness and ovicidal effects of artificially created breeding sites on ovipositing gravid *Anopheles gambiae* s.s.

Preparation of gravid *Anopheles* mosquitoes for semi-field experiments

Emerged adult female mosquitoes were raised in the insectary until they reached age of 2-4 days. These mosquitoes were blood fed on mice in the evening (1900 hrs), then left for two days until they became fully gravid. The fully gravid mosquitoes were randomly selected from the cage by using mouth aspirator and transferred to a new cage ready for experiments which assessed attractiveness and hatchability of eggs deposited in artificial breeding sites that contained attractants with or without vinegar. The vinegar was used in these experiments due to its safety, easily available and affordable.

3.2.4.4 Preparation of Treatments

I. Attractants

i) **Hay infusion (HI):** Hay from rice straw (*Oryza glaberrima*-African rice) which has previously been f used for preparation of infusion for attracting gravid *Anopheles* mosquitoes towards breeding site (Kweka *et al.*, 2009; Muturi *et al.*, 2007; Santana *et al.*, 2006; Polson *et al.*, 2002) was prepared from freshly harvested local rice field in Tanzania and sun-dried for five days. A 125 g of dried rice straw was ground and steeped into one liter of distilled water. The mixture was covered with net of mesh size 0.6 mm x 0.6 mm and left in the room at ambient temperature 25°C for three days to let the infusion ferment as described by Gopalakrishnan *et al* (2012); Reiter and Colon,(1991); with some modification. Thereafter, the infusion was filtered through a clean piece of cotton cloth to remove large debris. A 25% dilution of hay infusion was prepared for experiments (Reiter and Colon, 1991)

ii) **Cow urine**: Cow urine was collected from a single 3-year old cow. The fresh urine was collected in plastic container each day of experiment by the cow owner

in the morning between 0630 to 0730 hrs. For experiment which used fresh urine, the collected urine was taken straight to the experiment. For experiments in which fermented cow urine was used, fresh urine was poured into the container and left open for three days to allow fermentation. Then the fermented cow urine was used in the semi-field experiments.

II. Vinegar

One liter of household Heinz white vinegar (8% acetic acid in water with pH 2.4, expiry date February 2016) was purchased from a local shop at MRT premises and used to acidify water that also contained attractants for the purpose of halting mosquito metamorphosis process. A small amount (10 milliliters) of vinegar was mixed in distilled water that contained attractants (either hay infusion or cow urine) to obtain a dilution of 140 ppm and maintain the pH of 3.0. One hundred millilitre amounts of the mixture were poured into bowls to be used in experiments to assess attractiveness of selected attractants with or without vinegar to ovipositing gravid *Anopheles gambiae* s.s and hatchability of deposited eggs.

3.2.4.5 Ovicidal Bioassay for pH assortment (optimization of tool)

Experiments were done at insectary which was maintained at temperature of 28 ± 2°C, and relative humidity of 78 ± 10%. Ovicidal bioassay of vinegar on mosquito eggs was carried out following the method of Elango *et al*.(2009) with some modification. One hundred freshly-laid eggs of *Anopheles gambiae* s.s (Kisumu colonies) were exposed to water treated with vinegar at different concentrations (i.e. 0, 30.0, 80.0, 100.0, and 140.0 parts per million (ppm) contained in trays, with distilled water used as control. Each treatment was replicated four times as per WHOPES (2005) guidelines, with mortality in the control group being observed not to exceed 10%. The ovicidal activity was assessed

up to 168 hrs post-treatment by observing hatchability whereby every morning hatched and non-hatched eggs were counted using laboratory torch and magnifying hand lens. However after 168 hrs, non-hatched eggs were put under further observation for 4 weeks to assess if there will be a delayed egg hatching. For the hatched larvae, physical (shape, motility) assessment was conducted after which they were transferred to the new trays which carry the same labels to observe growth development for two weeks post treatment. Water of the same pH was added to the trays every three days and larvae were fed daily with ground TetraMin fish food (Tetra, Melle, Germany). For non- hatched eggs, percent egg mortality was calculated using the following formula (WHOPES, 2005):

$$\% \text{ of egg mortality} = \frac{\text{\# of non-hatched eggs}}{\text{Total \# of eggs}} \times 100$$

After performing ovicidal bioassay, LC_{50} and LC_{90} values were calculated using SPSS Probit analysis. Thereafter 140 ppm (pH 3) which gave lethal effect at LC_{90} was chosen for use in the experiment.

3.2.4.6 Optimization of chemo-attractants (hay infusion and cow urine)

Stock solution of crude extract of hay infusion (1000 ml) was taken for preparation of different dilutions (5%, 10%, 25% and 50%) which were used to find out a preferable dilution for attracting gravid *Anopheles* mosquitoes towards oviposition bowls. From the stock solution dilutions of 5%, 10%, 25% and 50% were prepared using 1000 ml of distilled water and make up 50 ml, 100ml, 250ml and 500ml of diluted stock solution of hay infusion. For cow urine (fresh urine and fermented); 500 mls of cow urine was diluted in 2500 mls of distilled water to make a solution with a ratio of 1:5 that was used in experiments (Kweka *et al.*, 2011).

Attraction of the oviposition substrate in each treatment was presented using Oviposition Activity Index (OAI). The oviposition activity index (OAI) was calculated by using the formula:

$$OAI = \frac{NT - NS}{NT + NS}$$

Where NT denotes the mean number of eggs in test treatment and NS denotes total number of eggs in control solution. All the index values ranged from -1 to +1. Positive OAI means that more eggs were laid in the treatment substrate than in control while, for the negative OAI, more eggs were laid in the control than in treatment substrate (Elango *et al.,* 2009; Kramer and Mulla, 1979).

3.2.4.7 Data collection

Fully gravid mosquitoes were used in five experiments which were conducted in parallel in an insectary and mosquito sphere (Plate 2). In the mosquito sphere one chamber with vegetation was chosen. Four similar mosquito cages with 1m x 1m x 1m measurements were used to represent 4 by 4 Latin square rotation. In 4 by 4 Latin square rotation, the bowls were rotated clockwise (Figure 3) to nullify any effect of position .Thus the arrangement of bowls were named relative to cage entrance (**E**) as front left (**FL**); front right (**FR**), back right (**BR**) and back left (**BL**).

In each cage four bowls (10x10x5cm) each containing 100 mls of different treatments (i.e. **1:** Bowl with attractant alone, **2:** Bowl with attractant +vinegar, **3:** Bowl with vinegar alone and **4:** Bowl with distilled water (control) were placed at each corner of the cage. After setting up bowls in the cages, 30 fully gravid mosquitoes were released once in each cage at 1500 hrs so that the mosquitoes can adopt the environment before oviposition which normally occurs during twilight. Similar set-ups were conducted in

insectary but the measurements of cage and bowls were different, being 30cm x 30cm x 30cm and 5cm x 5cm x 3cm respectively. Close observation was done and in each morning at 0900 hrs, bowls were taken out from the cage, all its contents including eggs if present poured into other bowls with the same label and the bowls taken to insectary to assess hatchability and attractiveness of treatments to gravid *Anopheles* mosquitoes. All bowls taken from the cage were rinsed with distilled water and the same treatment/mixture replaced ready for another experiment. The mosquitoes in the cages were given glucose to ensure survival for up to three days of observations to see if mosquitoes could continue laying eggs.

Plate 2: Experimental set up: (A) Mosquito sphere; (B) Vegetation chamber in mosquito sphere; (C) Arrangement of bowls in the cage; (D) Trays in insectary

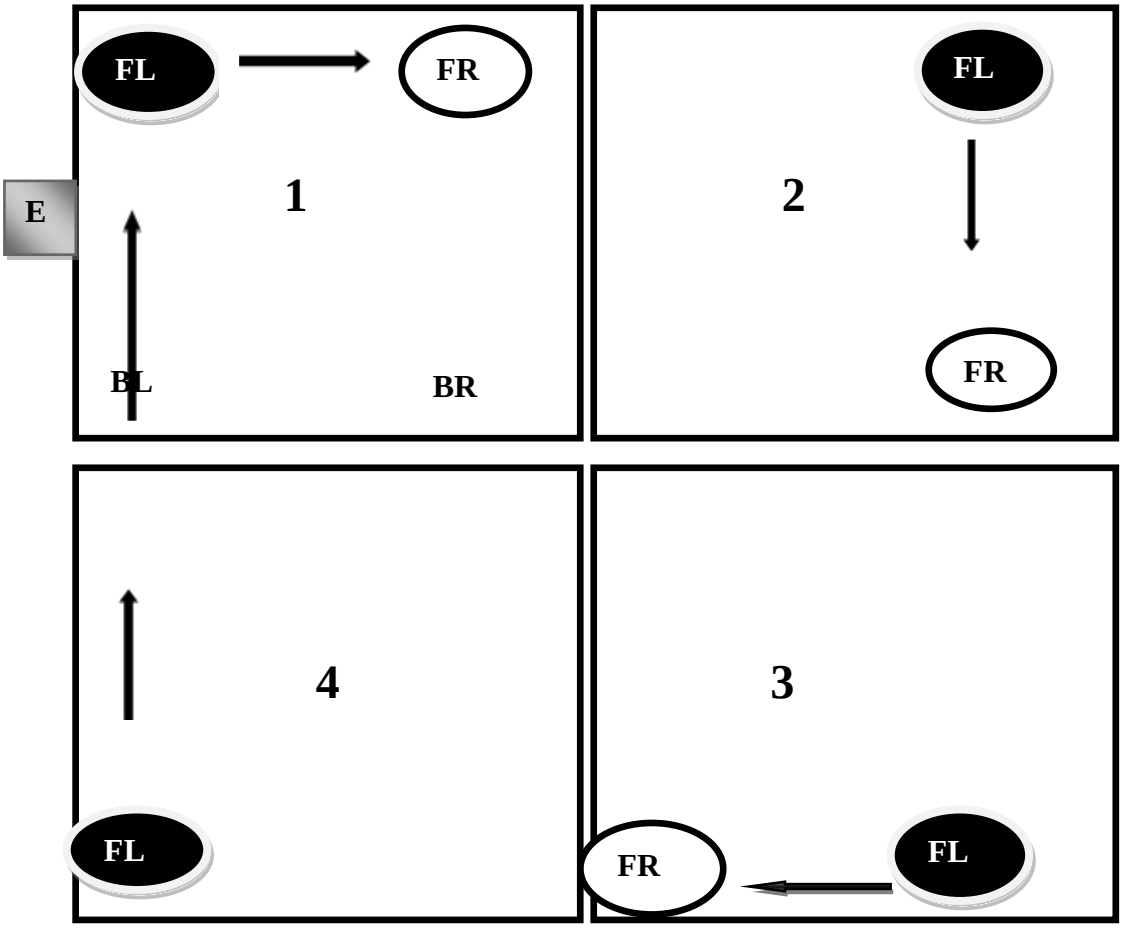


Figure 3: Illustration of the rotation of oviposition bowls inside the four cages to nullify position preference (4 by 4 Latin square)

3.2.4.7.1 Experiment I: Effect of attracting gravid *Anopheles gambiae s.s* to oviposit in hay infusion with or without vinegar

Four bowls in each cage (Figure 3) were used in the experiment. First bowl contained hay infusion, second bowl had vinegar, third bowl had hay infusion + vinegar and fourth bowl contained distilled water (as control). In the experiment, egg laying in the bowl was the indictor for the response (attractiveness) of gravid mosquitoes towards the four oviposition choices presented. Collection and counting of eggs were done on the next day and all collected eggs were taken to insectary to assess attractiveness of gravid *An. gambiae* s.s towards treatments andobserve hatchability of deposited eggs.

3.2.4.7.2 Experiment II & III: Effect of attracting gravid *Anopheles gambiae ss* to oviposit in fresh/fermented cow urine with or without vinegar

In this experiment cow urine (fresh and fermented) with or without vinegar was tested as attractant for gravid *An. gambiae* s.s to oviposit eggs. In this part, two experiments were conducted. First experiment was to assess the response of gravid mosquitoes toward fresh urine with or without vinegar. The second experiment was to assess the response of gravid mosquitoes toward fermented urine with or without vinegar. Four bowlscontaining different treatments were used in each experiment (i.e. II and III). In experiment II, first bowl contained fresh urine, second bowl had vinegar, third bowl contained fresh urine + vinegar and fourth bowl contained distilled water (as control). For experiment III, first bowl contained fermented urine, second bowl contained vinegar, third bowl had fermented urine + vinegar and fourth bowl contained distilled water (as control). The response of attraction and effects of vinegar on deposited eggs were assessed.

3.2.4.7.3 Experiment IV: Effect of attracting gravid *Anopheles gambiae ss* to oviposit in a combined treatments with or without vinegar

The experiment was carried out to assess the response of gravid *Anopheles* mosquitoes toward combined treatment i.e. Hay infusion, fresh urine + vinegar and control (water). Four bowls were set on the floor in one cage and gravid mosquitoes were released and observations made as to choice of where to deposit eggs. The first bowl contained hay infusion, second bowl contained fresh urine, third bowl had hay infusion + vinegar + fresh urine, fourth bowl contained distilled water (as control). Attraction and ovicidal effect were assessed after collection and counting of eggs. In this experiment fermented urine was excluded because of weak attraction to gravid *Anopheles gambiae s.s* observed in experiment III.

3.2.4.7.4 Experiment V: Residual effect of treatment with vinegar

Residual effects of cow urine + vinegar, and hay infusion+vinegar in attracting and inhibiting hatching of *Anopheles* mosquito eggs were assessed. Each morning 30 gravid mosquitoes were released in the cages containing ovipositing bowls with different treatments (cow urine + vinegar and hay infusion+vinegar). In these experiments, water (control) and treatments were not emptied but only the filter paper which contained eggs were removed from the bowls and the same bowls were set for another day experiment. These experiments were carried for 14 days. Every morning, eggs were collected and counted to determine whether they have increased or decreased in number. Thereafter the eggs were sent to the laboratory for hatchability assessment.

3.2.4.7.5 Comparison of experiment done in mosquito sphere and insectary

All the experiments which were done in mosquito sphere were also conducted in the insectary. The total number of eggs collected in the mosquito sphere and insectary were counted and compared.

3.2.4.8 Exclusion and inclusion criteria

Inclusion criteria

Fully gravid *Anopheles* mosquitoes were randomly selected from insectary cages and used in the experiments.

Exclusion criteria

Unfed *Anopheles* mosquitoes were not included in the experimental study.

3.2.4.9 Data processing and statistical analysis

All collected data were analyzed using statistical packages SPSS V.16. A 5% level of significance was used for independent variable with p-value less than 0.05 considered as significantly associated with outcome variable. Lethal concentration (LC50 and LC90) were determined by Probit analysis (SPSS program V.16). Multiple logistic regression model was used to find the effect of treatment used on hatching of the mosquito eggs while adjusting for the area where the experiments were conducted. The oviposition attraction of hay infusion and cow urine (fresh or fermented) were scored by oviposition activity index (OAI), which was calculated using the protocol used by Elango *et al.*,(2009) and Kramer and Mulla (1979).

3.2.5 General laboratory analysis

This section explains laboratory works which were conducted in two laboratories Macha Research Trust (MRT) and Ifakara Health Institute (IHI) (this is due to restrictions of sponsor). Each laboratory had its own protocol which was used to guide all activities for morphology and molecular analysis.

3.2.5.1 Morphological identification of mosquito samples

All collected adult mosquitoes from different source (field caught and from larval source) were sorted out under stereomicroscope. Female mosquitoes were identified by using morphological keys of Gilles and Coetzee (1987). The following were key features used to identify Anopheles; **Palps as long as proboscis** with smooth three pale rings including wide ring at the tip occasionally divided into two rings; **Wings:** Pale scales creamy yellow on the front margin of the wing; **Legs**: Irregular speckled (Narrow pale ring at the tip and base); **Abdomen:** Abdominal tergites are covered with hairs and last two segments have few scales.

3.2.5.2 Dissection of mosquito and DNA extraction

After morphological identification, samples of 600 female *Anopheles* mosquitoes were selected at random from a total number of 7584 female *Anopheles* mosquitoes collected from two wards. All selected female *Anopheles* were taken to the laboratory for DNA extraction for PCR analysis and for salivary gland extract for ELISA CSP test (sporozoite detection) while the abdomen of blood fed *Anopheles* were set for small blood meal species identification.

Based on Macha Research Trust (MRT) and Ifakara Health Institute (IHI) DNA extraction protocols (Appendix 6), DNA of each of the 600 *Anopheles* mosquito sample was extracted for PCR amplification. A 100 µl Bender Buffer (0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris-HCl, 0.05 M EDTA pH 9.1, and 0.5% SDS in DEPC water) was used to homogenize the head, thorax and legs until there were no recognizable mosquito parts. Eight moles of potassium acetate were added to the homogenized samples and incubated for one hour then spun. Thereafter 300 µl 100% ethanol was added to supernatant to precipitate DNA, then centrifuged to obtain small pellet of DNA. This DNA pellet was stored at -20°C until used for PCR analysis.

Polymerase chain reaction (PCR) analysis for differentiation of *Anopheles* species

The differentiation of the *Anopheles gambiae* complex by PCR was done by using MRT (Appendix 7) and IHI PCR protocols (Scott *et al.,* 1993). The PCR amplification was performed with universal and species specific primers for the *An. gambiae* complex. Molecular identification of *An. gambiae* complex was based on three deferentially sized amplicons of the species-specific nucleotide sequences in the ribosomal DNA intergenic spacer (IGS). The extracted product sizes were as follows: *An. gambiae* s.s. (~390 bp), *An. arabiensis* (~315 bp), and *An. quadriannulaus* (~150 bp) (Scott *et al.,* 1993). The

amplified DNA was separated on a 2.0% agarose gel stained with ethidium bromide and viewed on a UV transilluminator. The following are primers for *An. gambiae* complex:

Primers:

UN: 5'- GTG TGC CCC TTC CTC GAT GT -3'

GA: 5'- CTG GTT TGG TCG GCA CGT TT -3'

AR: 5'- AAG TGT CCT TCT CCA TCC TA -3'

QD: 5'- CAG ACC AAG ATG GTT AGT AT -3'

ELISA detection of *Plasmodium falciparum* circumsporozoite protein in mosquito samples

ELISA test for *Plasmodium falciparum* sporozoite infection rate in mosquitoes were performed on 150 *An. gambiae sensu lato* female mosquitoes randomly selected from 600 mosquitoes. Out of these, 100 samples of female mosquitoes were collected in hot-wet season and were analysed at IHI using IHI protocol (Appendix 8a) while 50 samples were collected in cold-dry season and analyzed at MRT using MRT protocol (Appendix 8b). The head and thorax of each mosquito was crushed in PBS (pH 7.4) and tested for circumsporozoite protein using double antibody ELISA (Burkot *et al.*, 1984). The results were scored visually and then photometrically at 405 nm using ELISA plate reader (Bioteck eLX300 (for MRT). Samples were considered positive if absorbance value exceeded 2X average absorbance of the four values of negative controls. Negative controls were uninfected colony mosquitoes.

Identification of blood meal source

DNA was extracted from mosquito abdomens and mosquitoes were homogenized in 100 µL of extraction buffer containing 0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris-HCl, 0.05 M EDTA, pH 9.1, and 0.5% sodium dodecyl sulfate (SDS) and incubated for one hour at

65°C. Fifteen µL of cold 8 M potassium acetate was added to each homogenate and DNA was precipitated by adding 250 µL of 100% ethanol to the transferred supernatant. DNA was pelleted by a 15-minute centrifugation. Finally, DNA extraction was evaluated by PCR amplification of a fragment of the mitochondrial NADPH dehydrogenase subunit 4 (ND4) using arthropod-specific primers (Appendix 9) (Kent and Norris, 2005).

Primers:

PIG573F: 5'- CCT CGC AGC CGT ACA TCT C -3'
HUMAN741F: 5'- GGC TTA CTT CTC TTC ATT CTC TCC T -3'
GOAT894F: 5'- CCT AAT CTT AGT ACT TGT ACC CTT CCT C -3'
DOG368F: 5'- GGA ATT GTA CTA TTA TTC GCA ACC AT -3'
COW121F: 5'- CAT CGG CAC AAA TTT AGT CG -3'
UNREV1025: 5'- GGT TGT CCT CCA ATT CAT GTT A -3'

Based on the results obtained from blood meal source detected by PCR, host feeding preference was determined. In this protocol, the proportion of blood-fed mosquitoes with human blood referred to as Human blood index (HBI) in female mosquitoes is used in determining the degree of anthropophily among species blood meal source detected.

HBI was calculated by the following formulae

$$HBI = \frac{\text{Number of mosquitoes with human blood meal}}{\text{Total number of mosquitoes with blood}}$$

3.3 Ethical Considerations

Ethical clearance for the studies was granted by the Sokoine University of Agriculture (SUA) Directorate for Research and Postgraduate studies. Participants (in objective number one) were briefed on details of the study and assured confidentiality and signed written informed consent (or oral in case illiterate respondent) to participate in the study.

Anonymity was assured by identifying respondents by numbers only in the research notebook, although the individual to which the number refered was confidentially kept in a separate notebook only accessed by the researcher. Permission to conduct the study was sought from the district and respective ward authorities (Appendix 10). Written consent to set up the experiments at selected house-holds to evaluate the Umbrella-topped Contaminating Device (UtMCD) was obtained after explaining objectives of the study.

CHAPTER FOUR

4.0 RESULTS

Chapter synopsis

This chapter presents the results of the study. First section describes findings on knowledge and awareness on malaria and its vectors. Sections two to four present the entomological results which describe spatial distribution of malaria vectors in study area as well as on evaluation of novel methods for reducation of population density of *Anopheles* mosquitoes.

4.1 Community Knowledge and Awareness on Malaria and Its Vectors

4.1.1 Demographic characteristics of study participants

A total of 400 respondents from 218 households agreed to participate in the study. Among these, 200 participants were from 112 households in Morogoro and 200 participants were from 106 households in Dodoma.Although it was intended that from each household there would be one adult and one child interviewed, no school pupil was available for interview in 50 households where only an adult (the household head) was interviewed. Therefore a total of 250 adult participants and 150 school children were interviwed during study period. From this study it was observed that the largest proportion (41.5%) of participants were aged 25-45 years old while the age groups 10-17, 18-24, and ≥ 45 years comprised 37.5%, 12.5% and 8.5% of the participants respectively. The ratio male to female was approximately 1:1.3 (176/224). Seventy per cent of participants had primary school level of education (Table 2).

Table 2: Socio-demographic characteristics of study participants in the two study regions (Dodoma and Morogoro): n=400

Variables	Dodoma n (%)	Morogoro n (%)	Both n (%)
Age group (years)			
<10-17	64 (32.0)	86 (43.0)	150 (37.5)
18-24	37 (18.5)	12 (6.0)	49 (12.5)
25-45	71 (35.5)	95 (47.5)	166 (41.5)
>45	28 (14.0)	7 (3.5)	35 (8.5)
Sex			
Male	96 (48.0)	80 (40.0)	176 (44.0)
Female	104 (52.0)	120 (60.0)	224 (56.0)
Education level			
Not attended school	34 (17.0)	0 (0)	34 (8.5)
Primary school	115 (57.5)	168 (84.0)	283 (70.7)
Secondary school	42 (21.0)	25 (12.5)	67 (16.8)
College/ university	9 (4.5)	7 (3.5)	16 (4.0)
Occupation			
Employed	36 (18.0)	32 (16.0)	68 (17.0)
Self employed	100 (50.0)	82 (41.0)	182 (45.5)
Studying	64 (32.5)	86 (43.0)	150 (37.5)

4.1.2 Knowledge on malaria and malaria vector

Table 3 presents results on respondent knowledge and awareness on malaria and malaria vector. In total, 250 household heads and 150 senior primary school children were interviewed during study period. It was found that majority of respondents confused causation and malaria transmission risk, with only9.0% participants correctly mentioning *Plasmodium* as a causative agent for malaria while the remainder (69.0%, 9.5%, 9.0% and 9.5%) mentioned mosquitoes, bacteria, worms and other factors respectively as the cause of malaria. Majority of respondents (86.2%) correctly associated malaria transmission with mosquito bites. When asked to name the vector transmitting malaria, more than three quarters (75.5%) of respondents correctly mentioned *Anopheles* while 11.5% and 7.3% mentioned *Culex* and *Aedes* respectively and 5.7% indicated they did not know. With respect to perceived health problems in their households, 86.7% of respondents

mentioned malaria as number one disease, while 13.3% of participants said typhoid, diarrhea and Urinary Tract Infection (UTI) and fungal infection were other health problems in their family. High fever, headache, and vomiting were mentioned by 38.7%, 37.3% and 13.4% of respondents as the signs/symptoms of malaria. A few participants pointed out joint pain and shivering (10.6%) as other symptoms of malaria. Sixty six per cent of respondents said they go to hospital when they encounter such symptoms while 15.5% and 15.8% reported taking pain killers and or using anti-malarial drugs without laboratory confirmation respectively. Regular use of bed nets for prevention of malaria was mentioned by 71.0% of the respondents, while other mentioned measures used to prevent mosquito bites were mosquito coils, mosquito spray, other (Wearing long sleeved clothes, burning local herbs) as mentioned by 12.8%, 11.8%and 3.4% of respondents respectively. With regards to main areas for mosquito breeding majority of respondents (57.3%) mentioned garbage and long grasses while less than one third (31.5%) mentioned stagnant water. With regards to which between HIV and malaria is the health information more frequently received at school, the vast majority (82.0%) of respondents who were primary school pupils at the time of the interviews mentioned HIV. (Table 3).

Table 3: Respondent knowledge on malaria, malaria vector and preventive measures

Variables	Dodoma n (%)	Morogoro n(%)	Both n (%)
Cause of malaria			
Mosquito	118 (59.0)	158 (79.0)	276 (69.0)
Bacteria	22 (11.0)	16 (8.0)	38 (9.5)
Worms	4 (2.0)	8 (4.0)	12 (3.0)
Plasmodium	33 (16.5)	3 (1.5)	36 (9.0)
Others	23 (11.5)	15 (7.5)	38 (9.5)
Risk of malaria transmission			
Mosquito bite	185 (92.5)	160 (80.0)	345 (86.2)
Don't know	4 (2.0)	33 (16.5)	37 (9.3)
Others	11 (5.5)	7 (3.5)	18 (4.5)
Name of the vector			
Anopheles	150 (75.0)	152 (76.0)	302 (75.5)
Culex	26 (13.0)	20 (10.0)	46 (11.5)
Aedes	17 (8.5)	12 (6.0)	29 (7.3)
Don't know	7 (3.5)	16 (8.0)	23 (5.7)
Household health problem			
Malaria	172 (86.0)	175 (87.5)	347 (86.7)
Others (typhoid, diarrhea, UTI, fungal infections)	28 (14.0)	25 (12.5)	53 (13.3)
Don't know	18 (9.0)	23 (11.5)	41 (10.2)
Malaria symptoms			
High fever	56 (28.0)	99 (49.5)	155 (38.7)
Headache	96 (48.0)	53 (26.5)	149 (37.3)
Vomiting	28 (14.0)	26 (13.0)	54 (13.4)
Others	20 (10.0)	22 (11.0)	44 (10.6)
Action taken when encounter these symptoms			
Visit hospital	119 (59.5)	145 (72.5)	264 (66.0)
Take pain killer	37 (18.5)	25 (12.5)	62 (15.5)
Consult a relative	4 (2.0)	2 (1.0)	6 (1.5)
Consult a traditional healer	2 (1.0)	3 (1.5)	5 (1.2)
Use antimalarial without checkup	38 (19.0)	25 (12.5)	63 (15.8)
Breeding sites			
Stagnant water	81 (40.5)	45 (22.5)	126 (31.5)
Garbage/Long grasses	100 (50.0)	129 (64.5)	229 (57.3)
Others	19 (9.5)	26 (13.0)	45 (11.2)
Methods used for personal protection			
LLINs	138 (69.0)	146 (73.0)	284 (71.0)
IRS	4 (1.0)	0 (0)	4 (1.0)
Mosquito coils	30 (15)	21 (10.5)	51 (12.8)
Mosquito spray	18 (9.0)	29 (14.5)	47 (11.8)
Others	10 (5.0)	4 (2.0)	14 (3.4)
For School Children			
What health information do you get more frequent at school			
HIV	48 (75.0)	75 (87.2)	123 (82.0)
Malaria	16 (25.0)	11 (12.8)	27 (18.0)

Table 4 presents results on respondent knowledge on malaria and malaria vector by sex. The findings showed that (81.4% of male in contrast to only 58.8% of female respondents while 11.9% of females in contrast to 6.2% of malescorrectly mentioned *Plasmodium* as the cause of malaria. A higher proportion of males than females (88.7% vs 83.6%) attributed risk of malaria transmission with mosquito bite while a higher proportion of females than males (79.9% of vs 69.9%) correctly mentioned *Anopheles* as the name of the mosquito which transmits malaria. Garbage/long grasses was mentioned by majority (66% of females and 46% of males as breeding sites for mosquitoes while stagnant water was mentioned as the breeding sites for mosquitoes 39.5% males and 25% of females. On personal protection against malaria, a vast majority (71%) of respondents (76.8% of females and 63.6% males) mentioned use of LLINs as the method for personal protection while 12.8% and 11.8% of respondents mentioned they use mosquito coils and mosquito sprays for personal protection.

Malaria was mentioned by 91.5% of females and 80.7% of males as a household health problem while high fever was mentioned by 43, 2% of male participants and 35.3% of female participants as the main symptom of malaria. A vast majority of females (72.7%) in contrast to only a slight majority (57.4%) of males indicated they visit health facilities upon suffering from malaria while. A quarter of males in contrast to only 8.5% of females indicated they use anti-malaria drugs without laboratory confirmation.

Table 4: Respondent knowledge on malaria, and malaria vector by sex

Variables	Male n (%)	Female n(%)	Both n (%)
Cause of malaria			
Mosquito	143 (81.4)	133 (58.8)	276 (69.0)
Bacteria	12 (6.8)	26 (11.5)	38 (9.5)
Worms	2 (1.1)	10 (4.4)	12 (3.0)
Plasmodium	11 (6.2)	27 (11.9)	36 (9.0)
Others	8 (4.5)	30 (13.3)	38 (9.5)
Risk of malaria transmission			
Mosquito bite	156(88.7)	189 (83.6)	345 (86.2)
Don't know	12 (6.8)	25 (11.1)	37 (9.3)
Others	8 (4.5)	10 (4.4)	18 (4.5)
Name of the vector			
Anopheles	123 (69.9)	179 (79.9)	302 (77.5)
Culex	19 (10.8)	27 (12.1)	46 (11.5)
Aedes	20 (11.4)	9 (4.0)	29 (7.3)
Don't know	14 (7.9)	9 (4.0)	23 (5.7)
Household health problem			
Malaria	142 (80.7)	205 (91.5)	347 (86.7)
Others (typhoid, diarrhea, UTI, fungal infections)	30 (17.1)	13 (5.8)	43(10.8)
Don't know	4(2.2)	6 (2.7)	10 (2.5)
Malaria symptoms			
High fever	76 (43.2)	79 (35.3)	155 (38.7)
Headache	58 (32.9)	91 (40.6)	149 (37.3)
Vomiting	18 (10.3)	36 (16.1)	54 (13.4)
Others	24 (13.6)	18 (8.0)	42 (10.6)
Action taken when encounter these symptoms			
Visit health facilities	101(57.4)	163(72.7)	264 (66.0)
Take pain killer	27 (15.4)	35 (15.8)	62 (15.5)
Consult a relative	2 (1.1)	4 (1.7)	6 (1.5)
Consult a traditional healer	2 (1.1)	3 (1.3)	5 (1.2)
Use antimalarial without checkup	44 (25.0)	19 (8.5)	63 (15.8)
Breeding sites			
Stagnant water	70 (39.8)	56 (25.0)	126 (31.5)
Garbage/Long grasses	81 (46.0)	148 (66.0)	229 (57.3)
Others	25(14.2)	20 (9.0)	45 (11.2)
Methods used for personal protection			
LLINs	112 (63.6)	172 (76.8)	284 (71.0)
IRS	3 (1.7)	1 (0.5)	4 (1.0)
Mosquito coils	23 (13.1)	28 (12.5)	51 (12.8)
Mosquito spray	30(17.1)	17 (7.5)	47 (11.8)
Others	8 (4.5)	6 (2.7)	14 (3.4)
For School Children			
What health information do you get more frequent at school			
HIV	53 (82.8)	70 (81.4)	123 (82.0)
Malaria	11 (17.2)	16 (18.6)	27 (18.0)

Figure 4 below shows respondent preferred sources of information on malaria and its vector. Adults preferred to get their information equally (50.6% vs 48.2%) from health facilities and radio/TV while in the case of school children, 56% preferred get their information from radio/TV while the remaining 31.4% and 12.6% indicated they refer prefer to get their health information from health facilities and school respectively. Only 1.2 % of adult respondents indicated they obtain their information from school.

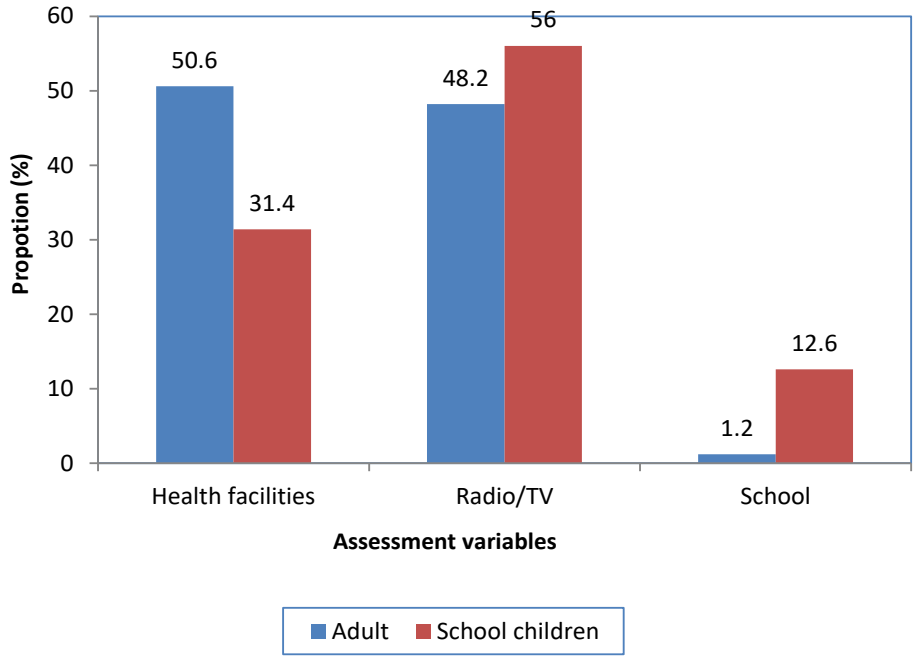


Figure 4: Respondent preferred source of information on malaria and its vector

4.1.3 Correct knowledge and awareness of malaria, malaria vectors and mosquito biting risk

Figures 5-7 present different responses between binary variables based on correct knowledge on mosquito breeding sites, risk of malaria transmission, identification of mosquito pictures as well as awareness on malaria vector control methods. Additional to figures, Chi-square test (χ^2) was performed to test for statistical significance between binary variables.

Adult and school children

The results in Figure 5 show that school children had more knowledge on mosquito breeding site compared to adult respondents ($\chi 2=19.79$, $p=0.0001$). Moreover more school children correctly identified adult and larval mosquito in pictures than adults ($\chi 2=9.65$, $p=0.002$). In comparison with adults, 21.6% of school children had correct knowledge on outdoor mosquitoes in relation to risk of malaria transmission. There were no statistically significant differences on awareness of malaria control methods, correct knowledge on midnight mosquito bites, and malaria transmission risk by early evening mosquito bites between age (adult/children) categories ($\chi 2=3.15$, $p>0.05$). Logistic regression analysis shows that lower age (school children) is significantly associated with knowledge on mosquito breeding sites (OR=2.46, 95% CI 1.5-3.9, p=0.0002)

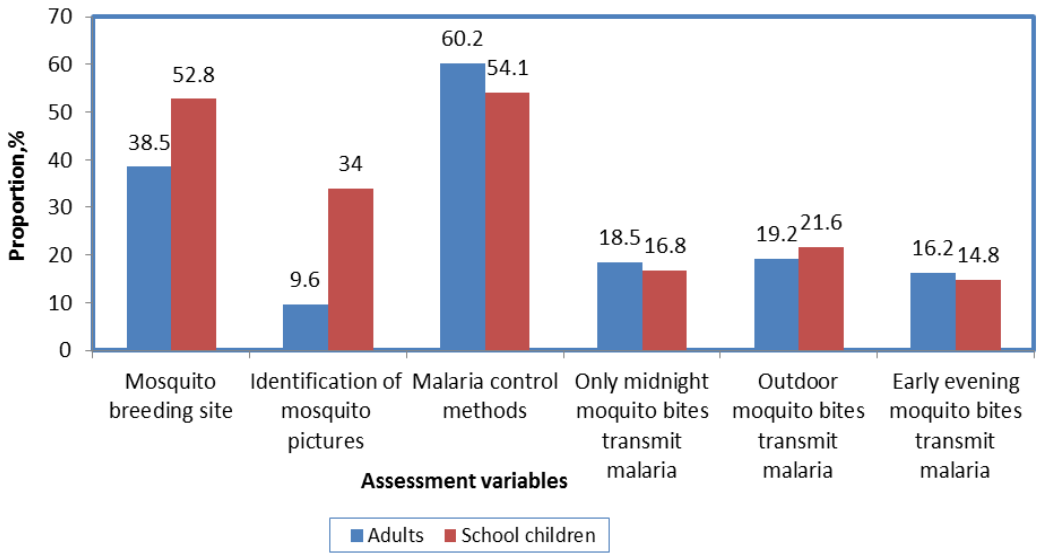


Figure 5: Correct knowledge and awareness on mosquitoes and malaria transmission among adult and school children

Education

Figure 6 shows that literate (had primary education) respondents had more knowledge of mosquito breeding sites compared to illiterate (had no primary education) respondents (χ^2=9.12; p=0.0025). Literate respondents correctly identified adult and larval mosquito pictures more than illiterates and this difference was statistically highly significant (χ^2=11.8, p=0.00056). The majority of literate respondents had correct knowledge on outdoor mosquito biting in relation to risk of malaria transmission (χ^2=9.9, p=0.0016). Furthermore, the results show that knowledge of malaria control and prevention methods was higher among literate than illiterate respondents (χ^2=13.69, p=0.00021). There were no statistically significant differences between literate and illiterate respondents on correct knowledge on biting peak hours of mosquitoes, as well as knowledge of malaria transmission risk by early evening mosquito bites (χ2=1.65, p>0.05). Logistic regression analysis shows that education (literate) is significantly associated with knowledge of malaria vector control and prevention methods (OR=3.9, 95% CI 1.8-8.2, p=0.0003). Other response variables were not associated with education (Figure 6).

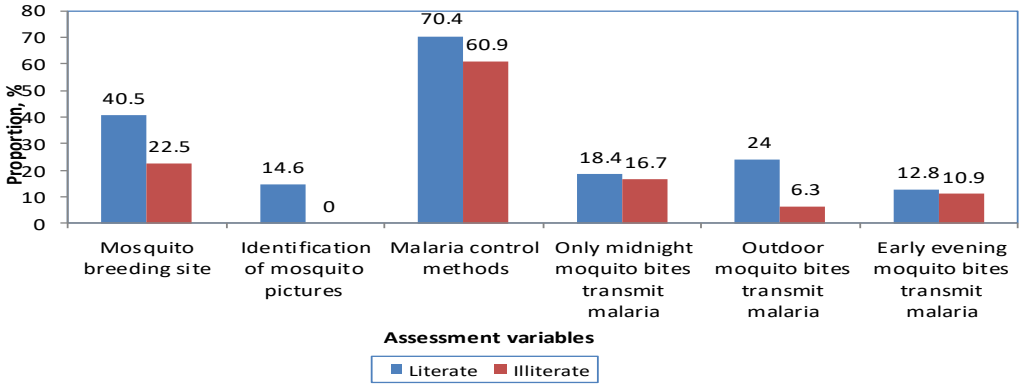


Figure 6: Correct knowledge and awareness on mosquito and malaria transmission risk by education level

Study area

Results in Figure 7 shows differences in response variables between the study area but these differences when analysed by the Chi-squire test were not statistically significant for most of response variables investigated ($\chi 2=2.06$, $p>0.05$). However correct knowledge related to risk of malaria transmission by outdoor mosquitoes was significantly higher in Dodoma than in Morogoro ($\chi^2=4.3$, $p=0.04$) (Figure 7).

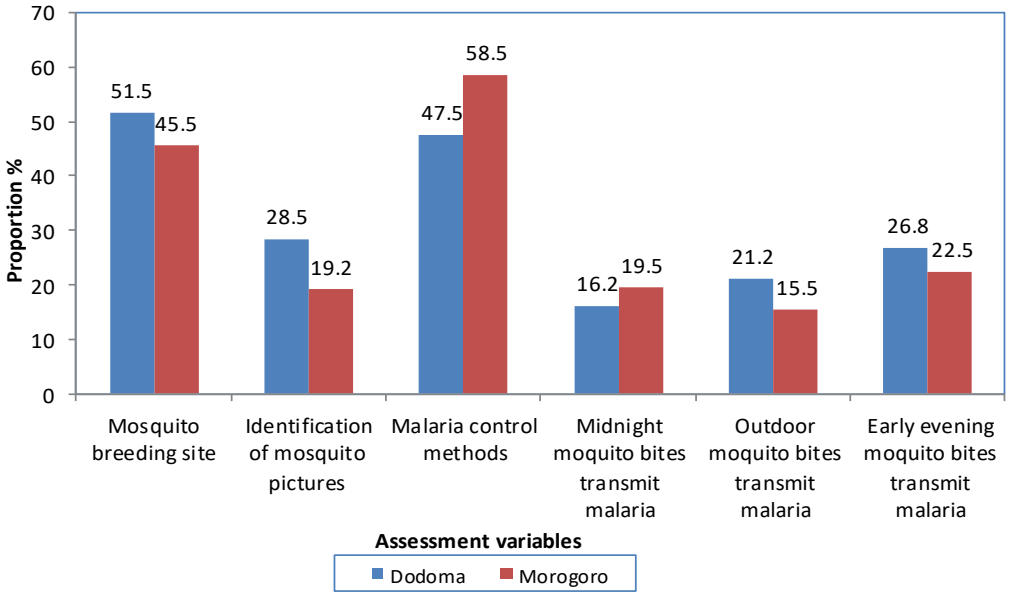


Figure 7: Correct knowledge and awareness on mosquito and malaria transmission risk by study area

4.1.4 Awareness on biting behavior of *Anopheles* mosquitoes

With regards to awareness on biting behavior of *Anopheles* mosquitoes, 64.5% of study participants were not aware of changed feeding behavior of malaria vectors. The majority (78.8%) of participants were not aware that early-evening *Anopheles* bites were associated with malaria transmission. Most (86.5%) participants believed that only midnight mosquito bites are responsible for malaria transmission (Figure 8).

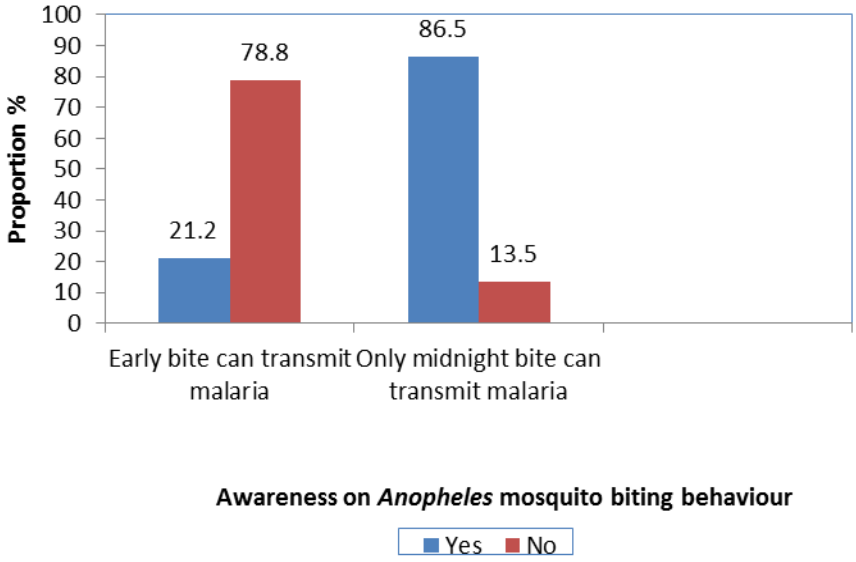


Figure 8: Community awareness of on risk of malaria transmission caused by *Anopheles* mosquito bite

" The findings of objective #1 has been published as: Mary M. Mathania, Sharadhuli L. Kimera and Richard S. Silayo: Knowledge and awareness of malaria and mosquito biting behaviour in selected sites within Morogoro and Dodoma regions Tanzania, *Malar J* (2016) 15:287"

4.2 Spatial and Temporal Distribution of Larvae and Adult Mosquitoes

The survey of spatial distribution of larval and adult *Anopheles* mosquitoes was carried out during cold-dry seasons (June to September 2014) and in hot-wet seasons (January to February 2015). Positive aquatic habitats for *Anopheles* larvae (potential breeding sites) and location where adult mosquitoes found were analyzed.

4.2.1 Spatial and temporal distribution of larvae

4.2.1.1 Spatial distribution of *Anopheles* larvae

This section gives details on distribution of *Anopheles* larvae in the study area. Figure 9 and Figure 10 are maps which show breeding sites for *Anopheles* larvae in Morogoro and Dodoma

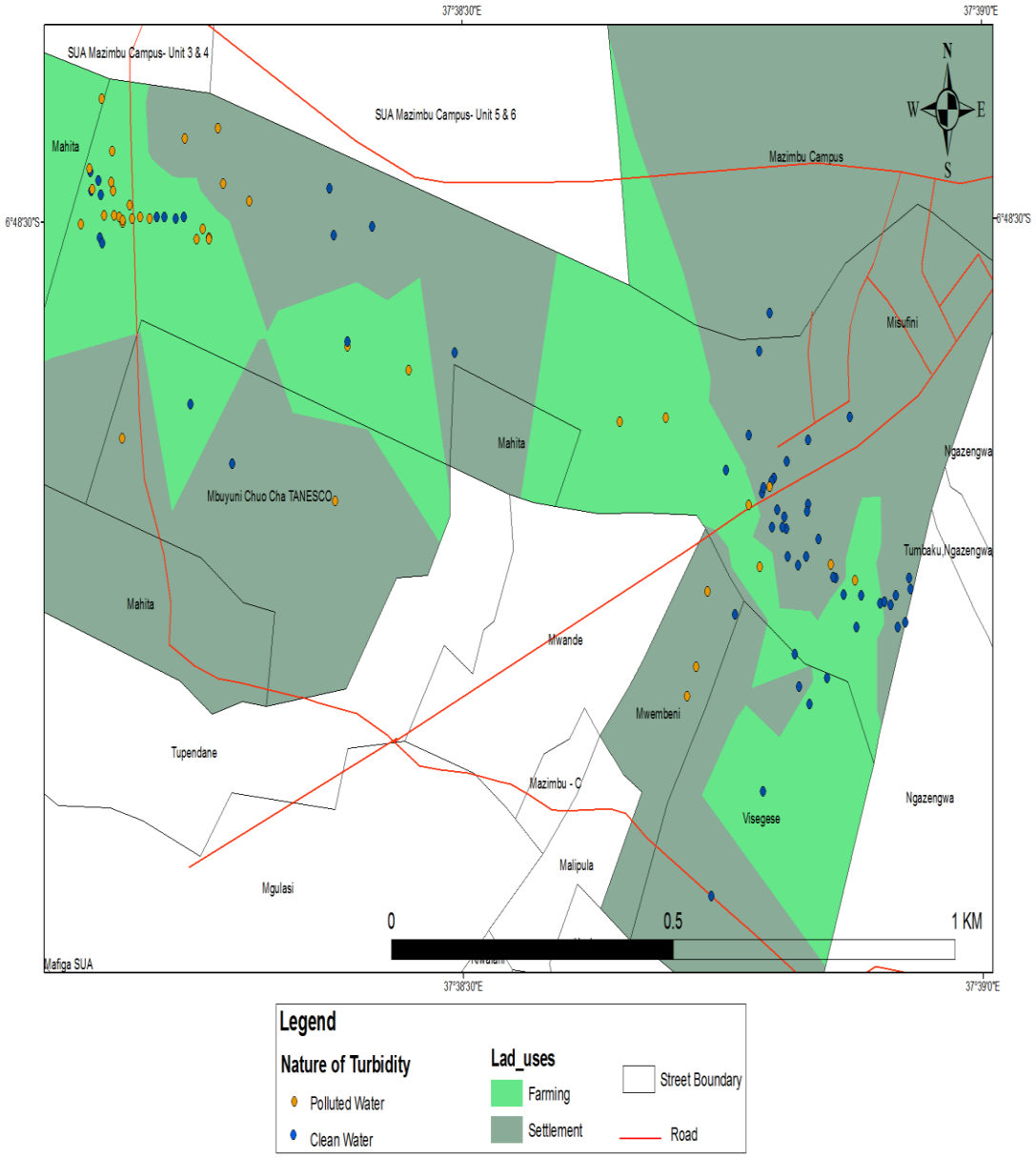


Figure 9: A map showing spatial distribution of potential breeding sites (positive for *Anopheles* larvae) in Morogoro based on type of water bodies

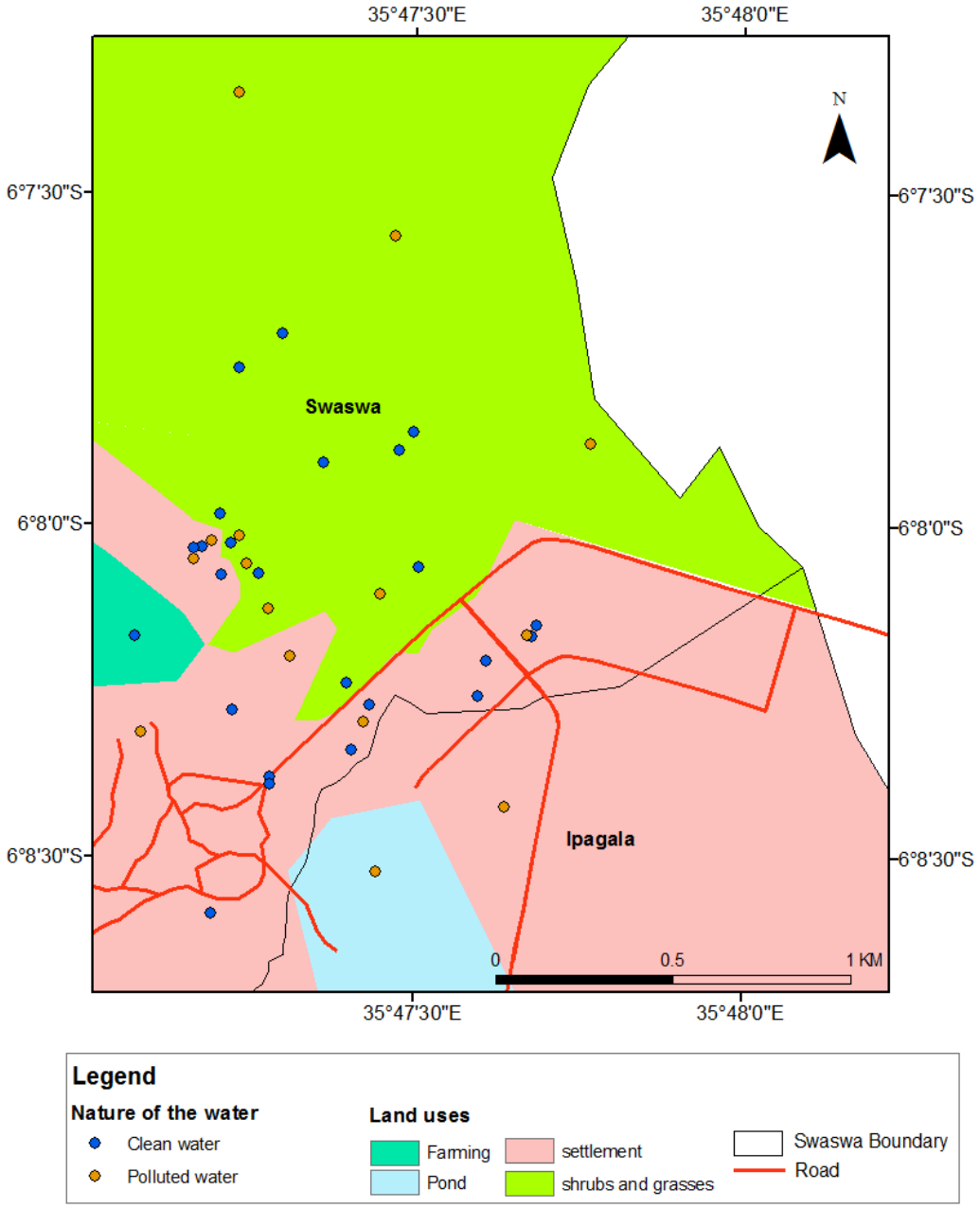


Figure 10: A map showing spatial distribution of potential breeding sites (positive for *Anopheles* larvae) in Dodoma based on type of water bodies

Distribution of *Anopheles* larvae by study characteristics

Characteristics of aquatic habitats

Table 5 shows the aquatic characteristics of potential water bodies surveyed in the study areas. A total of 724 water bodies were analysed in two study areas during dry and rainy seasons. Out of these, 576 (79.6%) which were potential breeding sites for mosquito larvae were analyzed. Among the surveyed potential breeding sites, 69.8% were found in Morogoro of which 72.2% of potential habitats were found during rainy season. In Dodoma positive aquatic habitat for *Anopheles* larvae were reported to be 30.2% and majority (68.4%) of those habitats were visited during rainy season. On the occasions when study were conducted, it was observed that 96.2% of potential breeding habitats were manmade in origin (e.g. brick pits) while only 3.8% were naturally occurring (e.g swamps). Interestingly, the study found out that, 18.8% water bodies that were positive for *Anopheles* larvae were polluted with either human or animal excreta. Moreover, 38.9% of potential polluted breeding sites were observed during the rainy season.

Table 5: Aquatic habitat characteristics of surveyed potential mosquito breeding sites in Dodoma and Morogoro according to seasons (n=576)

Variable	Total n (%)	Rainy Season n (%)	Dry Season n (%)
Surveyed Potential breeding sites			
Dodoma	174 (30.2)	119 (68.4)	55 (31.6)
Morogoro	402 (69.8)	290 (72.2)	112 (27.8)
Origin of habitat			
Manmade	554 (96.2)	389 (70.2)	165 (29.8)
Natural	22 (3.8)	20 (90.9)	2 (9.1)
Type of water bodies			
Clean	468 (81.2)	367 (78.4)	101 (21.6)
Polluted	108 (18.8)	42 (38.9)	66 (61.1)

Characteristics of larval habitats

Table 6 shows the characteristic of potential breeding sites with respect to presence of *Anopheles* larvae. The results show that *Anopheles* larvae were spatially distributed in different aquatic habitats. Out of the 576 sampled habitats that had *Anopheles* larvae, 25.2% were rice paddies, 23.3% were ditches, 21.8% were containers and 18.8% were septic tanks/pits. Furthermore, 59.5% of the habitats had size less than five meters diameter while 24% were of diameter 5-10 meters and 16.5% had diameter greater than 10 meters. On distance of breeding site to human settlements, this was found to be 10-100m for 50% while 37.2% and 12.8% were less than 10m and more than 100m respectively from the human settlement. Slightly more than half (51.9%) of the breeding sites were covered by short and long grasses while 40.5% were covered by short grass. On light intensity on the breeding sites, 34.2% were shaded while 45.8% had partial sunlight and 20% had full sunlight.. It was observed that all (100%) of the aquatic habitats which contained *Anopheles* larvae were stagnant water. Additionally, during the survey it was found that in 33.9% of the breeding sites, *Anopheles* and Culicine species occurred concurrently of which 49.2% of coexistence was observed during rainy season and 50.8% during cold-dry season

Table 6: Characteristics of potential breeding sites with respect to sampling frequency of *Anopheles* mosquitoes collected (n=576)

Variable	Total (576) n (%)	Rain Season (Total 409) n (%)	Dry Season (Total 167) n (%)
Habitat types			
Brick pits	18 (3.1)	18 (100)	0 (0)
Containers	126 (21.8)	79 (62.7)	47 (37.3)
Ditch	134 (23.3)	76 (56.7)	58 (43.3)
Foot print	20 (3.6)	20 (100)	0 (0)
Rice paddies	145 (25.2)	145 (100)	0 (0)
Septic tanks/pits	108 (18.8)	46 (42.6)	62 (57.4)
Swamps	21 (3.6)	21 (100)	0 (0)
Tyre tracks	4 (0.6)	4 (100)	0 (0)
Size of habitat (m)			
< 5	343 (59.5)	202 (58.9)	141 (41.1)
5-10	138 (24.0)	114 (82.6)	24 (17.4)
>10	95 (16.5)	93 (97.9)	2 (2.1)
Distance from house to breeding site (m)			
<10	214 (37.2)	125 (58.4)	89 (41.6)
10-100	288 (50.0)	220 (76.4)	68 (23.6)
>100	74 (12.8)	64 (45.9)	10 (54.1)
Light intensity			
Shaded	197 (34.2)	147 (74.6)	50 (25.4)
Partial Sunlight	264 (45.8)	202 (76.5)	62 (23.5)
Full Sunlight	115 (20)	60 (52.2)	55 (47.8)
Land Cover			
Short grasses (≤ 30 cm)	233 (40.5)	124 (53.2)	109 (46.8)
Combination (Short and long grasses)	299 (51.9)	246 (82.3)	53 (17.7)
Under tree	44 (7.6)	39(88.6)	5 (11.4)
Water Current			
Running	0 (0)	0 (0)	0(0)
Stagnant	576 (100)	409 (100)	167 (100)
Coexistence of mosquito species			
Anopheles alone	381 (66.1)	313 (82.2)	68 (17.8)
Both-*Anopheles* and culicine	195 (33.9)	96 (49.2)	99 (50.8)

Table 7 shows the characteristic of potential breeding sites for *Anopheles* larvae in Dodoma and Morogoro. The findings show that majority (68.4%) and (72.1 %) of breeding sites in Dodoma and Morogoro respectively were visited during wet seasons, of which rice paddies were the major (33.4%) breeding sites encountered in Morogoro and containers (40.3%) in Dodoma. During dry seasons most of breeding sites were dried off thus the main breeding habitats in Morogoro were ditches (41.1%) and septic tanks (35.7%) while in Dodoma were containers (40.3%) and septic tanks (40.0%). Furthermore, it was also observed that during dry and wet seasons majority of surveyed breeding site had size of ≤ 5m, and most of them 42.5% in Dodoma and 57.2% in Morogoro had a distance of 10-100m from human settlement. With respect to co-existence of *Anopheles* with other species, it was revealed that during wet season Dodoma and Morogoro did not differ much in terms of breeding sites either *Anopheles* alone or a mixture of *Anopheles* and Culicines. While in dry season most (68.7%) breeding sites in Morogoro displayed coexistence of *Anopheles* and Culicnes in compared with Dodoma where most (60%) of the breeding sites had *Anopheles* larvae alone.

Table 7: Characteristics of potential breeding sites with respect to sampling frequency of *Anopheles* mosquitoes collected in Dodoma(n=174) and Morogoro (n=402)

Variables	DODOMA			MOROGORO		
	% Total (n= 174)	% Rain Season (n=119)	% Dry Season (n= 55)	% Total (n= 402)	% Rain Season (n=290)	% Dry Season (n= 112)
Habitat types						
Brick pits	4.5	6.7	0	2.5	3.4	0
Containers	28.2	40.3	38.2	19.2	17.6	23.2
Ditches	17.8	16.0	21.8	24.1	19.7	41.1
Foot print	3.5	5.0	0	3.5	4.8	0
Rice paddies	27.6	23.5	0	25.6	33.4	0
Septic tanks/pits	18.4	8.5	40.0	18.9	12.5	35.7
Swamps	0	0	0	5.2	7.3	0
Tyre tracks	0	0	0	1.0	1.3	0
Size of habitat (m)						
≤ 5	47.1	52.9	56.4	64.9	52.1	98.2
5-10	25.3	26.8	43.6	23.4	32.4	0
≥10	27.6	20.3	0	11.7	15.5	1.8
Distance from house to breeding site (m)						
≤10	33.3	37.8	32.7	34.8	27.5	44.6
10-100	42.5	33.6	52.7	57.2	62.1	53.6
≥100	24.2	28.6	14. 6	8.0	10.4	1.8
Light intensity						
Shaded	25.9	31.9	12.7	37.8	37.6	38.4
Partial Sunlight	43.6	50.4	58.2	52.5	62.4	26.8
Full Sunlight	30.5	17.6	29.1	9.7	0	34.8
Land Cover						
Short grasses (≤30 cm)	42.5	27.7	74.5	39.6	31.4	60.7
Combination (Short and long grasses)	44.8	57.2	18.2	54.9	61.4	38.4
Under tree	12.6	15.1	7.3	5.5	27.2	0.9
Water Current						
Running (slow)	0	0	0	0	0	0
Stagnant	100	100	100	100	100	100
Coexistence of mosquito species						
Anopheles alone	70.1	74.8	60.0	64.4	77.3	31.3
Both-*Anopheles* and culicine	29.9	25.2	40.0	35.6	22.7	68.7

Anopheles larval density

The results of univariate analysis using Chi-square test (Table 8) showed that the *Anopheles* larval density was significantly associated with season (χ^2=5.7, p=0.017), type of water (χ^2 =5.4, p=0.020), light intensity (χ^2 =13.7, p=0.001), and habitat origin (χ^2

=12.1, p=0.001). Of the 576 aquatic habitats with *Anopheles* larvae, 59.4% in rain season and 70.1% in dry season were respectively having high density of *Anopheles* larvae (χ^2 =5.7, p=0.017). Proportion of clean water habitats that had high density of *Anopheles* larvae was 59.0% while the proportion of polluted habitats that had high density of *Anopheles* was 68.7% and the difference was statistically significant (χ^2 =5.4, p=0.02).

Table 8: The distribution of larval density according to breeding sites characteristics

Variable	High Density n (%)	Low density n (%)	X^2	P-Value
Season				
Rain	243 (59.4)	166 (40.6)	5.74	0.017
Dry	117 (70.1)	50 (29.9)		
Type of water				
Clean	217 (59.0)	151 (41.0)	5.43	0.020
Polluted	143 (68.7)	65 (31.3)		
Distance from house to breeding site (m)				
10	184 (63.9)	104 (36.1)		
10-100	126 (58.9)	88 (41.1)	2.25	0.3254
>100	50 (67.6)	24 (32.4)		
Light intensity				
Shaded	116 (58.9)	81 (41.1)		
Partial sunlight	185 (70.1)	79 (29.9)	13.71	0.001
Full Sunlight	59 (51.3)	56 (48.7)		
Land Cover				
Combination (Short and long grasses)	176(58.9)	123(41.1)	4.71	0.0949
Under tree	26 (59.1)	18 (40.9)		
Short grasses (≤30 cm)	158 (67.8)	75 (32.2)		
Habitat Origin				
Manmade	354 (63.9)	200 (36.1)	12.11	0.001
Natural	6 (27.3)	16 (72.7)		
Surveyed sites				
Dodoma	101 (58.0)	73 (42.0)	2.11	0.146
Morogoro	259 (64.4)	143 (35.6)		

As much as 70.1% and 58.9% of the habitats in partial sunlight and in shaded light had high mosquito larval density prevalence respectively in contrast to habitats in full sunlight of which only 51.3% had high mosquito larval density. This difference was statistically highly significant (χ^2 =13.7, p=0.001). As high as 63.9% of manmade habitats had high larval density of *Anopheles* in contrast to only 27.3% of natural habitats that had low *Anopheles* larval density. The difference was statistically highly significant (χ^2=12.1, p=0.001). With regards to the breeding sites surrounded by grasses, 67.8% of habitants with short grasses had high *Anopheles* larval density compared 58.9% of those with combination of short and long grass, and 59.1% of those under tree. The difference was however not statistically significant (χ^2 =4.7, p=0.09).

Multiple Logistic regression models

The analysis of multiple logistic regression model adjusting for season, type of water, size of habitats,distance from house to breeding site**,** light intensity , land cover, habitants origin, surveyed sites and water current demonstrated that the effect of season on larval density was no longer significant (p=0.21). Water current (p=0.9365), land cover (p=0.6162), size of habitat (p=0.6428), and surveyed site (p=0.1958) were also not significantly associated with larval density so were removed from the final model. The adjusted odds ratios of the final fitted model for the risk factors associated with larval density are presented in Table 9. Aquatic habitats with clean water were significantly more likely to have high density of *Anopheles* mosquito larvae than those with polluted water (AOR=1.5, p=0.0340). Habitats at a distance of 10-100m from the house had almost two times higher odds of having high density of mosquito larvae than habitats more than 100m from a house (AOR=1.9, p=0.03). With respect to light intensity, habitats in shaded (AOR=2.16,p=0.0014) and partial sunlight (AOR=1.78,p=0.0047) were significantly more likely to have high density of mosquito larvae than habitants in

full sunlight. The odds of having high density of mosquito larvae for natural habitats was 4.09 times that of manmade habitats (AOR=4.09, p=0.0059).

Table 9: Adjusted odds ratio (AOR), 95% confidence interval and p-value for factor associated with larval density

Variable	AOR	95% CI	P-Value
Type of water			
Clean	1.50	[1.031, 2.185]	0.0340
Polluted	Reference		
Distance from house to breeding site (m)			
<10	1.20	[0.685, 2.114]	0.5185
10-100	1.90	[1.064, 3.405]	0.0300
>100	Reference		
Light intensity			
Shaded	2.16	[1.348, 3.468]	0.0014
Partial Sunlight	1.78	[1.193, 2.650]	0.0047
Full Sunlight	Reference		
Habitat Origin			
Manmade	Reference		
Natural	4.09	[1.501, 11.156]	0.0059

Molecular identification

Polymerase chain reaction (PCR) analysis

Out of 1426 *Anopheles* mosquitoes which emerged from collected *Anopheles* larvae, a sample of 200 *Anopheles* mosquitoes (100 from dry season and 100 rainy season) were processed for species identification using PCR. The PCR analysis showed that 72.5% were *An. arabiensis,* 4.5% *An. gambiae s.s,* 0.5% *An. coustani* and 20% *An. quadrianulatus* while 2.5% of the samples could not be identified because DNA was not amplified. Most (96.1%) of those identified as *An. arabiensis* had emerged from dry season samples while all *An. quadrianulatus* and *An. coustani* were from rainy season samples (Table 10 and Appendix 11 and 12).

Table 10: Species composition of emerged A*nopheles* mosquitoes collected from larvae collection in study sites

Species	**No**	**Percentage**
Anopheles arabiensis	145	72.5
Anopheles gambiae s.s	9	4.5
An. coustani	1	0.5
An. quadrianulatus	40	20.0
Not amplified	5	2.5
Total	200	100.0

4.2.2 Spatial and temporal distribution of adult mosquitoes

4.2.2.1 Spatial distribution of adult *Anopheles* mosquitoes

This section gives details on the distribution of adult *Anopheles* mosquitoes in the study area. Figure 11 and Figure 12 are maps which show spatial distribution of adult *Anopheles* mosquitoes in study area

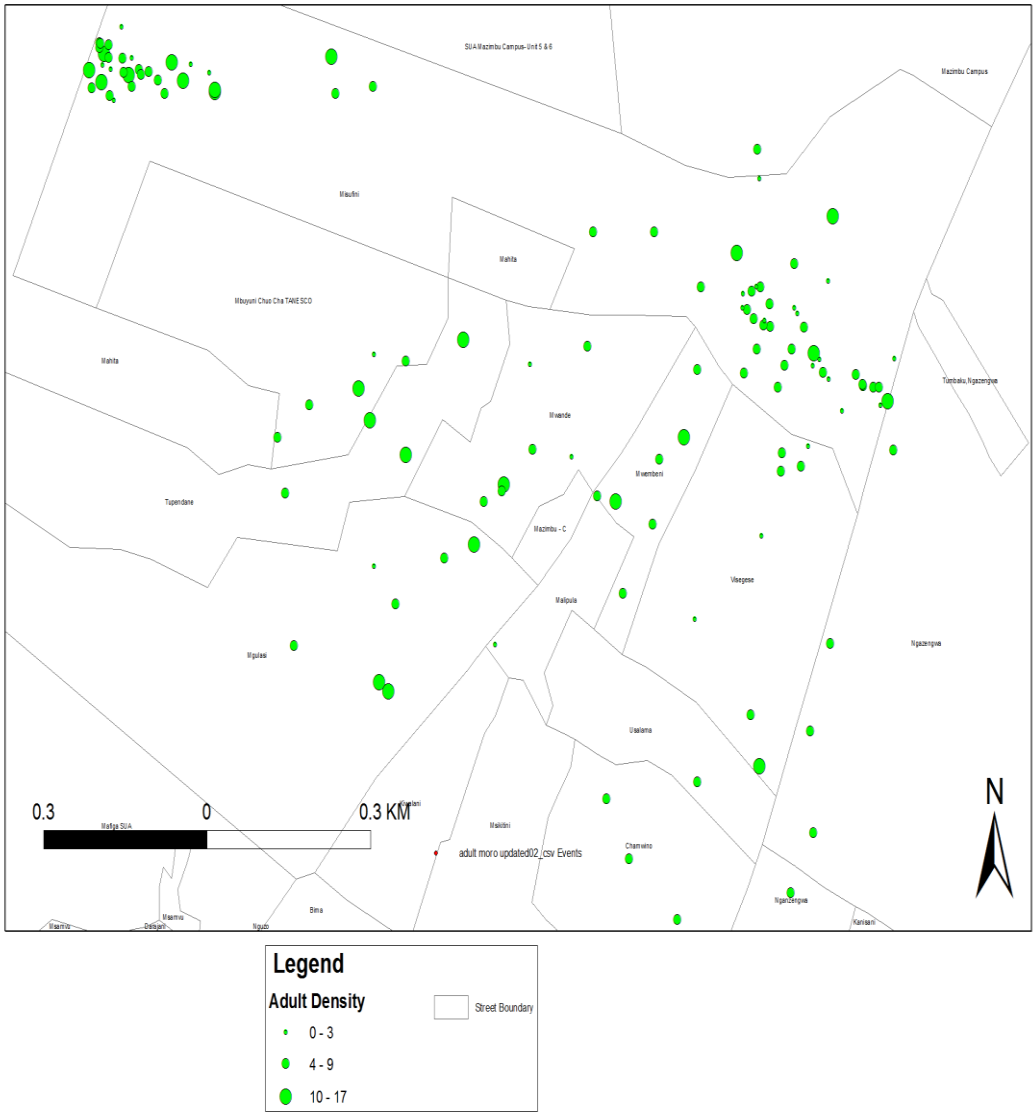


Figure 11: Map showing spatial distribution of adult *Anopheles* mosquitoes in study sites Morogoro

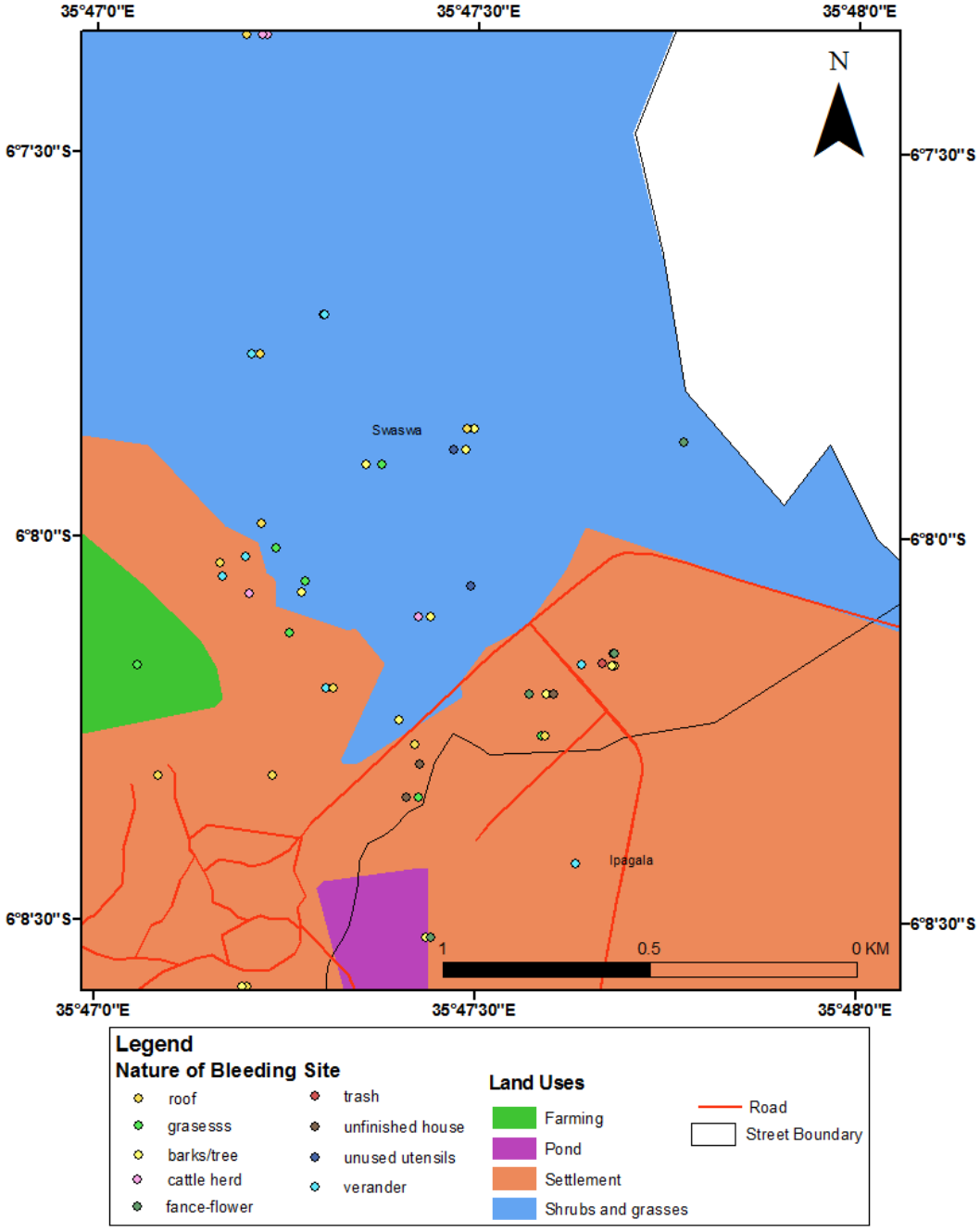


Figure 12: Map showing spatial distribution of adult *Anopheles* mosquitoes in study sites Dodoma

Distribution of adult *Anopheles* mosquitoes by study characteristics

Table 11 shows the distribution of *Anopheles* mosquitoes caught during spatiotemporal survey. A total of 2742 *Anopheles* mosquitoes with overall mean of 18.21±1.12 per day were collected outside human dwellings of which a higher number, 1717 (10.51±1.17), were collected in Morogoro. With respect to seasons, only 302 (4.72±1.04) were collected in the cold-dry season (June-September) compared to 2440 (12.96±1.52) collected in warm-wet season (March-May). When the number of mosquitoes collected from different streets were analyzed, it was found that highest numbers of mosquitoes were collected from Mahita in Morogoro 621(21.64±2.74) and Swaswa in Dodoma 576 (18.87±2.59).

Table 11: Distribution of adult *Anopheles* mosquito by study characteristics

Variable	Total Mosquitos	Mean ± SE
Region		
Morogoro	1717	10.51±1.17
Dodoma	1025	8.42±1.41
Season		
Wet	2440	12.96±1.52
Dry	302	4.72±1.04
Area		
Morogoro		
Visegese	164	8.37±1.47
Tupendane	272	7.20±1.54
Mwembeni	447	13.55±2.15
Mahita	621	21.64±2.74
Misufini	213	11.65±2.07
Dodoma		
Swaswa	576	18.87±2.59
Mnalani	449	14.97±2.34

Distribution of adult *Anopheles* mosquitoes in different locations

Figure13 shows the distribution of collected adult mosquitoes according to location. Out of 2742 of *Anopheles* mosquitoes caught, 42.33% were found on the ceiling, followed by stored junks 16.33%, verandas 14.96% and the smallest proportion 4.27% of the adult *Anopheles* mosquitoes was collected from barks/tree.

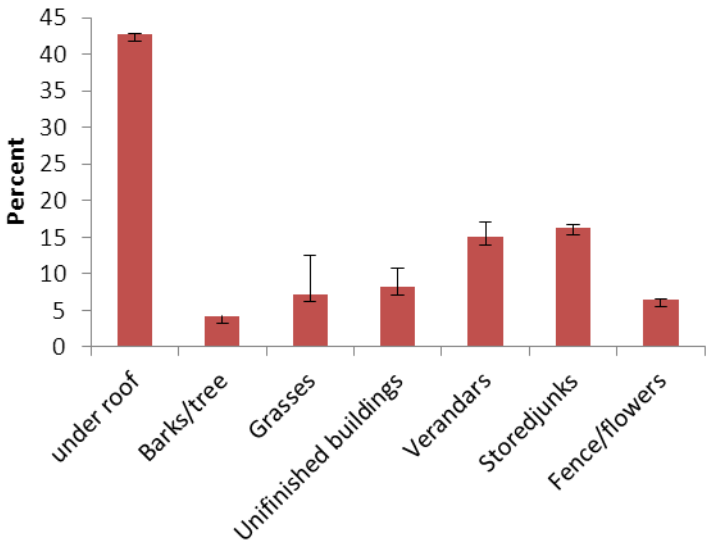


Figure 13: Percentage distribution of adult *Anopheles* mosquitoes by location. Error bars represent the standard error of the mean

The distribution of adult mosquitoes by location varied significantly with respect to season ($\chi^2 = 168.79$, $p =< 0.0001$) . The results displayed in Figure 14 shows that, in cold-dry season (June-September) a total of 320 *Anopheles* mosquitoes were caught outside human dwellings of which 36.47% were collected from ceiling followed by stored junks 22.43%, verandas 16.11% and inside unfinished buildings 8.55% . In wet-warm season (March-May), out of 2440 *Anopheles* mosquitoes caught, the highest percent (49.03%) were found on ceiling ceiling followed by verandas 13.64%, stored junks 9.35%) and fence/flower 9.28%.

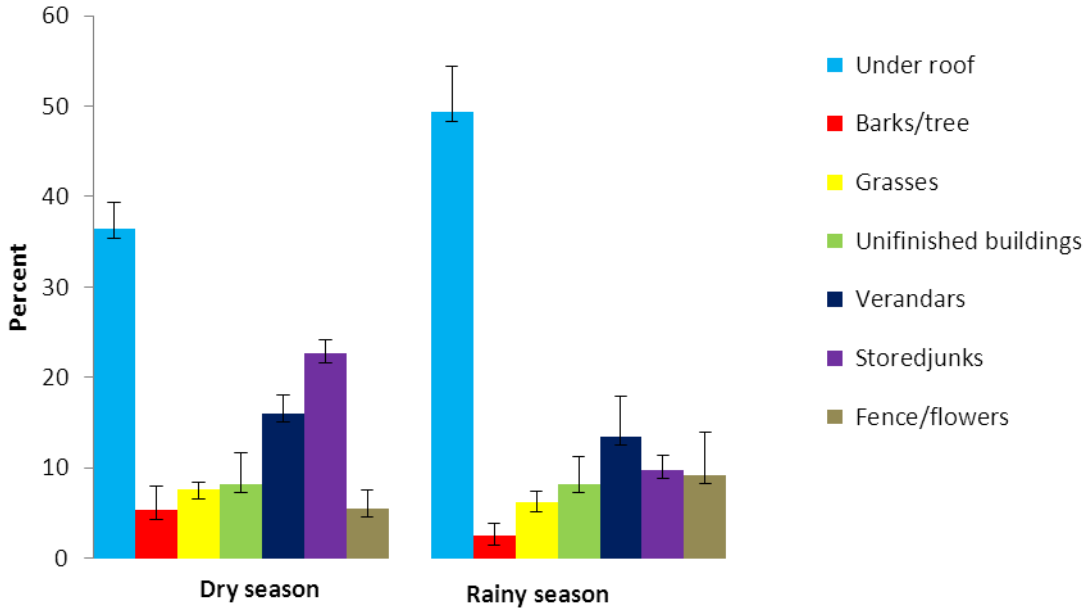


Figure 14: Percentage distribution of adult mosquitoes by season and location. Error bars represent the standard error of the mean

A poisson regression model was fitted to assess the effect of season on the distribution of the collected adult mosquitoes. The results of the model which are presented in Table 12 reveal that after adjusting for the surveyed area, the distribution of the adult mosquitoes was significantly associated with season ($p<0.0001$), whereby mean number of mosquitoes collected during cold-dry season (June-September) was almost two times that of rain season(March-May) (AMR=1.6, $p<0.0001$). Moreover, the average number of adult mosquitoes collected in Morogoro (AMR=1.34, $p<0.0001$) was significantly greater than in Dodoma.

Table 12: Parameter estimates, adjusted mean ratio (AMR), and significance levels of the Poisson regression model for the effect of region and season on distribution of the adult mosquitoes.

Parameter	Parameter Estimate ($\hat{\beta}$)	Standard Error	P-Value	AMR
Intercept	2.47	0.04	<0.0001	11.85
Region				
Morogoro	0.29	0.04	<0.0069	1.34
Dodoma				
Season				
Dry	0.47	0.03	<0.0001	1.6
Rain				

Species composition of collected adult mosquitoes

A total of 100 mosquitoes randomly selected from a total of 2742 morphologically identified adult female *Anopheles* mosquitoes were identified further using PCR. Out of the total, 89.0% were identified as *An. arabiensis* while only 10.0% were identified as being *An. gambiae ss* and 1.0% as *An.quadrianulatus* (Table 13 and Appendix 13&14).

Table 13: Species composition of adult A*nopheles* mosquitoes caught in study sites

Species	No	Percentage
Anopheles arabiensis	89	89.0
Anopheles gambiae	10	10.0
An.quadrianulatus	1	1.0
Total	100	100.0

4.2.2.2 Sheltering places of*Anopheles* mosquitoes during cold- dry seasons

A total of 1541 mosquitoes were collected during cold-dry season (June –September) inside human dwelling with overall mean daily collection of 19.26±1.81. It was observed that mosquitoes shelter in different places inside the house. From the findings, higher mean number of collected mosquitoes from sheltering places were from Dodoma

(26.63±2.37) of which the highest mean *Anopheles* catches was from Swaswa street (32.07±3.33) (Table 14).

Table 14: The mean distribution of *Anopheles* mosquito caught sheltering in different palces in Morogoro and Dodoma.

Variable	Mean ± SE
Region	
Morogoro	14.84 ± 1.03
Dodoma	26.63 ± 2.37
Study area (streets)	
Morogoro	
Visegese	12.40±1.52
Tupendane	14.60±1.93
Mwembeni	12.80±1.81
Mahita	17.00±2.39
Misufini	15.40±1.98
Dodoma	
Swaswa	32.07±3.33
Mnalani	21.20±2.08

Percentage distribution of*Anopheles*mosquitoes according to sheltering places

Figure 15 shows the percentage distribution of the collected mosquitoes by sheltering places. Out of 1541 *Anopheles* caught, majority were collected under beds (32.06%) followed by behind undisturbed curtains (25.31%) and store room/piled bags (23.75%) while the lowest percentage (4.48%) of *Anopheles* mosquitoes was from ceilings.

Figure 15: Percentage distribution of *Anopheles* mosquitoes with respect to sheltering places inside the house (n=1541). Error bars represent the standard error of the mean.

Poisson regression model

The results of the Poisson regression model (Table 15) for the effect of the region on the distribution of the *Anopheles* in sheltering period during dry season revealed that, the mean number of *Anopheles* mosquitoes collected in Morogoro (MR=0.56, p=0.0002) was significantly lower than that collected in Dodoma.

Table 15: Parameter estimates, adjusted mean ratio (MR), and significance levels of the Poisson regression model for the effect of region on distribution of sheltering *Anopheles* mosquitoes collected during cold-dry season

Parameter	Parameter Estimate	Standard error	P-Value	MR
Intercept	3.28	0.11	<0.0001	26.63
Region				
Morogoro	-0.58	0.16	0.0002	0.56
Dodoma	Reference			

Species identification of mosquitoes from sheltering places

Hundred mosquitoes randomly selected from a total of 1541 morphologically identified *Anopheles* mosquitoes collected from different sheltering places were subjected to PCR for molecular identification. The results showed that the predominant *Anopheles* species were *An. arabiensis* 76(*7*6.0%), *An. gambiae ss* 23 (23.0%) while 1 (1.0%) of the mosquito sample was not identified because DNA was not amplified (Table 16 and Appendix 15).

Table 16: Species composition of A*nopheles* mosquitoes caught in sheltering places

Species	No	Percentage
Anopheles arabiensis	76	76.0
Anopheles gambiae	23	23.0
Not amplified	1	1.0
Total	100	100.0

4.3 "Attract and Kill" (Combined Use of Chemo-Attractants and Non-Excito Repellant Insecticide on Mosquito Contaminating Device

4.3.1 Mosquito contaminating device (UtMCD) analysis

A total of 1875 *Anopheles* mosquitoes were caught in two wards of Dodoma and Morogoro during the entire study period, with daily means catch of 11 ± 1.22. Table 17 shows that UtMCD set-up D (Device with insecticide and attractants) had higher number 607 (16.15±1.35) of *Anopheles* caught compared to other UtMCD set-ups (set-up **A**: Device alone, set-up **B**: Device with attractants alone, set-up **C**: Device with insecticide alone and set-up **E**: Device without topped umbrella but containing insecticide and attractants). Furthermore, the UtMCD which were set in Morogoro caught more 1273 (12.79±1.05) *Anopheles* mosquitoes than those set in Dodoma 602 (6.25 ±0.94), whereas UtMCD mounted at Mahita street (in Morogoro) collected more *Anopheles* mosquitoes 801(9.14 ±0.76) than those set in other streets. The findings also revealed that the UtMCD

collected more *Anopheles* mosquitoes in households which possessed LLINs 1582 (10.96±1.04) than those without LLINs.

Table 17: The mean distribution of *Anopheles* caught under different study conditions

Variable	Total Anopheles	Mean	± SE
UtMCD set-up			
A	197	4.68	0.76
B	454	10.48	1.57
C	171	2.5	0.12
D	607	16.15	1.35
E	446	11.4	1.14
Season			
Dry	468	5.24	0.39
Wet	1407	14.8	2.00
Region			
Dodoma	602	6.25	0.94
Morogoro	1273	12.79	1.05
Study area			
Morogoro			
Mahita	801	9.14	0.76
Misufini	472	6.44	1.41
Dodoma			
Mnalani	235	4.48	0.20
Swaswa	367	5.02	0.52
House with LLIN			
No	293	9.0	1.66
Yes	1582	10.96	1.04

*UtMCD set-ups: **A**: Device alone, **B**: Device with attractants alone, **C**: Device with insecticide alone, **D**: Device with insecticide and attractants and **E**: Device without topped umbrella but contained insecticide and attractants*

Poisson regression analysis

The results of Poisson regression model (Table 18) revealed that adjusting for season of sampling and study location, and presence of LLINs inside human dwellings, the number of *Anopheles* mosquitoes caught were significantly associated with type of UtMCD set-up used. The variables that were significantly associated with number of *Anopheles* caught were season of sampling and study region. The mean number of *Anopheles* caught during

wet season was almost three times that of dry season (AMR=2.72, p<0.0001). Comparing Dodoma and Morogoro, the average number of *Anopheles* caught in Morogoro was significantly higher, being two times that caught in Dodoma (AMR=1.81, p<0.0001). Average number of *Anopheles* mosquitoes caught in the different UtMCD set-up in comparison with UtMCD set-up A, showed that the average number of *Anopheles* caught using UtMCD set-up B (AMR=2.01, p=0.0003), UtMCD set-up D (AMR=2.96, p<0.0001) and device set-up E (AMR=2.17, p<0.0001) were significantly higher. However, the estimated average number caught by UtMCD set-up C (AMR=0.93, p=0.7631) was statistically not significantly different from that of UtMCD set-up A.

Table 18: Parameter estimates, adjusted mean ratio (AMR), and significance levels of the Poisson regression model for the factors associated with number of the *Anopheles* caught

Effect	Parameter Estimates $(\hat{\beta})$	Standard Error	P-Value	AMR
Intercept	-0.08	0.20	0.6749	
Season				
Wet	0.99	0.11	<0.0001	2.72
Dry	Reference			
Region				
Morogoro	0.59	0.10	<0.0001	1.81
Dodoma	Reference			
UtMCD set-up				
A	Reference			
B	0.69	0.19	0.0003	2.01
C	-0.07	0.23	0.7631	0.93
D	1.51	0.17	<0.0001	2.96
E	1.02	0.18	<0.0001	2.17
House with LLIN				
No	0.12	0.18	0.5239	1.12
Yes	Reference			

*UtMCD set-ups: **A**: Device alone, **B**: Device with attractants alone, **C**: Device with insecticide alone, **D**: Device with insecticide and attractants and **E**: Device without topped umbrella but contained insecticide and attractants*

4.3.2 Morphological identification of mosquitoes

Relative mosquito abundance

Overall a total of 4991 female mosquitoes were collected from different UtMCD set-ups outdoor during the entire study period. Out of these 4991, 37.6% were *Anopheles* species, 61.9% were *Culex* species, and 0.5% were *Aedes* species. (Figure 16).

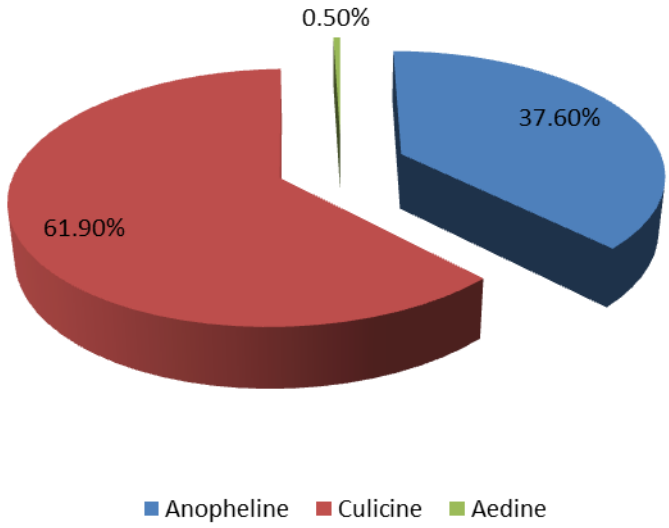


Figure 16: *Anopheles, Culex* and *Aedes* collected from device set-ups

Species composition

PCR identification of *Anopheles* mosquitoes

A total of 200 mosquitoes randomly selected from 1875 morphologically identified adult female *Anopheles* mosquitoes were further identified to species level using PCR. The predominant *Anopheles* species were *An. arabiensis* (96.0%) *An. gambiae s.s* (3.0%) while 1.0% of the mosquito samples were not amplified (Table: 19, Appendix 16 and 17).

Table 19: Species composition of A*nopheles* mosquitoes caught in study sites

Species	N	Dodoma n (%)	Morogoro n (%)
Anopheles arabiensis	192	101 (52.6)	91 (47.4)
Anopheles gambiae	6	2 (33.3)	4 (66.7)
Not amplified	2	2 (100.0)	0 (0)
Total	200	105 (52.5)	95 (47.5)

Analysis of abdominal contents of *Anopheles* for determination of feeding preferences

Before performing blood meal source using PCR, mosquitoes were *g*rouped as fed or unfed. Out of 1875 female *Anopheles* caught 3.6% were bloodfed while the rest were not bloodfed. Of 68 that were bloodfed, 77.9% were caught during warm/wet season and 22.1% in dry/cold season. The results of blood meal analysis (Plate 3) showed that, out of the 68 bloodfed mosquitoes, 92.6% were human malaria transmitting vectors (i.e *An. arabiensis and An gambiae s.s*) and had no fragments for human, pig, goat, cat or dog blood while 7.4% were zoonotic vector (*An. quadriannulatus)* and were positive for goat blood.

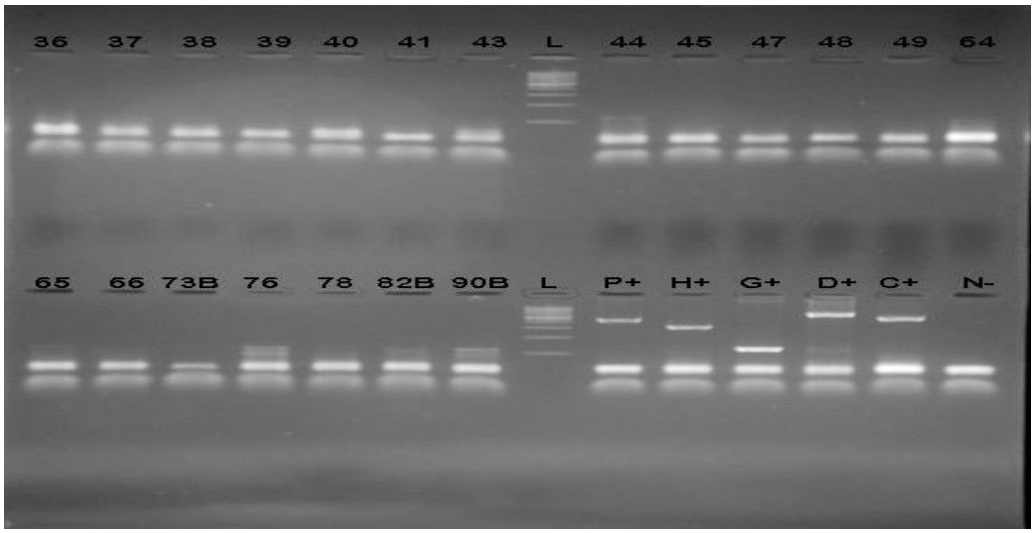


Plate 3: Agarose gel showing host-specific cytochrome b PCR product from blood engorged *Anopheles* mosquitoes caught in dry and rainy season

Detection of sporozoite infection rates for *Anopheles* mosquitoes

One hundred and fifty samples randomly selected from the 1875 *Anopheles* mosquitoes caught in dry and rainy seasons were subjected to ELISA-CSP for detection of sporozoite rate in *Anopheles* mosquitoes. The results showed that all samples tested negative for *Plasmodium falciparum* circumsporozoite antigen

Mortality rate of *Anopheles* mosquitoes landed on the UtMCDs

Mortality was assessed on all mosquitoes which landed on different UtMCD set-ups (with or without insecticide and/or chemo-attractants). A total of 1875 *Anopheles* mosquitoes were caught during the study period and out of these, 1179 (62.9%) died after visiting the UtMCD with or without insecticide (chlorfenapyr). Figure 17 shows the percentage distribution of number of dead *Anopheles* according to the type of UtMCD set-up used. Almost equal proportion of *Anopheles* caught using UtMCD set-ups D, (87.7%); UtMCD set-up C, (89.4%) and device set-up E, (84.9%) died within five days (120 hrs) of the study period. In contrast mortality rate for UtMCD set-up A, (19.2%) and UtMCD set-up B (17.4%) occurred within five days (120hrs). Based on the observed mortality rate data, the UtMCDs which contained insecticide (Chlorfenapyr) had higher mortality (i.e. UtMCD set-up C (89.4%); UtMCD set-up C (87.7%) and Device set-up E (84.9%) than UtMCDs which did not contain insecticide (i.e. UtMCD set-up A (19.2%) and UtMCD set-up B (17.4%).

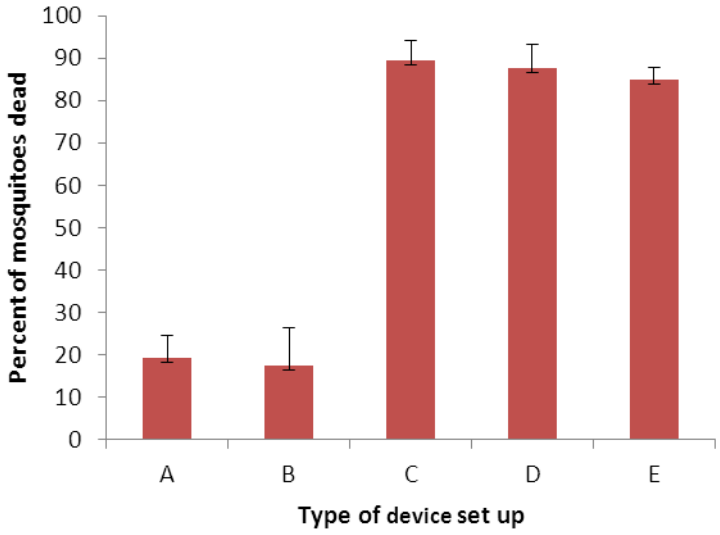


Figure 17: The percentage distribution of number of dead *Anopheles* according to type of device set-up. Error bars represent the standard error of the mean

Figure 18 shows the mean mortality rates of *Anopheles* according to type UtMCDs against holding post-exposure time (in days) (24-120 hrs). It was observed that the highest percentage mortality occurred after 48 and 72 hrs (Day2 & Day3) compared to other days (Day 1, Day 4 and Day 5).

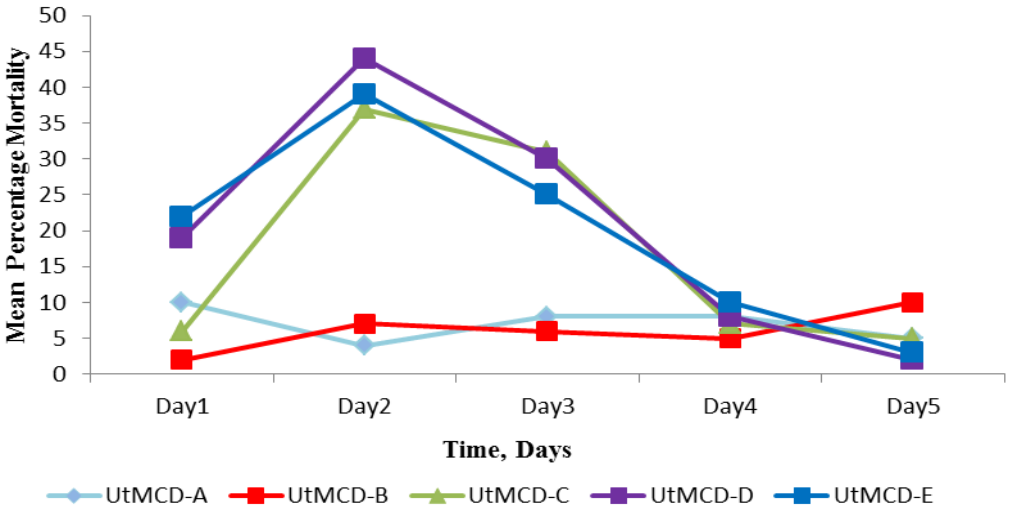


Figure 18: The mean percentage mortality of *Anopheles* against holding post exposure time for each UtMCD set-ups

Survival analysis

The survival functions of the *Anopheles* mosquitoes for five UtMCDs presented in figure 19 showed that, at any time t, the expected survival probability of the mosquitoes in UtMCD set-ups A and B were longer than that of mosquitoes in UtMCD set-ups C, D and Device E. In addition mosquitoes in UtMCD set-ups A and B had almost similar survival functions. The survival functions of mosquitoes in UtMCD set-ups C, D and Device E seemed to be similar.

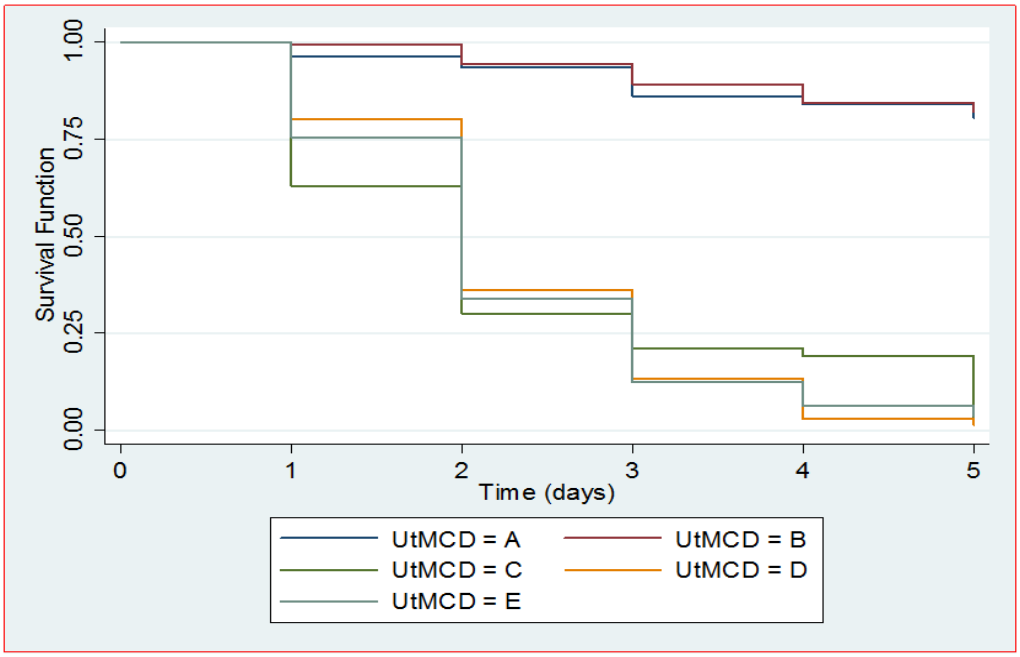


Figure 19: Kaplan-Meier estimates of survival function of *Anopheles* mosquito at time t (Days) by UtMCD set-up

The effect of UtMCD set-ups on survival time of *Anopheles* Mosquitoes

The results of the fitted proportion hazard model presented in Table 20 revealed that, type of UtMCD set-up has a significant effect on survival duration of the *Anopheles* mosquitoes. Mosquitoes in UtMCD set-ups C ($\hat{\beta}$ =2.4633,p<0.0001), D ($\hat{\beta}$ =2.4737,p<0.0001) and E($\hat{\beta}$ =2.4763, p<0.0001) have increased hazard , and hence have shorter expected survival time than those in UtMCD set-up A.

The hazard ratio between UtMCD set-up C and UtMCD set-up A is 11.74. This means that, mosquitoes in UtMCD set-up C were 11.74 times more likely than those in UtMCD set-up A to have shorter survival duration. Similar findings were found for mosquitoes in UtMCD set-up D (hazard ratio=11.87) and Device set-up E (hazard ratio=11.90). Though not significant (p=0.90), mosquitoes in UtMCD set-up B ($\hat{\beta}$ = -0.1040) appeared to have decreased hazard, and have longer expected survival time than those UtMCD set-up A.

Table 20: The Parameter estimates and corresponding Hazard ratio of the proportion hazard model for the effect of UtMCDs on survival time of the *Anopheles* mosquitos

UtMCD set-up	Parameter Estimate($\hat{\beta}$)	Standard Error	Z-Value	P-Value	Hazard Ratio
A	Reference				Reference
B	-0.11	0.27	-0.38	0.702	0.90
C	2.46	0.24	10.20	<0.0001	11.74
D	2.47	0.23	10.94	<0.0001	11.87
E	2.48	0.22	10.83	<0.0001	11.90

UtMCD set-ups: ***A****: Device alone,* ***B****: Device with attractants alone,* ***C****: Device with insecticide alone,* ***D****: Device with insecticide and attractants and* ***E****: Device without topped umbrella but contained insecticide and attractants*

With respect to residual effects of insecticide, it was observed that mortality rate of *Anopheles* mosquitoes dropped from 94 (75.9%), 32(33.6%) to 13 (12.1%) once mosquitoes landed on the UtMCD which stayed with insecticide for three months, six months and nine months respectively (Table 21).

Table 21: Residual effects of Chlorfenapyr sprayed on UtMCD set-up within a specified period of time

#months a UtMCD stayed with insecticide	Total # of mosquitoes collected(N)	Total # of mosquito died	Mortality rate (%)
3 months	124	94	75.9
6 months	97	32	33.6
9 months	102	13	12.1

4.4 Luring Gravid *Anopheles*towards Acidified Breeding Sites

A total of ten experiments were conducted in MRT premises; five in mosquito sphere and five in insectary. The experiments used 1230, 2-5 days old laboratory reared gravid *Anopheles gambiae s.s* Kisumu strain (600 in mosquito sphere and 630 in insectary). At the end of the experiments, a total of 10,306 mosquito eggs were collected, of which 3620 (35.2%) were collected in the mosquito sphere and 6683 (64.8%) in the insectary (Table 22).

Table 22: Distribution of mosquitoes and eggs collected in the experiments

Experiments	**#of Eggs collected**	**%**
Insectary	6683	64.8
Mosquito sphere	3620	35.2
Total	10 306	100

To address the effect of position of bowls rotated in the cages on the number of mosquito eggs collected, the Poisson regression model was used. The results of the model displayed in Table 23 showed that, the average number of eggs collected at position BL(MR=1.15,p=0.0636), BR (MR=0.99,p=0.2167) and FL (MR=1.11,p=0.0958) were not statistically significant in comparison to position FR (*Arrangement of bowls: Front left (**FL**); Front right (**FR**), Back left (**BL**) and Back right (**BR**) with respect to cage entrance (**E**)*.

Table 23: Parameter estimates, unadjusted mean ratio (MR), and significance levels of the Poisson regression model for the effect of position of bowls rotated in the cages on number of mosquitoes eggs collected

Effect	Parameter Estimates	Standard Error	P-Value	MR
Intercept	4.11	0.03	<0.0601	
Position				
BL	0.14	0.04	0.0636	1.15
BR	-0.06	0.05	0.2167	0.99
FL	0.11	0.04	0.0958	1.11
FR	Reference			

*Note: Arrangement of bowls: Front left (**FL**); Front right (**FR**), Back left (**BL**) and Back right (**BR**) with respect to cage entrance (**E**)*

4.4.1 Ovicidal bioassay for pH assortment (optimization of tool)

Table 24 shows ovicidal effect of different concentrations of vinegar on *Anopheles gambiae* eggs. High (90%) egg mortality (ovicidal effect) was observed in treatment containing 140.0 ppm concentration, whereas 8% egg mortality occurred in treatment containing the lowest concentration (30.0 ppm) of vinegar. The ovicidal effect was evaluated after *Anopheles* eggs had been exposed for 168 hours. The ninety percent, lethal concentration (LC_{90}) was observed to range from 118.4 ppm to 203.4 ppm thus percent hatchability was inversely proportional to the concentration of vinegar. Figure 20 shows the toxicity of vinegar on *Anopheles* eggs which were obtained when the eggs were exposed to treatment for 168 hrs. High toxicity was observed at 140 ppm (log pH of 2.1). In addition to the observed high mortality with treatment at LC_{90}, it was observed that some of the larvae which hatched from remaining eggs (9.1%) had abnormal physical appearance (very slender and weak larvae). When the larvae were followed for two weeks, it was found that 100% of the larvae did not enter to third instar, and they died before completion of metamorphosis while in distilled water (control) hatchability of eggs to larvae (95.8%) was observed to be normal.

Table 24: Percentage mortality of *Anopheles gambiae* eggs 168 hrs after exposure to different concentrations (ppm) of vinegar

Mosquito species	Concentration ppm (pH)	Lethal concentration (LC %)	Ovicidal effect (% mean mortality ±SE)
Anopheles gambiae s.s	30.0 (5.0)	6 (Lower -32.5 to Upper 53.)	Mean 8.175±0.4
	80.0 (4.5)	40 (Lower 59.1 to Upper 100.1)	Mean 28.5 ±1.3
	100.0 (4.0)	60 (Lower 91.4 to Upper 113.4.)	Mean 53.1 ±1.1
	140.0 (3)	90 (Lower 118.4 to Upper 203.4)	Mean 90.9 ±0.9
	Control (0.0ppm)	0	Mean 4.2 ±0.2

Values are mean of four replicates SE: Standard error

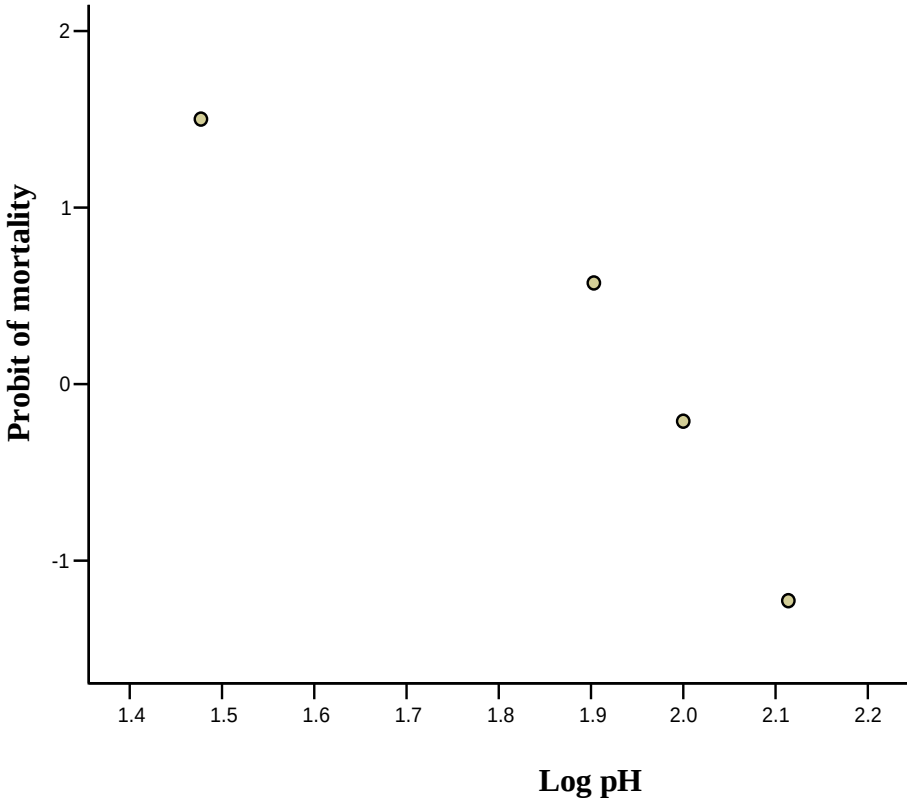


Figure 20: Probity Mortality of *Anopheles* eggs due to exposure to pH of 5, 4.5, 4, and 3 after 168 hrs treatment period as determined by linear regression equation.

Oviposition activity index (OAI) of *Anopheles* mosquitoes in different treatments

OAI for *An. gambiae s.s* ranged from -1 to + 1 in treatment during the course of all experiments tested for oviposition. The results showed that, *An. gambiae s.s* laid more

eggs in hay infusion than in control and the OAI was positive in both places experiments were conducted (mosquito sphere and insectary). The same results were observed with fresh cow urine. However in treatments with fermented urine, *An. gambiae s.s* laid fewer eggs in the treatment than in control in both mosquito sphere and insectary showing a negative OAI (Table 25).

Table 25: Oviposition activity index of *Anopheles* mosquitoes in different treatment substrates

	OAI	
Treatments	**Mosquito sphere**	**Insectary**
Hay infusion	0.22	0.3
Fresh urine	0.01	0.12
Fermented urine	-0.43	-0.39

4.4.2 Results for the different experiments

Results on effect of attracting gravid *Anopheles gambiae* to oviposit in hay infusion with or without vinegar (Experiment I)

The numbers of mosquito eggs collected during Experiment I were 3467. A total of 1141 (32.9%) and 1076 (31.0%) eggs were collected from hay infusion and hay infusion with vinegar, whereas 599 (17. 3%) and 651(18.8%) eggs were collected from vinegar and distilled water respectively. Majority, 2373 (68.45%) of these eggs were collected from insectary and the rest 1094 (31.55%) were from mosquito sphere (Table 26).

Ovicidal activity of vinegar on deposited *Anopheles* eggs

Results from the study revealed that vinegar was associated with reduced hatchability of *Anopheles* mosquito eggs. It was observed that hay infusion with vinegar prevented hatchability of 949 eggs (88.2%) while vinegar alone prevented hatchability of 563 eggs (93.9%) after 168 hrs post-oviposition. In contrast, in the case of the eggs on hay infusion

alone and on distilled water alone, only 133(11.6) % and 55(8.5%) respectively remained unhatched 168 hrs post-oviposition. Results from studies carried out in the insectary were statistically not different from those carried out in mosquito sphere in that 1187 (50.02%) and 513 (46.89%) of the eggs collected in insectary and mosquito sphere respectively did not hatch (p=0.09). The results of simple logistic regression model for the effect of treatment and area where experiments were conducted on hatching of the eggs presented in Table 26 reveal that, the risk of eggs not being hatched was significantly higher on vinegar (OR=169.37, p<0.0001), hay infusion with vinegar (OR=80.97, p=0.0338) and hay infusion alone (OR=1.43, p<0.0001) as compared to water. The association between the area where the experiment was conducted and the hatching of the eggs was not statistically significant (p=0.0871).

Table 26: Unadjusted odd ratios (OR) for association between treatment, area and hatching of mosquito eggs in Experiment I

Variable	Total Eggs n (%)	Eggs not Hatched n (%)	OR	P-Value
Treatment				
Hay infusion alone	1141 (32.9)	133 (11.6)	1.43	<0.0001
Hay infusion+ vinegar	1076 (31.0)	949 (88.1)	80.97	0.0338
Vinegar	599 (17.2)	563 (93.9)	169.37	<0.0001
Water	651 (18.8)	55 (8.4)	Reference	
Area of Experiment				
Insectary	2373 (68.5)	1187 (50.1)	1.13	0.0871
Mosquito sphere	1094 (31.5)	513 (46.9)	Reference	

Multivariate analysis for the effect of treatment and area on hatching of the mosquito eggs

Table 27 shows the results of multiple logistic regression models. Adjusting for the area where experiments were conducted, it was shown that the odds of not hatching for eggs collected in hay infusion (AOR=1.44, p=0.0319), in hay infusion with vinegar (AOR=80.6, p<0.001) and in vinegar alone (AOR=174.63, p=<0.0001) were statistically

significantly greater than that of eggs in water alone. Although not statistically significant, the proportion of egg collected in insectary area which were not hatched was lower than those collected in mosquito sphere (AOR=0.9, p=0.3767).

Table 27: Adjusted odd ratio (AOR) for association between treatment, area and hatching of mosquito eggs in Experiment I

Variable	Parameter Estimate	Standard Error	AOR	P-Value
Treatment				
Hay infusion	0.36	0.17	1.44	0.0319
Hay infusion+ vinegar	4.39	0.16	80.60	<0.0001
Vinegar	5.16	0.23	174.63	<0.0001
Water	Reference			
Areaof experiment				
Insectary	-0.11	0.12	0.90	0.3767
Mosquito sphere	Reference			

Results on effect of attracting gravid *Anopheles gambiae* to oviposit in fresh cow urine with or without vinegar (Experiment II)

A total 2359 eggs were collected in experiment II. The distribution of eggs collected by treatment and area of the experiment as presented in Table 28 shows that majority of the eggs, 811 (34.38%) were collected in distilled water followed by fresh urine alone, 637 (27%) and fresh urine with vinegar, 549 (23.27%). A total of 1424 (60.36%) eggs were collected in insectary while 935 (39.64%) were collected from mosquito sphere.

Ovicidal activity of vinegar on deposited *Anopheles* eggs

Table 28 shows that for treatments which contained vinegar, eggs which did not hatch were 507 (92.6%) for eggs collected in fresh urine with vinegar and 335(92.5%) for the eggs collected in vinegar alone. In contrast, for treatments without vinegar i.e fresh urine alone and in distilled water only 230 (36.1%) and 110 (13.6%) of eggs respectively were

not hatched. When hatchability was assessed with regards to area where experiments were conducted, 729 (51.6%) of eggs in insectary and 453(48.45%) of eggs in mosquito sphere did not hatch. The results of simple logistic regression model (Table 28) showed that the eggs in fresh urine (OR=3.6,p<0.0001), in fresh urine with vinegar (OR=76.92, p<0.0001) and in vinegar (OR=79.06, p<0.0001) were significantly more likely not to hatch in comparison to those in water. The effect of the area was not significant (p=0.1924).

Table 28: Unadjusted odd ratio (OR) for association between treatment, area and hatching of mosquito eggs in Experiment II

Variable	Total Eggs n (%)	Eggs not Hatched n (%)	OR	P-Value
Treatment				
Fresh urine	637(27)	230(36.11)	3.60	<0.0001
Fresh urine+ vinegar	549(23.27)	507(92.35)	76.92	<0.0001
Vinegar	362(15.35)	335(92.54)	79.06	<0.0001
Water	811(34.38)	110(13.56)	Reference	
Area of Experiment				
Insectary	1424(60.36)	729(51.59)	1.12	0.1924
Mosquito sphere	935(39.64)	453(48.41)	Reference	

Multivariate analysis for the effect of treatment and area on hatching of the mosquito eggs

In experiment II, the results of multiple logistic regression model (Table 29) revealed that, adjusting for the area where experiments were conducted, the odds of not hatching for eggs collected in fresh urine was almost four times that of eggs deposited in water (AOR=3.52 , p<0.0001). Similarly, the risk of eggs not being hatched was significantly higher in eggs deposited on fresh urine with vinegar almost 77 times that of eggs deposited in water (AOR=77.2, p <0.0001) and 83 times higher in eggs deposited on vinegar alone than in eggs deposited in water (AOR=83.19, p<0.0001). Adjusting for the treatment the results showed that the effect of the area was significant, with eggs collected

from insectary having significantly lower chance of not hatching than those eggs collected in mosquito sphere (AOR=0.79, p=0.0497).

Table 29: Adjusted odd ratio (AOR) for association between treatment, area and hatching of mosquito eggs in Experiment II

Variable	Parameter Estimate	Standard Error	AOR	P-Value
Treatment				
Fresh urine	1.26	0.13	3.52	<0.0001
Fresh urine+ vinegar	4.35	0.19	77.20	<0.0001
Vinegar	4.42	0.23	83.19	<0.0001
Water	Reference			
Area of Experiment				
Insectary	-0.23	0.12	0.79	0.0497
Mosquito sphere	Reference			

Results on effect of attracting gravid *Anopheles gambiae* to oviposit in fermented cow urine with or without vinegar (Experiment III)

In experiment III, a total 1491 of eggs were collected and the distribution of eggs collected by treatment and area of the experiment is presented in Table 30. The table shows that majority of the eggs, 486 (32.6 %) were collected in water followed by fermented urine, 372 (24.9%), fermented urine with vinegar 318 (21.3%) and vinegar, 315 (21.2%). A total of 982 (65.9%) eggs were collected in insectary while 509 (34.1%) were from mosquito sphere.

Ovicidal activity of vinegar on deposited *Anopheles* eggs

Table 30 shows that treatments which contained vinegar, prevented egg hatchability. It was observed that 294 (92.5%) eggs were not hatched in fermented urine with vinegar and 280 (88.9%) in vinegar alone compared to 154(41.4%) eggs and 49 eggs (10.1%) in treatments without vinegar i.e in fermented urine alone and in distilled water respectively. When hatchability was assessed with regards to area where experiments were conducted,

535 (54.5%) eggs in insectary and 242(47.5%) eggs in mosquito sphere did not hatch. The results of the fitted simple logistic regression model displayed in Table 30 revealed that, in comparison to the eggs collected in water the odds of not hatching among eggs collected in fermented urine (OR=6.3, p<0.0001), in fermented urine with vinegar (OR=109.24, p<0.0001) and in vinegar (OR=71.35, p<0.0001) were significantly greater statistically.

Table 30: Unadjusted odd ratio (OR) for association between treatment, area and hatching of mosquito eggs in Experiment III

Variable	Total Eggs n (%)	Eggs not Hatched n (%)	OR	P-Value
Treatment				
Fermented urine	372(24.9)	154 (41.4)	6.30	<0.0001
Fermented urine+ vinegar	318 (21.3)	294 (92.5)	109.24	<0.0001
Vinegar	315 (21.2)	280 (88.8)	71.35	<0.0001
Water	486 (32.6)	49 (10.1)	Reference	

Multivariate Analysis for the effect of treatment and area on hatching of the mosquito eggs

The fitted multiple logistic regression model (Table 31) for the effect of treatment and area, revealed that, the risk of eggs not hatching was significantly higher in fermented urine (AOR=6.93, p<0.0001), fermented urine with vinegar (AOR=119.91, p<0.0001) and vinegar alone (AOR=78.43,p<0.0001) as compared to water. Accounting for area where experiments were conducted, the chance of eggs collected in insectary not hatching was significantly lower than the eggs collected in mosquito sphere (AOR=0.69, p=0.0249).

Table 31: Adjusted odd ratio (AOR) for association between treatment, area and hatching of mosquito eggs in Experiment III

Variable	Parameter Estimate	Standard Error	AOR	P-Value
Treatment				
Fermented urine	1.94	0.19	6.93	<0.0001
Fermented urine+ vinegar	4.79	0.27	119.91	<0.0001
Vinegar	4.36	0.24	78.43	<0.0001
Water	Reference			
Area Of Experiment				
Insectary	-0.37	0.16	0.69	0.0249
Mosquito sphere	Reference			

Results on effect of attracting gravid *Anopheles gambiae* to oviposit in combined treatments (Experiment IV)

In experiment IV, a total of 2989 eggs were collected in different treatments. The distribution of eggs collected by treatment and area of the experiment are presented in Table 32. The table shows that majority of the eggs, 918 (30.7%) were collected in hay infusion followed by hay infusion with fresh urine combined with vinegar, 763 (25.5%), distilled water 658 (22.3%) and fresh urine 650 (21.5%). Total number of eggs collected in insectary and mosquito sphere were 982 (65.9%) and 509(34.1%), respectively.

Ovicidal effect of vinegar on deposited eggs

With regards to ovicidal activity of vinegar on deposited eggs, it was found that 689 (90.2%) of eggs deposited in hay infusion+fresh urine combined with vinegar did not hatch, while the treatments without vinegar fewer eggs were not hatched; with hay infusion alone 112 (12.2%) did not hatch, with fresh urine alone 121 (18.5%) and distilled water 60 (9.1%). Almost the same percent of eggs collected in insectary 577 (32.07%) and eggs collected in mosquito sphere 405 (34.03%) did not hatch. When the results were fitted in simple logistic regression model for the association between treatment, area where experiments were conducted and hatching of the eggs (Table 32) it was found

that, the odds of eggs not being hatched was significantly higher in hay infusion with fresh urine combined with vinegar (OR=92.18, p<0.0001) than with water. The area where the experiments were conducted had no significant association with eggs hatching (p=0.2641).

Table 32 : Unadjusted odd ratio (OR) for association between treatment, area and hatching of mosquito eggs in Experiment IV

Variable	Total Eggs n (%)	Eggs not Hatched n (%)	OR	P-Value
Treatment				
Hay infusion+ vinegar	918 (30.7)	112 (12.2)	1.38	0.0589
Fresh urine+ vinegar	650 (21.5)	121 (18.5)	2.25	0.0521
Hay infusion+ fresh urine+ vinegar	763 (25.5)	689 (90.3)	92.18	<0.0001
Water	658 (22.3)	60 (9.0)	Reference	
Area of experiment				
Insectary	1799 (60.2)	577 (32.0)	0.92	0.2641
Mosquito sphere	1190 (39.8)		Reference	

Multivariate Analysis

The results of multiple logistic regression model (Table 33) revealed that both treatment and area where the experiments were conducted were significantly associated with hatching of the mosquito eggs. The eggs collected in hay infusion alone (AOR=1.55, p=0.0110), in fresh urine alone (AOR=2.31, p<0.0001) and hay infusion +fresh urine combined with vinegar (AOR=104.89, p<0.0001) were significantly more likely not to hatch than those in water. On the other hand, the odds of not hatching among the eggs collected in insectary was significantly lower than that collected in mosquito sphere (AOR=0.60, p<0.0001).

Table 33: Adjusted odd ratio (OR) for association between treatment, area and hatching of mosquito eggs in Experiment IV

Variable	Parameter Estimate	Standard Error	AOR	P-Value
Treatment				
Hay infusion	0.4363	0.17	1.55	0.0110
Fresh urine	0.8388	0.16	2.31	<0.0001
Hay infusion+ fresh urine+ vinegar	4.6530	0.19	104.89	<0.0001
Water	Reference			
Area of experiment				
Insectary	-0.51	0.12	0.60	<0.0001
Mosquito sphere	Reference			

Results on residual activity of treatment with vinegar (Experiment V)

Distribution of number of collected mosquito eggs by treatment and area

An average of 229 (SE ± 0.08) eggs were collected in the treatment containing hay infusion with vinegar and 62 (SE ± 0.01) in fermented urine with vinegar. The average numbers of eggs collected in insectary and mosquito sphere were 149 (SE ± 1.28) and 142 (SE ± 1.12), respectively (Table 34).

Table 34: The distribution of mean number of mosquito eggs collected by treatment and area of experiment

Variable	Mean ± Std Error	Minimum	Maximum
Treatment			
Hay infusion+ vinegar	229.29 ± 0.08	123	326
Fermented urine+ vinegar	62.14 ± 0.01	23	104
Area of experiment			
Insectary	148.79 ± 1.28	27	326
Mosquito sphere	142.64 ± 1.12	23	321

Poisson regression model for number of eggs collected over time (days)

Table 35 shows the parameter estimates and adjusted mean ratios of the Poisson regression model fitted for the effect of treatment, time (days), area of the experiments

were conducted and temperature on number of mosquito eggs collected. The results showed that the number of eggs collected was significantly associated with treatment, time and temperature. On the other hand, the effect of area on the number of mosquito eggs was not significant (p=0.0611). Adjusting for other three variables in the model , the mean number of mosquito eggs collected in treatment containing hay infusion with vinegar (AMR=3.69, p<0.0001) was significantly greater than that of eggs collected in fermented urine with vinegar. In addition, the predicted mean number of eggs collected significantly decreased with increase in time (days) (AMR=0.91, p=0.0126). The other factor associated with number of eggs collected was the daily temperature. The average number of eggs collected increase with an increase in temperature (AMR=1.27, p=0.02).

Table 35: Parameter estimates, adjusted mean ratios (AMR), and significance levels of the Poisson regression model for the effect of treatment, time, area of the experiment and temperature on number of the mosquito eggs collected

Effect	Parameter estimates	Standard error	P-Value	AMR
Intercept	0.28	1.13	0.8941	1.33
Treatments				
Hay infusion+ vinegar	1.31	0.16	<0.0001	3.69
Fermented urine+ vinegar	Reference			
Area of Experiment				
Insectary	-2.59	1.38	0.0611	0.07
Mosquito sphere	Reference			
Time(Days)	-0.09	0.04	0.0126	0.91
Temperature	0.24	0.10	0.02	1.27

CHAPTER FIVE

5.0 DISCUSSIONS

Synopsis of the chapter

This chapter presents a discussion on findings from the different studies. It covers firstly community knowledge and awareness on malaria and its vectors in relation to existing shift of indoor to oudoor biting behaviour of *Anopheles* mosquitoes and its effect on malaria transmission. Secondly the chapter discusses the spatial distribution of malaria vectors, both larvae and adult mosquitoes found in the study area and thirdly it discusses findings on the evaluated novel tools against adult and immature *Anopheles* mosquitoes that can contribute to control of immature and adult stagesof outdoor biting *Anopheles* mosquitoes

5.1 Community Knowledge and Awareness on Malaria and Its Vectors

Findings of this study indicate that malaria is a public health problem in many households in the study areas. The present study revealed that majority of respondents had good knowledge on malaria transmission risk, signs and symptoms. It was pointed out by majority of participants that malaria is transmitted through the bite of *Anopheles* mosquitoes and the common signs and symptoms were mentioned to be headache, high fever and vomiting. Similar findings on good knowledge on malaria transmission, signs and symptoms were previously reported from studies carried out in Tanzania, Bangladesh and Swaziland (Ahmed *et al.,* 2009; Hlongwana *et al.,* 2009; Mazigo *et al.,* 2010). The observed better knowledge about vector transmission risks, signs and symptoms may have been influenced by health information (such as “malaria is transmitted by *Anopheles* mosquitoes that bites at midnight”) respondents are receiving through radio, television. Several studies on knowledge, awareness and practice (KAP-studies) conducted in

Tanzania have reported same findings that information, education and communication (IEC) facilities eg radio, television have contributed in raising malaria knowledge and awareness among community members (Kamugisha *et al.*, 2005; Kinung'hi *et al.,* 2010; Mazigo *et al.,* 2010; Rumisha *et al.*, 2014).

The present studies found out that health facilities were the most preferred source of health information by slight majority (50.6%) of adult participants whereas 56% of respondents who were school children indicated radio and television as source of information on health. This finding on adult respondent preference is in agreement with previous findings from elsewhere in Africa which have reported health facilities as a good place for disseminating health information to those who visit health facilities (Obrist *et al*., 2007; Kiwanuka *et al*., 2008). Usually in health facilities clients get health information through health providers or posters while they are waiting for services (Mboera *et al.,* 2007). Frequent utilization of health facilities by adults in seeking health care and time spent at home by schoolchildren after school hours could explain the differences between adults and children in terms of where they get source of health information. In addition 12.6% of children indicated school as the best source of health information corroborating findings by Edson and Kayombo, (2007) who reported that primary school teachings and television are the most preferred ICE materials for young children while hospitals were mentioned as source of information for adults.

Despite better understanding of malaria transmission risk, signs and symptoms, the study participants demonstrated knowledge gap on mosquito breeding sites. Majority of respondents in the present study were found to have low level of knowledge on malaria breeding sites as most of them mentioned that garbage and long grasses were potential breeding sites for malaria vector. This finding is contrary to the study done in Geita where

majority of participants said stagnant water was the breeding sites for *Anopheles* mosquitoes (Mazigo *et al.,* 2010). Additionally, the present study found out that most of respondents were not aware of transmission risks brought by early mosquito bites as majority reported that malaria is transmitted by *Anopheles* mosquitoes which bite in the midnight. This response could be brought by continuing media message from radio and television which says "malaria is transmitted by *Anopheles* mosquito which bites at midnight". This media message is only partially correct since there are *Anopheles* mosquitoes which bite early outdoor contributing to residual malaria transmission (Martins-Campos*et al.,* 2012; Russell *et al*., 2013; Chareonviriyaphap *et al.,* 2013; Killeen, 2014).

From the present study it was revealed that predominant *Anopheles* species in study areas is *An. arabiensis* which mostly exhibit early outdoor feeding. Therefore this finding points to the need to give correct information on malaria to participants as Appiah-Darkwah and Badu-Nyarko (2011) reported that good community knowledge and awareness on malaria and its vectors contribute in the fight against malaria. The right information will empower people to make informed choices on available malaria preventive measures.

It was observed from the present study that more than 70% of participants used ITNs as their personal protection against mosquito bites. The use of insecticide treated nets (ITNs) has been reported in many studies as a major recognized method of personal protection against mosquitoes bite in malaria endemic areas (Hlongwana *et al.,* 2009; Govella *et al.,* 2010; Kinung'hi *et al.,* 2010; Mazigo *et al.,* 2010). Although ITN use still provides useful protection, its protective efficacy is limited to those who sleep under ITNs and exclude those who are active outdoors early evening. Therefore community should be told not to

depend only on to ITNs/LLINs but also to use other personal protective measures such as wearing long sleeved clothes and use of mosquito repellents, e.g mosquito coils, aerosol sprays (Frances and Cooper, 2007; Kiszewski and Darling, 2010; Wilson *et al.,* 2014; Crawshaw *et al.,* 2017).

On other hand, particpants were given additional question on mosquito pictures of different species. Only about a quarter of participants correctly identified pictures of adult and larval stage of *Anopheles* mosquitoes in contrast to 75.5% of participants being able to correctly give the name (*Anopheles*) of the vector of malaria. This probably emanates from the radio message "Malaria is transmitted by a mosquito called *Anopheles* that bites at midnight" that had been on air for a considerable length of time in Tanzania. It would appear therefore that many people know *Anopheles* without known the entity to which this name applies because they were not given images of this entity. If they are to be engaged in control of the vector it is imperative that they will be able recognize it in its immature as well as its adult stage. When further analysis was done, school children scored higher than adults showing correct understanding on breeding sites and risk of malaria transmission by the bite of outdoor *Anopheles*. Health information given to pupils at school could explain the difference of this score (Edson and Kayombo, 2007). Although majority of pupils did not point out school as main source of malaria information, this study points to the fact that school children have better knowledge on malaria and mosquitoes than adults. Perhaps these findings could alarm malaria intervention officers to invest into school children; since children are future generation so imparting malaria knowledge to them will be of great value. Therefore school teachers and pupils can be involved in malaria intervention programmes. In Tanzania most children attend day school and therefore they could be a good channel for disseminating health information among household members when they go back home. Thus school

teachers should be empowered with appropriate health knowledge i.e. malaria through seminars and other communication means so that they can deliver correct information to pupils (Edson and Kayombo, 2007). Furthermore school children pointed out that they receive more information on HIV/AIDS than on malaria, while malaria was mentioned by majority of participants to be the number one disease among top five diseases in their households. This observation would call for revision of school curricula so that malaria could be given emphasis similar to that given to HIV/AIDS.

5.2 Spatial Distribution of Larval and Adult *Anopheles* Mosquitoes

Studies on spatial and temporal distribution patterns of malaria vectors were conducted to determine species of *Anopheles* mosquitoes, their abundance and sheltering behaviours in the areas where the UtMCDs were to be tested. The ARcGIS analysis showed that mosquito distribution was spatially aggregated around human settlements. As expected, higher abundances of *Anopheles* mosquitoes were found in houses a few meters (100-500 meters) from rice paddies as compared to houses far away from rice paddies. Mwangangi *et al.* (2007b) and others Kweka *et al.* (2009) reported that rice plantations are major mosquito-breeding habitats and constitute the most important factor determining aggregated distribution of *Anopheles* mosquitoes along an irrigation canal (e.g. rice irrigation scheme) (Mwangangi *et al.,* 2007b; Kweka *et al.,* 2009). Other studies also found that households which were near mosquito temporary or permanent breeding sites had exhibited higher mosquito abundance (Konradsen *et al.,* 2003; Lindsay *et al*., 2003; Cano *et al.,* 2006). In the present study breeding sites with positive *Anopheles* mosquito larvae were found to be rice paddies, ditches, containers, swamps, brick pits, foot print and tyre tracks. Interestingly *Anopheles* larvae (mostly *An. arabiensis*) were also found in septic tanks/pits (polluted water) during rainy and dry season. The explanation for this could be due unplanned urban and peri-urban cities which had an impact on natural

ecosystems that favour the adaptation of *Anopheles* mosquitoes to expand their niches to polluted habitants.This findingis are inline with Minakawa *et al.* (2001); Sattler *et al.* (2005); and Awolola *et al.* (2007) who did their study during dry season and reported that polluted breeding sites were positive for *Anopheles* larvae. Thus in control of *Anopheles* mosquitoes through environmental manipulation or modification, it is important to take note of the breeding of the malaria vector in polluted water.

The present study also observed substantial spatial-temporal variations in mosquito abundances which were mainly caused by rainfall changes. Expectedly, there were more mosquito abundances during rainy season than during dry/cold season as a result of higher number of larval habitats in rainy season than in the cold/dry season. During all seasons *Anopheles arabiensis* were major malaria vector in the study area. The higher abundance of *An. arabiensis* could probably be attributed to use LLINs/ITNs and IRS which diminish the highly anthropophilic *Anopheles* species *An. gambiae s.s* which is now being replaced by *An. arabiensis* (Russell *et al.,* 2011*).* For instance in many places in Tanzania there is a great shift from *An. gambiae ss* to *An. arabiensis* (Russell *et al.,* 2010; Russell *et al.,* 2011; Kitau *et al.,* 2012; Derua *et al.,* 2012). This has also been observed in the study area especially Morogoro where in previous years *An. gambiae ss* were predominant species (Russell *et al.,* 2010; Kitau *et al.,* 2012) but in the present study two wards surveyed showed over 80% of *Anopheles* caught were *An. arabiensis.*.

It is not surprising that during dry cold season mosquitoes disappear from vicinity to aestivate/hibernate (Robich *et al.,* 2005; Huestis *et al.,* 2012; Lehmann *et al.,* 2014). From the findings of the present study, it was observed that during dry/cold season majority of mosquitoes hide inside the houses in the dark and/ or places with little or no disturbances. The results showed that the main places where mosquitoes hide during dry/cold period are

under beds, behind undisturbed curtains, store rooms/stored junks and under furnitures. Merdic *et al.* (2005) found out in their study that during cold season *Anopheles* species hid in cellars, deep basement, dark rooms with small windows and on dirt floor made of bricks. According to reports presented by many studies which explain how mosquitoes become immobile during dry/cold season (Robich, *et al.,* 2005; Huestis *et al.,* 2012; Yaro *et al.,* 2012; Lehmann *et al.,* 2014), spraying methods which knock down mosquitoes can be conducted during cold season and kill many mosquitoes subsequently reducing population density in the community before warm/ wet seasons when mosquitoes come out from hiding places.

5.3 "Attract and kill "

UtMCD analysis

An "Attract and kill technique" is one among the tools which have been studied for past decades in disease-transmitting insects including tsetse flies, horse flies and mosquitoes (Knols *et al.,* 1993; Day and Sjogren, 1994; Copping and Menn, 2000; Okumu *et al.,* 2010a; Hiscox *et al.,* 2012; Matowo *et al.,* 2013; Mmbando *et al.,* 2015). The present study used the technique of luring malaria vectors to the UtMCD sprayed with Chlorfenapyr, a non excito-repellent insecticide. The findings revealed that UtMCD set-ups with chemo-attractants caught higher number of *Anopheles* mosquitoes than UtMCD set-ups without chemo-attractants. Chemo-attractants included carboxylic acid emanating from unwashed socks as well as ammonia and CO_2 which were incooperated in the UtMCDs. In previous studies it was reported that unwashed nylon socks which contained human foot odours attract *Anopheles* species and *Culex quinqfasciatius* (Mboera and Takken, 1997; Schmied *et al.,* 2008). Similarly several studies have reported that ammonia and CO_2 are potent chemical ques used by blood seeking mosquitoes while searching for host to feed upon (Smallegange *et al.,* 2005; Carey *et al.,* 2010; Verhulst *et*

al., 2011; Takken, and Verhulst, 2013). In line with those findings, the present study confirmed the synergistic effects of odour plumes released by the chemo-attractants incorporated in the UtMCD following higher catches of mosquitoes in the UtMCD containing chemo-attractants especially during warm season. The findings of this study showed that seasonal variations had influences on the mosquito collection, as the total number of mosquitoes caught during warm season was higher than in cold-dry season. Nevertheless, the UtMCD captured higher number of mosquitoes in the courtyard of houses which are constructed in close proximity to the larval habitats such as rice paddies (e.g in Mahita and Swaswa areas) than the device E. Umbrella which was on top of mosquito contaminating device could probably explain the higher catch since the umbrella increased surface area where mosquitoes hide as it mimicked thatched roof. This suggests that the UtMCD can perform very well in catching *Anopheles* mosquitoes especially shortly after rainy seasons.

Following the observed outdoor malaria vectors in the study areas, use of additional malaria intervention methods which use inexpensive chemo-attractants in combination with insecticide that has different mechanism of action could be of great help (Smallegange *et al.*, 2005; Okomu *et al.*, 2010; Matowo *et al.*, 2013; Mmbando *et al.*, 2015). It was demonstrated in the present study that UtMCDs with attractants and insecticides were effective in reducing survival rate of mosquitoes which came into contact with the treated UtMCD. The findings showed that UtMCD set-up D (tested UtMCD with chemo-attractants and insecticide i.e chlorfenapyr) caused higher mortality contrary to low mortality observed in odour baited landing box sprayed with pirimiphos methyl organophospate used in the study conducted in Ifakara by Matowo *et al.*, 2013. Therefore UtMCD set-up D (that has chemoattractants and insecticide-Chlorfenapyr) would be a suitable outdoor tool for controlling outdoor mosquitoes because of its

potential to catch higher number of *Anopheles* mosquitoes and cause higher mortality to *Anopheles* mosquitoes (>85%) landed on it due to Chlorfenapyr.

The present study used chlorfenapyr as a killing agent that has different mode of action (it acts on the oxidative pathway in the mitochondria of the mosquito) in contrast to existing frontline insecticides (i.e pyrethroids) which are neurotoxic insecticides (Tomlin, 2000; Mosha *et al*., 2008; Raghavendra *et al.,* 2011; WHOPES, 2013). The findings from the present study revealed that mortality rate was 87% for *Anopheles gambiae* complex landed on all UtMCDs treated with chlorfenapyr. This suggests that the malaria vectors (i.e *Anopheles* species), are susceptible to novel insecticide (chlorfenapyr) (Pridgeon *et al*., 2008; N'Guessan,*et al.,* 2009; Oliver *et al.,* 2010; Oxborough *et al.,* 2010; Mosha *et al.,* 2010; Ngufor, *et al.,* 2011; Raghavendra *et al.,* 2011; Bhatt *et al*, 2013).

It is also known that chlorfenapyr causes delayed mortality when the mosquitoes come into contact with the insecticide (N'Guessan *et al*., 2009; Oxborough *et al.,* 2010; Raghavendra *et al.,* 2011). This delayed mortality is caused by slow depletion of ATP once oxidative phosphorylation in the insect mitochondria is disrupted and consequently the organism will lose energy and subsequently die (N'Guessan *et al*., 2009; Oxborough *et al.,* 2010; Raghavendra*et al.,* 2011). It was observed in the present study that higher mortality was in day 2 and day 3 (i.e. within 72 hrs post-insecticide exposure). However, about 15% of *Anopheles* mosquitoes caught in different UtMCDs did not die. It is difficult to pin point the reasons for this could probably be due to the fact that these mosquitoes could have landed on the UtMCDs which were not sprayed with insecticide. The results showed that UtMCD without insecticide supported longer expected mosquito survival than UtMCD with insecticide.

Following the observed good performance of the chlorfenapyr used in the UtMCD, it appeared worthwhile to study the residual effect of chlorfenapyr for 9 months post insecticide spray on UtMCD set-up D as many previous studies on chlorfenapyr were conducted inside human dwellings (Mosha *et al.,* 2008; N'Guessan *et al*., 2009; Oliver *et al.*, 2010; Oxborough *et al.,* 2010; Ngufor, *et al.,* 2011 Raghavendra *et al.*, 2011; Bhatt *et al.*, 2013). It was found out that relatively higher mortality of *Anopheles* mosquitoes occurred in a UtMCD which had stayed with insecticide for three months compared to UtMCD that had stayed with the insecticide for six and nine months. The mortality rates declined from 75.9% at three months to 33.6% at six months and finally to 12.1% at nine months. This result is in contrast to findings from other studies in which chlorfenapyr was applied indoors on the walls and in mosquito nets resulting in a residual effect of up to 18 months (Mosha *et al.,* 2008). The different results from the two different studies could be due to location of UtMCD outside human dwellings in the present studies in contrast to the studies by Mosha *et al.* (2008). Placement of chorfenapyr oustside subjects the chemical to detrimental weather elements including wind, sunlight and humidity.

Molecular (PCR) identification of *Anopheles* spp, ELISA CSP detection and blood meal analysis

A sample of 600 *Anopheles* mosquitoes caught in the present study was subjected to the Polymerase Chain Reaction (PCR) for species identification and ELISA CSP for detection of sporozoites infection rate. According to the PCR results, *Anopheles arabiensis* was by far the most dominant malaria vector in the study areas (Dodoma and Morogoro). This finding is in agreement with the results reported by Russell *et al.* (2010) Derua *et al.* (2012) and Kabula *et al.* (2014) that in many regions of Tanzania *An. gambiae s.s.,* the predominant malaria vector species which previously colonialized the areas is now largely overtaken by *An. arabiensis.* This dramatic shift has been suggested

to emanate from scale-up of indoor insecticidal interventions particularly ITNs (Bayoh *et al.*, 2010; Russell *et al.*, 2010; Russell *et al*., 2011; Derua *et al.*, 2012; Kitau *et al.*, 2012; Futami *et al.*, 2014).

In Tanzania there are reports of an increase in the national malaria prevalence from 9.5% in 2012 to 14.1% in 2016 with the prevalence remaining higher even in the places where the LLINs/ITNs coverage is almost above 90% (TDHS-MIS, 2016). The possible explanation would be that the present interventions (LLINs/ITNs and IRS) are not effective against exophilic and/or exophagic malaria vectors. Therefore residual malaria is becoming more common in the areas where *An. arabiensis* is important malaria vector. Thus additional important alternatives to prevent outdoor and/or early biting *Anopheles* mosquitoes such as UtMCD which attract and kill mosquitoes, and larviciding, oviciding could be integrated in malaria programmes to complement the current interventions (LLINs/ITNs and IRS). An interesting observation from the present study was that a high proportion of *Anopheles* mosquitoes captured were not blood fed. This suggested that these mosquitoes visited the UtMCDs before they had bitten a human. This finding concurs with the results reported by Matowo *et al.* (2013) and Mmbando *et al.* (2015) that their odour-baited devices protected people against outdoor mosquitoes bite. With regards to blood meal analysis, this study found out that malaria transmitting vector had no fragments of blood host tested. This was probabily due to post-exposure holding time after been exposed to chlorfenapyr thus the mosquito consumed the blood. Kent and Norris (2005) explained that enzyme digest which are used in analysis allow identification of mammalian host sources from partially digested mosquito blood meals up to 60 hours post-feeding only.

5.4 Effect of acid (vinegar) on *Anopheles* eggs

Ovicidal activity of vinegar on deposited eggs

The findings revealed that breeding habitats with water acidified to pH 3.0 by vinegar possessed ovicidal activity against deposited *Anopheles gambiae s.s* eggs. Similar findings were reported by Clement (2000); Simşek (2004); Thamer and Abdulsamad (2005); Govindarajan *et al.* (2011); Khandagle *et al.* (2011); Panneerselvam *et al.* (2013) when they did experiments on eggs of *Anopheles stephensi*, *Aedes aegypti* and*Culex quinquefasciatus*. Their findings showed how plant extracts with acid and pH of less than 4 prevented eggs hatching. Broadbent and Pree (1984); Sutcliffe and Hildrew (1989); Simşek (2004) and Thamer and Abdulsamad (2005) have explained the effect of acid on the embryo that as the acid penetrates inside the egg shell membrane through chorion, it inactivates growth and choriolytic enzyme consequently suppresses further embryonic development and eventually the developing larva dies.

The present study also revealed that at pH level of 3 resulting from vinegar 140 ppm, the ovicidal effect of the vinegar on *An. gambiae s.s* eggs deposited in the treatment for 168 hr was above 85%. Furthermore the hatched larvae had abnormal physical appearances including being slender and having very weak body movements. Moreover these larvae did not enter into 3rd instar after being followed for two weeks and they eventually died. These findings are in agreement with those from studies by Clement (2000); Clark *et al*. (2004); Chenniappan and Kadarkarai (2008); Thamer and Abdulsamad (2005) and Reegan *et al.* (2015) who showed that low pH of plant extract caused nearly zero hatchability of An. *stephensi Liston, Cx. Quinquefasciatus Say,* Ae. *aegypti* eggs. Other previous reported studies on the aquatic environment for mosquitoes reported that pH less than 4 or higher than 10 do not support development of eggs, larvae and pupa of almost

all mosquitoes (Strange *et al.,* 1982; Truchot, 1987; Sutcliffe and Hildrew, 1989; Cooper, 1994; Clement, 2000; Patrick *et al*., 2002; Clark *et al.*, 2004).

Oviposition attraction using hay infusion *(Oryza sativa, L)* and cow urine

Several bioassays have been developed and used to investigate oviposition substrate preferences of gravid mosquitoes. Most of these bioassays were mixed with insecticide to kill gravid *Anopheles* before they lay eggs or kill the deposited eggs and prevent hatchability (Simşek, 2004; Thamer and Abdulsamad, 2005; Govindarajan *et al.,* 2011; Khandagle *et al.,* 2011). The findings showed that gravid *Anopheles* mosquitoes were more attracted to bowls treated with hay infusion as compared to cow urine and distilled water. This attractiveness of hay infusion is thought to be contributed to by its 3-methylindole and 4-ethylphenol components which have been shown to have electro-antennographical effects on mosquitoes inducing positive oviposition response in *An. gambiae* s.s and other mosquito vectors (Reiter and Colon, 1991; Allan and Kline, 1995; Polson *et al.*, 2002; Santana *et al*., 2006). During experimental period it was observed that gravid mosquitoes showed a strong preference to oviposit on bowls which contained either hay infusion alone with/ or without acidified water than in bowls containing cow urine or distilled water as more eggs were collected in hay infusion bowls (OAI was positive). It was previously reported that gravid *Anopheles* like to oviposit in clean water but it was not the case in this study, probably because distilled water did not have microbial fauna which attract the gravid mosquitoes to oviposit (Clements, 2000; Dieng *et al.,* 2002; Sumba *et al.,* 2004; Munga *et al.,* 2005; Yaro *et al.*, 2006; Ponnusamy *et al.,* 2008).

When fresh cow urine (which contains low content of 4-methylphenol, 3-n-propylphenol) was used as chemo-attractant, a good number of eggs were collected in the bowls which

contained fresh cow urine but for fermented urine *An. gambiae* ss laid fewer eggs compared to eggs collected in distilled water (OAI was negative) (Kweka *et al.,* 2011; Mihok,and Mulye, 2010). These results suggest that fresh cow urine can be used as chemo-attractant for *Anopheles* species unlike fermented cow urine which deters ovipostion as found in the present studies as well as other previous studies (Mahande *et al.,* 2010; Kweka *et al.,* 2011).

With regards to residual effects of chemo-attractants used (hay infusion and cow urine) results have demonstrated that after 14 days of egg collection, treatment which contained acidified water with hay infusion had significantly greater number of deposited eggs compared to the eggs collected in bowls containing cow urine (Polson *et al.,* 2002; Santana *et al.,* 2006; Muturi *et al.,* 2007; Kweka *et al.,* 2011). No doubt the declines in eggs collected from bowls with cow urine corroborate the above studies which showed that fermented urine deters ovipositing mosquitoes. However even in case of hay infusion the mean number of eggs collected started to decrease significantly as time went by. This could be perhaps due to depletion of compound released by hay infusion,which is in line with findings reported by Reiter and Colon, (1991) and Gopalakrishnan *et al.* (2012) who showed in their studies that CDC ovitrap with hay infusions which contained strong infusions (dilution of 50-100%) contribute to longer range attraction, than the weaker solutions (dilution 10-30%).

Following the findings on effects of hay infusion and partly cow urine as has been discussed above; the combination of an oviposition attractants with ovicidal (vinegar) can be used to control of mosquito disease vectors through destruction of immature stages of *An. gambiae* complex and in the end reduce the population density of malaria vectors.

Combination of attractants and ovicidal agent (vinegar) for malaria vector control

In the present study the combination of chemo-attractants (hay infusion and cow urine) and ovicidal agent (acidified water at pH 3, 140ppm) was evaluated for of its luring effect on gravid *Anopheles* mosquitoes and the hatchability of the deposited egg. The finding of the study revealed that, bowls with acidified water containing hay infusion had significantly more eggs deposited than distilled water and acidified water with cow urine. This higher oviposition is likely to have resulted from effect of volatile chemical compounds (e.g. 3-methylindole) released by hay infusion from oviposition bowls (Polson *et al.*, 2002; Santana *et al*., 2006; Muturi *et al.,* 2007; Kweka *et al.,* 2009). Furthermore for eggs which were exposed to acidified water, the study showed that the eggs did not hatch and it can be speculated that the ovicidal compound (acetic acid-(CH3COOH) present in the oviposition bowls containing vinegar affected the embryo development inside the chorions therefore hatchability was not possible. Kaiser *et al.* (2014) in their study on embryonic development and rates of metabolic activity in early and late hatching eggs of the major malaria vector *Anopheles gambiae*, used acetic acid to inhibit hatchability during embryo development assessment. Again it was shown in the present study that bowls which contained acidified water alone had nearly zero eggs hatched although gravid *Anopheles* mosquitoes did not prefer to lay eggs in them as few eggs were collected from these bowls. This finding is similar to that of Elango *et al.,* (2009) who studied the effect of acetonic leaf extract of *Aegle marmelos* on *Anopheles subpictus* and reported egg mortality of 100%. The same was reported by Chenniappan and Kadarkarai (2008); Govindarajan *et al.* (2008) Khandagle (2011) and Cheah *et al.* (2013) who used ethanolic and acetonic leaf extracts and reported nearly zero hatching of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* eggs. Similarly, prevention of hatchability was higher in acidified water containing cow urine although mean number of eggs collected were very few. This could be due to chemical compounds

such as 4-methylphenol, 3-n-propylphenol released by cow urine which have been shown to deter oviposition response of *An. gambiae ss* (Mahande *et al.*, 2010; Mihok and Mulye, 2010; Kweka *et al.*, 2011).

Although the hatchability reduction was observed in almost all treatments which contained acidified water, for malaria vector control it is important to note what lures gravid mosquitoes to lay their eggs (e.g. hay infusion). In the present study, a combination of hay infusion as attractant and an ovidical agent such as vinegar at pH3 can be used as a tool of controlling juvenile stage of *Anopheles* mosquitoes and ultimately reduce the population of outdoor and indoor malaria vectors.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the present studies, the following were significant findings:

i. *An. arabiensis*, which basically exhibits outdoor feeding, is the predominant malaria vector in Dodoma and Morogoro urban districts

ii. Respondents in the study area had poor knowledge and awareness of breeding sites and mosquito biting times but were moderately knowledgeable on malaria and its vectors.

iii. School children were more knowledgeable on malaria and its vectors than adults and therefore there is a need to empower teachers with appropriate information on malaria and its vectors so that they can increase school children's awareness that can be passed onto their parents for improvement of malaria control.

iv. Majority of participants were not aware that outdoor early *Anopheles* mosquito bites increase the risk of residual malaria transmission.

v. Hiding places where mosquitoes found during cold-dry season were under bed, behind curtains, in undisturbed places such as store rooms.

vi. A fabricated Umbrella-topped Mosquito Contaminating Device (UtMCD) that was tested for efficacy in attracting and killing *Anopheles* mosquitoes showed potential for use in mosquito control.

vii. Artificial breeding sites with chemo-attractants (hay infusion) and an ovicidal agent (acetic acid from vinegar) proved effective in luring gravid *Anopheles* mosquito to oviposit eggs which then did not hatch as aresult of the acidity.

viii. Co- existence of *Anopheles* and culicine species in breeding habitats was recorded and it was also shown that polluted water supported breeding of *Anopheles*

mosquitoes unlike what is currently known (that *Anopheles* species prefer to breed in clean water).

ix. An additional method of mosquito control that can be incorporated in intergrated Vector Management (IVM) in urban and peri urban settings is attracting gravid *Anopheles* to oviposit in acidified artificial breeding sites (bowls). This was shown to prevent egg hatching.

6.2 Recommendations

1. National malaria control program should endeavour to increase awareness on changed feeding behavior of *Anopheles* species previously endophilic endophagic. People still have perception that malaria is only transmitted by the midnight feeding *Anopheles* mosquitoes. Therefore awareness creation through information, educational and communication (ICE) materials, such as television, radio, posters, schools, health centre should be used to disseminate information on changed feeding behaviour of malaria vector so people can protect themselves against early outdoor mosquito bite by using mosquito repellents, wearing long sleeved cloths, and these person protective methods should be practiced early in the evening.
2. There should be more teaching on malaria and its vector *Anopheles* at schools and the school children should be used to disseminate information on mosquitoes and malaria.
3. There is a need of considering chlorfenapyr as alternative insecticide for control of the malaria mosquito vector since it has different mode of action with non- excito repellence. Therefore the insecticide can be used in IRM strategies for LLINs intervention (in rotation and mosaic spraying)

4. Since contaminating device (UtMCD) have shown great potential of attracting and killing outdoor mosquitoes, this device (which is cheap) shuld be further optimized and promoted for field use to complement the existing indoor mosquito control interventions (ITNs/ILLNs &IRS)
5. Further research for trapping and killing outdoor early biting *Anopheles* mosquitoes should be conducted to improve UtMCD in terms of size,
6. Acidified artificial breeding sites (bowls) can be incorporated into integrated vector management (IVM) in urban and peri urban settings
7. There is a need to carry out studies on An. *arabiensis* to determine effectiveness of combination of chemoattractant (cow urine) and vinegar in luring gravid females for oviposition. The present studies only used *Anopheles gambiae* s.s
8. In larval source management (LSM), it is important to consider all breeding sites (whether clean or polluted) as potential breeding sites for *Anopheles* mosquitoes as it has been shown that *Anopheles* can also breed in polluted water.
9. Malaria, mosquitoes hiding in various places (store rooms, behind undisturbed curtains, under bed, under furniture) should be targeted with knock down aerosol spray insecticides which when appled at time of lowest mosquito prevalence (cold-dry season will result in reduced mosquito population when the climatic conditions become favourable for mosquito breeding.

Limitations of the study

i) The fact that the umbrella top of the fabricated UtMCDs was made of cotton cloth likely resulted in some wash off of sprayed Chlorfenapyr whenever there was a rain shower since the UtMCDs were set shortly after rain season at which time there were occasional rain drizzles. Improved version of the UtMCD will have a plastic sheet designed as a shed to protect the device or better still the umbrella top

will be made large enough and with plastic sheet sawn onto the upper surface to protect the rest of the device from rain.

ii) The impact of different weather conditions (temperature, rain, sunlight, humidity and wind) on the residual effect of Chlorfenapyr sprayed on the UtMCDs was not evaluated.

iii) Experiments for evaluating the attractiveness of acidified artificial breeding sites to ovipositing gravid *Anopheles gambiae s.s* were conducted during cold-dry season only instead of also during the warm-wet season. Cold weather could affect oviposition especially in mosquito sphere

iv) Use of one species of *Anopheles* mosquitoes i.e. *An gambiae s.s* (in objective 3) affected the outcome since *An. gambiae s.s* are not much attracted to cow urine compared to *An. arabiensis*

REFERENCES

Abate, A., Degarege, A. and Erko, B. (2013). Community knowledge, attitude and practice about malaria in a low endemic setting of Shewa Robit Town, northeastern Ethiopia. *BioMed Central Public Health* 13(312): 1 – 8.

Abdalla, H., Matambo, T. S., Koekemoer, L. L., Mnzava, A. P., Hunt, R. H. and Coetzee, M. (2008). Insecticide susceptibility and vector status of natural populations of Anopheles arabiensis from Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 102(3): 263 – 271.

Acheson, E. S., Plowright, A. A. and Kerr, J. T. (2015). Where have all the mosquito nets gone? Spatial modelling reveals mosquito net distributions across Tanzania do not target optimal Anopheles mosquito habitats. *Malaria Journal* 14(322): 1 – 14.

Adongo, P. B., Kirkwood, B. and Kendall, C. (2005). How local community knowledge about malaria affects insecticide-treated net use in northern Ghana. *Tropical Medicine and International Health* 10(4): 366 – 378.

Ahbirami, R., Zuharah, W. F., Yahaya, Z. S., Dieng, H., Thiagaletchumi, M., Fadzly, N. and Bakar, S. A. (2014). Oviposition deterring and oviciding potentials of Ipomoea cairica L. leaf extract against dengue vectors. *Tropical BioMed* 31(3): 456 – 465.

Ahmed, S. M., Haque, R., Haque, U. and Hossain, A. (2009). Knowledge on the transmission, prevention and treatment of malaria among two endemic populations of Bangladesh and their health-seeking behaviour. *Malaria Journal* 8(173): 1 – 11.

Aïkpon, R., Agossa, F., Ossè, R., Oussou, O., Aïzoun, N., Oké-Agbo, F. and Akogbéto, M. (2013). Bendiocarb resistance in Anopheles gambiae sl. populations from Atacora department in Benin, West Africa: A threat for malaria vector control. *Parasites and Vectors* 6(192): 1 – 7.

Aïzoun, N., Aïkpon, R., Gnanguenon, V., Oussou, O., Agossa, F., Padonou, G. G. and Akogbéto, M. (2013). Status of organophosphate and carbamate resistance in Anopheles gambiae sensu lato from the south and north Benin, West Africa. *Parasites and Vectors* 6(274): 1 – 6.

Allan, S. A. and Kline, D. L. (1995). Evaluation of organic infusions and synthetic compounds mediating oviposition inAedes albopictus andAedes aegypti (Diptera: Culicidae). *Journal of Chemical Ecology* 21(11): 1847 – 1860.

Anderson, M. T., Kiesecker, J. M., Chivers, D. P. and Blaustein, A. R. (2001). The direct and indirect effects of temperature on a predator prey relationship. *Canadian Journal of Zoology* 79(10): 1834 – 1841.

Appiah-Darkwah, I. and Badu-Nyarko, S. K. (2011). Knowledge of malaria prevention and control in a sub-urban community in Accra, Ghana. *International Journal of Tropical Medicine* 6(3): 61 – 69.

Awolola, T. S., Oduola, A. O., Obansa, J. B., Chukwurar, N. J. and Unyimadu, J. P. (2007). Anopheles gambiae s.s breeding in polluted water bodies in urban Lagos, southwestern Nigeria. *Journal of Vector Borne Diseases* 44: 241 – 244.

Balkew, M., Ibrahim, M., Koekemoer, L. L., Brooke, B. D., Engers, H., Aseffa, A. and Elhassen, I. (2010). Insecticide resistance in Anopheles arabiensis (Diptera: Culicidae) from villages in central, northern and south west Ethiopia and detection of kdr mutation. *Parasites and Vectors* 3(1): 40 – 49.

Bang, Y. H., Mrope, F. M. and Sabuni, I. B. (1977). Changes in mosquito populations associated with urbanization in Tanzania. *East African Medical Journal* 54(7): 403 – 410.

Barat, L. M. (2006). Four malaria success stories: how malaria burden was successfully reduced in Brazil, Eritrea, India, and Vietnam. *The American Journal of Tropical Medicine and Hygiene* 74(1): 12 – 16.

Barber, J. A., Greer, M. and Coughlin, J. (2007). Field tests of malathion and permethrin applied via a truck-mounted cold fogger to both open and vegetated habitats. *Journal of the American Mosquito Control Association* 23(1): 55 – 59.

Barnard, D. R. and Xue, R. D. (2004). Laboratory evaluation of mosquito repellents against Aedes albopictus, Culex nigripalpus, and Ochlerotatus triseriatus (Diptera: Culicidae). *Journal of Medical Entomology* 41(4): 726 – 730.

Baume, C. A., Reithinger, R. and Woldehanna, S. (2009). Factors associated with use and non-use of mosquito nets owned in Oromia and Amhara regional states, Ethiopia. *Malaria Journal* 8(64): 1 – 11.

Bayoh, M. N. (2001). Studies on the development and survival of Anopheles gambiae sensu stricto at various temperatures and relative humidities. Thesis for Award of PhD Degree at Durham University, Durham, 151pp.

Bayoh, M. N., Mathias, D. K., Odiere, M. R., Mutuku, F. M., Kamau, L., Gimnig, J. E. and Walker, E. D. (2010). Anopheles gambiae: historical population decline associated with regional distribution of insecticide-treated bed nets in western Nyanza Province, Kenya. *Malaria Journal* 9(62): 1 – 12.

Beck-Johnson, L. M., Nelson, W. A., Paaijmans, K. P., Read, A. F., Thomas, M. B. and Bjørnstad, O. N. (2013). The effect of temperature on Anopheles mosquito population dynamics and the potential for malaria transmission. *PLOS One* 8(11): e79276.

Betson, M., Jawara, M. and Awolola, T. S. (2009). Status of insecticide susceptibility in Anopheles gambiae sl from malaria surveillance sites in The Gambia. *Malaria Journal* 8(189): 1 – 8.

Bhatt, S., Weiss, D. J., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U. and Wenger, E. A. (2015). The effect of malaria control on Plasmodium. *Malaria Journal* 6(1): 1 – 12.

Blackwell, A. and Johnson, S. N. (2000). Electrophysiological investigation of larval water and potential oviposition chemo-attractants for Anopheles gambiae ss. *Annals of Tropical Medicine and Parasitology* 94(4): 389 – 398.

Bohbot, J. D., Fu, L., Le, T. C., Chauhan, K. R., Cantrell, C. L., and Dickens, J. C. (2011). Multiple activities of insect repellents on odorant receptors in mosquitoes. *Medcidal Veterinary Entomology* 25(1): 436–444.

Broadbent, A. B. and Pree, D. J. (1984). Effects of diflubenzuron and BAY SIR 8514 on the oriental fruit moth (Lepidoptera: Olethreutidae) and the obliquebanded leafroller (Lepidoptera: Tortricidae). *Journal of Economic Entomology* 77(1): 194 – 197.

Brown, Z.S., Dickinson, K.L. and Kramer, R.A. (2013). Insecticide Resistance and Malaria Vector Control: The Importance of Fitness Cost Mechanisms in Determining Economically Optimal Control Trajectories. *Journal of Economic Entomology* 106(1): 366 – 374.

Burkot, T. R., Williams, J. L. and Schneider, I. (1984). Identification of Plasmodium falciparum-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. *The American Journal of Tropical Medicine and Hygiene* 33(5): 783 – 788.

Busula, A. O., Takken, W., Loy, D. E., Hahn, B. H., Mukabana, W. R. and Verhulst, N. O. (2015). Mosquito host preferences affect their response to synthetic and natural odour blends. *Malaria Journal* 14(1):133 – 142.

Cano, J., Descalzo, M. Á., Moreno, M., Chen, Z., Nzambo, S., Bobuakasi, L. and Benito, A. (2006). Spatial variability in the density, distribution and vectorial capacity of anopheline species in a high transmission village (Equatorial Guinea). *Malaria Journal* 5(21): 1 – 10.

Carey, A. F., Wang, G., Su, C-Y., Zwiebel, L. J. and Carlson, J. R. (2010). Odorant reception in the malaria mosquito Anopheles gambiae. *Nature* 464: 66 – 71.

Castro, M. C., Yamagata, Y., Mtasiwa, D., Tanner, M., Utzinger, J., Keiser, J. and Singer, B. H. (2004). Integrated urban malaria control: a case study in Dar es Salaam, Tanzania. *The American Journal of Tropical Medicine and Hygiene* 71(2): 103 – 117.

Castro, M.C., Kanamori, S., Kannady, K., Mkude, S., Killeen, G. F. and Fillinger, U. (2010). The importance of drains for the larval development of lymphatic filariasis and malaria vectors in Dar es Salaam, United Republic of Tanzania. *PLoS Neglected Tropical Disease* 4(5): e693.

Chaki, P. P., Govella, N. J., Shoo, B., Hemed, A., Tanner, M., Fillinger, U. and Killeen, G. F. (2009). Achieving high coverage of larval-stage mosquito surveillance: challenges for a community-based mosquito control programme in urban Dar es Salaam, Tanzania. *Malaria Journal* 8(311): 1 – 12.

Chaki, P. P., Kannady, K., Mtasiwa, D., Tanner, M., Mshinda, H., Kelly, A. H. and Killeen, G. F. (2014). Institutional evolution of a community-based programme for malaria control through larval source management in Dar es Salaam, United Republic of Tanzania. *Malaria Journal* 13(245): 1 – 13.

Chanda, E., Hemingway, J., Kleinschmidt, I., Rehman, A. M., Ramdeen, V., Phiri, F. N., Coetzer, S., Mthembu, D., Shinondo, C. J., Chizema-Kawesha, E. and Kamuliwo, M. (2011). Insecticide resistance and the future of malaria control in Zambia. *PLoS One* 6(9): e24336.

Chareonviriyaphap, T., Bangs, M. J., Suwonkerd, W., Kongmee, M., Corbel, V. and Ngoen-Klan, R. (2013). Review of insecticide resistance and behavioral avoidance of vectors of human diseases in Thailand. *Parasite Vectors* 6(280): 1 – 28.

Cheah, S. X., Tay, J. W., Chan, L. K. and Jaal, Z. (2013). Larvicidal, oviposition, and ovicidal effects of Artemisia annua (Asterales: Asteraceae) against Aedes aegypti, Anopheles sinensis, and Culex quinquefasciatus (Diptera: Culicidae). *Parasitology Research* 112(9): 3275 – 3282.

Chenniappan, K. and Kadarkarai, M. (2008). Oviposition deterrent, ovicidal and gravid mortality effects of ethanolic extract of Andrographis paniculata Nees against the malarial vector Anopheles stephensi Liston (Diptera: Culicidae). *Entomological Research* 38(2): 119 – 125.

Choi, K. S., Christian, R., Nardini, L., Wood, O. R., Agubuzo, E., Muleba, M. and Hunt, R. H. (2014). Insecticide resistance and role in malaria transmission of Anopheles funestus populations from Zambia and Zimbabwe. *Parasites and Vectors* 7(464): 1 – 8.

Chouaibou, M., Etang, J., Brevault, T., Nwane, P., Hinzoumbe, C. K., Mimpfoundi, R. and Simard, F. (2008). Dynamics of insecticide resistance in the malariavector *Anopheles gambiae s.l.* from an area of extensive cotton cultivation inNorthern Cameroon. *Tropical Medicine and International Health* 13(1): 476–486.

Clark, T. M., Flis, B. J. and Remold, S. K. (2004). pH tolerances and regulatory abilities of freshwater and euryhaline Aedine mosquito larvae. *Journal of Experimental Biology* 207(13): 2297 – 2304.

Clement, A. N. (2000). *The Biology of Mosquitoes. Development, Nutrition and Reproduction.* Commonwealth for Agriculture Bureau International, Wallingford, UK. 740pp.

Cohuet, A., Simard, F., Wondji, C. S., Antonio-Nkondjio, C., Awono-Ambene, P. and Fontenille, D. (2004). High malaria transmission intensity due to Anopheles funestus (Diptera: Culicidae) in a village of savannah–forest transition area in Cameroon. *Journal of medical entomology 41*(5), 901-905.

Coleman, M., Casimiro, S., Hemingway, J. and Sharp, B. (2008). Operational impact of DDT reintroduction for malaria control on Anopheles arabiensis in Mozambique. *Journal of Medical Entomology* 45(5): 885 – 890.

Conlon, J. (2011). Control of mosquitoes in the United States. *Outlooks on Pest Management* 22(1): 32 – 37.

Cooke, M. K., Kahindi, S. C., Oriango, R. M., Owaga, C., Ayoma, E., Mabuka, D. and Drakeley, C. (2015). A bite before bed': exposure to malaria vectors outside the times of net use in the highlands of western Kenya. *Malaria Journal* 14(259): 1 – 15.

Cooper, P. D. (1994). Mechanisms of hemolymph acid-base regulation in aquatic insects. *Physiological and Biochemical Zoology* 67(1): 29 – 53.

Copping, L. G. and Menn, J. J. (2000). Biopesticides: a review of their action, applications and efficacy. *Pest Management Science* 56(8): 651 – 676.

Corbel, V., N'guessan, R., Brengues, C., Chandre, F., Djogbenou, L., Martin, T., Akogbeto, M., Hougard, J. M. and Rowland, M. (2007). Multiple insecticide resistance mechanisms in Anopheles gambiae and Culex quinquefasciatus from Benin, West Africa. *Acta Tropica* 101(3): 207 – 216.

Cox-Singh, J., Davis, T.M., Lee, K.S., Shamsul, S.S., Matusop, A., Ratnam, S., Rahman, H.A., Conway, D.J. and Singh, B. (2008). Plasmodium knowlesi malaria in humans is widely distributed and potentially life threatening. *Clinical Infectious Diseases* 46(2): 165 – 171.

Crawshaw, A., Maung, T., Shafique, M., Sint, N., Nicholas, S., Li, M. and Roca-Feltrer, A. (2017). Acceptability of insecticide-treated clothing for malaria prevention among migrant rubber tappers in Myanmar: a cluster-randomized non-inferiority crossover trial. *Malaria Journal* 16(1): 92 – 109.

Czeher, C., Labbo, R., Arzika, I. and Duchemin, J. B. (2008). Evidence of increasing Leu-Phe knockdown resistance mutation in Anopheles gambiae from Niger following a nationwide long-lasting insecticide-treated nets implementation. *Malaria Journal* 7(189): 1 – 12.

Dagne, G. and Deressa, W. (2008). Knowledge and utilization of insecticide treated mosquito nets among freely supplied households in Wonago Woreda, Souther Ethiopia. *Ethiopian Journal of Health Development* 22(1): 34 – 41.

Davies, T. G. E., Field, L. M., Usherwood, P. N. R. and Williamson, M. S. (2007). DDT, pyrethrins, pyrethroids and insect sodium channels. *IUBMB Life* 59(3): 151 – 162.

Day, J. and Sjogren, R.(1994). Vector control by removal trapping. *American Journal of Tropical Medicine and Hygiene* 50(6): 126 – 133.

Deressa, W., Ali, A. and Enquoselassie, F. (2003). Knowledge, attitude and practice about malaria, the mosquito and antimalarial drugs in a rural community. *Ethiopian Journal of Health Development* 17(2): 99 – 104.

Derua, Y. A., Alifrangis, M., Hosea, K. M., Meyrowitsch, D. W., Magesa, S. M., Pedersen, E. M. and Simonsen, P. E. (2012). Change in composition of the Anopheles gambiae complex and its possible implications for the transmission of malaria and lymphatic filariasis in north-eastern Tanzania. *Malaria Journal* 11(188): 1 – 9.

Dieng, H., Mwandawiro, C., Boots, M., Morales, R., Satho, T., Tuno, N., Tsuda, Y. and Takagi, M. (2002). Leaf litter decay process and the growth performance of Aedes albopictus larvae (Diptera: Culicidae). *Journal of Vector Ecology* 27: 31 – 38.

Dohar, J.E (2003). Evolution of management approaches for otitis externa. *Pediatric Infectious Disease Journal* 22(1): 299–308.

Doolan, D.L., Dobaño, C. and Baird, J.K. (2009). Acquired immunity to malaria. *Clinical Microbiology Reviews* 22(1): 13 – 36.

Dunson, W. A. and Connell, J. (1982). Specific inhibition of hatching in amphibian embryos by low pH. *Journal of Herpetology* 16(3): 314 – 316.

Edillo, F. E., Tripét, F., Touré, Y. T., Lanzaro, G. C., Dolo, G. and Taylor, C. E. (2006). Water quality and immatures of the M and S forms of Anopheles gambiae ss and An. arabiensis in a Malian village. *Malaria Journal* 5(35): 1 – 10.

Edson, F. and Kayombo, E. J. (2007). Knowledge on malaria transmission and its prevention among schoolchildren in Kyela District, South Western Tanzania. *Tanzania Journal of Health Research* 9(3): 207 – 210.

Eisele, T. P., Larsen, D. and Steketee, R. W. (2010). Protective efficacy of interventions for preventing malaria mortality in children in Plasmodium falciparum endemic areas. *International Journal of Epidemiology* 39(1): 88 – 101.

Elango, G., Bagavan, A., Kamaraj, C., Zahir, A. A. and Rahuman, A. A. (2009). Oviposition-deterrent, ovicidal, and repellent activities of indigenous plant extracts against Anopheles subpictus Grassi (Diptera: Culicidae). *Parasitology Research* 105(6): 1567 – 1576.

Elimam, A. M., Elmalik, K. H. and Ali, F. S. (2009). Efficacy of leaves extract of Calotropis procera Ait.(Asclepiadaceae) in controlling Anopheles arabiensis and Culex quinquefasciatus mosquitoes. *Saudi Journal of Biological Sciences* 16(2): 95 – 100.

Elliott, R. (1972). The influence of vector behavior on malaria transmission. *The American Journal of Tropical Medicine and Hygiene* 21(5): 755 – 763.

Enayati, A. and Hemingway, J. (2010). Malaria management: past, present, and future. *Annual Review of Entomology* 55: 569 – 591.

Ernst, K. C., Adoka, S. O., Kowuor, D. O., Wilson, M. L. and John, C. C. (2006). Malaria hotspot areas in a highland Kenya site are consistent in epidemic and non-epidemic years and are associated with ecological factors. *Malaria Journal* 5(78): 1 – 10.

Esu, E., Lenhart, A., Smith, L. and Horstick, O. (2010). Effectiveness of peridomestic space spraying with insecticide on dengue transmission; systematic review. *Tropical Medicine and International Health* 15(5): 619 – 631.

Etang, J., Manga, L., Toto, J.C., Guillet, P., Fondjo, E. and Chandre, F. (2007). Spectrum of metabolic-based resistance to DDT and pyrethroids in Anopheles gambiae sl populations from Cameroon. *Journal of Vector Ecology* 32(1): 123 – 133.

Ferguson, H. M., Dornhaus, A., Beeche, A., Borgemeister, C., Gottlieb, M., Mulla, M. S., Gimnig, J. E., Fish, D. and Killeen, G. F. (2010). Ecology: a prerequisite for malaria elimination and eradication. *PLoS Med 7*(8): e1000303.

Feyereisen, R. (2005). Insect Cytochrome P450 in: *Comprehensive Molecular Insect Science - Biochemistry and Molecular Biology*. (Edited by Gilbert, L. I., Iatrou, K. and Gill, S. S.), Elsevier Publishers, Oxford. pp. 1 – 77.

Fillinger, U. and Lindsay, S. W. (2006). Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. *Tropical Medicine and International Health* 11(11): 1629 – 1642.

Fillinger, U., Sombroek, H., Majambere, S., van Loon, E., Takken, W. and Lindsay, S. W. (2009). Identifying the most productive breeding sites for malaria mosquitoes in The Gambia. *Malaria Journal* 8(62): 1 – 14.

Foster, W.A. and Walker, E.D. (2002). Mosquitoes (Culicidae). In: Mullen, G. and L. Durden (Eds.) *Medical and Veterinary Entomology*. Academic Press, New York, 12:78 – 82.

Fradin, M. S. and Day, J. F. (2002). Comparative efficacy of insect repellents against mosquito bites. *New England Journal of Medicine* 347(1): 13 – 18.

Frances, S. P. and Cooper, R. D.(2007). Personal protective measures against mosquitoes: insecticide-treated uniforms, bednets and tents. *ADF Health* 8(1):50–6.

Fukuto, T. R. (1990). Mechanism of action of organophosphorus and carbamate insecticides. *Environmental Health Perspectives* 87: 245 – 254.

Futami, K., Dida, G. O., Sonye, G. O., Lutiali, P. A., Mwania, M. S., Wagalla, S. and Minakawa, N. (2014). Impacts of insecticide treated bed nets on Anopheles gambiae sl populations in Mbita district and Suba district, Western Kenya. *Parasites and Vectors* 7(63): 1 – 13.

Gage, K. L., Burkot, T. R., Eisen, R. J. and Hayes, E. B. (2008). Climate and vectorborne diseases. *American Journal of Preventive Medicine* 35(5): 436 – 450.

Gatton, M. L., Chitnis, N., Churcher, T., Donnelly, M. J., Ghani, A. C., Godfray, H. C. J., Gould, F., Hastings, I., Marshall, J., Ranson, H. and Rowland, M. (2013). The importance of mosquito behavioural adaptations to malaria control in Africa. *Evolution* 67(4): 1218 – 1230.

Geissbuhler, Y., Kannady, K., Chaki, P., Emidi, B., Govella, N., Mayagaya, V., Kiama, M., Mtasiwa, D., Mshinda, H., Lindsay, S., Tanner, M., Fillinger, U, de Castro, M. C. and Killeen, G. F. (2009). Microbial larvicide application by a large-scale, community-based program reduces malaria infection prevalence in urban Dar es Salaam, Tanzania. *PLoS One* 4: e5107.

Geissbühler, Y., Chaki, P., Emidi, B., Govella, N. J., Shirima, R., Mayagaya, V., Mtasiwa, D., Mshinda, H., Fillinger, U., Lindsay, S. W. and Kannady, K. (2007). Interdependence of domestic malaria prevention measures and mosquito-human interactions in urban Dar es Salaam, Tanzania. *Malaria Journal* 6(126): 1 – 17.

Gerald, N., Mahajan, B. and Kumar, S. (2011). Mitosis in the human malaria parasite Plasmodium falciparum. *Eukaryotic Cell* 10(4): 474 – 482.

Gillies, M.T.(1954). Studies of house leaving and outside resting of *Anopheles gambiae* Giles and *Anopheles funestus* Giles in East Africa. 1. The outside resting population. *Bulletin of Entomology Research* 45 (1): 361 – 373.

Gillies, M. T. and Coetzee, M. (1987). A Supplement to the Anophelinae of Africa South of the Sahara. *Publications of the South African Institute for Medical Research* 55: 1 – 143.

Gillies, M. T. and De Meillon, B. (1968). *The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region).* South Africa Institute for Medical Research, South Africa. 343pp.

Githeko, A. K., Adungo, N. I., Karanja, D. M., Hawley, W. A., Vulule, J. M., Seroney, I. K., Ofulla, A. V., Atieli, F. K., Ondijo, S. O., Genga, I.O. and Odada, P. K. (1996). Some Observations on the Biting Behavior ofAnopheles gambiae ss, Anopheles arabiensis, andAnopheles funestusand Their Implications for Malaria Control. *Experimental Parasitology* 82(3): 306 – 315.

Gopalakrishnan, R., Das, M., Baruah, I., Veer, V. and Dutta, P. (2012). Studies on the ovitraps baited with hay and leaf infusions for the surveillance of dengue vector, Aedes albopictus, in northeastern India. *Tropical BioMed* 29(4): 598 – 604.

Gouagna, L. C., Rakotondranary, M., Boyer, S., Lempérière, G., Dehecq, J. S. and Fontenille, D. (2012). Abiotic and biotic factors associated with the presence of Anopheles arabiensis immatures and their abundance in naturally occurring and man-made aquatic habitats. *Parasites and Vectors* 5(96): 1 – 12.

Govella, N. J., Chaki, P. P. and Killeen, G. F. (2013). Entomological surveillance of behavioural resilience and resistance in residual malaria vector populations. *Malaria Journal* 12(124): 1 – 9.

Govella, N. J., Okumu, F. O. and Killeen, G. F. (2010). Insecticide-treated nets can reduce malaria transmission by mosquitoes which feed outdoors. *The American Journal of Tropical Medicine and Hygiene* 82(3): 415 – 419.

Govindarajan, M. and Karuppannan, P. (2011). Mosquito larvicidal and ovicidal properties of Eclipta alba (L.) Hassk (Asteraceae) against chikungunya vector, Aedes aegypti (Linn.) (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine* 4(1): 24 – 28.

Govindarajan, M., Mathivanan, T., Elumalai, K., Krishnappa, K. and Anandan, A. (2011). Ovicidal and repellent activities of botanical extracts against Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine* 1(1): 43 – 48.

Greenwood, B. M., Fidock, D. A., Kyle, D. E., Kappe, S. H., Alonso, P. L., Collins, F. H. and Duffy, P. E. (2008). Malaria: progress, perils, and prospects for eradication. *The Journal of Clinical Investigation* 118(4): 1266 – 1276.

Gu, W., Regens, J. L., Beier, J. C. and Novak, R. J. (2006). Source reduction of mosquito larval habitats has unexpected consequences on malaria transmission. *Proceedings of the National Academy of Sciences* 103(46): 17560 – 17563.

Gu, W., Utzinger, J. and Novak, R. J. (2008). Habitat-based larval interventions: a new perspective for malaria control. *The American Journal of Tropical Medicine and Hygiene* 78(1): 2 – 6.

Hargreaves, K., Koekemoer, L. L., Brooke, B. D., Hunt, R. H., Mthembu, J. and Coetzee, M. (2000). Anopheles funestus resistant to pyrethroid insecticides in South Africa. *Medical and Veterinary Entomology* 14(2): 181 – 189.

Hemingway, J. (2014). The role of vector control in stopping the transmission of malaria: threats and opportunities. *Philosophical Transactions of the Royal Society* 369: 1 – 5.

Hemingway, J., Field, L. and Vontas, J. (2002). An overview of insecticide resistance. *Science* 298(5591): 96 – 97.

Herrera-Varela, M., Lindh, J., Lindsay, S. W. and Fillinger, U. (2014). Habitat discrimination by gravid Anopheles gambiae sensu lato–a push-pull system. *Malaria Journal* 13(133): 1 – 15.

Hiscox, A., Maire, N., Kiche, I., Silkey, M., Homan, T., Oria, P. and Sawa, P. (2012). The SolarMal Project: innovative mosquito trapping technology for malaria control. *Malaria Journal* 11(1): 1 – 2.

Hlongwana, K. W., Mabaso, M. L., Kunene, S., Govender, D. and Maharaj, R. (2009). Community knowledge, attitudes and practices on malaria in Swaziland: a country earmarked for malaria elimination. *Malaria Journal* 8(29): 1 – 8.

Homan, T., Hiscox, A., Mweresa, C. K., Masiga, D., Mukabana, W. R., Oria, P., Takken, W. and Bousema, T. (2016). The effect of mass mosquito trapping on malaria transmission and disease burden (SolarMal): A stepped-wedge cluster-randomised trial. *The Lancet* 388(10050): 1193 – 1201.

Huang, J., Walker, E. D., Giroux, P. Y., Vulule, J. and Miller, J. R. (2005). Ovipositional site selection by Anopheles gambiae: influences of substrate moisture and texture. *Medical and Veterinary Entomology* 19(4): 442 – 450.

Hudson, E.J. and Esozed, S. (1971). The effect of smoke from mosquito coils on Anopheles gambiae Gillies and Mansonia uniformis (Theo.) in verandah-trap huts at Magugu, Tanzania. *Bull Entomology Research* 61(1): 247–265

Huestis, D. L., Traoré, A. I., Dieter, K. L., Nwagbara, J. I., Bowie, A. C., Adamou, A. and Lehmann, T. (2012). Seasonal variation in metabolic rate, flight activity and body size of Anopheles gambiae in the Sahel. *Journal of Experimental Biology* 215(12): 2013 – 2021.

IRAC (2010). Management of insecticide resistance in vectors of public health importance. Crop life international, insecticide resistance action committee. [http://whqlibdoc.who.int/publications/2011/9789241502177_eng.pdf] site visited on 19/5/2015.

IRAC (2011). Prevention and management of insecticide resistance. [http://www.irac-online.org/about/resistance] site visited on 19/5/2015.

Jima, D., Getachew, A., Bilak, H., Steketee, R. W., Emerson, P. M., Graves, P. M. and Hwang, J. (2010). Malaria indicator survey 2007, Ethiopia: coverage and use of major malaria prevention and control interventions. *Malaria Journal* 9(58): 1 – 12.

Jones, M. K. and Good, M. F. (2006). Malaria parasites up close. *Nature Medicine* 12(2): 170 – 171.

Jung, H.H., Sho, S. D., Yoo CK, Lim, H. H. and Chae, S.W. (2002). Vinegar treatment in the management of granular myringitis. *Journal of Laryngology and Otology* 116(1): 176–180.

Kabbale, F.G, Anne. M., Akol, A.M., Kaddu, J. B. and Onapa, A. W. (2013). Biting patterns and seasonality of *Anopheles gambiae sensu lato* and *Anopheles funestus* mosquitoes in Kamuli District, Uganda. *Parasites and Vectors* 6(1): 340-349

Kabula, B., Derua, Y. A., Tungu, P. K., Massue, D. J., Sambu, E., Mosha, F. W. and Kisinza, W. N. (2011). Malaria entomological profile in Tanzania from 1950 to 2010: a review of mosquito distribution, vectorial capacity and insecticide resistance. *Tanzania Journal of Health Research* 13(1): 1 – 14.

Kabula, B., Kisinza, W., Tungu, P., Ndege, C., Batengana, B., Kollo, D. and Magesa, S. (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 and International Health* 19(3): 331 – 341.

Kaiser, M. L., Duncan, F. D. and Brooke, B. D. (2014). Embryonic development and rates of metabolic activity in early and late hatching eggs of the major malaria vector anopheles gambiae. *PloS One* 9(12): e114381.

Kamugisha, M. L., Maxwell, C. and Curtis, C. F. (2005). Characteristics of malaria among children living in lowland and highlands of Muheza District, north-east Tanzania. *Tanzania Journal of Health Research* 7(2): 67 – 72.

Keating, J., Macintyre, K., Mbogo, C. M., Githure, J. I. and Beier, J. C. (2004). Characterization of potential larval habitats for Anopheles mosquitoes in relation to urban land-use in Malindi, Kenya. *International Journal of Health Geographics* 3(9): 1 – 13.

Keiser, J., Singer, B. H. and Utzinger, J. (2005). Reducing the burden of malaria in different eco-epidemiological settings with environmental management: A systematic review. *The Lancet Infectious Diseases* 5(11): 695 – 708.

Kelly-Hope, L. A. and McKenzie, F. E. (2009). The multiplicity of malaria transmission: a review of entomological inoculation rate measurements and methods across sub-Saharan Africa. *Malaria Journal* 8(19): 1 – 16.

Kent, R.J. and Norris, D. E. (2005). Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome b. *American Journal of Tropical Mededicine and Hygiene 73*(1): 336 – 342.

Khandagle, A. J., Tare, V. S., Raut, K. D. and Morey, R. A. (2011). Bioactivity of essential oils of Zingiber officinalis and Achyranthes aspera against mosquitoes. *Parasitology Research* 109(2): 339 – 343.

Killeen, G. F. (2014). Characterizing, controlling and eliminating residual malaria transmission. *Malaria Journal* 13(330): 1 – 22.

Killeen, G. F. and Chitnis, N. (2014). Potential causes and consequences of behavioural resilience and resistance in malaria vector populations: a mathematical modelling analysis. *Malaria Journal* 13(97): 1 – 16.

Killeen, G. F., Fillinger, U. and Knols, B. G. (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): 1 – 7.

Kim, D., Fedak, K. and Kramer, R. (2012). Reduction of malaria prevalence by indoor residual spraying: a meta-regression analysis. *The American Journal of Tropical Medicine and Hygiene* 87(1): 117 – 124.

Kinung'hi, S. M., Mashauri, F., Mwanga, J. R., Nnko, S. E., Kaatano, G. M., Malima, R. and Mboera, L. E. (2010). Knowledge, attitudes and practices about malaria among communities: comparing epidemic and non-epidemic prone communities of Muleba district, North-western Tanzania. *BioMed Central Public Health* 10(395): 1 – 11.

Kirby, M. J. and Lindsay, S. W. (2009). Effect of temperature and inter-specific competition on the development and survival of Anopheles gambiae sensu stricto and An. arabiensis larvae. *Acta Tropica* 109(2): 118 – 123.

Kiszewski, A.E., and Darling, S.T.(2010). Estimating a mosquito repellent's potential to reduce malaria in communities. *Journal of Vector Borne Diseases* 47(1):217–221

Kiszewski, A., Mellinger, A., Spielman, A., Malaney, P., Sachs, S.E. and Sachs, J. (2004). A global index representing the stability of malaria transmission. *The American Journal of Tropical Medicine and Hygiene* 70(5): 486 – 498.

Kitau, J., Oxborough, R. M., Tungu, P. K., Matowo, J., Malima, R. C., Magesa, S. M., Malima, R. C., Magesa, S. M., Bruce, J., Mosha, F. W. and Rowland, M. W. (2012). Species shifts in the Anopheles gambiae complex: do LLINs successfully control Anopheles arabiensis?. *PLoS One 7*(3): e31481.

Kiwanuka, S. N., Ekirapa, E. K., Peterson, S., Okui, O., Rahman, M. H., Peters, D. and Pariyo, G. W. (2008). Access to and utilisation of health services for the poor in Uganda: a systematic review of available evidence. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 102(11): 1067 – 1074.

Kleinschmidt, I., Schwabe, C., Shiva, M., Segura, J. L., Sima, V., Mabunda, S. J. A. and Coleman, M. (2009). Combining indoor residual spraying and insecticide-treated net interventions. *The American Journal of Tropical Medicine and Hygiene* 81(3): 519 – 524.

Knols, G. J., Njiru, B. N., Mathenge, E. M., Mukabana, W.R., Beier, J. C. and Killeen, G. F. (2002). MalariaSphere: A greenhouse-enclosed simulation of a natural *Anopheles gambiae* (Diptera: Culicidae) ecosystem in western Kenya. *Malaria Journal* 21(1): 19 – 32.

Knols, B. G., Willemse, L. U. C., Flint, S. and Mate, A. (1993). A trial to control the tsetse fly, Glossina morsitans centralis, with low densities of odour-baited targets in west Zambia. *Medical and Veterinary Entomology* 7(2): 161 – 169.

Konradsen, F., Amerasinghe, P., Van Der Hoek, W. I. M., Amerasinghe, F., Perera, D. and Piyaratne, M. (2003). Strong association between house characteristics and malaria vectors in Sri Lanka. *The American Journal of Tropical Medicine and Hygiene* 68(2): 177 – 181.

Kramer, W. L. and Mulla, M. S. (1979). Oviposition attractants and repellents of mosquitoes: oviposition responses of Culex mosquitoes to organic infusions. *Environmental Entomology* 8(6): 1111 – 1117.

Kramer, R. A., Mboera, L. E. G., Miranda, M. L., Senkoro, K., Lesser, A.,Shayo, E. H. and Paul, C. J. (2014). A randomized longitudinal factorial design to assess malaria vector control and disease management interventions in rural Tanzania. *International Journal of Environmentand Public Health* 11(1): 5117 – 5132.

Kulkarni, M. A., Desrochers, R. E. and Kerr, J. T. (2010). High resolution niche models of malaria vectors in northern Tanzania: a new capacity to predict malaria risk?. *PLoS One* 5(2): e9396.

Kulkarni, M. A., Malima, R., Mosha, F. W., Msangi, S., Mrema, E., Kabula, B., Lawrence, B., Kinung'hi, S., Swilla, J., Kisinza, W. and Rau, M. E. (2007). Efficacy of pyrethroid-treated nets against malaria vectors and nuisance-biting mosquitoes in Tanzania in areas with long-term insecticide-treated net use. *Tropical Medicine and International Health* 12(9): 1061 – 1073.

Kweka, E. J., Mwang'onde, B. J., Kimaro, E., Msangi, S., Massenga, C. P. and Mahande, A. M. (2009). A resting box for outdoor sampling of adult Anopheles arabiensis in rice irrigation schemes of lower Moshi, northern Tanzania. *Malaria Journal* 8(82): 1 – 6.

Kweka, E. J., Owino, E. A., Mwang'onde, B. J., Mahande, A. M., Nyindo, M. and Mosha, F. (2011). The role of cow urine in the oviposition site preference of culicine and Anopheles mosquitoes. *Parasit Vectors* 4(184): 1 – 6.

Lahondère, C. and Lazzari, C. R. (2012). Mosquitoes cool down during blood feeding to avoid overheating. *Current Biology* 22(1): 40 – 45.

Lehmann, T., Dao, A., Adamou, A., Kassogue, Y., Diallo, M., Sékou, T. and Coscaron-Arias, C. (2010). Aestivation of the African malaria mosquito, Anopheles gambiae in the Sahel. *The American Journal of Tropical Medicine and Hygiene* 83(3): 601 – 606.

Lehmann, T., Dao, A., Yaro, A. S., Diallo, M., Timbiné, S., Huestis, D. L. and Traoré, A. I. (2014). Seasonal variation in spatial distributions of Anopheles gambiae in a Sahelian village: evidence for aestivation. *Journal of Medical Entomology* 51(1): 27 – 38.

License, A., Malaria, G. and Program, E. (2011). A research agenda for malaria eradication: Vector Control. *PLoS Med* 8: e1000401.

Lindsay, S. W., Jawara, M., Paine, K., Pinder, M., Walraven, G. E. L. and Emerson, P. M. (2003). Changes in house design reduce exposure to malaria mosquitoes. *Tropical Medicine and International Health* 8(6): 512 – 517.

Liu, W., Zhang, J., Hashim, J. H., Jalaludin, J., Hashim, Z. and Goldstein, B. D.(2003). Mosquito coil emissions and health implications. *Environmental Health Perspective* 111(12): 1454 – 1460.

Logan, J. G. and Birkett, M. A. (2007). Semiochemicals for biting fly control: their identification and exploitation. *Pest Management Science* 63(7): 647 – 657.

Lothrop, H., Lothrop, B., Palmer, M., Wheeler, S., Gutierrez, A., Gomsi, D. and Reisen, W. K. (2007). Evaluation of pyrethrin and permethrin ground ultra-low volume applications for adult Culex control in rural and urban environments of the Coachella Valley of California. *Journal of the American Mosquito Control Association* 23(2): 190 – 207.

Lovell, J. B., Wright Jr, D. P., Gard, I. E., Miller, T. P., Treacy, M. F., Addor, R. W. and Kamhi, V. M. (1990). AC 303,630-An insecticide/acaricide from a novel class of chemistry. *Brighton Crop Protection Conference, Pests and Diseases* 1: 43 – 48.

Lynch, P. A., Grimm, U., Thomas, M. B. and Read, A. F. (2012). Prospective malaria control using entomopathogenic fungi: comparative evaluation of impact on transmission and selection for resistance. *Malaria Journal* 11(1): 383-391.

Madne, G., Jindal, A. K., Patel, B. B., Sharma, R. and Kant, R. (2014). Knowledge and practices concerning malaria in rural community of Pune district. *Medical Journal* 7(4): 450 – 453.

Magesa, S. M., athumani, Y., Barongo, V., Sambu, E. Z., Senkoro, K. P., Mboera, L. E. G., Sandra, M. R., Reynaldo, G. V., Aramis, R.G. and Kitua, A.Y. (2009). Efficacy of Bacillus Thuringiensis var. Israelensis (Bactivec) and Bicillus (Griselesf) for control of malaria larvae. *A field trial in Mvomero and Bagamoyo districts, Tanzania*. Final Report , January 2009

Mahande, A., Mosha, F., Mahande, J. and Kweka, E. (2007). Feeding and resting behaviour of malaria vector, Anopheles arabiensis with reference to zooprophylaxis. *Malaria Journal* 6(100): 1174– 1186.

Mahande, A. M., Mwang'onde, B. J., Msangi, S., Kimaro, E., Mnyone, L. L., Mazigo, H. D., Mahande, M. J. and Kweka, E. J. (2010). Is aging raw cattle urine efficient for sampling Anopheles arabiensis Patton? *BioMed Central Infection Disease* 10(172): 1 – 5.

Maheu-Giroux, M. and Castro, M. C. (2013). Impact of community-based larviciding on the prevalence of malaria infection in Dar es Salaam, Tanzania. *PLoS One* 8(8): e71638.

Maheu-Giroux, M. and Castro, M. C. (2014). Cost-effectiveness of larviciding for urban malaria control in Tanzania. *Malaria Journal* 13(477): 1 – 12.

Majambere, S., Lindsay, S. W., Green, C., Kandeh, B. and Fillinger, U. (2007). Microbial larvicides for malaria control in The Gambia. *Malaria Journal* 6(76): 1 – 14.

Martins-Campos, K. M., Pinheiro, W. D., Vítor-Silva, S., Siqueira, A. M., Melo, G. C., Rodrigues, Í. C., Fé, N. F., Barbosa, M., Tadei, W. P., Guinovart, C. and Bassat, Q. (2012). Integrated vector management targeting Anopheles darlingi populations decreases malaria incidence in an unstable transmission area, in the rural Brazilian Amazon. *Malaria Journal* 11(1): 1 – 10.

Mathenge, E. M., Gimnig, J. E., Kolczak, M., Ombok, M., Irungu, L. W. and Hawley, W. A. (2001). Effect of permethrin-impregnated nets on exiting behavior, blood feeding success, and time of feeding of malaria mosquitoes (Diptera: Culicidae) in western Kenya. *Journal of Medical Entomology* 38(4): 531 – 536.

Matowo, N. S., Moore, J., Mapua, S., Madumla, E. P., Moshi, I. R., Kaindoa, E. W. Stephen, P. M., Deogratius R. K., Robert, D. S., Dickson, W. L. Okumu, F. O. (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 and Vectors* 6(137): 1 – 16.

Mazigo, H. D., Obasy, E., Mauka, W., Manyiri, P., Zinga, M., Kweka, E. J. and Heukelbach, J. (2010). Knowledge, attitudes, and practices about malaria and its control in rural northwest Tanzania. *Malaria Research and Treatment* 2010: 1 – 9.

Mboera, L. E. G. and Takken, W. (1997). Carbon dioxide chemotropism in mosquitoes (Diptera: Culicidae) and its potential in vector surveillance and management programmes. *Review ofMedical and Veterinary Entomology* 7: 355 – 368.

Mboera, L. E., Rumisha, S. F., Senkoro, K. P., Mayala, B. K., Shayo, E. H. and Kisinza, W.N. (2007). Knowledge and health information communication in Tanzania. *East African Journal of Public Health* 4(1): 33 – 39.

Mboera, L. E., Mazigo, H. D., Rumisha, S. F. and Kramer, R. A. (2013). Towards malaria elimination and its implication for vector control, disease management and livelihoods in Tanzania. *Malaria World Journal* 4(19): 1 – 14.

Mboera, L. E. G., Kramer, R. A., Miranda, M. L., Kilama, S. P., Shayo, E. H., and Lesser, A. (2014). Community knowledge and acceptance of larviciding for malaria control in rural district of east-central Tanzania. *International Journal of Environmental Researchand Public Health* 11(1): 5137 – 5154.

McCahon, C. P. and Pascoe, D. (1989). Short-term experimental acidification of a Welsh stream: toxicity of different forms of aluminium at low pH to fish and invertebrates. *Archives of Environmental Contamination and Toxicology* 18(2): 233 – 242.

McMeniman, C. J., Corfas, R. A., Matthews, B. J., Ritchie, S. A. and Vosshall, L. B. (2014). Multimodal integration of carbon dioxide and other sensory cues drives mosquito attraction to humans. *Cell* 156(5): 1060 – 1071.

Merdic, E. and Vujicic-Karlo, S. D. (2005). Two types of hibernation of Culex pipiens complex (Diptera: Culicidae) in Croatia. *Entomology Croatia* 9: 71 – 76.

Mereta, S. T., Yewhalaw, D., Boets, P., Ahmed, A., Duchateau, L., Speybroeck, N. and Goethals, P. L. (2013). Physico-chemical and biological characterization of anopheline mosquito larval habitats (Diptera: Culicidae): implications for malaria control. *Parasites and Vectors* 6(320): 1 – 16.

Mihok, S. and Mulye, H. (2010). Responses of tabanids to Nzi traps baited with octenol, cow urine and phenols in Canada. *Medical and Veterinary Entomology* 24(3): 266 – 272.

Minakawa, N., Githure, J. I., Beier, J. C. and Yan, G. (2001). Anopheline mosquito survival strategies during the dry period in western Kenya. *Journal of Medical Entomology* 38(3): 388 – 392.

Minakawa, N., Munga, S., Atieli, F., Mushinzimana, E., Zhou, G., Githeko, A. K. and Yan, G. (2005). Spatial distribution of anopheline larval habitats in Western Kenyan highlands: effects of land cover types and topography. *The American Journal of Tropical Medicine and Hygiene* 73(1): 157 – 165.

Ministry of Health and Social Welfare (MoHSW) (2015). *National Malaria Control Programme Database*. Health Management Information System, Dar es Salaam, Tanzania.

Mmbando, A. S., Okumu, F. O., Mgando, J. P., Sumaye, R. D., Matowo, N. S., Madumla, E. and Lwetoijera, D. W. (2015). Effects of a new outdoor mosquito control device, the mosquito landing box, on densities and survival of the malaria vector, Anopheles arabiensis, inside controlled semi-field settings. *Malaria Journal* 14(494): 1 – 13.

Mng'ong'o, F. C., Sambali, J. J., Sabas, E., Rubanga, J., Magoma, J., Ntamatungiro, A. J., Turner, E. L., Nyogea, D., Ensink, J. H. and Moore, S. J. (2011). Repellent plants provide affordable natural screening to prevent mosquito house entry in tropical rural settings--results from a pilot efficacy study. *PLoS One* 6: e25927.

Mnzava, A. E. P. (1984). Studies on ecological difference among members of *Anopheles gambiae* group of species in Tanzania. *Annual Report of the National Institute for Medical Research* 3(1): 16 – 33.

Moonen, B., Cohen, J. M., Snow, R. W., Slutsker, L., Drakeley, C., Smith, D.L., Abeyasinghe, R. R., Rodriguez, M. H., Maharaj, R., Tanner, M. and Targett, G. (2010). Operational strategies to achieve and maintain malaria elimination. *The Lancet* 376(9752): 1592 – 1603.

Moore, S. J., Davies, C. and Cameron, M. M. (2007). Are mosquitoes diverted from repellent-using individuals to non-users? Results of a field study in Bolivia. *Tropical Medicine and International Health 1*2(1): 1–8

Mosha, F. W., Lyimo, I. N., Oxborough, R. M., Malima, R., Tenu, F., Matowo, J. and Rowland, M. (2008). Experimental hut evaluation of the pyrrole insecticide chlorfenapyr on bed nets for the control of Anopheles arabiensis and Culex quinquefasciatus. *Tropical Medicine and International Health* 13(5): 644 – 652.

MTUHA (2014a). Prevalence of Malaria, Upper and lower respiratory infection, Gastrointestinal infection and Urinary infection in Dodoma region (unpublished data).

MTUHA (2014b). Prevalence of Malaria, Upper and lower respiratory infection, Gastrointestinal infection and Urinary infection in Morogoro region (unpublished data).

Mullai, K. and Jebanesan, A. (2006). Larvicidal and ovicidal activity of the leaf extract of two cucurbitaceous plants against filarial vector, Culex quinque fasciatus Say. *Indian Journal of Environment Ecoplan* 12: 611 – 615.

Munga, S., Minakawa, N., Zhou, G., Barrack, O. O. J., Githeko, A. K. and Yan, G. (2005). Oviposition site preference and egg hatchability of Anopheles gambiae: Effects of land cover types. *Journal of Medical Entomology* 42(6): 993 – 997.

Munhenga, G., Masendu, H. T., Brooke, B. D., Hunt, R. H. and Koekemoer, L. K. (2008). Pyrethroid resistance in the major malaria vector Anopheles arabiensis from Gwave, a malaria-endemic area in Zimbabwe. *Malaria Journal* 7(247): 1 – 11.

Murray, C. J., Rosenfeld, L. C., Lim, S. S., Andrews, K. G., Foreman, K. J., Haring, D. andLopez, A. D. (2012). Global malaria mortality between 1980 and 2010: a systematic analysis. *The Lancet* 379(9814): 413 – 431.

Muthukrishnan, J. and Pushpalatha, E. (2001). Effects of plant extracts on fecundity and fertility of mosquitoes. *Journal of Applied Entomology* 125(2): 31 – 35.

Mutuku, F. M., King, C. H., Mungai, P., Mbogo, C., Mwangangi, J., Muchiri, E. M. and Kitron, U. (2011). Impact of insecticide-treated bed nets on malaria transmission indices on the south coast of Kenya. *Malaria Journal* 10(356): 1 – 14.

Muturi, E. J., Joseph, M., Josephat, S., Simon, M., Benjamin, J., Charles, M., Mbogo, G. J. and John, G. N. (2007).Evaluation of Four Sampling Techniques for Surveillance of Culex quinquefasciatus (Diptera: Culicidae) and Other:Mosquitoes in African Rice Agroecosystems. *Journal of Medicine Entomology* 44(3): 503 – 508.

Mwangangi, J. M., Mbogo, C. M., Muturi, E. J., Nzovu, J. G., Githure, J. I., Yan, G. and Beier, J. C. (2007a). Spatial distribution and habitat characterisation of Anopheles larvae along the Kenyan coast. *Journal of Vector Borne Diseases* 44(1): 44 – 51.

Mwangangi, J. M., Muturi, E. J., Shililu, J. I., Muriu, S., Jacob, B., Kabiru, E. W. and Novak, R. J. (2007b). Environmental covariates of Anopheles arabiensis in a rice agroecosystem in Mwea, Central Kenya. *Journal of the American Mosquito Control Association* 23(4): 371 – 377.

N'Guessan, R., Boko, P., Odjo, A., Knols, B., Akogbeto, M. and Rowland, M. (2009). Control of pyrethroid-resistant Anopheles gambiae and Culex quinque fasciatus mosquitoes with chlorfenapyr in Benin. *Tropical Medicine and International Health* 14(4): 389 – 395.

NBS, (2012) National Bureau of statistics (2012). *Tanzania Population and Housing Census. Population Distribution by Administrative Area*. National Bureau of Statistics, Dar es Salaam, Tanzania.

Namountougou, M., Simard, F., Baldet, T., Diabaté, A., Ouédraogo, J. B., Martin, T. and Dabiré, R. K. (2012). Multiple insecticide resistance in *Anopheles gambiae* sl populations from Burkina Faso, West Africa. *PLoS One* 7(11): e48412.

Natera, R., Castro, R., Garcia-Moreno, M., Hernandez, M. and Garcia-Barroso, C. (2003). Chemometric studies of vinegars from different raw materials and processes of production. *Journal of Agricuture Food Chemistry* 51(1): 3345–3351

Nauen, R. (2007). Insecticide resistance in disease vectors of public health importance. *Pest Management Science* 63(1): 628–633

Ndjemaï, H. N., Patchoké, S., Atangana, J., Etang, J., Simard, F., Bilong, C. F. B. and Fondjo, E. (2009). The distribution of insecticide resistance in Anopheles gambiae sl populations from Cameroon: an update. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 103(11): 1127 – 1138.

N'Guessan, R., Boko, P., Odjo, A., Chabi, J., Akogbeto, M. and Rowland, M. (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. *Malaria Journal* 9(44): 1 – 8.

N'Guessan, R., Corbel, V., Akogbéto, M. and Rowland, M. (2007). Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerging Infectious Diseases* 13(2): 199 – 206.

Ngufor, C., N'Guessan, R., Boko, P., Odjo, A., Vigninou, E., Asidi, A., Akogbeto, M. and Rowland, M. (2011). Combining indoor residual spraying with chlorfenapyr and long-lasting insecticidal bed nets for improved control of pyrethroid-resistant Anopheles gambiae: an experimental hut trial in Benin. *Malaria Journal* 10(343): 1174– 1186.

Nkhoma, S. C., Nair, S., Cheeseman, I. H., Rohr-Allegrini, C., Singlam, S., Nosten, F. and Anderson, T. J. (2012). Close kinship within multiple-genotype malaria parasite infections. *Proceedings of the Royal Society of Biological Sciences* 279(1738): 2589 – 2598.

Obrist, B., Iteba, N., Lengeler, C., Makemba, A., Mshana, C., Nathan, R. and Schulze, A. (2007). Access to health care in contexts of livelihood insecurity: a framework for analysis and action. *PLoS Medicine* 4(10): e308.

Oduola, A. O., Idowu, E. T., Oyebola, M. K., Adeogun, A. O., Olojede, J. B., Otubanjo, O. A. and Awolola, T. S. (2012). Evidence of carbamate resistance in urban populations of Anopheles gambiae ss mosquitoes resistant to DDT and deltamethrin insecticides in Lagos. *Parasites and Vectors* 5(116): 1 – 8.

Oesterholt, M. J. A. M., Bousema, J. T., Mwerinde, O. K., Harris, C., Lushino, P., Masokoto, A. and Drakeley, C. J. (2006). Spatial and temporal variation in malaria transmission in a low endemicity area in northern Tanzania. *Malaria Journal* 5(98): 1 – 7.

Ogama, S. B., Moore S.J. and Maia, M.F. (2012). A systematic review of mosquito coils and passive emanators: defining recommendations for spatial repellency testing methodologies.Parasite and Vector 5(1): 287 – 297.

Okal, M. N., Francis, B., Herrera-Varela, M., Fillinger, U. and Lindsay, S. W. (2013). Water vapour is a pre-oviposition attractant for the malaria vector Anopheles gambiae sensu stricto. *Malaria Journal* 12(365): 1 – 8.

Okumu, F. O., Madumla, E. P., John, A. N., Lwetoijera, D. W. and Sumaye, R. D. (2010a). Attracting, trapping and killing disease-transmitting mosquitoes using odor-baited stations-The Ifakara Odor-Baited Stations. *Parasites and Vectors* 3(12): 1 – 10.

Okumu, O. F., Killeen, F. G., Ogoma, S., Biswaro, L., Smallegange, C. R., Mbeyela, E., Titus, E., Munk, C., Ngonyani, H. and Takken, W. (2010b). Development and Field Evaluation of a Synthetic Mosquito Lure That Is More Attractive than Humans. *PLos One* 5(1): e8951.

Okumu, F., Sumaye, R. D., Matowo, N.S., Mwangungulu, S.P., Kaindoa, E.W. and Moshi, I. R. (2013). Outdoor mosquito control using odour-baited devices: development and evaluation of a potential new strategy to complement indoor malaria prevention methods. *Malaria World Journal* 4(1): 8 – 21.

Oliver, S. V., Kaiser, M. L., Wood, O. R., Coetzee, M., Rowland, M. and Brooke, B. D. (2010). Evaluation of the pyrrole insecticide chlorfenapyr against pyrethroid resistant and susceptible *Anopheles funestus* (Diptera: Culicidae). *Tropical Medicine International Health* 15(1): 127 – 131

Oxborough, R. M., Kitau, J., Matowo, J., Mndeme, R., Feston, E., Boko, P. and Mosha, F. W. (2010). Evaluation of indoor residual spraying with the pyrrole insecticide chlorfenapyr against pyrethroid-susceptible Anopheles arabiensis and pyrethroid-resistant Culex quinquefasciatus mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 104(10): 639 – 645.

Paaijmans, K. P., Takken, W., Githeko, A. K. and Jacobs, A. F. G. (2008). The effect of water turbidity on the near-surface water temperature of larval habitats of the malaria mosquito *Anopheles gambiae*. *International Journal of Biometeorology* 52(8): 747 – 753.

Panneerselvam, C. and Murugan, K. (2013). Adulticidal, repellent, and ovicidal properties of indigenous plant extracts against the malarial vector, *Anopheles stephensi* (Diptera: Culicidae). *Parasitology Research* 112(2): 679 – 692.

Parham, P. E., Pople, D., Christiansen-Jucht, C., Lindsay, S., Hinsley, W. and Michael, E. (2012). Modeling the role of environmental variables on the population dynamics of the malaria vector *Anopheles gambiae sensu stricto*. *Malaria Journal*11(271): 1 – 3.

Pask, G. M., Jones, P. L., Rützler, M., Rinker, D. C. and Zwiebel, L. J. (2011). Heteromeric anopheline odorant receptors exhibit distinct channel properties. *PLoS One* 6(12): e28774.

Pates, H., Takken, W., Curtis, C., Huisman, P., Akinpelu, O. and Gill, G (2001). Unexpected anthropophagic behaviour in *Anopheles quadriannulatus*. *Medical and Veterinary Entomology* 15(3): 293-298

Pates, H. and Curtis, C. (2005). Mosquito behavior and vector control. *Annual Review Entomology* 50: 53 – 70.

Patrick, M. L., Gonzalez, R. J., Wood, C. M., Wilson, R. W. and Val, A. L. (2002). The characterization of ion regulation in Amazonian mosquito larvae: evidence of phenotypic plasticity, population-based disparity, and novel mechanisms of ion uptake. *Physiological and Biochemical Zoology* 75(3): 223 – 236.

Paul, A., Harrington, L. C. and Scott, J. G. (2006). Evaluation of novel insecticides for control of dengue vector Aedes aegypti (Diptera: Culicidae). *Journal of Medical Entomology* 43(1): 55 – 60.

Penilla, R. P., Rodríguez, A. D., Hemingway, J., Trejo, A., López, A. D. and Rodríguez, M. H. (2007). Cytochrome P450-based resistance mechanism and pyrethroid resistance in the field *Anopheles albimanus* resistance management trial. *Pesticide Biochemistry and Physiology* 89 (1): 111–117.

Pimprale, S. S., Besco, C. L., Bryson, P. K. and Brown, T. M. (1997). Increased susceptibility of pyrethroid-resistant tobacco budworm (Lepidoptera: Noctuidae) to chlorfenapyr. *Journal of Economic Entomology* 90(1): 49 – 54.

Pitts, R. J., Rinker, D. C., Jones, P. L., Rokas, A. and Zwiebel, L. J. (2011). Transcriptome profiling of chemosensory appendages in the malaria vector Anopheles gambiae reveals tissue-and sex-specific signatures of odor coding. *BioMed Central Genomics* 12(271): 1 – 17.

Pluess, B., Tanser, F. C., Lengeler, C. and Sharp, B. L. (2010). Indoor residual spraying for preventing malaria. *Cochrane Database of Systematic Reviews* 4: 1 – 4.

Polson, K. A., Curtis, C., Seng, C. M., Olson, J. G., Chantha, N. and Rawlins, S. C. (2002). The use of ovitraps baited with hay infusion as a surveillance tool for Aedes aegypti mosquitoes in Cambodia. *Dengue Bulletin* 26: 178 – 184.

Ponnusamy, L., Xu, N., Stav, G., Wesson, D. M., Schal, C. and Apperson, C. S. (2008). Diversity of bacterial communities in container habitats of mosquitoes. *Microbial Ecology* 56(4): 593 – 603.

Pridgeon, J. W., Pereira, R. M., Becnel, J. J., Allan, S. A., Clark, G. G. and Linthicum, K. J. (2008). Susceptibility of Aedes aegypti, Culex quinquefasciatus Say, and Anopheles quadrimaculatus Say to 19 pesticides with different modes of action. *Journal of Medical Entomology* 45(1): 82 – 87.

Pushpanathan, T., Jebanesan, A. and Govindarajan, M. (2006). Larvicidal, ovicidal and repellent activities of Cymbopogan citratus Stapf (Graminae) essential oil against the filarial mosquito Culex quinquefasciatus (Say) (Diptera: Culicidae). *Tropcal Biomedicine Journal* 23(2): 208 – 212.

Qiu, Y. T., Smallegange, R. C., Hoppe, S., Van Loon, J. J. A., Bakker, E. J. and Takken, W. (2004). Behavioural and electrophysiological responses of the malaria mosquito Anopheles gambiae Giles sensu stricto (Diptera: Culicidae) to human skin emanations. *Medical and Veterinary Entomology* 18(4): 429 – 438.

Raghavendra, K., Barik, T. K., Sharma, P., Bhatt, R. M., Srivastava, H. C., Sreehari, U. and Dash, A. P. (2011). Chlorfenapyr: a new insecticide with novel mode of action can control pyrethroid resistant malaria vectors. *Malaria Journal* 10(16): 1 – 7.

Raghavendra, K., Verma, V., Srivastava, H. C., Gunasekaran, K., Sreehari, U. and Dash, A. P. (2010). Persistence of DDT, malathion and deltamethrin resistance in Anopheles culicifacies after their sequential withdrawal from indoor residual spraying in Surat district, India. *Indian Journal of Medical Research* 132: 260 – 264.

Rahman, R., Lesser, A., Mboera L. and Kramer, R. (2016). Cost of microbial larviciding for malaria control in rural Tanzania. *Tropical Medicine and international Health* 21(11): 1468 – 1475.

Rahuman, A. A., Gopalakrishnan, G., Venkatesan, P. and Geetha, K. (2008). Larvicidal activity of some Euphorbiaceae plant extracts against Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae). *Parasitology Research* 102(5): 867 – 873.

Rajkumar, S. and Jebanesan, A. (2007). Repellent activity of selected plant essential oils against the malarial fever mosquito Anopheles stephensi. *Tropical BioMedicine Journal* 24(2): 71 – 75.

Rand, G. M. (2004). Fate and effects of the insecticide–miticide chlorfenapyr in outdoor aquatic microcosms. *Ecotoxicology and Environmental Safety* 58(1): 50 – 60.

Ranson, H. and Lissenden, N. (2016). Insecticide resistance in African Anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. *Trends in Parasitology* 32(3): 187 – 196.

Ranson, H., Claudianos, C., Ortelli, F., Abgrall, C., Hemingway, J., Sharakhova, M. V., Unger, M. F., Collins, F. H. and Feyereisen, R. (2002). Evolution of supergene families associated with insecticide resistance. *Science* 298(5591): 179 – 181.

Rapley, L. P., Johnson, P. H., Williams, C. R., Silcock, R. M., Larkman, M., Long, S. A., Russell, R. and Ritchie, S. A. (2009). A lethal ovitrap-based mass trapping scheme for dengue control in Australia: Impact on populations of the mosquito Aedes aegypti. *Medical and Veterinary Entomology* 23(4): 303 – 316.

Read, A. F., Lynch, P. A. and Thomas, M. B. (2009). How to Make Evolution-Proof Insecticides for Malaria Control. *American Journal of Tropical Medicine and Hygiene*. 81(1): 165 – 176.

Reddy, M. R., Overgaard, H. J., Abaga, S., Reddy, V. P., Caccone, A., Kiszewski, A. E. and Slotman, M. A. (2011). Outdoor host seeking behaviour of Anopheles gambiae mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. *Malaria Journal* 10(184): 1 – 10.

Reegan, A. D., Kinsalin, A. V., Paulraj, M. G. and Ignacimuthu, S. (2015). Larvicidal, ovicidal and repellent activities of marine sponge Cliona celata (Grant) extracts against Anopheles stephensi Liston (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine* 8(1): 29 – 34.

Reiter, P. and Colon, M. N. (1991). Enhancement of the CDC ovitrap with hay infusions for daily monitoring of Aedes aegypti?/ populations, *Journal of American Mosquito Control Association* 7: 52 – 55.

Renggli, S., Mandike, R., Kramer, K., Patrick, F., Brown, N. J., McElroy, P. D., Rimisho, W., Msengwa, A., Mnzava, A., Nathan, R., Mtung'e, R., Mgullo, R., Lweikiza, J. and Lengeler, C. (2013). Design, implementation and evaluation of a national campaign to deliver 18 million free long-lasting insecticidal nets to uncovered sleeping spaces in Tanzania. *Malaria Journal* 12: 85 - 94

Rinker, D. C., Pitts, R. J., Zhou, X., Suh, E., Rokas, A. and Zwiebel, L. J. (2013). Blood meal-induced changes to antennal transcriptome profiles reveal shifts in odor sensitivities in Anopheles gambiae. *Proceedings of the National Academy of Sciences* 110(20): 8260 – 8265.

Ritchie, S. A., Rapley, L. P., Williams, C., Johnson, P. H., Larkman, M., Silcock, R. M., Long, S. and Russell, R. C. (2009). A lethal ovitrap-based mass trapping scheme for dengue control in Australia: I. Public acceptability and performance of lethal ovitraps. *Medical and Veterinary Entomology* 23(4): 295 – 302.

Rivero, A., Vezilier, J., Weill, M., Read, A.F. and Gandon, S. (2010). Insecticide control of vector-borne diseases: when is insecticide resistance a problem?. *PLoS Pathogy* 6(8): e1001000.

Robich, R. M. and Denlinger, D. L. (2005). Diapause in the mosquito Culex pipiens evokes a metabolic switch from blood feeding to sugar gluttony. *Proceedings of the National Academy of Sciences of the United States of America* 102(44): 15912 – 15917.

Ross, R. (1897). On some peculiar pigmented cells found in two mosquito’s fed on malarial blood. *British Medical Journal* 18: 1786 – 1788.

Rumisha, S.F., Zinga, M. M., Fahey, C. A., Wei, D., Bwana. V.M., Mlozi, M. R., Shayo, E. L., Malima, R.C., Mayala, B. K., Stanley, G., Mlacha, T. and Mboera, L. E. (2014). Accessibility, availability and utilisation of malaria interventions among women of reproductive age in Kilosa district in central Tanzania. *BMC Health Services Research* 14 (1): 452 – 462.

Rund, C.R. (1996). Nonconventional topical therapies for wound care. *Ostomylitis Wound Management* 42(1): 22–24

Rutala, W. A., Barbee, S. L., Agular, N. C., Sobsey, M. D. and Weber, D. J. (2000). Antimicrobial activity of home disinfectants and natural products against potential human pathogens. *Infection Control Hospital Epidemiology* 21(1): 33–38.

Russell, T. L., Govella, N. J., Azizi, S., Drakeley, C. J., Kachur, S. P. and Killeen, G. F. (2011). Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malaria Journal* 10(80): 1 – 10.

Russell, T. L., Lwetoijera, D. W., Maliti, D., Chipwaza, B., Kihonda, J., Charlwood, J. D. and Knols, B. G. (2010). Impact of promoting longer-lasting insecticide treatment of bed nets upon malaria transmission in a rural Tanzanian setting with pre-existing high coverage of untreated nets. *Malaria Journal* 9(187): 1 – 14.

Russell, T. L., Beebe, N. W., Cooper, R. D., Lobo, N. F. and Burkot, T. R. (2013). Successful malaria elimination strategies require interventions that target changing vector behaviours. *Malaria Journal* 12(56): 1 – 5.

Saitoh, Y., Hattori, J., Chinone, S., Nihei, N., Tsuda, Y., Kurahashi, H. and Kobayashi, M. (2004). Yeast-generated CO2 as a convenient source of carbon dioxide for adult mosquito sampling. *Journal of the American Mosquito Control Association* 20(3): 261 – 264.

Santana, A. L., Roque, R. A. and Eiras, A. E. (2006). Characteristics of grass infusions as oviposition attractants to Aedes (Stegomyia) (Diptera: Culicidae). *Journal of Medical Entomology* 43(2): 214 – 220.

Sattler, M. A., Mtasiwa, D., Kiama, M., Premji, Z., Tanner, M., Killeen, G. F. and Lengeler, C.(2005). Habitat characterization and spatial distribution of Anopheles sp. mosquito larvae in Dar es Salaam Tanzania during an extended dry period. *Malaria Journal* 4(4): 1 – 15.

Schmied, W. H., Takken, W., Killeen, G. F., Knols, B. G. and Smallegange, R. C. (2008). Evaluation of two counterflow traps for testing behaviour-mediating compounds for the malaria vector Anopheles gambiae ss under semi-field conditions in Tanzania. *Malaria Journal* 7(230): 1 – 9.

Scott, J. A., Brogdon, W. G. and Collins, F. H. (1993). Identification of single specimens of the Anopheles gambiae complex by the polymerase chain reaction. *The American Journal of Tropical Medicine and Hygiene* 49(4): 520 – 529.

Sengun. I.,Y., and Karapinar, M. (2005). Effectiveness of household natural sanitizers in the elimination of Salmonella typhimurium on rocket (Eruca sativa Miller) and spring onion (Allium cepa L.) *International Journal of Food Microbiology* 98 (1):319–323

Service, M. W. (2000). *Medical Entomology for Students.* Cambridge University Press, Liverpool.

Sharma, V. K. (2003). Period responses to Zeitgeber signals stabilize circadian clocks during entrainment. *Chronobiology International 20*(3): 389 – 404.

Shaukat, A. M., Breman, J. G. and McKenzie, F. E. (2010). Using the entomological inoculation rate to assess the impact of vector control on malaria parasite transmission and elimination. *Malaria Journal* 9(122): 1 – 12.

Sheppard, C. D. and Joyce, J. A. (1998). Increased susceptibility of pyrethroid-resistant horn flies (Diptera: Muscidae) to chlorfenapyr. *Journal of Economic Entomology* 91(2): 398 – 400.

Shililu, J., Mbogo, C., Ghebremeskel, T., Githure, J. and Novak, R. (2007). Mosquito larval habitats in a semiarid ecosystem in Eritrea: impact of larval habitat management on Anopheles arabiensis population. *The American Journal of Tropical Medicine and Hygiene* 76(1): 103 – 110.

Simşek, F. M. (2004). Seasonal larval and adult population dynamics and breeding habitat diversity of Culex theileri Theobald, 1903 (Diptera: Culicidae) in the Gölbaşı District, Ankara, Turkey. *Turkish Journal of Zoology* 28(4): 337 – 344.

Smallegange, R. C., Knols, B. G. and Takken, W. (2010a). Effectiveness of synthetic versus natural human volatiles as attractants for *Anopheles gambiae* (Diptera: Culicidae) sensu stricto. *Journal of Medical Entomology* 47(3): 338 – 344.

Smallegange, R. C., Schmied, W. H., van Roey, K. J., Verhulst, N. O., Spitzen, J., Mukabana, W. R. Takken, W. (2010b). Sugar-fermenting yeast as an organic source of carbon dioxide to attract the malaria mosquito Anopheles gambiae. *Malaria Journal* 9(292):1 – 15.

Smallegange, R. C., Qiu, Y. T., van Loon, J. J. and Takken, W. (2005). Synergism between ammonia, lactic acid and carboxylic acids as kairomones in the host-seeking behaviour of the malaria mosquito Anopheles gambiae sensu stricto (Diptera: Culicidae). *Chemical Senses* 30(2): 145 – 152.

Smith, A., Hudson, E. J.and Esozed, S. (1972). Trials with pyrethrum mosquito coils against Anopheles gambiae Gillies, Mansonia uniformis Theo. and Culex fatigans Wied. entering verandah-trap huts. *Pyrethroids* 11(1):111–115

Smith, D. L., Cohen, J. M., Chiyaka, C., Johnston, G., Gething, P. W., Gosling, R. and Tatem, A. J. (2013). A sticky situation: the unexpected stability of malaria elimination. *Philosophical Transactions of the Royal Society* 368 (1623): e20120145.

Smith, T. G., Lourenco, P., Carter, R., Walliker, D. and Ranford-Cartwright, L. C. (2000). Commitment to sexual differentiation in the human malaria parasite, Plasmodium falciparum. *Parasitology* 121(02): 127 – 133.

Steketee, R. W. and Campbell, C. C. (2010). Impact of national malaria control scale-up programmes in Africa: magnitude and attribution of effects. *Malaria Journal* 9(299): 1 – 15.

Strange, K., Phillips, J. E. and Quamme, G. A. (1982). Active HCO3-Secretion in the Rectal Salt Gland of a Mosquito Larva Inhabiting NaHCO3-CO3 Lakes. *Journal of Experimental Biology* 101(1): 171 – 186.

Sugiyama, M., Tang, A.C., Wakaki, Y. and Koyama, W. (2003). Glycemic index of single and mixed meal foods among common Japanese foods with white rice as a reference food. *Europe Journal of Clinical Nutrition* 57(1):743–752.

Sumba, L. A., Guda, T. O., Deng, A. L., Hassanali, A., Beier, J. C. and Knols, B. G. (2004). Mediation of oviposition site selection in the African malaria mosquito Anopheles gambiae (Diptera: Culicidae) by semiochemicals of microbial origin. *International Journal of Tropical Insect Science* 24(03): 260 – 265.

Sutcliffe, D. W. and Hildrew, A. G. (1989). Invertebrate communities in acid streams. *Acid Toxicity and Aquatic Animals* 34: 13 – 28.

Takken, W. and Verhulst, N.O. (2013). Host preferences of blood-feeding mosquitoes. *Annual Review of Entomology* 58: 433 – 453.

Thomas, M. B., Godfray, H. C. J., Read, A. F., Berg, H. Van Den, Tabashnik, B. E., Lenteren, J. C. Van, Waage, J. K. and Takken, W. (2012). Lessons from Agriculturefor the Sustainable Management of Malaria Vectors. *PLoS Medicine*9(1): 7–10

TDHS-MIS (2016). Tanzania Demographic and Health Survey and Malatia indicator survet; 2015-2016: National Bureau of Statistics .[www.nbs.go.tz] site visited on 19/12.2016.

Teklehaimanot, A., Sachs, J. D. and Curtis, C. (2007). Malaria control needs mass distribution of insecticidal bednets. *The Lancet* 369(9580): 2143 – 2146.

Texier, G., Machault, V., Barragti, M., Boutin, J. P. and Rogier, C. (2013). Environmental determinant of malaria cases among travellers. *Malaria Journal* 12(1): 87-96

Thamer, A. and Abdul -Samad, S. (2005). The Effect of Different NaCl and pH Levels on the Survival of Culex sp. (Diptera; Culicidae) Larvae and Eggs in Basrah, *Journal of Basrah Researches Sciences* 31(2): 31 – 36.

THMIS (2008). Tanzania HIV and malaria indicator survey 2007/08. [http//www.nbs. gotz/THMIS2011/12THIMS2007/2008htm] site visited on 5/7/2014.

THMIS (2012).Tanzania HIV/AIDS and Malaria Indicator Survey 2011-12. TACAIDS, ZAC, NBS, OCGS and ICF International, March 2013.

Tomlin CDS (2000). *A World Compendium. In the Pesticide Manual.* British Crop Protection Council, London, UK.

Truchot, J. P. (1987). *Comparative Aspects of Extracellular Acid-Base Balance.* Springer Science and Business Media, New York, 247pp.

Utzinger, J., Tozan, Y. and Singer, B. H. (2001). Efficacy and cost-effectiveness of environmental management for malaria control. *Tropical Medicine and International Health* 6(9): 677 – 687.

Van Loon, J. J., Smallegange, R. C., Bukovinszkiné-Kiss, G., Jacobs, F., De Rijk, M., Mukabana, W. R. and Takken, W. (2015). Mosquito attraction: crucial role of carbon dioxide in formulation of a five-component blend of human-derived volatiles. *Journal of Chemical Ecology* 41(6): 567 – 573.

Vanek, M. J., Shoo, B., Mtasiwa, D., Kiama, M., Lindsay, S. W., Fillinger, U., Kannady, K., Tanner, M. and Killeen, G. F. (2006). Community-based surveillance of malaria vector larval habitats: a baseline study in urban Dar es Salaam, Tanzania. *BioMed Central Public Health* 6(154): 1 – 8.

Vangenechten, J. H. D., Witters, H. and Vanderborght, O. L. J. (1989). *Laboratory Studies on Invertebrate Survival and Physiology in Acid Waters. Acid Toxicity and Aquatic Animals.* Cambridge University Press, Cambridge. 169pp.

Verhaeghen, K., Van Bortel, W., Roelants, P., Okello, P. E., Talisuna, A. and Coosemans, M. (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(4): 566 – 573.

Verhulst, N. O., Mbadi, P. A., Kiss, G. B., Mukabana, W. R., van Loon, J. J., Takken, W. and Smallegange, R. C. (2011). Improvement of a synthetic lure for Anopheles gambiae using compounds produced by human skin microbiota. *Malaria Journal* 10(28): 1 – 9.

Verhulst, N. O., Qiu, Y. T., Beijleveld, H., Maliepaard, C., Knights, D., Schulz, S., Berg-Lyons, D., Lauber, C. L., Verduijn, W., Haasnoot, G. W. and Mumm, R. (2011). Composition of human skin microbiota affects attractiveness to malaria mosquitoes. *PloS One* 6(12): e28991.

Walker, K. and Lynch, M. (2007). Contributions of Anopheles larval control to malaria suppression in tropical Africa: review of achievements and potential. *Medical and Veterinary Entomology* 21(1): 2 – 21.

Webster, B., Lacey, E. S. and Cardé, R. T. (2015). Waiting with bated breath: opportunistic orientation to human odor in the malaria mosquito, Anopheles gambiae, is modulated by minute changes in carbon dioxide concentration. *Journal of Chemical Ecology* 41(1): 59 – 66.

White, N. J., Pukrittayakamee, S., Hien, T. T., Faiz, M. A., Mokuolu, O. A. and Dondorp, A. M. (2014). Malaria. *Lancet* 383(9918): 723 – 735.

Wilson, A.L., Chen-Hussey, V., Logan, J. G., Lindsay, S.W. (2014). Are topical insect repellents effective against malaria in endemic populations? A systematic review and meta-analysis. *Malaria Journal* 13(1): 446 – 454.

WHO (2008). World Malaria. [http://www.who.int/ma laria/publications/world_malaria_ report_2012/en/index.html] site visited on 23/08/2013.

WHO (2009). *Global Insecticide Use for Vector-Borne Disease Control: A 10 -Year Assessment 2000-2009.* (5th Ed.) World Health Organization, Geneva. 41pp.

WHO (2013). World malaria. Geneva. [http://www.who.int/malaria/publi cations/world_ malaria_report_2012/en/index.html] site visited on 7/9/2014.

WHO (2014). *Malaria Vector Control and Personal Protection*. Technical Report Series No. 936. World Health Organization, Geneva.

WHO (2015). *Global Malaria Programme; World Malaria Report*. Geneva, World Health Organization, Geneva. 280pp.

WHOPES (2013). *Guidelines for Laboratory and Field Testing of Mosquito Larvicides*. World Health Organization, Geneva, Switzerland. 195pp.

WHOPES (2014). Pesticide Evaluation Scheme. [http://www.who.int/whopes/en/] site visited on 14/06/2014.

Wirth, D. F. (2002). Biological revelations. *Nature* 419(6906): 495 – 496.

Wondji, C. S., Irving, H., Morgan, J., Lobo, N. F., Collins, F. H., Hunt, R. H., Coetzee, M., Hemingway, J. and Ranson, H. (2009). Two duplicated P450 genes are associated with pyrethroid resistance in Anopheles funestus, a major malaria vector. *Genome Research* 19(3): 452 – 459.

Yaro, A. S., Traoré, A. I., Huestis, D. L., Adamou, A., Timbiné, S., Kassogué, Y. and Lehmann, T. (2012). Dry season reproductive depression of Anopheles gambiae in the Sahel. *Journal of Insect Physiology* 58(8): 1050 – 1059.

Yaro, AS., Dao, A., Adamou, A., Crawford, J. E., Ribeiro, J. M., Gwadz, R., Traoré, S. F. and Lehmann, T. (2006).The distribution of hatching time in Anopheles gambiae, *Malaria Journal* 5(19): 1 – 7.

Yawson, A. E., McCall, P. J., Wilson, M. D. and Donnelly, M. J. (2004). Species abundance and insecticide resistance of *Anopheles gambiae* in selected areas of Ghana and Burkina Faso. *Medical and Veterinary Entomology18*(4): 372 – 377.

ZABS, (2010). Zambia Population and housing Post Enumeration Survey Lusaka: Central Statistical Office. [http://www./en https://www.zabs.org.zm/Zambian_ census] site visited on 14/06/2014.

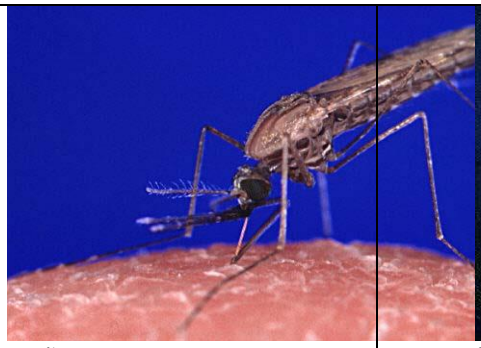
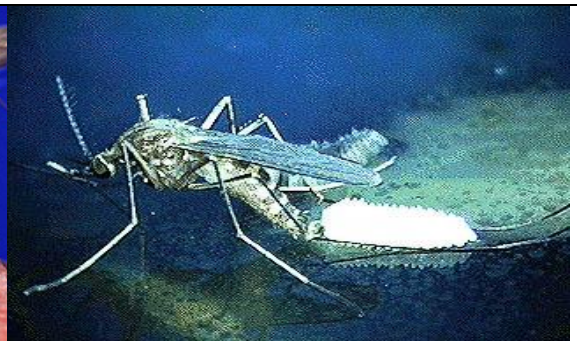
Zaim, M., Aitio, A. and Nakashima, N. (2000). Safety of pyrethroid-treated mosquito nets. *Medical and Veterinary Entomology* 14(1): 1 – 5

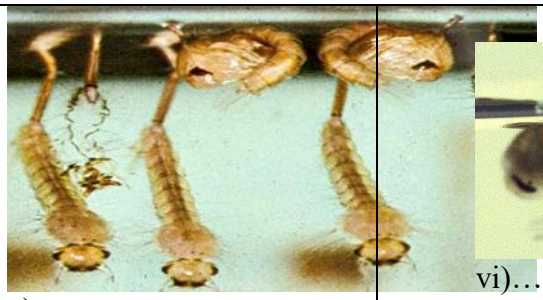
APPENDICES

Appendix 1: Household questionnaire: English version

Knowledge and awareness on malaria, malaria vector controls and mosquito biting behavior in selected sites within Dodoma and Morogoro regions Tanzania

	Variables	**Response**	
1.	**Region ……**		
2.	**Questionnaire #**		
	SOCIODEMOGRAPHIC CHARACTERISTICS		
3.	Age	…….	
4.	Sex	1. Male	2. Female
5.	Ward and street	1. Ward…….	2. Street……
6.	Level of education	1. No formal education 2. Primary education 3. Secondary education	4. College /university 5. Others (specify)……
7.	Marital status	1. Single 2. Married 3. Cohabited	4. Divorced 5. Widowed 6. Others(specify)
8.	Occupation status	1. Self employed 2. Employed	3. Studying 4. Others (specify}……
	MALARIA, MALARIA VECTOR CONTROLS AND MOSQUITOES-(KNOWLEDGE) Choose appropriate answer(s)		
9.	What causes malaria?	1.Mosquito 2. Bacteria 3. Worms	5. Plasmodium 6. Others, explain…..……
10.	What is a risk mode of malaria transmission?	……..	
11.	What is risk time malaria transmission	…..……	
12.	What is the name of the mosquito which transmits malaria?	1. Anopheles 2. Culex	3. Aedes 4. Don't know
13.	What are the breeding sites for*Anopheles* mosquitoes?	1. Garbage/trash 2. Running water	3. Stagnant water 4. Long grasses
14.	What is the most important health problem affecting your household	…… ……	
15.	Have you suffered from malaria?	1. Yes	2. No
16.	What are symptoms and signs of malaria?	1. High fever 2. Headache 3. Vomiting	4. Joint pain 5. Shivering/Rigors 6. Other (specify)…….

17.	When you get these symptoms above what do you do?	1. Go to hospital for check up and treatment 2. Use antimalarial without check up	3. Go to traditional healer 4. Use pain killer 5. Other (specify)…..
18.	What is your preferred source of information for Malaria and its vector	1. Health Centre 2. Radio	3. Television 4. At school
MALARIA, MALARIA VECTOR CONTROLS AND MOSQUITOES - (Awareness) Choose one correct answer			
19.	What time can malaria vector start biting?	1. Midnight 2. Early evening	3. Anytime
20.	Is midnight mosquito only responsible for malaria transmission?	1. Yes	2. No
21.	Can early *Anopheles* bite transmit malaria?	1. Yes	2. No
22.	Can outdoor mosquito bite transmit malaria?	1. Yes	2.No
23.	Convenient protection methods against mosquito bite outdoor do use in your household	1. LLINs 2. IRS 3. Mosquito spray	4. Mosquito coils 5. Others (specify)…..
24.	In your household do you own mosquito nets	1.Yes	2.No
25.	Who sleeps under mosquito nets?	1.Pregnant mother 2.Mothers and under-five children	3.Father only 4.All people in the house 5.Others (specify)
26.	Where did you get this net?	1. Mass campaign 2. Retail shop	3. Health facility 4. Others(specify)…
	KNOWLEDGE OF MALARIA VECTOR **Put tick (√) to the pictures of mosquito which transmit malaria, (X) to pictures of mosquito which do not transmit malaria and (0) if you don't know**		
	 i)……		 ii)……..

	 v)……	 vi)……	
	ADDITIONAL QUESTION FOR PUPILS ONLY		
27.	What health information do you get more frequent at school	1.HIV `	2.Malaria

Appendix 2: Household questionnaire: Swahili version

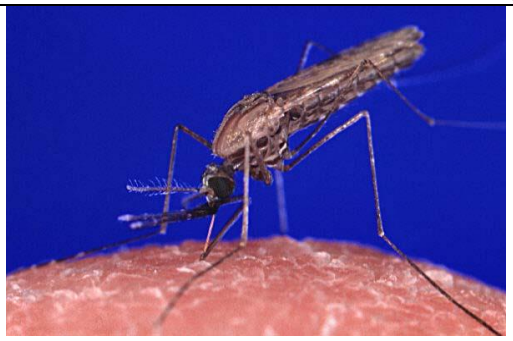
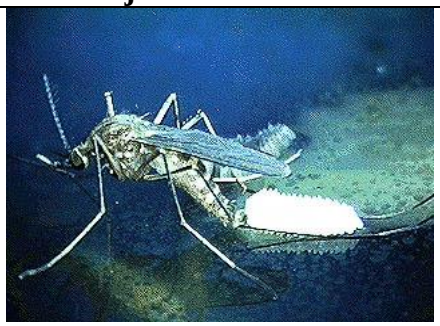
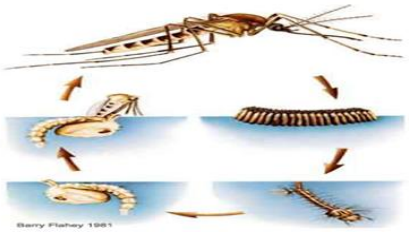
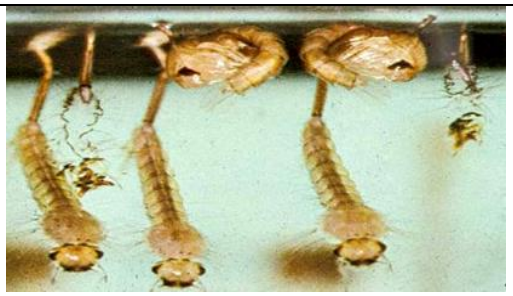
Dodoso la kupima uelewa juu ya malari000a, mbu wangatao mapema/jion na njia za kujikinga na mbu hao

	Maelezo	**Majibu**	
1.	Mkoa……..		
2.	Namba ya dodoso		
Taarifa ya mshiriki			
3.	Umri (Miaka)		
4.	Jinsia	1.Me	2.Ke
5.	Kata na Mtaa unamoishi	Kata ……	Mtaa ……
6.	Kiwango cha elimu	1.Sijasoma 2.Elimu ya msingi	3.Elimu ya sekondari 4.Chuo/ Chuo kikuu 5. Majibu mengine……
7.	Hali ya ndoa	1.Sijaowa /Sijaolewa 2.Nimeowa /Nimeolewa 3.Tunaishi bila ndoa	4. Tumeachana 5. Nimefiwa mke/mme 6. Mengineyo...
8.	Ajira	1.Nimeajiajiri 2. Nimeajiriwa	3. Nasoma 4. Menyineyo...
Elimu juu ya malaria, mbu na njia za kujikinga dhidi ya malaria (Chagua jibu au majibu sahihi)			
9.	Ugonjwa wa malaria unasababishwa na nini?	1. Mbu 2. Bakteria 3. Minyoo	4.Plasmodiam 5. Fangasi 6. Nyingine (elezea)……
10.	Ni njia gani hatarishi inayoweza kusambaza malaria?	……	
11.	Muda gani ni hatari kupata maambukizo ya malaria	……..	
12.	Taja jina la mbu anayesambaza ugonjwa wa malaria	1. Anofelesi 2. Kiuleksi	3. Aidesi 4. Sijui
13.	Ni mazingira gani yanasababisha mbu kuzaliana?	1. Sehemu ya kutunza takataka 2. Maji yanayotiririka	3. Maji yaliyotuama 4. Majani marefu
14.	Je ni magonjwa gani yanasumbua sana familia yako?	…… ……	
15.	Umeshawahi kuugua malaria	1.Ndio	2.Hapana
16.	Taja dalili za malaria	1.Homa kali 2. Kichwa kuuma 3.Kutapika	4.Kutetemeka 5.Maumivu ya viungo 6.Nyingine (elezea) ……..

17.	Ukiona dalili hizi za malaria anafanya nini?	1.Ninaenda hospitali 2.Nakunywa dawa za malaria bila kupima 3.Ananipeleke kwa mganga wa jadi	4.Ananipa dawa za kupunguza maumivu 5. Majibu mengine……
18.	Chagua sehemu ungependa ikupatie taarifa kuhusu malaria na mbu	1. Kituo cha afya 2. Radio	3. Kwenye Runinga 4. Shuleni
Mtazamo juu ya malaria, mbu na njia za kujikinga dhidi ya malaria (Chagua jibu au majibu sahihi)			
19.	Muda gani ambao mbu wanaoambukiza malaria wanaanza kungata watu	1.Usiku wa manane 2. Mapema jioni	3.Muda wowote
20.	Mbu wanaonang'ata usiku wa manane ndio mbu pekee wanaoasambaza malaria?	1. Ndio	2. Hapana
21.	Mbu wanaong'ata mapema jioni wanaweza kusambaza malaria	1. Ndio	2. Hapana
22.	Mbu wanaong'ata nje ya nyumba wanaweza kuambukiza malaria?	1.Ndio	2.Hapana
23.	Njia zipi rahis unazotumia nyumban kujikinga na mbu wangatao mapema wanaoasambaza malaria?	1. Chandarua kilichoweke wa dawa ya muda mrefu 2. Dawa za kunyunyiza kwenye ukuta 3. Dawa za kupuliza	4. Dawa za mbu za kuchoma 5. Majibu mengine, (elezea)……
24.	Je kwenye familia yako mnamiliki chandaruakujikinga na malaria?	1. Ndio	2. Hapana
25.	Je vyandarua hivyo mnavitoa wapi ?	1. Kwenye kampeni za kugawa vyandarua 2. Tunanunua dukani	3. Tunapewa hospitali 4. Majibu mengine, (elezea)……
26.	Nani analala kwenye chandarua?	1. Mama mjamzito 2. Mama na watoto chini ya miaka mitano	3. Baba 4. Watu wote 5. Majibu mengine, (elezea)…… ……

UELEWA JUU YA MBU ANAYEENEZA MALARIA.

Weka alama ya tiki (√) kwenye picha za mbu wanaoeneza malaria, alama (X)atika picha ya mbu wasioeneza malaria na alama (0) kama haujuiako

 i)……	 ii)……
 iii)□……□……	 iv) …□……□…□…
 v) ……□……□……□…	 vi)…… …□…

SWALI LA NYONGEZA KWA WANAFUNZI TU

27.	Ni habari zipi mnaazofundishwa zaidi pewa shuleni	1. Virusi vya ukimwi na ukimwi.	2. Malaria

Asante kwa ushiriki

Appendix 3: Consent form

TITLE: KNOWLEDGE AND AWARENESS ON MALARIA AND MOSQUITO BITING BEHAVIOR IN SELECTED SITES WITHIN MOROGORO AND DODOMA REGIONS TANZANIA

PURPOSE: To assess knowledge and awareness on malaria and mosquito biting behavior in selected sites within Morogoro and Dodoma regions Tanzania

SPONSOR: The Higher Education students loan Boardand St. John's University of Tanzania-Dodoma and INTRA-ACP

PROCEDURES: Your participation in the study will be at your own decision and you are free to decide without any adverse reactions. Participation will require you to answer questions on knowledge, and awareness on malaria and mosquito biting behavior. And households with primary school children the head/second adult in-change will be asked to consent for their children to be involved in the study.

RISK: The study will not harm you in any way.

BENEFIT: This study will help to provide information about knowledge and awareness on malaria and mosquitoes in study area. Recommendations will be given to the ward leaders following the findings obtained from the study to enhance community participation toward environmental sanitation, personal & family protection, and health education on mosquito feeding behaviors.

CONFIDENTIALITY: All information collected will be maintained, anonymity will be assured to all study participants.

THE RIGHT TO PARTICIPATE/ REFUSE PARTICIPATION

Participation in the study is voluntarily and you have the right to reject to participate or quite from the study at any time exclusive of any adverse.

CONSENT: I have read and understood this consent form.

I agree/ allow my child to participate in this study.

Signature of the participant /parent/guardian…….. Date……..

For any Question please don't hesitate to contact

Student: Mary Mathew Mathania-0764698175

Supervisor: Prof RS. Silayo-0754879139

Thank you for your participation!

Appendix 4: Larvae collection form

Field collection form mosquitoes and breeding habitats

Season	Study area	Grid Utm X	Grid Utm Y	land cover	Light intensity	larval type	Larval density	Size habitat	Distance	Water type	Habitat origin

Appendix 5: Adult mosquito collection form

Individual female *Anopheles* form for contaminating device (UtMCD)

to be filled while in field							to be filled while in the Laboratory								
							By stereomicroscope				Mortality/Day				
Mosquito ID	Season	HH ID	Trap ID	Study area	Collection method	LLINs inside	Total # of mosq per device	Blooded	#Anoph caught	#Culicine caught	1	2	3	4	5

Individual female *Anopheles* form for spatial distribution of Adult *Anopheles* mosquitoes

Mosquito ID	**Season**	**Study area**	**storeroom**	**Under bed**	**Behind curtain**	**Under furniture**	**ceiling**	**Barks/tree**	**Veranda**	**Junks**	**Buildings**

Appendix 6: DNA extraction protocol (MRT)

Marriott DNA Extraction Procedure

Materials: Bender Buffer 0.1 M NaCl (5 mL from a 1M stock solution—need to make this stock solution) 0.2 M sucrose (3.42 grams) 0.1 M Tris-HCl (5 mL from a 1M stock) 0.05 M EDTA pH 9.1 (5 mL from a 0.5M stock) 0.5% SDS in DEPC water (0.25 mL from a 0.1M stock).For 50 mL Bender Buffer: Add 3.42 grams dry sucrose to a 50 mL conical tube. Add the proper amounts of the other ingredients, listed above in parentheses. Fill to a final volume with DEPC water. Be sure to add SDS last, after mixing, otherwise the detergent will foam. Filter-sterilize with a 0.2 micron filter before using. Store at room temperature.

To make 1 M NaCl stock solution, add 2.9 grams dry NaCl into 50 mL HPLC H2O and vortex. Stock solutions of the liquid reagents should come in the molar concentrations listed.

8M Postassium acetate To make an 8 molar stock solution, add 19.63 grams into 25 mL HPLC H2O. Store at 4°C.

Extraction Protocol:

1. If specimens are dry, rehydrate them in a 1.5 mL microfuge tube containing 20 µl HPLC H2O for 10 minutes. If specimens are frozen, begin the procedure from Step 2.

2. Add 100 µl Bender Buffer directly into the tube with the specimen and homogenize until there are no recognizable mosquito parts. Place used pestle in 1M NaOH.

3. Incubate homogenized samples at 65°C for 1 hour.

4. Add 15 µl cold 8M potassium acetate to each sample. Mix gently and incubate on ice for 45 minutes. (Procedure may be stopped here overnight.)

5. Spin samples in a microcentrifuge (14,000 rpm) for 10 minutes, and then transfer the supernatant to a new 1.5 mL microfuge tube.

6. Add 300 µl 100% ethanol (2X volume) to each supernatant to precipitate DNA. Mix well by inverting the tube. Incubate samples at room temperature for 5 minutes.

7. Centrifuge samples (14,000 rpm) for 15 minutes. Following this spin there should be a small pellet of DNA at the bottom of the tube.

8. Carefully remove the supernatant and discard it, leaving the pellet behind in the tube. Let the pellets dry completely before resuspending—residual ethanol can interfere with PCR later.

9. Resuspend pellets in 50 µl HPLC H2O for head/thorax or abdomen extractions (100 µl for whole mosquitoes). Ideally, store overnight at 4°C before use. Store DNA permanently at -20°C.

Pestle washing: To prevent DNA contamination in PCR-based analyses, pestles should be soaked in 1M NaOH after use. They should then be washed in soapy water, rinsed off in distilled water, and autoclaved before they are used again.

Appendix 7: PCR protocol for *An. gambiae* complex kit (MRT)

Differentiation of the *Anopheles gambiae* complex by PCR

This PCR uses 4 primers that in combination produce three differentially-sized amplicons of the ribosomal DNA spacer region of *An. gambiae* complex mosquiotoes. The expected product sizes are as follows: *An. gambiae* s.s. (~390 bp), *An. arabiensis* (~315 bp), and *An. quadriannulaus* (~150 bp).

Primers:

UN: 5'- GTG TGC CCC TTC CTC GAT GT -3'
GA: 5'- CTG GTT TGG TCG GCA CGT TT -3'
AR: 5'- AAG TGT CCT TCT CCA TCC TA -3'
QD: 5'- CAG ACC AAG ATG GTT AGT AT -3'

PCR Program: (SCOTT)

1.	94ºC	2 min
2.	94ºC	30 sec
3.	50ºC	30 sec
4.	72ºC	30 sec
5.	Go to step 2	29x
6.	72ºC	7 min
7.	4ºC	forever

Reaction Mixture:	**25 µL**	**20 µL**	**12 µL**
10X	2.5 µL	2.0 µL	1.25 µL
dNTPs 2.5 mM	2.0 µL	1.6 µL	1.0 µL (final conc. 200 µM each)
AR	3.0 µL	2.4 µL	1.5 µL (150 pmol)
QD	3.0 µL	2.4 µL	1.5 µL (150 pmol)
GA	0.5 µL	0.4 µL	0.25 µL (25 pmol)
UN	1.0 µL	0.8 µL	0.5 µL (50 pmol)
Taq	1.5 U	1.2 U	0.9 U
dH_20	fill to total reaction mix volume		

Use between 0.5 and 1 µL of template DNA.

Reference: Scott, J.A., W.G. Brogdon and F.H. Collins. 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am. J. Trop. Med. Hyg. 49(4): 520-529.

Appendix 8: ELISA detection of *Plasmodium falciparum* circumsporozoite protein protocol

ELISA TESTING PROTOCOL for IHI

CIRCUMSPOROZOITE PROTEIN EXTRACTION

Add 50µl of grinding buffer (pH=8.0) into the tube containing mosquito thorax. Using a small sterile plastic pestle, gently grind the thorax making sure that the thorax is completely crushed and well mixed with the buffer, and that no parts are left on the tube wall. Add 150µls of the grinding buffer. Close tightly the tube containing the protein and store at -200C until time of use. Use another sterile pestle to extract protein from the next specimen. Used pestles are kept in adifferent container and these shall be sterilized by autoclaving before next use.

CIRCUMSPOROZOITE PROTEIN STORAGE

Store extracted protein in grinding buffer in well closed tubes at -200C. Storage should be done immediately after extraction.

PRECAUTIONS

Wear gloves when handling reagents to avoid contamination and any harm from hazardous chemicals. Wear lab coat while working in the lab. Wash hands with soap and dry with clean towel every after work.

ELISA STEPS

1. Coat 96- well ELISA plate with 50µl of Mab solution (4µg/ml) and incubate for 30 minutes to 1 hour at room temperature. To make enough Mab for coating 1 plate, mix 40µls of Mab with 5mls of PBS (pH 7.4). Cover plates with another plate (lid) during all incubations to prevent evaporation. Use separate plate for each sporozoite species.
2. Remove well contents by aspiration. Fill wells with 200µls of BB. Incubate for 1 hour at room temperature.
3. Drain well contents by aspiration or banging plate on a paper tissue. Add 50µl of mosquito homogenate (sample) per well. Add 50µls of positive controls (vial III) in

wells H1-H12 and Multiple negative controls (at least 7), add negative controls in wells H2-H8. Incubate at room temperature for 2 hours.

4. Near the end of incubation prepare Mab-peroxidase conjugate-0.025µg/50µl BB. To make enough for 1 plate, dissolve 5µls of conjugate in 5mls of BB. Vortex well.
5. Aspirate well contents by banging on tissue paper several times. Wash wells twice with 200µls of washing buffer and bang the plate on tissue paper.
6. Add 50µls of the conjugate solution to each well. Incubate for 1 hour at room temperature.
7. Near the end of incubation period, prepare the ABTS substrate solution. This solution should be prepared fresh. Mix solution A and B in 1:1. If you are doing 1 plate prepare 10mls of this solution. Confirm enzyme activity by mixing 5µl of the conjugate with 100µl of ABTS. There should be a rapid color change indicating that the peroxidase conjugate and the substrate are functional.
8. Remove conjugate by washing four times with 200µls of washing buffer and banging plate on tissue paper.
9. Add 100µl of ABTS to each well. Incubate for 30-60 minutes at room temperature.
10. Read plates at OD 405-414nm after 30 and 60 minutes using plate reader. Samples with OD above the negative cut-off value are considered positive while those with OD below the negative cut-off value are negative.
11. Record the OD of the positive samples in the database.

(b)CSP (Circumsporozoite protein) ELISA for(MRT)

This assay detects *Plasmodium falciparum* CSP protein in mosquito samples. CSP is only expressed during the sporozoite stage of malaria development, so this assay detects only sporozoite-positive mosquitoes, which are capable of transmitting malaria. The monoclonal capture antibody nonspecifically binds to the ELISA plate, after which the addition of blocking buffer prevents nonspecific binding of other proteins. After the addition of mosquito homogenate, the capture antibody binds to CSP and holds it during subsequent wash steps. After the monoclonal antibody is added, it also binds CSP and remains after washing. This antibody is conjugated to a peroxidase which catalyzes ABTS indicator solution, turning the solution green, while negative samples remain uncolored.

Materials

PBS (phosphate buffered saline, available from MMI Dept.)

BSA (bovine serum albumin) (A7906)

Casein (Sigma C7078)

Phenol red (Sigma P4758)

IGEPAL CA-630 (Sigma I3021)

Tween (Fisher BP337)

P.f. capture MAb (MR #890)

P.f. conjugate MAb (MR #890)

P.f. CSP positive control (MR #890)

Glycerol (Sigma G6279)

ABTS solution (Kirkegaard Perry)

10% SDS (sodium dodecyl sulfate) (Gibco #15553-035)

96-well U bottom vinyl ELISA plates (Corning #2797)

Solutions

Blocking Buffer (BB): 250 mL

250 mL PBS

2.5 g BSA

1.25 g casein

50 µl 0.1 g/mL phenol red stock, Stir ~3 hours until dissolved. Store overnight at 4°C or freeze for future use. Store BSA at 4°C.

BB: IG-630 (mosquito grinding buffer): 5 mL

5 mL BB

25 µl IGEPAL CA-630 detergent

PBS: Tween (wash buffer): 500 mL

500 mL PBS (*500ml H20 + PBS powder 4.8g*)

0.25 mL Tween

MAb (monoclonal antibody) stock

Dissolve lyophilized antibody in 1:1 dH_2O: glycerol, following instructions on the bottle. Store antibody at -20°C. Make the following antibody dilutions immediately prior to use:
Capture antibody: 40 µl stock in 5 mL PBS—this is enough for one 96-well plate
Conjugated antibody: 10 µl stock in 5 mL BB—this is enough for one 96-well plate

P.f. positive control stock

Resuspend *Plasmodium falciparum* CSP protein in 250 µl BB (vial I) Take 10 µl from vial I, dissolve in 990 µl BB (vial II, 100x dilution) Take 10 µl from vial II, dissolve in 990 µl BB for working stock (vial III, 10,000x dilution).

For the positive control serial dilution, add 100 µl from vial III to a plate well. Transfer 50 µl of this to the next well down, mix well with 50 µl BB. Using a new pipet tip, transfer 50 µl to the next well down, mix well with 50 µl BB, etc., resulting in 1X, 2X, 4X, 8X, 16X, 32X, 64X, and 128X positive control dilutions.

Mosquito homogenate

Grind each whole mosquito in 50 µl BB: IG-630. Rinse pestle twice with 100 µl BB, for a total of 250 µl mosquito homogenate. Mosquito homogenates can be prepared in advance and stored at -20°C.

Negative controls

Homogenize uninfected colony mosquitoes as above for negative controls.

ABTS solution

Immediately before use, mix 1:1 Solution A (ABTS) and Solution B (Hydrogen Peroxide), 100 µl per well, 10 mL total per 96-well plate. Store at 4°C, throw away remaining solution after assay is finished.

Stop Solution

1% SDS (1 mL 10% SDS in 9 mL dH_2O for one 96-well plate)

ELISA Protocol

Note: All incubations are carried out at room temperature.

1. Add 50 µl Capture MAb solutions to each well (40 µl MAb in 5 mL PBS). Cover and incubate overnight.
2. Remove solution by knocking plates upside-down. Fill wells with BB (~220-250 µl) and incubate for 1 hour.
3. Remove solution and add 50 µl mosquito homogenate, positive controls, and negative controls to their respective wells. Run all mosquito samples in duplicate. Add 50 µl BB to any empty wells. Incubate for 2 hours.

4. During the 2 hour incubation:
    - Prepare the ABTS solution (mix solutions A and B)
    - Dilute the conjugate (coagulase antibody) MAb in BB as described above (10 µl MAb in 5 mL BB).
    - Confirm enzyme activity by mixing 5 µl conjugate MAb with 100 µl ABTS. A dark green color should begin developing within a few minutes.
5. Remove mosquito homogenate. Wash plate 7 times with PBS-Tween using a plate washer.
6. Add 50 µl conjugate MAb to each well, incubate for 1 hour.
7. Remove conjugate MAb, wash 7 times with PBS-Tween.
8. Add 100 µl ABTS solution to each well and incubate for 60 minutes.
9. Add 100 µl Stop Solution to each well and read plate absorbance at 405 nm.
10. The absorbance cut-off for positive samples in 2X the average absorbance of the negative controls.

CSP Elisa technique has been developed from these reference papers.

Burkot, T. R., Williams, J. L., & Schneider, I. (1984). Identification of Plasmodium falciparum-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. *The American journal of tropical medicine and hygiene*, *33*(5), 783-788.

Appendix 9: PCR protocol for identification of blood meal source

This multiplexed PCR diagnostic differentiates between potential mammal host bloods in engorged mosquitoes. Species-specific products are amplified from the cytochrome b gene of the mitochondrial genome. Expected product sizes are as follows: Human (334 bp), Cow (561 bp), Dog (680 bp), Goat (132 bp), Pig (453 bp). Host source can be detected out to 30 hours post feeding.

Primers:

PIG573F:	5'- CCT CGC AGC CGT ACA TCT C -3'
HUMAN741F:	5'- GGC TTA CTT CTC TTC ATT CTC TCC T -3'
GOAT894F:	5'- CCT AAT CTT AGT ACT TGT ACC CTT CCT C -3'
DOG368F:	5'- GGA ATT GTA CTA TTA TTC GCA ACC AT -3'
COW121F:	5'- CAT CGG CAC AAA TTT AGT CG -3'
UNREV1025:	5'- GGT TGT CCT CCA ATT CAT GTT A -3'

PCR Program: (BLOOD)

1.	95ºC	5 min
2.	95ºC	1 min
3.	56ºC	1 min
4.	72ºC	1 min
5.	Go to step 2	39x
6.	72ºC	7 min
7.	4ºC	forever

Reaction Mixture:	**25 µL**
10X	2.5 µL
dNTPs 2.5 mM	1.0 µL (final conc. 100 µM each)
UNREV1025	0.5 µL (50 pmol of each primer)
PIG573F	0.5 µL
HUMAN741F	0.5 µL
GOAT894F	0.5 µL
DOG368F	0.5 µL
COW121F	0.5 µL
Taq	2.0 U
dH_20	fill to 25 µL

Use up to 3 µL of template DNA (from abdomen extraction eluted in 50 µL dH_20).

Reference: Kent RJ, Norris DE, 2005. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome b. *Am J Trop Med Hyg* *73:* 336-342.

Appendix 10: Permission to conduct study

SOKOINE UNIVERSITY OF AGRICULTURE
FACULTY OF VETERINARY MEDICINE
Department of Veterinary Microbiology & Parasitology
P.O BOX 3019 CHUO KIKUU, MOROGORO - TANZANIA
TEL. GEN. 255 23 2603511-4, EXT. 4513. FAX. 255 23 2604647
EMAIL: rssilayo@suanet.ac.tz ; rssilayo@yahoo.co.uk

OUR REF: VET/MP/PF 1152 **Date: 12/03/2014**

Mkurugenzi,
Manispa ya Dodoma
DODOMA, TANZANIA.

kk **Mkuu wa Idara,**
Idara ya Mikrobiologia na Parasitologia,
SLP 3019, CHUO KIKUU,
MOROGORO, TANZANIA.

Inapitishwa asaidiwe ni muhimu kwa maoni yake
E.N. Mhimbira
12/3/2014

Ndugu Mkurugenzi,

YAH: MAOMBI YA RUHUSA KWA DR. M MATHANIA
KUFANYA UTAFITI KUHUSU MBU KATIKA MAKAZI YA WATU

Mtajwa hapo juu ni mfanya kazi wa St John's University, Dodoma ambaye pia ni mwanafunzi wetu wa digrii ya uzamivu. Anatakiwa afanye utafiti kuhusu mbu katika makazi ya watu na amechagua kuanzia hiyo kazi karibu na maeneo anayoishi huko Dodoma.

Kusudi la barua hii ni kuomba kibali cha kufanya huu utafiti. Ni matumaini yetu kwamba utamruhusu afanye huu utafiti ambao utaweza kuongeza mafanikio katika vita dhidi ya mbu na malaria. Tutashukuru pia kwa msaada wowote atakaopata kutoka manispa ya Dodoma atakapohitaji. Utafiti wake utazingatia mashariti yote ya utafiti unaohusu binadamu. Uwekaji wa kumbukumbu utazingatia ulindaji wa siri za mtu mmoja mmoja na pia watakaoshiriki wataombwa kukubali kushiriki baada ya kuelezwa kikamilifu juu ya huo utafiti.

Wako katika ujenzi wa taifa,

RSilayo

Prof RS Silayo,
PhD Supervisor.

HALMASHAURI YA MANISPAA DODOMA
(Barua zote zipelekwe kwa Mkurugenzi wa Manispaa)

MKOA WA DODOMA

Tel.: 2354817/2321550
Fax: 2321550

Ofisi ya Mkurugenzi wa Manispaa
S.L.P. 1249
Dodoma
E-mail: dodomamunicipality@yahoo.co.uk

Unapojibu tafadhali taja:

Kumb. Na. HMD/T.40/6/VOL.II/ Tarehe: 3/4/2014

Mkuu wa Chuo,
Chuo cha SUA
MOROGORO

YAH: KUFANYA MAZOEZI KWA VITENDO/UTAFITI

Tafadhali rejea somo hapo juu,

Napenda kukujulisha kuwa ombi lako la mwanachuo/~~wanachuo~~ wako DR. M. MATHANDA limekubaliwa.

Mtajwa afike ofisi ya KAIA YA IPAGALA.

Kwa ajili ya kuanza ~~mazoezi hayo~~/kufanya utafiti huo, kwa tarehe husika.

Aidha Manispaa haitakuwa na fungu lolote la kumlipa mwanachuo/ ~~wanachuo~~ hao.

Nakutakia kazi njema.

Kny: MKURUGENZI WA MANISPAA
DODOMA.

MKURUGENZI WA MANISPAA DODOMA

Nakala:- Mkuu wa Idara ya —
Mwanachuo/Wanachuo ndugu
Mtendaji wa Kata/~~Kijiji~~/Mtaa wa IPAGALA.

SOKOINE UNIVERSITY OF AGRICULTURE
FACULTY OF VETERINARY MEDICINE
Department of Veterinary Microbiology & Parasitology
P.O BOX 3019 CHUO KIKUU, MOROGORO - TANZANIA
TEL. GEN. 255 23 2603511-4, EXT. 4513. FAX. 255 23 2604647
EMAIL: rssilayo@suanet.ac.tz ; rssilayo@yahoo.co.uk

OUR REF: VET/MP/PF 1152 **Date: 14/05/2014**

Mkurugenzi,
Manispa ya Morogoro
MOROGORO, TANZANIA.

kk **Mkuu wa Idara,**
Idara ya Mikrobiologia na Parasitologia,
SLP 3019, CHUO KIKUU,
MOROGORO, TANZANIA.

Imepitishwa, asaidiwe kufanya utafiti huo
15/5/2014

Ndugu Mkurugenzi,

YAH: MAOMBI YA RUHUSA KWA DR. Mary MATHANIA
KUFANYA UTAFITI KUHUSU MBU KATIKA MAKAZI YA WATU

Mtajwa hapo juu ni mfanya kazi wa St John's University, Dodoma ambaye pia ni mwanafunzi wetu wa digrii ya uzamivu. Anatakiwa afanye utafiti kuhusu mbu katika makazi ya watu na amechagua kufanya utafiti huo katika manispa ya Morogoro.

Kusudi la barua hii ni kuomba kibali chako ili utafiti huu uweze kufanyika kama ulivyoidhinishwa na chuo. Ni matumaini yetu kwamba utamruhusu afanye huu utafiti ambao utaweza kuongeza mafanikio katika vita dhidi ya mbu na malaria. Tutashukuru pia kwa msaada wowote atakaopata kutoka manispa ya Morogoro atakapohitaji. Utafiti wake utazingatia masharti yote ya utafiti unaohusu binadamu. Uwekaji wa kumbukumbu utazingatia ulindaji wa siri za mtu mmoja mmoja na pia watakaoshiriki wataombwa kukubali kushiriki baada ya kuelezwa kikamilifu juu ya huo utafiti.

Wako katika ujenzi wa taifa,

Prof RS Silayo,
PhD Supervisor.
Nakala: Dr Mary MATHANIA.

HALMASHAURI YA MANISPAA MOROGORO

Simu/Nukushi Na: 023 - 2614727

Barua Pepe: info@morogoromc.go.tz
Tovuti: www.morogoro.go.tz.
Unapojibu taja:

Ofisi ya Mkurugenzi wa Manispaa,
S.L.P. 166,
MOROGORO
TANZANIA

Kumb.Na.R.10/MMC- 24/..... Tarehe: 24/05/2014

Mkuu wa Chuo,
Chuo Kikuu cha Sokoine cha Kilimo (SUA)
S.L.P. 3038
MOROGORO

Yah: MAOMBI YA KUFANYA MAFUNZO KWA VITENDO

Tafadhali husika na kichwa cha habari hapo juu.
Napenda kukujulisha kuwa kibali kimetolewa kwa Mwanachuo MARY MATHEW MATHANIA ambaye ni mwanafunzi katika Chuo chako kufanya mafunzo kwa vitendo katika Halmashauri ya Manispaa ya Morogoro kuanzia 25/5/2014 hadi 26/8/2014 kwa gharama zake mwenyewe.

Tunamtakia mafunzo mema.

Kim[illegible]ela H.S.
Kny: Mkurugenzi wa Manispaa
MOROGORO
K.N.Y: MKURUGENZI WA MANISPAA
MOROGORO

Nakala: AFISA MISITU KATA CHAMWINO
P.O. BOX 166,
MOROGORO *-Apokelewe na apatiwe ushirikiano*

MARY MATHEW MATHANIA
S.L.P. 3038
MOROGORO

Appendix 11: PCR results for Anopheles species emerged from larvae collected in rainy season (Resuts from MRT laboratory)

Lane#/Sample id	PCR results	Lane#/Sample id	PCR results
1	*An. quadriannulatus*	31	*An. quadriannulatus*
2B	*An. arabiensis*	32	*An. quadriannulatus*
5	*An. arabiensis*	33	*An. quadriannulatus*
7	*An. quadriannulatus*	34	*An. quadriannulatus*
8	*An. quadriannulatus*	35	*An. quadriannulatus*
9	*An. quadriannulatus*	36	*An. arabiensis*
10	*An. quadriannulatus*	37	*An. quadriannulatus*
11	*An. arabiensis*	38	*An. quadriannulatus*
12B	Not amplified	39	*An. quadriannulatus*
13B	*An. arabiensis*	40	*An. quadriannulatus*
14B	*An. quadriannulatus*	41	*An. quadriannulatus*
15	*An. quadriannulatus*	43	*An. quadriannulatus*
16	*An. quadriannulatus*	44	*An. quadriannulatus*
17	*An. quadriannulatus*	45	*An. coustani*
18	*An. quadriannulatus*	47	*An. quadriannulatus*
19B	Not amplified	48	*An. quadriannulatus*
20	*An. quadriannulatus*	49	*An. arabiensis*
21B	Not amplified	64	*An. quadriannulatus*
22	*An. quadriannulatus*	65	*An. quadriannulatus*
24	*An. quadriannulatus*	66	*An. quadriannulatus*
25	*An. quadriannulatus*	73B	*An. quadriannulatus*
26	*An. quadriannulatus*	76	*An. quadriannulatus*
28B	Not amplified	78	*An. quadriannulatus*
29B	*An. quadriannulatus*	82B	*An. quadriannulatus*
30	*An. quadriannulatus*	90D	*An. quadriannulatus*

Appendix 12: PCR results for Anopheles species emerged from larvae collected in dry season (Resuts from IHI laboratory)

Lane#	Sample id	PCR results	Lane#	Sample id	PCR results
1	LDM301	*An. arabiensis*	31	LDM3	*An. arabiensis*
2	LDM2	*An. arabiensis*	32	LDM146	*An. arabiensis*
3	LDD3A	*An. arabiensis*	33	LDD78	*An. arabiensis*
4	LDD4	*An. arabiensis*	34	LDD171	*An. arabiensis*
5	LDM5	*An. arabiensis*	35	LDM98	*An. arabiensis*
6	LDM6A	*An. arabiensis*	36	LDD81	*An. arabiensis*
7	LDM7	*An. arabiensis*	37	LDD186	*An. arabiensis*
8	LDM108	*An. arabiensis*	38	LDM23	*An. arabiensis*
9	LDD9	*An. arabiensis*	39	LDM84	*An. arabiensis*
10	LDD10	*An. arabiensis*	40	LDM85	*An. arabiensis*
11	LDM11	*An. arabiensis*	41	LDD105	*An. arabiensis*
12	LDM22	*An. arabiensis*	42	LDM74	*An. arabiensis*
13	LDM13	*An. gambiae*	43	LDM188	*An. arabiensis*
14	LDD14A	*An. arabiensis*	44	LDM89	*An. arabiensis*
15	LDD15A	*An. arabiensis*	45	LDD32	*An. arabiensis*
16	LDM16B	*An. arabiensis*	46	LDM149	*An. arabiensis*
17	LDM171	*An. arabiensis*	47	LDD92	*An. arabiensis*
18	LDM1	*An. arabiensis*	48	LDM73	Not amplified
19	LDD19A	*An. arabiensis*	49	LDM94	*An. arabiensis*
20	LDD20	*An. arabiensis*	50	LDD67	*An. arabiensis*
21	LDM402	*An. arabiensis*	51	LDD190	*An. arabiensis*
22	LDM22A	*An. arabiensis*	52	LDM97	*An. arabiensis*
23	LDM83	*An. arabiensis*	53	LDM80	*An. arabiensis*
24	LDD24	*An. arabiensis*	54	LDD99	*An. arabiensis*
23	LDD100	*An. arabiensis*	55	LDM150A	*An. arabiensis*
26	LDD139	*An. arabiensis*	56	LDM101	*An. arabiensis*
27	LDM27	*An. arabiensis*	57	LDD102	*An. arabiensis*
28	LDM103	*An. arabiensis*	58	LDM28	*An. arabiensis*
29	LDM324	*An. arabiensis*	59	LDM104	*An. gambiae*
30	LDM30	*An. arabiensis*	60	LDD86	*An. arabiensis*
61	LDM31	*An. arabiensis*	97	LDM216A	*An. gambiae*
62	LDM90	*An. gambiae*	98	LDM107	*An. arabiensis*
63	LDM108	*An. arabiensis*	99	LDD33	*An. arabiensis*
64	LDM214	*An. arabiensis*	100	LDM309	*An. arabiensis*
65	LDD352	*An. arabiensis*	101	LDM110	*An. arabiensis*
66	LDM36	*An. arabiensis*	102	LDD38	*An. arabiensis*
67	LDM37	*An. arabiensis*	103	LDD112	*An. arabiensis*
68	LDD111	*An. arabiensis*	104	LDM160	*An. arabiensis*
69	LDM39	*An. arabiensis*	105	LDD114	*An. arabiensis*
70	LDM65	*An. arabiensis*	106	LDM115	*An. arabiensis*
71	LDM41	*An. arabiensis*	107	LDM106A	*An. arabiensis*
72	LDD120	*An. arabiensis*	108	LDM117	*An. arabiensis*
73	LDD43	*An. arabiensis*	109	LDD118	*An. arabiensis*
74	LDD244	*An. arabiensis*	110	LDM53	*An. arabiensis*
75	LDM45	*An. arabiensis*	111	LDD42	*An. arabiensis*

76	LDD132	*An. arabiensis*	112	LDD121	*An. arabiensis*
77	LDM47	*An. gambiae*	113	LDM220	*An. arabiensis*
78	LDM127	*An. arabiensis*	114	LDM161	*An. arabiensis*
79	LDM49	*An. arabiensis*	115	LDM124	*An. arabiensis*
80	LDD50	*An. gambiae*	116	LDM55	*An. arabiensis*
81	LDD130	*An. gambiae*	117	LDD126	*An. arabiensis*
82	LDD52	*An. arabiensis*	118	LDD48	*An. arabiensis*
83	LDM119	*An. arabiensis*	119	LDM128	*An. arabiensis*
84	LDM54	*An. arabiensis*	120	LDD129	*An. arabiensis*
85	LDD125	*An. arabiensis*	121	LDM51	*An. gambiae*
86	LDM142	*An. arabiensis*	122	LDM131	*An. arabiensis*
87	LDM57	*An. arabiensis*	123	LDM46	*An. arabiensis*
88	LDD58	*An. arabiensis*	124	LDD61	*An. arabiensis*
89	LDM59	*An. arabiensis*	125	LDD134	*An. arabiensis*
90	LDD60	*An. arabiensis*	126	LDM135	*An. arabiensis*
91	LDM133	*An. gambiae*	127	LDM70	*An. arabiensis*
92	LDM62	*An. arabiensis*	128	LDD237	*An. arabiensis*
93	LDD363	*An. arabiensis*	129	LDM138	*An. arabiensis*
94	LDD64	*An. arabiensis*	130	LDM27	*An. arabiensis*
95	LDM40	*An. arabiensis*	131	LDM140	*An. arabiensis*
96	LDM66	*An. arabiensis*	132	LDD244	*An. arabiensis*
133	LDD95	*An. arabiensis*	142	LDM56	*An. arabiensis*
134	LDD147	*An. arabiensis*	143	LDM143	*An. arabiensis*
135	LDM69	*An. arabiensis*	144	LDD144A	*An. arabiensis*
136	LDM136A	*An. arabiensis*	145	LDD145	*An. arabiensis*
137	LDM71	*An. arabiensis*	146	LDM77	*An. arabiensis*
138	LDD270	*An. arabiensis*	147	LDD68	*An. arabiensis*
139	LDM93	*An. arabiensis*	148	LDM148	*An. arabiensis*
140	LDM87	*An. arabiensis*	149	LDM91	*An. arabiensis*
141	LDM75	*An. arabiensis*	150	LDM25	*An. arabiensis*

Appendix 13 : PCR results for Adult *Anopheles* species collected during dry season

(Resuts from IHI laboratory)

Lane#	Sample id	PCR results	Lane#	Sample id	PCR results
1	SDM28	*An. arabiensis*	*26*	*SDD88*	*An. arabiensis*
2	*SDM64*	*An. arabiensis*	*27*	*SDD70A*	*An. arabiensis*
3	*SDD1*	*An. arabiensis*	*28*	*SDM29*	*An. arabiensis*
4	*SDD10*	*An. arabiensis*	*29*	*SDD67*	*An. arabiensis*
5	*SDM38*	*An. quadrianulatus*	*30*	*SDM66*	*An. arabiensis*
6	*SDM2*	*An. arabiensis*	*31*	*SDM153*	*An. arabiensis*
7	*SDD4*	*An. arabiensis*	*32*	*SDD50*	*An. arabiensis*
8	*SDD51*	*An. arabiensis*	*33*	*SDM99*	*An. arabiensis*
9	*SDD43*	*An. arabiensis*	*34*	*SDD73*	*An. arabiensis*
10	*SDM42*	*An. arabiensis*	*35*	*SDD71*	*An. arabiensis*
11	*SDD79*	*An. gambiae*	*36*	*SDD64*	*An. arabiensis*
12	*SDM81*	*An. arabiensis*	*37*	*SDM135*	*An. arabiensis*
13	*SDD3*	*An. arabiensis*	*38*	*SDM206*	*An. arabiensis*
14	*SDD11*	*An. arabiensis*	*39*	*SDD44*	*An. arabiensis*
15	*SDD94*	*An. arabiensis*	*40*	*SDM115*	*An. arabiensis*
16	*SDM93*	*An. gambiae*	*41*	*SDM91*	*An. arabiensis*
17	*SDD133*	*An. arabiensis*	*42*	*SDD83*	*An. arabiensis*
18	*SDD126*	*An. arabiensis*	*43*	*SDM129*	*An. arabiensis*
19	*SDD14*	*An. arabiensis*	*44*	*SDM130*	*An. arabiensis*
20	*SDM18*	*An. arabiensis*	*45*	*SDD45*	*An. arabiensis*
21	*SDM20*	*An. arabiensis*	*46*	*SDM71*	*An. arabiensis*
22	*SDD19*	*An. arabiensis*	*47*	*SDD96A*	*An. arabiensis*
23	*SDD63*	*An. arabiensis*	*48*	*SDD39*	*An. arabiensis*
24	*SDM60*	*An. arabiensis*	*49*	*SD3D7A*	*An. arabiensis*
25	*SDD9*	*An. gambiae*	*50*	*SDM89*	*An. arabiensis*

Appendix 14: PCR results for Adult Anopheles species collected during wet/warm season (Resuts from IHI laboratory)

Lane#	Sample id	PCR results	Lane#	Sample id	PCR results
1	*SWM12*	*An. arabiensis*	*26*	*SWM86*	*An. arabiensis*
2	*SWM13*	*An. arabiensis*	*27*	*SWD24*	*An. arabiensis*
3	*SWD16*	*An. arabiensis*	*28*	*SWD26*	*An. arabiensis*
4	*SWM47*	*An. arabiensis*	*29*	*SWD111*	*An. gambiae*
5	*SWM17*	*An. arabiensis*	*30*	*SWM132*	*An. arabiensis*
6	*SWD90*	*An. arabiensis*	*31*	*SWM127*	*An. arabiensis*
7	*SWD49*	*An. arabiensis*	*32*	*SWD116*	*An. arabiensis*
8	*SWM21*	*An. gambiae*	*33*	*SWD35*	*An. arabiensis*
9	*SWM23*	*An. arabiensis*	*34*	*SWM58*	*An. arabiensis*
10	*SWD15A*	*An. arabiensis*	*35*	*SWM138*	*An. arabiensis*
11	*SWD22*	*An. arabiensis*	*36*	*SWM114*	*An. arabiensis*
12	*SWM28*	*An. arabiensis*	*37*	*SWD59*	*An. gambiae*
13	*SWM29*	*An. arabiensis*	*38*	*SWM72*	*An. arabiensis*
14	*SWD112*	*An. gambiae*	*39*	*SWM141*	*An. arabiensis*
15	*SWD98*	*An. arabiensis*	*40*	*SWM136*	*An. gambiae*
16	*SWM30*	*An. arabiensis*	*41*	*SWD76*	*An. arabiensis*
17	*SWM32*	*An. arabiensis*	*42*	*SWM75A*	*An. arabiensis*
18	*SWD7*	*An. gambiae*	*43*	*SWD117*	*An. arabiensis*
19	*SWM31*	*An. arabiensis*	*44*	*SWD130*	*An. arabiensis*
20	*SWD36*	*An. arabiensis*	*45*	*SWM97*	*An. arabiensis*
21	*SWM77*	*An. arabiensis*	*46*	*SWD204*	*An. arabiensis*
22	*SWM128*	*An. arabiensis*	*47*	*SWD146*	*An. arabiensis*
23	*SWD92*	*An. gambiae*	*48*	*SWM171*	*An. arabiensis*
24	*SWM84A*	*An. arabiensis*	*49*	*SWD33*	*An. arabiensis*
25	*SWM153*	*An. arabiensis*	*50*	*SWD87*	*An. arabiensis*

Appendix 15: PCR results for Anopheles species collected from hibernating places during dry season (Resuts from IHI laboratory)

Lane#	Sample id	PCR results	Lane#	Sample id	PCR results
1	HPD 2	*An. arabiensis*	31	HPD 10	*An. arabiensis*
2	HPD27	*An. arabiensis*	32	HPD 15	*An. arabiensis*
3	HPM 13	*An. arabiensis*	33	HPM 28	*An. arabiensis*
4	HPD 24	*An. gambiae*	34	HPD 124	*An. gambiae*
5	HPM 1	*An. arabiensis*	35	HPM 86	*An. arabiensis*
6	HPM 31	*An. arabiensis*	36	HPM 206	*An. gambiae*
7	HPD 5	*An. gambiae*	37	HPM 107	*An. arabiensis*
8	HPD 108	*An. arabiensis*	38	HPM 23	*An. arabiensis*
9	HPM 14	*An. arabiensis*	39	HPD 109	*An. arabiensis*
10	HPD 52	*An. gambiae*	40	HPM 19	*An. arabiensis*
11	HPM 6	*An. arabiensis*	41	HPD 38	*An. arabiensis*
12	HPM 37	*An. arabiensis*	42	HPD 102	*An. arabiensis*
13	HPM 11	*An. gambiae*	43	HPD 16	*An. arabiensis*
14	HPM 55	*An. arabiensis*	44	HPD 85	*An. arabiensis*
15	HPD 45	*An. arabiensis*	45	HPM29	*An. arabiensis*
16	HPM 41	*An. gambiae*	46	HPD 149	*An. arabiensis*
17	HPD 121	*An. arabiensis*	47	HPD 617	*An. arabiensis*
18	HPD43	*An. arabiensis*	48	HPM 188	*An. gambiae*
19	HPD 44	*An. arabiensis*	49	HPM 53	*An. arabiensis*
20	HPM 17	*An. arabiensis*	50	HPD 142	*An. arabiensis*
21	HPD 22	*An. gambiae*	51	HPM 421	*An. gambiae*
22	HPM 47	*An. gambiae*	52	HPD 210	*An. arabiensis*
23	HPM 57	*An. arabiensis*	53	HPD 161	*An. gambiae*
24	HPD 649	*An. arabiensis*	54	HPD123	*An. arabiensis*
25	HPD 450	*An. gambiae*	55	HPM 59	*An. arabiensis*
26	HPD 130	*An. gambiae*	56	HPD 116	*An. arabiensis*
27	HPM 56	*An. arabiensis*	57	HPM 48	*An. arabiensis*
28	HPD 119	*An. arabiensis*	58	HPD111	*An. arabiensis*
29	HPD 54	*An. arabiensis*	59	HPM 129	*An. arabiensis*
30	HPD 125	*An. arabiensis*	60	HPD 350	*Not amplified*

61	HPM 742	*An. arabiensis*	81	HPD 137	*An. An. gambiae*
62	HPD257	*An. arabiensis*	82	HPM 21	*An. An. gambiae*
63	HPM 581	*An. arabiensis*	83	HPD 61	*An. arabiensis*
64	HPD 191	*An. gambiae*	84	HPD 18	*An. arabiensis*
65	HPM 60	*An. arabiensis*	85	HPM 135	*An. arabiensis*
66	HPM133	*An. gambiae*	86	HPM 70	*An. arabiensis*
67	HPM 627	*An. arabiensis*	87	HPD 237	*An. arabiensis*
68	HPD 313	*An. arabiensis*	88	HPM 138	*An. gambiae*
69	HPD 164	*An. gambiae*	89	HPM 27	*An. arabiensis*
70	HPD 464	*An. arabiensis*	90	HPD 131	*An. arabiensis*
71	HPM 66	*An. arabiensis*	91	HPD 141	*An. arabiensis*
72	HPM 95	*An. arabiensis*	92	HPD 56	*An. gambiae*
73	HPM 147	*An. arabiensis*	93	HPM 41	*An.gambiae*
74	HPD 69	*An. arabiensis*	94	HPM 12	*An. arabiensis*
75	HPD 136	*An. arabiensis*	95	HPD 115	*An. arabiensis*
76	HPD 171	*An. gambiae*	96	HPD 77	*An. arabiensis*
77	HPM 270	*An. arabiensis*	97	HPD 68	*An. arabiensis*
78	HPM 193	*An. arabiensis*	98	HPM 108	*An. arabiensis*
79	HPD 87	*An. arabiensis*	99	HPM 919	*An. arabiensis*
80	HPM 90	*An. arabiensis*	100	HPM 213	*An. arabiensis*

Appendix 16: PCR results for *Anopheles* species caught by UtMCD during wet season (Resuts from IHI laboratory)

Lane#	Sample id	PCR Results	Lane#	Sample id	PCR Results
1	DWMS433	*An. gambiae*	34	DWM 2	*An.arabiensis*
2	DWM4	*An. arabiensis*	35	DWD44	*An. arabiensis*
3	DWD31	*An. arabiensis*	36	DWD50	*An. arabiensis*
4	DWM29	*An. arabiensis*	37	DWM7	*An. arabiensis*
5	DWM54	*An. arabiensis*	38	DWD 401	*An. arabiensis*
6	DWM 35	*An. arabiensis*	39	DWD 255	*An. arabiensis*
7	DWM 109	*An. arabiensis*	40	DWM 210	*An. arabiensis*
8	DWM 21	*An. gambiae*	41	DWM 604	*An. arabiensis*
9	DWD65	*An. arabiensis*	42	DWD 567	*An. arabiensis*
10	DWM38	*An. arabiensis*	43	DWM 131	*An. arabiensis*
11	DWD 203	Not amplified	44	DWM 19	*An. arabiensis*
12	DWD 39	*An. arabiensis*	45	DWD 88	*An. arabiensis*
13	DWM 11	*An. arabiensis*	46	DWD 108	*An. arabiensis*
14	DWM 9	*An. arabiensis*	47	DWM 10	*An. arabiensis*
15	DWD 267	*An. arabiensis*	48	DWD 64	*An. arabiensis*
16	DWM 153	*An. arabiensis*	49	DWM 138	*An. gambiae*
17	DWM 66	*An. arabiensis*	50	DWD 119	Not amplified
18	DWD 74	*An. arabiensis*	51	DWM 444	*An. arabiensis*
19	DWM 100	*An. arabiensis*	52	DWM 264	*An. arabiensis*
20	DWD 110	*An. arabiensis*	53	DWM202	*An. arabiensis*
21	DWM 62	*An. arabiensis*	54	DWD 37	*An. arabiensis*
22	DWM 61	*An. arabiensis*	55	DWM 101	*An. arabiensis*
23	DWD 14	*An. arabiensis*	56	DWD 123	*An. arabiensis*
24	DWM 28	*An. arabiensis*	57	DWM 234	*An. arabiensis*
25	DWM 3	*An. arabiensis*	58	DWM 531	*An. arabiensis*
26	DWD99	*An. arabiensis*	59	DWD701	*An. arabiensis*
27	DWM323	*An. arabiensis*	60	DWD424	*An. arabiensis*
28	DWM413	*An. arabiensis*	61	DWM204	*An. arabiensis*
29	DWD501	*An. gambiae*	62	DWD18	*An. arabiensis*
30	DWM106	*An. arabiensis*	63	DWD23	*An.arabiensis*

31	DWM78	*An. arabiensis*	64	DWM45	*An. arabiensis*
32	DWD15	*An. arabiensis*	65	DWM59	*An. arabiensis*
33	DWD6	*An. arabiensis*	66	DWM196	*An. arabiensis*
67	DWD70	*An. arabiensis*	84	DWD411	*An. arabiensis*
68	DWM17	*An. arabiensis*	85	DWM93	*An. arabiensis*
69	DWD116	*An. arabiensis*	86	DWD117	*An. arabiensis*
70	DWM48	*An. arabiensis*	87	DWM191	*An. arabiensis*
71	DWM556	*An. arabiensis*	88	DWM183	*An. arabiensis*
72	DWD26	*An. arabiensis*	89	DWD261	*An. arabiensis*
73	DWM164	*An. arabiensis*	90	DWM5	Not amplified
74	DWD90	*An. arabiensis*	91	DWM27	Not amplified
75	DWM 30	*An. arabiensis*	92	DWD 322	*An. arabiensis*
76	DWD12	*An. arabiensis*	93	DWD 256	*An. arabiensis*
77	DWM421	*An. arabiensis*	94	DWM 145	*An. arabiensis*
78	DWD 320	*An. arabiensis*	95	DWM 105	*An. arabiensis*
79	DWM 513	*An. arabiensis*	96	DWM 77	*An. arabiensis*
80	DWM1	*An. arabiensis*	97	DWD 8	*An. arabiensis*
81	DWD 301	*An. arabiensis*	98	DWM13	*An. arabiensis*
82	DWD 55	*An. arabiensis*	99	DWM22	*An. arabiensis*
83	DWM 16	*An. arabiensis*	100	DWD20	*An. arabiensis*

Appendix 17: PCR results for Anopheles species caught by UtMCD during dry season (Resuts from IHI laboratory)

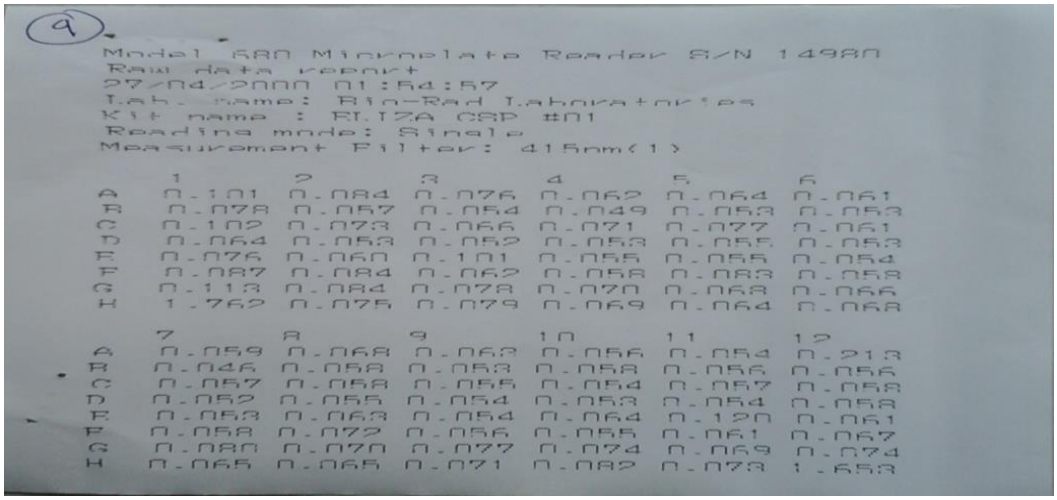
Lane#	Sample id	PCR Results	Lane#	Sample id	PCR Results
1	DDMS42	*An. arabiensis*	34	DDM 46	*An. arabiensis*
2	DDM126	*An. arabiensis*	35	DDD140	*An. arabiensis*
3	DDD50	*An. arabiensis*	36	DDD90	*An. arabiensis*
4	DDM144	*An. arabiensis*	37	DDM12	*An. arabiensis*
5	DDM702	*An. arabiensis*	38	DDD 164	*An. arabiensis*
6	DDD 3	*An. arabiensis*	39	DDD 413	*An. arabiensis*
7	DDD 97	*An. arabiensis*	40	DDM 510	*An. arabiensis*
8	DDM 63	*An. arabiensis*	41	DDM 99	*An.arabiensis*
9	DDD16	*An. arabiensis*	42	DDD 62	*An. arabiensis*
10	DDMS78	*An. arabiensis*	43	DDM 11	*An. arabiensis*
11	DDD 129	*An. arabiensis*	44	DDM 80	*An. arabiensis*
12	DDD 9	*An. arabiensis*	45	DDD 130	*An. arabiensis*
13	DDM 59	*An. arabiensis*	46	DDD 34	*An. arabiensis*
14	DDM 324	*An. arabiensis*	47	DDM 19	*An. arabiensis*
15	DDD 83	*An. arabiensis*	48	DDD 6	*An. arabiensis*
16	DDM 51	*An. arabiensis*	49	DDM 5	*An. arabiensis*
17	DDM 1	*An. arabiensis*	50	DDD 58	*An. arabiensis*
18	DDD 4	*An. arabiensis*	51	DDM 191	*An. arabiensis*
19	DDD 612	*An. arabiensis*	52	DDM 106	*An. arabiensis*
20	DDD 28	*An. arabiensis*	53	DDM29	*An. arabiensis*
21	DDM 131	*An. arabiensis*	54	DDD 27	*An. arabiensis*
22	DDM 76	*An. arabiensis*	55	DDD 85	*An. arabiensis*
23	DDD 10	*An. arabiensis*	56	DDD 13	*An. arabiensis*
24	DDM 15	*An. arabiensis*	57	DDM 603	*An. arabiensis*
25	DDM 65	*An. arabiensis*	58	DDM 88	*An. arabiensis*
26	DDD129	*An. arabiensis*	59	DDD7091	*An. arabiensis*
27	DDM223	*An. arabiensis*	60	DDD224	*An. arabiensis*
28	DDM419	*An. arabiensis*	61	DDM139	*An. arabiensis*
29	DDD302	*An. gambiae*	62	DDD128	*An. arabiensis*

30	DDM21	*An. arabiensis*	63	DDD323	*An. arabiensis*
31	DDM95	*An. arabiensis*	64	DDM145	*An. arabiensis*
32	DDD81	*An. arabiensis*	65	DDM259	*An. arabiensis*
33	DDD48	*An. arabiensis*	66	DDM52	*An. arabiensis*
67	DDD177	*An. arabiensis*	84	DDD121	*An. arabiensis*
68	DDM107	*An. arabiensis*	85	DDM136	*An. arabiensis*
69	DWD149	*An. arabiensis*	86	DDD60	*An. arabiensis*
70	DDM250	*An. arabiensis*	87	DDM190	*An. arabiensis*
71	DDM516	*An. arabiensis*	88	DDM145	*An. arabiensis*
72	DDD260	*An. arabiensis*	89	DDD316	*An. arabiensis*
73	DDM112	*An. arabiensis*	90	DDM153	*An. arabiensis*
74	DDD148	*An. arabiensis*	91	DDM132	*An. arabiensis*
75	DDM 7	*An. arabiensis*	92	DDD 26	*An. arabiensis*
76	DDD119	*An. arabiensis*	93	DDD 856	*An. arabiensis*
77	DDD 420	*An. arabiensis*	94	DDM 91	*An. arabiensis*
78	DDD 125	*An. gambiae*	95	DDM 92	*An. arabiensis*
79	DDM 666	*An. arabiensis*	96	DDM 72	*An. arabiensis*
80	DDM8	*An. arabiensis*	97	DDD 55	*An. arabiensis*
81	DDD 14	*An. arabiensis*	98	DDM143	*An. arabiensis*
82	DDD 555	*An. arabiensis*	99	DDM150	*An. arabiensis*
83	DDM 124	*An. arabiensis*	100	DDD82	*An. arabiensis*

Appendix 18: ELISA- CSP results for detecting infection rate of *Anopheles* mosquitoes caught by UtMCD during wet season

Molecula assay: ELISA- CSP

Malaria institute: Ifakara Health Institute (IHI)-Tanzania**.**

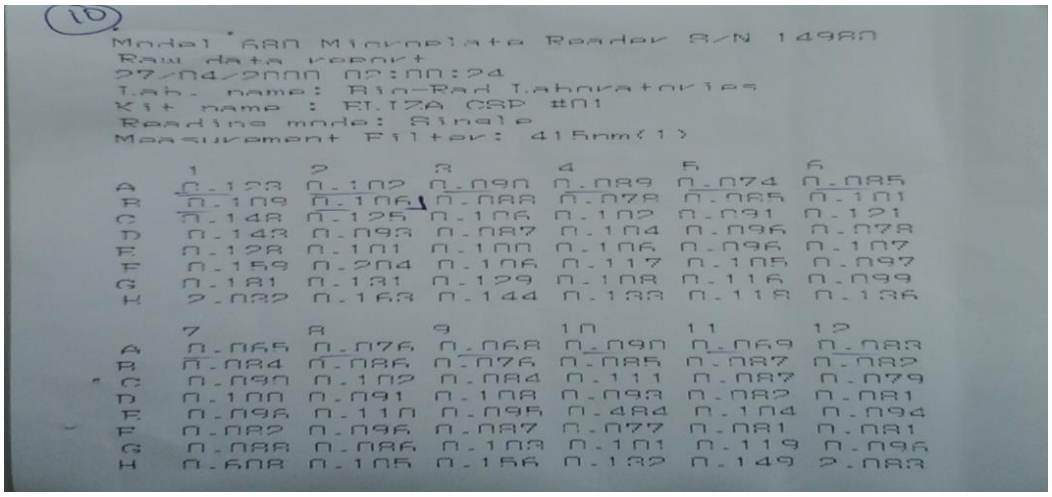


(9)

Model ARM Microplate Reader S/N 14980
Raw data report
27/04/2000 01:54:57
Lab. name: Bio-Rad Laboratories
Kit name : ELISA CSP #01
Reading mode: Single
Measurement Filter: 415nm(1)

	1	2	3	4	5	6
A	0.101	0.084	0.076	0.062	0.064	0.061
B	0.078	0.057	0.054	0.049	0.053	0.053
C	0.102	0.073	0.066	0.071	0.077	0.061
D	0.064	0.053	0.052	0.053	0.055	0.053
E	0.076	0.060	0.101	0.055	0.055	0.054
F	0.087	0.084	0.062	0.058	0.083	0.058
G	0.113	0.084	0.078	0.070	0.068	0.066
H	1.762	0.075	0.079	0.069	0.064	0.068

	7	8	9	10	11	12
A	0.059	0.068	0.063	0.056	0.054	0.213
B	0.046	0.058	0.053	0.058	0.056	0.056
C	0.057	0.058	0.055	0.054	0.057	0.058
D	0.052	0.055	0.054	0.053	0.054	0.058
E	0.053	0.063	0.054	0.064	0.120	0.061
F	0.058	0.072	0.056	0.055	0.061	0.067
G	0.080	0.070	0.077	0.074	0.069	0.074
H	0.065	0.065	0.071	0.082	0.073	1.653



(10)

Model ARM Microplate Reader S/N 14980
Raw data report
27/04/2000 02:00:24
Lab. name: Bio-Rad Laboratories
Kit name : ELISA CSP #01
Reading mode: Single
Measurement Filter: 415nm(1)

	1	2	3	4	5	6
A	0.123	0.102	0.090	0.089	0.074	0.085
B	0.109	0.106	0.088	0.078	0.085	0.101
C	0.148	0.125	0.106	0.102	0.091	0.121
D	0.143	0.093	0.087	0.104	0.096	0.078
E	0.128	0.101	0.100	0.106	0.096	0.107
F	0.159	0.204	0.106	0.117	0.105	0.097
G	0.181	0.131	0.129	0.108	0.116	0.099
H	2.032	0.163	0.144	0.133	0.118	0.136

	7	8	9	10	11	12
A	0.085	0.076	0.068	0.090	0.069	0.083
B	0.084	0.086	0.076	0.085	0.087	0.082
C	0.090	0.102	0.084	0.111	0.087	0.079
D	0.100	0.091	0.108	0.093	0.082	0.081
E	0.096	0.110	0.095	0.484	0.104	0.094
F	0.082	0.096	0.087	0.077	0.081	0.081
G	0.088	0.086	0.103	0.101	0.119	0.096
H	0.608	0.105	0.156	0.132	0.149	2.083

Appendix 19: ELISA- CSP results for detecting infection rate of *Anopheles* mosquitoes caught by UtMCD during dry season

Molecular assay: ELISA- CSP

Malaria institute: Macha Malaria Research Trust (MRT)-Zambia

1	2	3	4	5	6	7	8	9	10	11	12	
3.334	3.415	0.278	0.246	0.268	0.221	0.24	0.214	0.228	0.188	0.229	0.199	405
2.182	1.855	0.256	0.278	0.247	0.206	0.231	0.191	0.188	0.218	0.179	0.202	405
1.347	1.182	0.279	0.271	0.23	0.216	0.266	0.218	0.205	0.305	0.182	0.194	405
0.792	0.715	0.252	0.252	0.228	0.217	0.207	0.184	0.184	0.229	0.187	0.179	405
0.475	0.448	0.255	0.288	0.246	0.243	0.221	0.214	0.258	0.212	0.228	0.194	405
0.423	0.327	0.249	0.259	0.21	0.207	0.203	0.192	0.229	0.315	0.271	0.242	405
0.389	0.356	0.287	0.298	0.212	0.227	0.293	0.223	0.2	0.278	0.259	0.207	405
0.359	0.264	0.258	0.219	0.217	0.227	0.203	0.223	0.377	0.293	0.27	0.219	405
1	2	3	4	5	6	7	8	9	10	11	12	
3.493	3.637	0.295	0.272	0.294	0.283	-	-	-	-	-	-	405
2.379	2.072	0.292	0.247	0.303	0.285	-	-	-	-	-	-	405
1.44	1.331	0.281	0.27	0.29	0.284	-	-	-	-	-	-	405
0.863	0.896	0.294	0.271	0.303	0.324	-	-	-	-	-	-	405
0.609	0.635	0.28	0.292	0.314	0.326	-	-	-	-	-	-	405
0.483	0.447	0.3	0.28	0.297	0.281	-	-	-	-	-	-	405
0.417	0.401	0.32	0.275	0.306	0.305	-	-	-	-	-	-	405
0.36	0.344	0.278	0.292	0.318	0.318	-	-	-	-	-	-	405

*" I can do all things through him who gives me strength"***(Philipians 4: 13)**