EFFECTIVENESS OF EAVE NETS IN PREVENTION OF MALARIA TRANSMISSION AND MOSQUITO HOUSE ENTRY IN CHILIKO AND CHILORE VILLAGES, ZOMBA DISTRICT

MSc. (ENVIRONMENTAL SCIENCE) THESIS

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CHANCELLOR COLLEGE
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MARCH, 2017

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MSc. (ENVIRONMENTAL SCIENCE) THESIS

By

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MARCH, 2017

DECLARATION

I, the undersigned, hereby declare that this thesis is my own original work which has not been submitted to any other institution for similar purposes. Where other people's work has been used acknowledgements have been made.

Full Legal Name

Signature

Date

CERTIFICATE OF APPROVAL

The undersigned certify that this thesis represents the student's own work and effort and has been submitted with our approval.

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DEDICATION

To my lovely children Takudzwa and Tanatswa, my parents and the rest of my family.

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All praises to God, the provider of all that is good in my life.

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ABSTRACT

Malaria is a highly infectious disease, in Malawi it is mainly transmitted by *Anopheles gambiae, Anopheles arabiensis* and *Anopheles funestus.* It is of the most significant causes of morbidity and mortality in Malawi and remains a leading killer in Africa. Malaria is responsible for 90% of the deaths in Sub-Saharan region, 20.3% in Africa and 35% in Malawi. The study was conducted to identify environmental factors that may affect mosquito populations in the study area and to assess how eave nets affect malaria prevalence and vectors in the study area. Eave nets were installed in 200 randomly selected houses; the study was conducted as part of monitoring of a larger project and for this purpose it sampled 45 households. Environmental factors of the study area such as temperature, rainfall, land use and distance from swamps to houses were identified and mosquito collection and identification were done. Distance was measured using google earth and mosquitoes were caught using light traps and Pyrethroid Spray Catch Sheet (PSSC), identification was done microscopically and confirmed using Polymerase Chain Reaction (PCR). To evaluate malaria transmission, parasitaemia tests were done on the people living in the study area. Mosquito collection and parasitaemia tests were done and compared among individuals from houses that had eave nets and those from houses without eave nets. Results showed that eave nets were effective in reducing entry of mosquitoes into houses. Malaria transmission was reduced by the use of bed nets and not so much by presence of eave nets. The distance from houses to the nearest swampy area had no significant effect on malaria prevalence.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACT	:	Arteminisinin-based Combination Therapy
ADD	:	Agricultural Development Division
CDC	:	Center for Disease Control
DNA	:	Deoxyrivonucleic Acid
EHP	:	Essential Health Package
ELISA	:	Enzyme – Linked Immunosorbent Assay
GoM	:	Government of Malawi
GPS	:	Geographical Positioning System
ITNs	:	Insecticide Treated Nets
HAS	:	Health Surveillance Assistant
LLINs	:	Long lasting Insecticide Nets
MES	:	Masters in Environmental Science
MSOER	:	Malawi State of Environment and Outlook Report
NGO	:	Non Governmental Organisation
NMCP	:	National Malaria Control Programme
NMSP	:	National Malaria Strategic Plan
PCR	:	Polymer Chain Reaction
PSSC	:	Pyrethroid Spray Catch Sheet
RDTs	:	Rapid Diagnostic Test
RBM	:	Roll Back Malaria
SPSS	:	Statistical Package for Social Scientists

TA	:	Traditional Authority
WHO	:	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background Information

Malaria is a highly infectious disease that is transmitted by mosquitoes, and the principal vectors in Africa are *Anopheles gambiae, Anopheles arabiensis* and *Anopheles fenestus* (Yukich, et al., 2008). It remains one of the most significant causes of morbidity and mortality in Malawi (MOH, 2003). Malaria is responsible for 90%of the deaths in Sub-Saharan region, 20.3% in Africa and 35% in Malawi (MOH, 2003).The use of Insecticidal Treated Nets (ITNs) has been shown to reduce these morbidity and mortality rates (Spiers, et al., 2002). The introduction of mosquito nets in most parts of Africa has raised hopes of a new method of combating malaria; numerous studies attest to the fact that nets significantly reduce the transmission and infection in the region especially among very young children (Spiers, et al., 2002).

However, there are challenges that are associated with the use of bed nets as a malaria preventive method. Some people find it tiresome to set mosquito nets every night and some people do not like to use mosquito nets in the hot season which just happens to be the season during which mosquitoes are abundant. Even though the number of bed nets is enough for the whole household, children who sleep on the floor in living rooms do not often use bed nets (Iwashita, et al., 2010). Older children more likely sleep on the floor in the living room and usually without an ITN. Because these are living rooms, the bedding and beddings have to be removed every morning and be set again at bed time; this may cause the children to not sleep under a bed net since this is a complicated process for them. Even when they manage to hang the nets, the children are not fully secure since there are no bed frames to properly spread the nets. Thus children's body parts may be out of the net, exposing them to mosquitoes.

An effective way of preventing malaria transmission other than use of bed nets, is through understanding the behaviour of the mosquito species that transmit it; this can help in finding an effective way of preventing them from coming into contact with human beings. *Anopheles gambiae* mosquitoes are well adapted for entering houses; they fly upwards upon encountering a vertical surface, and then enter the house through the eave gap between the wall and the roof. Studies have actually demonstrated that the eaves are the major route by which *Anopheles gambiae* enters houses. By contrast, *Culicine* mosquitoes enter largely through doors and windows. Sealing the eave gap is an important method for reducing malaria transmission in homes where doors and windows are screened. (Njie, et al., 2009). This observation is reinforced by studies showing that houses with open eaves and those without ceilings are associated with high mosquito numbers and higher levels of malaria as compared to those with closed eaves and those that have ceilings (Geissbühler, et al., 2007). In the Gambia, children who lived in houses with closed eaves and metal roofs but slept without bed nets had less malaria infection than the children who slept in houses with open eaves and also had no bed nets (Ogoma, et al., 2009).

The closing of eaves is commonly known as screening, this involves using a netting material to cover the gap between the roof and the wall with a net stretching down between the wall and the ceiling (eave). This mosquito-proof method prevents mosquitoes from entering into the entire interior, which is effective for all residents who sleep indoors; it is not only effective during the night but also throughout the day.

1.2 Problem Statement

Studies have shown that using bed nets consistently significantly reduces the transmission of malaria and infection in the Sub-Saharan region especially among very young children. (Spiers, et al., 2002). Malawi had hoped to fight the disease by giving one million insecticide-treated nets to young children and pregnant women but it has been reported that net distribution programs sometimes fall short of ensuring that nets are actually used and kept appropriately, and often vulnerable members of the households e.g. children do not benefit from the use of the nets since priority is given to adults (Pemba, et al., 2008).

The introduction of eave nets is meant to reduce malaria. Studies in Kenya, Gambia and Tanzania have shown that eave nets are an effective way of preventing mosquito house entry and reducing malaria prevalence. Studies in these countries were conducted to compare prevalence of malaria and anemia among children sleeping in houses with eave nets and those sleeping in houses without eave nets. The studies revealed that children who slept in houses with eave nets had lower malaria prevalence and low cases of anemia than those from houses without eave nets (Kirby, et al., 2009).

Eave nets have also been shown to be a widely accepted method by most people (Ogoma, et al., 2009). In Malawi screening of eaves has only been introduced and its effectiveness is yet to be examined.

1.3 Justification of the Study

This study will determine the protective efficacy of eave nets in reducing prevalence of malaria and the entry of mosquitoes into houses. The study area is within Lake Chilwa catchment area, one of the areas with high malaria prevalence in Zomba district.

It will focus on effectiveness of eave nets in reducing vector-host contact hence reducing malaria transmission. This strategy has not been given much attention by both researchers and policy makers in Malawi. The study will inform further studies that will create an opportunity and provide evidence for scaling up the intervention to the rest of the district and possibly the rest of the country.

1.4 Objectives

1.4.1 Main Objective

The objective of the study was to evaluate effectiveness of eave nets on prevention of malaria transmission and mosquito entry into houses.

1.4.2 Specific Objectives

To identify environmental factors that may affect mosquito populations in the study area

To assess how eave nets affect indoor mosquito populations with more interest on *Anopheles gambiae.*

To assess how eave nets affect malaria prevalence in the study area

1.4.3 Expected Results

In line with the stated objectives, the study had the following expected results:

- It was expected that the study would identify environmental factors that are conducive to breeding of mosquitoes; i.e. environmental factors which contribute to high mosquito populations in the area.
- It was expected that the study would reveal that eave nets prevent entry of mosquitoes into houses. Meaning that houses with eave nets would have less mosquitoes in them than those that did not have eave nets. This would be so since *Anopheles gambiae* mosquitoes usually enter houses through open eaves because they fly upwards in search h for open spaces.
- It was expected that the presence of eave nets would reduce malaria cases in the area. Since eave nets would prevent entry of mosquitoes into houses, this would in turn reduce malaria transmission and therefore malaria cases would go down.

CHAPTER 2

LITERATURE REVIEW

This chapter gives an overview of the epidemiology of malaria, the life cycle of *plasmodium*, environmental factors that affect *Anopheles gambiae,* and how eave nets work.

2.1 Epidemiology of Malaria

Malaria is one of the most important infectious diseases in the world. About 14% of the world's population is at risk of malaria (throughout most of the tropical region) and each year, 350-500 million people are infected with malaria and over one million people die (Mali, et al., 2009) Malaria is caused by *Plasmodium* spp and four species are known. These are *P. falciparum, P. vivax, P. ovale, and P. malariae.* Malaria is endemic throughout Malawi and continues to be a major public health problem. Malaria is the leading cause of morbidity and mortality in children under age 5 and among pregnant women. It is estimated that Malawi experiences about 4 million episodes of malaria annually (MOH, 2003). Transmission is mainly determined by climatic factors; temperature humidity and rainfall. The extent and distribution of these factors influence malaria prevalence. Transmission is highest in areas of high temperature and frequent rainfall from the months of October through April (NMCP, 2014). There are few (0.05%) Malawians who live in areas regarded as free from malaria.

Efforts to control malaria are currently being scaled up through coordination of Roll Back Malaria (RBM) partners. Malaria is one of the main public health priorities in the Essential Health Package (EHP). The Ministry of Health (MoH), in collaboration with its development partners, has developed the National Malaria Strategic Plan 2011-2016 (NMSP 2011-2016). The vision of the National Malaria Control Programme (NMCP) is for all people in Malawi to be free from the burden of malaria. The programme's mission is to reduce the burden of malaria to a level of no public health significance in Malawi. This can be achieved through improved diagnosis, appropriate treatment, integrated vector management; supply chain management; behavior change communication and advocacy, and a robust monitoring and surveillance system. The main objective of the NMSP is to minimize the exposure of individuals to malaria vectors through integrated vector management (NMCP, 2010), understanding the natural history of Malawian malaria vectors is essential to achieving this objective. The study area i.e. Chiliko and Chilore villages are within the Lake Chilwa catchment area and malaria prevalence is 35% in this area. Ranking in order of prevalence, the commonest illnesses are as follows: malaria, bilharzia, skin infections, eye infections and cholera (Mtilatila, et al., 2003)

2.2 Life Cycle of the Malaria Parasite

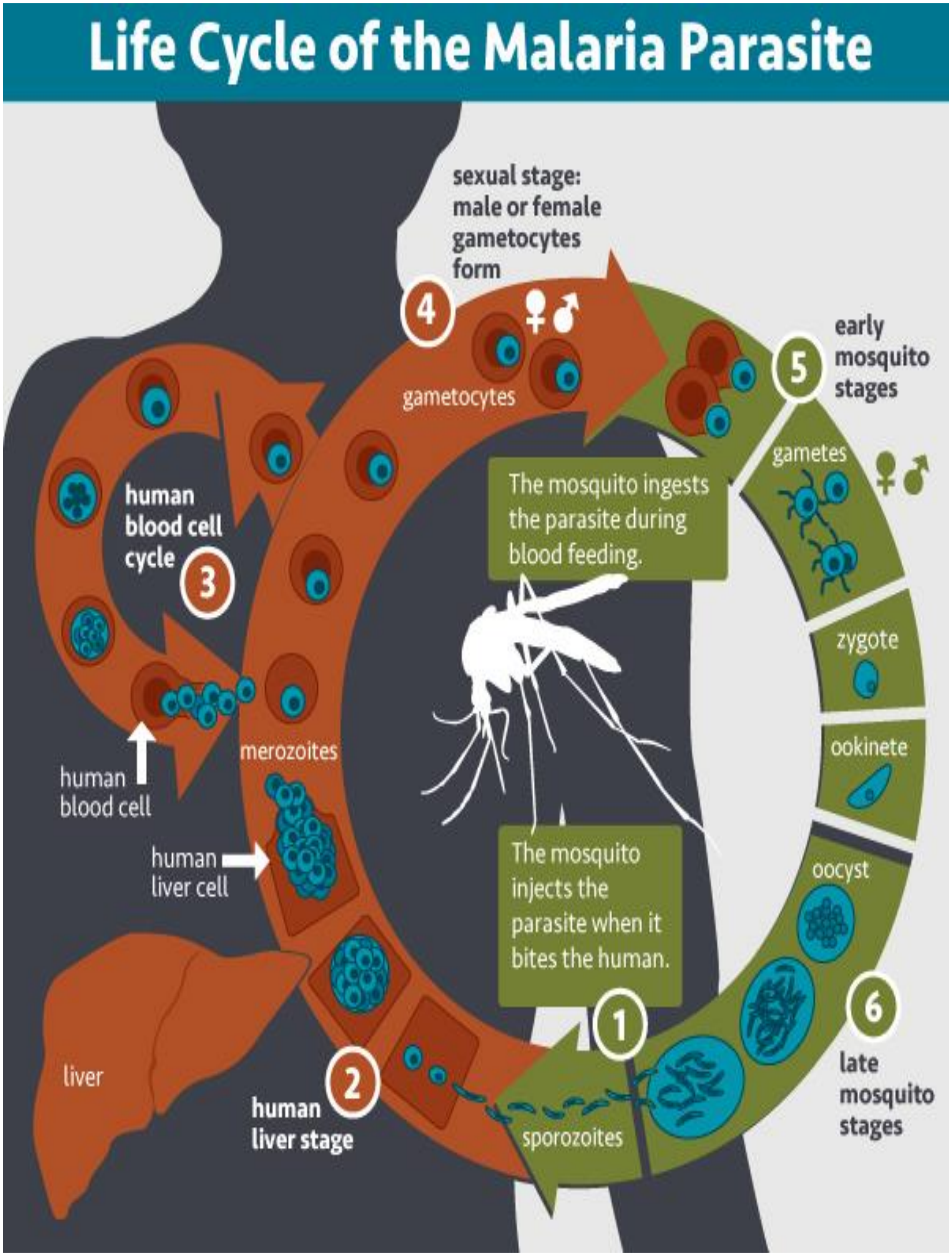
The malaria parasite life cycle involves two hosts i.e. humans and mosquitoes and it goes through the following stages:

1. A female Anopheles mosquito carrying malaria-causing parasites feeds on a human and injects the parasites in the form of sporozoites into the bloodstream. The sporozoites travel to the liver and invade liver cells.

2. Over 5-16 days, the sporozoites grow, divide, and produce tens of thousands of haploid forms, called merozoites, per liver cell. Some malaria parasite species also produce hypnozoites in the liver that remain dormant for extended periods, causing relapses weeks or months later.
3. The merozoites exit the liver cells and re-enter the bloodstream, beginning a cycle of invasion of red blood cells, known as asexual replication. In the red blood cells they develop into mature schizonts, which rupture, releasing newly formed merozoites that then reinvade other red blood cells. This cycle of invasion and cell rupture repeats every 1-3 days and can result in thousands of parasite-infected red blood cells in the host bloodstream, leading to illness and complications of malaria that can last for months if not treated.
4. Some of the merozoite-infected blood cells leave the cycle of asexual replication. Instead of replicating, the merozoites in these cells develop into sexual forms of the parasite, called male and female gametocytes. In some malaria species, young gametocytes sequester in the bone marrow and some organs while late stage (stage V) gametocytes, circulate in the bloodstream.
5. When a mosquito bites an infected human, it ingests the gametocytes. In the mosquito midgut, the infected human red blood cells burst, releasing the gametocytes, which develop further into mature sexual forms called gametes. Male and female gametes fuse to form diploid zygotes, which develop into actively moving ookinetes that burrow through the mosquito midgut wall and form oocysts on the other side.

6. Growth and division of each oocyst produces thousands of active haploid forms called sporozoites. After 8-15 days, the oocyst bursts, releasing sporozoites into the body cavity of the mosquito, from which they travel to and invade the mosquito salivary glands. The cycle of human infection re-starts when the mosquito takes a blood meal, injecting the sporozoites from its salivary glands into the human bloodstream (CDC, 2006).

The time frame depends on the malaria parasite species. The cycle can be summarized and well understood in Figure 1 below.



[Source: www.cdc.gov.]

Figure 1: Life Cycle of the Malaria parasite

2.3 Environmental Factors and *Anopheles* Mosquitoes

No insect population remains the same over time, their number vary and they are subject to fluctuations. The main cause of these fluctuations is the instability of the environment and its subsequent influence on the insect's ecology (Barrera, et al., 2006) In Malawi, the growing demand for food due to increase in human population has increased the alteration of natural ecosystems to agrarian ones causing changes in local microclimates. Since insects are col-blooded, they are extremely sensitive to temperature changes. They are more active at higher temperatures and less so when temperatures become low. Increases in temperatures positively correlate to increase in insect feeding, faster growth rates and rapid reproduction. *Anopheles* mosquitoes are no exception to this, thus many physical and chemical parameters in the environment affect their population dynamics. A study by (Yukich, et al., 2008) revealed that *Anopheles* mosquitoes breed only in clean, sunlit water which is not originally polluted with feces, rotting vegetation, garbage e.t.c. The mosquitoes also like to breed in small natural (as opposed to artificial) breeding sites. Common breeding sites include animal footprints, small areas of flooded grass, wet rice fields and borrow pits. Since all water in the immediate vicinity of human dwellings is polluted, *Anopheles* does not generally breed in this peri-domestic environment. For this reason, cleaning or draining puddles around the house has no impact on Anopheles density or malaria. Consequently density is very low in urban areas and very high in rural areas resulting in higher malaria transmission. Another reason is that Anopheles can breed in tiny amounts of water e.g. animal hoof prints. Hence, it is generally difficult to control in rural areas through destruction of breeding sites since the sites are simply too many and too dispersed.

Anopheles species, with very few exceptions, have a crepuscular and nocturnal activity; during the day they rest in favorable places. Flight activities are performed in a daily rhythm which is governed by climatological factors (temperature and humidity) and physiological needs such as mating, feeding, resting and egg laying (WHO, 1981)With regard to biting, some species have intense activity early in the evening, others the whole night increasing after 22:00 hours. The majority of species have two peaks of activity: the first, the highest peak of activity, is before midnight and the second, at dawn. These might change with temperature and humidity and wind which might increase or decrease the biting activity. Mosquito densities and behavior are therefore highly affected by ecological factors such as temperature, humidity, wind and vegetation or land use.

Cultivation in the study area takes place throughout the year, with rice as the main crop in the rainy season. Rice varieties grown in the area include hybrids, such as faya, pusa, kilombero, and taichuni, and local varieties, such as amanda, kawasala, and tuwengane. Ecological variability across areas results in different cropping patterns. Thus, some people plant rice in the dry season because there is adequate moisture. In the dry season, from April to October, people grow maize, sweet potatoes, tomatoes, beans, pumpkins, cowpeas, green leafy vegetables, onions, and watermelons, plots are usually intercropped. The cultivation calendar is such that the first crop is planted at the beginning of the rainy season, from October to November, and harvested February. From March to April gardens are prepared for the dry season crop to be planted. Usually crops planted during this time grow on residual moisture, and it is only when there is critical moisture stress that people dig wells and use watering cans, pails, and plates for irrigation. Cultivation is the main source of livelihood, followed by fishing (Kambewa, 2005)

2.4 House Screening (Eave Nets)

For many years the fight against malaria has been done through the use of Long Lasting Insecticide Treated Nets (LLINs). This has helped some part of Africa to experience a decline in malaria (Meyrowitsch, et al., 2011), however other parts throughout most of the tropical region are still facing high malaria incidence and risk of infection (CDC, 2006) The reason is that the use of bed nets is sometimes difficult and does not protect those sleeping outside the bed nets from mosquito bites. Some young people do not use bed nets because older people are given priority to use bed nets. Thus, making homes mosquito-proof is a key aspect of environmental management to prevent residents from malaria (Alonso, et al., 2000). House screening can reduce contact with mosquitoes for all the people in the house all day thereby avoiding issues of inequity within the household. In Gambia, Kirby et al. (2009) assessed whether house screening could reduce house entry of malaria vectors and frequency of anemia in children in an area of seasonal malaria transmission during 2006 and 2007. Screening was not treated with insecticide, 462 houses were included in the analysis. The mean number of *Anopheles gambiae* caught in houses without screening was higher compared with houses with screening. Screened houses had 47% fewer mosquitoes than unscreened houses (Kirby, et al., 2009).In the early twentieth century, improved housing and screening were regarded as priority methods of controlling malaria. Italian field experiments on proofing houses against mosquitoes were the first successful malaria control trials (Alonso, et al., 2000). The same study also found that house screening reduces mosquito human biting rates as well as malaria infections in settings as diverse as the United States, Italy and Greece. Clinical trials have shown that both house screening and ceilings alone provide valuable protection against anaemia and malaria transmission in rural parts of the Gambia (Kirby, et al., 2009) .The broader potential of window screening, closed eaves and ceilings for preventing a variety of mosquitoes into houses has recently been established in both West and East Africa (Ogoma, et al., 2009). In the same study, it was concluded that people had readily accepted house screening as a way of protecting themselves from mosquito bites and perhaps inadvertently in many cases, reducing malaria transmission. Screening therefore has been shown to be one of the alternative ways to reducing mosquito human bites and also malaria transmission in many parts of the world and Africa. A study in Tanzania revealed that high coverage and spending on screens and ceilings by individuals implies that these techniques are highly acceptable and excellent uptake can be achieved in urban settings like Dar es Salaam. Effective models for promotion and subsidization can be used for installation of ceilings and screening materials that prevent entry via the eaves, which are the most important entry point for mosquitoes that cause malaria, a variety of neglected tropical diseases and the nuisance which motivates uptake (Ogoma, et al., 2009).

[Source: www.google com] **[Source: Photo by R. Makoko, (2012)]**

Figure 2: Picture of a Bed Net and an Eave Net

As is seen in Figure 2 above, a bed net covers the bed to protect the person sleeping under it from mosquito bites while an eave net covers the open eaves of a house to prevent entry of mosquitoes into the house.

CHAPTER 3

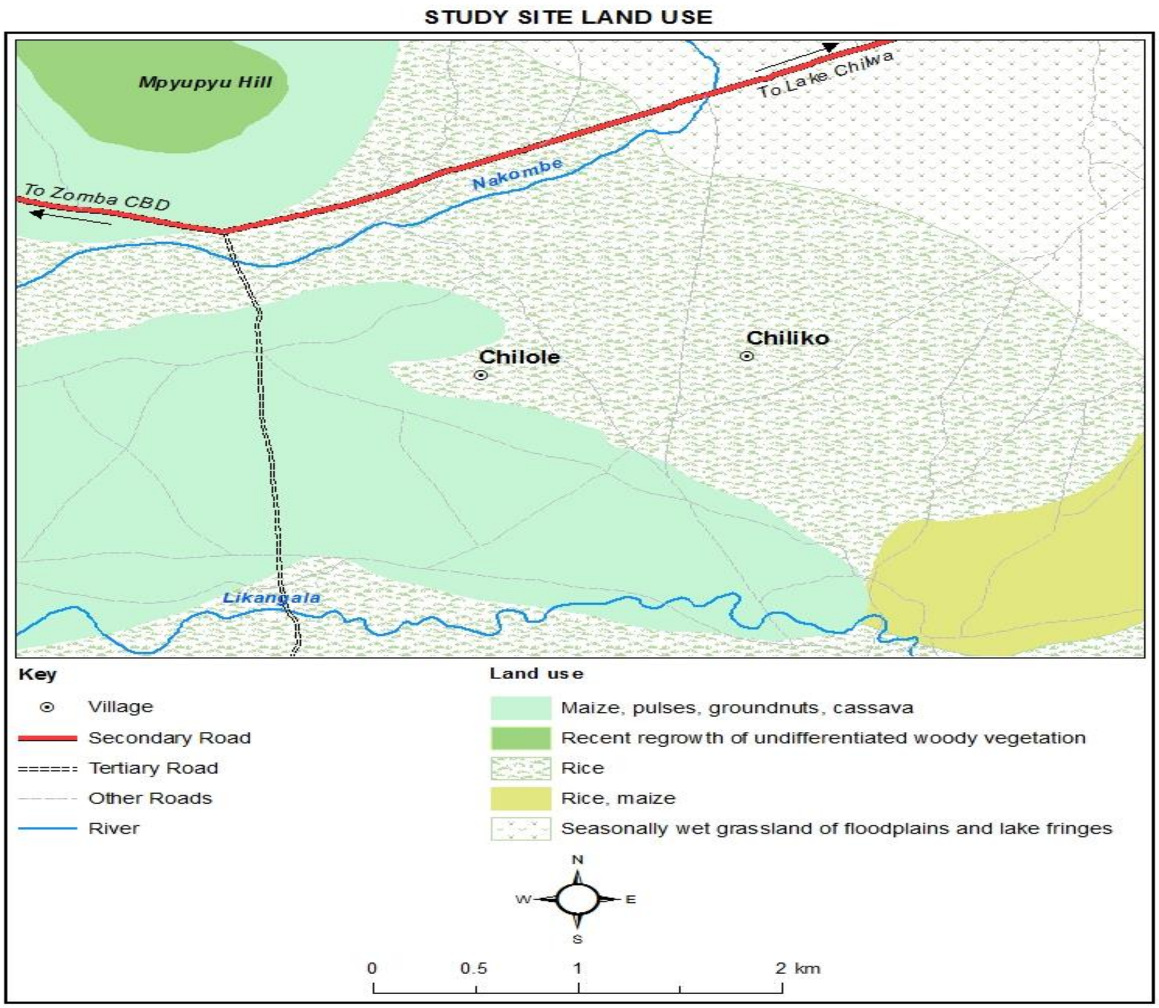
METHODOLOGY

This chapter presents the methodological approach used in the study. It gives a description of the study area, data collection and analysis techniques employed in the study.

3.1 Study Area

This research was conducted in Chiliko and Chilore villages, Traditional Authority (T/A) Mwambo in Zomba district in the South Eastern part of Malawi. The study area consists of approximately 321 households and a population of approximately 900, 89% of which are male-headed households and 11% are female-headed households. A large proportion of the houses are constructed with bricks and have grass thatched roofs, only a few houses have corrugated iron roofs. The major income sources of the people in the area are fishing and rice farming.

The main land use at the study site is agriculture; hence vegetation is mainly rice, maize and some scattered bare land. Rice is the main crop they grow in this area and it is mainly grown in the swampy areas. The part of land that is used for rice cultivation usually becomes large pools of water during the rainy season and that part of land which is used for maize cultivation is comparatively dry. The figure below shows land use of the area.



[Source: Image by R. Makoko and Gwaligwali, (2012)]

Figure 3: Map Showing Land Use/Vegetation of Study Site

3.2 Sampling Techniques

The study used simple random sampling technique to select households. Two different types of treatment were compared i.e. houses with eave nets and houses without eave nets. There were 200 houses that had eave nets installed. This study was conducted as part of monitoring a larger project, for this purpose, it was decided to use 150 households as population size i.e. 75 from those that had eave nets and 75 from those that did not have eave nets. According to (Edriss, 2006), for the sample to be representative, there is need to have 30% and above of the total population elements as the sample size. Hence, for monitoring 45 households were sampled for mosquito collection and 397 individuals for malaria testing. Installation of eave nets involved having a netting material the same as a bed net stretched from wall to wall below the eave gap inside the house. The sample size for monitoring households was determined by using the following statistical formulae:

$$n = \frac{30 \times N}{100} = \frac{30 \times 150}{100} = 45 \text{ Households}$$

Where: n = Sample size and

N = Population size

3.3 Data Collection Techniques

3.3.1 Demographic Data

Demographic data was collected using a standard questionnaire, this included data on names of household heads and all other members, age, sex, level of education, source of income, design of house in terms of type of roof and whether the house had open eaves or closed eaves. The questionnaire also asked about the people's behavior in protecting themselves from mosquito bites; individuals were asked if they had used a bed net the previous night.

3.3.2 Environmental Factors

Environmental factors of the study area were examined so as to identify factors that may affect mosquito populations. To identify these factors, data were collected on the following parameters; elevation, vegetation, rainfall and temperature. Primary data were obtained from different locations in the area and Secondary data were obtained from Likangala Agricultural Development Division (ADD) which collects and stores meteorological data among other things. Another physical factor that was taken into consideration was the distance from swampy areas to houses. Distance from houses to the nearest swampy area was measured and recorded this was done using Google Earth.

3.3.3 Bed Net Distribution

After demographic data were collected, bed nets were distributed to all households in the study area i.e. one bed net per two individuals; bed nets were distributed to all households since it would have been unethical to leave others without bed nets.

Eave nets were installed in 200 sampled houses i.e. 50 in Chikilo village and 150 in Chilole village. This study was conducted as part of monitoring of a larger study, hence the collection of demographic data in only 45 households, despite installing eave nets in 200 houses.

3.3.4 Entomological (Mosquito) Data

The houses were cleared of mosquitoes one week after installation of eave nets; this was done by spraying a pyrethroid insecticide inside the houses. The aim was to make sure that mosquitoes that were to be caught after eave-net installation were those that got there after the intervention and not before as this would not have shown the real effect of the eave nets. Indoor resting mosquitoes were caught using Center for Disease Control (CDC) light traps and PSSC. The CDC light traps were place at a corner in every room of a house; these were placed in the evening and left to trap mosquitoes throughout the night and were removed the following morning. Mosquitoes were emptied from the traps into labeled jars and taken to the lab for identification. Jars had house identity numbers labeled on them to help to know whether the mosquitoes were from a house with eave nets or not. PSSC used a pyrethrum aerosol which in this case was Doom; prior to spraying, doors and windows of houses were closed, and the floors were covered with white sheets. Then the pyrethrum aerosol was sprayed inside the house while at the same time it was being sprayed from outside the house directed toward the eaves (See Figure 4).

[Source: Photos by R. Makoko, (2012)]

Figure 4: Catching mosquitoes using PSSC Method

((A) Eave Net seen from outside, B) spraying of a pyrethroid through eaves from outside a house, C) eave net inside a house, D) Collecting dead mosquitoes from sheets)

After spraying was completed, the houses remained closed for 15 minutes (recommended time is 10-20 minutes), and later all knocked down mosquitoes were collected from the sheets. Mosquitoes were sampled both before and after installation of eave nets for comparison.

Both methods of collection were done twice before installation and twice after installation. First in early January, 2012 before eave net installation and then in July, 2012 after eave nets were installed.

3.3.5 Mosquito Identification

Data on the type (species) of mosquitoes was also collected since the study was most interested in *Anopheles gambiae.* To collect such data, all mosquitoes collected from houses were identified one by one using morphological features basing on taxonomic (morphological) keys developed by Edwards (year). To further confirm the identity of *Anopheles gambiae* Polymerase Chain Reaction (PCR) was used to see if their Deoxyribonucleic Acid (DNA) was really that of *A. gambiae*. PCR is a powerful and sensitive technique for DNA amplification (Saiki et al. 1985). DNA was extracted from mosquitoes by using a column purification kit: each mosquito sample was placed into a microcentrifuge tube containing DNA lysis buffer and finely ground with disposable DNase-free pestles. After homogenization, samples were processed according to established protocols, washed, and eluted in DNase-free water. Individual DNA samples were combined in pools of less than 20 mosquitoes (the recommended number), based on homestead trap for DNA synthesis and PCR. The PCR reaction setup is summarized in Table 1below.

Table 1: PCR Reaction Setup

Component	**25 µl reaction**	**50 µl reaction**	**Final Concentration**
10X Standard *Taq* Reaction Buffer	2.5 µl	5 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
Template DNA	variable	Variable	<1,000 ng
Taq DNA Polymerase	0.125 µl	0.25 µl	1.25 units/50 µl PCR
Nuclease-free water	to 25 µl	to 50 µl	

The reaction was gently mixed, all liquid was collected to the bottom of the tube by a quick spin. PCR tubes were then transferred from ice to a PCR machine with the block preheated to 95°C thermocycling begun. Thermocycling conditions used are shown in Table 2 below.

Table 2: Thermocycling conditions for PCR

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
30 Cycles	95°C 45-68°C 68°C	15-30 seconds 15-60 seconds 1 minute/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	

3.3.6 *Plasmodium falciparum* Parasitaemia (Malaria)

Data on parasitaemia were collected by testing individuals for malaria. Parasitaemia testing and questionnaire administration involved 397 individuals. The interview was done using a standard questionnaire and it was designed to collect data on basic demographic characteristics, the numbers of residents per household, number of bed nets owned, usage of a bed net the previous night and income generating activities. Parasitaemia was established using malaria Rapid Diagnostic Tests (RDTs) SD BIO LINE malaria Antigen pf. These are immunochromatographic tests that detect *Plasmodium* antigens in the blood by an antigen-antibody reaction on a nitrocellulose strip.

Malaria testing was done twice; it was first conducted early January, 2012 before eave nets were installed but after bed nets were distributed, the second round of malaria testing was conducted in July, 2012 after eave nets were installed. Eave nets were installed end of January, 2012 therefore malaria testing was done six months after installation of eave nets.

3.4 Data Analysis

Logistic Regression analysis was used to examine effectiveness of eave nets in prevention of malaria transmission. The Logistic Regression is used to estimate the probability of a binary response based on one or more independent variables. It measures the relationship between an independent variable and one or more variables (Hosmer, 2000). For this study, the dependent variable was malaria infection and independent variables were eave nets, net use, age, sex and distance (m) between house and swamp.

A poisson generalized linear model was used to determine whether eave nets are effective in reducing entry of mosquitoes into houses. The poisson distribution is a discrete probability distribution that expresses the probability of a given number of events occurring in a fixed interval of time or space if these events occur with a known average rate and independently of the time since the last event (Haight, 2000). In the case of this study it was used to establish the distribution of mosquitoes per house in relation to the two treatments i.e. eave nets or not eave nets. The number of malaria vectors in each house was the dependent variable, and the independent variables were presence of eve nets, the number of people per house, the number of bed nets per house, and distance between house and swamp. The significance level was 5% for all analyses.

Data was analyzed using R Package; this package implements a wide variety of standard and graphical techniques including linear and nonlinear modeling, classical statistical tests and many others (Dalgaard, 2002). R was used for this study because it has standard functions written in R itself which made it effective and efficient to follow.

3.5 Ethical Consideration

The raw data from the study is not to be made available to those not directly involved in it as this will be a breach of the ethical agreement made between the researchers and study participants. The results however, can be made available to the public since these are anonymized further. The data collection form used codes instead of names of participants so as to conceal the identity of the participants. All participants were asked to make an informed decision and give their consent before their houses were entered into and before a malaria test was carried out on them. To further ensure that there was indeed ethical consideration, malaria testing was carried out only by registered nurses and clinical officers.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter presents the findings of the study and discusses the results that have been obtained through the analysis of the results and observations. Section 4.1 presents the sample demographic characteristics. Section 4.2 presents environmental factors of the study area. Section 4.3 discusses mosquito indoor populations in relation to eave nets. Section 4.4. Presents malaria prevalence in relation to eave nets and finally, section 4.5. Presents malaria prevalence and bed nets use in Chiliko and Chilore villages.

4.1 Sample Demographic Characteristics

The study had 397 respondents for a questionnaire and malaria testing. Out of these, 67% (n=264) respondents were females while 34% (n=133) were males. There were 182 (46%) individuals from Village A (Chiliko) and 215 (54%) were from Village B (Chilole). The mean age of the whole study sample was 19 years. Out of the 397 individuals 58% (n=230) were from houses that had eave nets while 42% (n=167) were from houses without eave nets. When parasitaemia test were done, results showed that 79% (n=312) people tested negative while 22% (n=85) tested positive for malaria. When asked if they had used a bed net the previous night, 88 (n=348) of the respondents said they had used a bed net and 12% (49) said they had not used a bed net the previous night. Table 3 below summarizes the sample demographic characteristics.

Table 3: Sample Demographic Characteristics

Variable	Value	Observations	Percent
Sex	Female	264	67
	Male	133	34
Age Group	Under 18	220	55
	18 or Above	177	50
Eave Nets	Houses with Eave nets	230	58
	Houses without Eave nets	167	42
Village Code	A-Chiliko	182	45
	B-Chilore	215	54
Parasitaemia Results	Positive	85	22
	Negative	312	79
Bed Net use Previous Night	Used bed net	348	88
	Did not use bed net	49	12
Total		397	100

4.2 Environmental Factors

The study concentrated on one major environmental factor which is vegetation (land use) since the study area mainly uses its land for rice cultivation which has in the long run created swamps which are possible mosquito breeding sites. For analytical purposes, distance from houses to the swampy areas was measured to examine if it had an effect on parasiteamia results and mosquito numbers inside houses.

The study area is approximately 9 Kilometers away from Lake Chilwa, the longest distance from the nearest swampy area to a house is 1130.1 m, the shortest is 58.1 m. The mean distance from the swampy area to the house was 393.6 m. The study revealed that distance did not have a significant effect on Parasitaemia with P-value=0.091. The odds ratio suggested that the further a house is away from a swampy area, the risk of being infected gets reduced by 59%. This effect however, is not statistically significant since P-Value=0.091. This could be explained by the use of bed nets since they were distributed to all households. Results also showed that there was no significant difference (P-value = 0.642) in the number of in-door mosquito numbers between houses that were very far from the swampy areas and houses that are near the swampy areas. Results also showed that the area's mean temperature is 26.4 degrees Celsius per year, which is comparatively warm. The average amount of rainfall for the area is 126.6mm per year during the wet season. The area generally has a flat terrain with the lowest point at 638.2m and the highest point at 660.8m. This combination of a warm temperature and high rainfall amounts is very conducive for mosquito breeding.

The literature reviewed suggested that land use affects mosquito breeding and their populations indoors. Land use that promotes pools of stagnant water such as rice farming is conducive for mosquito breeding. This can also cause malaria prevalence in such an area to be high. The study area has rice farming as one of the major uses of land. However, the study established that there was also no significant difference in the number of mosquitoes in houses that were far from swampy areas and those that were close to the swampy areas.

This may be due to the presence of the eave nets which blocked their entry into houses. Consequently, there was also no significant difference in parasitaemia results between houses that were close to swampy areas and those that were far from the swampy areas. This can be attributed to the use of bed nets by household members. However the odds ratio suggested that the further away from the swamps one moves, the lesser the risk of being infected by malaria by 59%, this could be attributed to a high rate of transmission of malaria caused by an increased presence of mosquitoes in such areas which are usual breeding sites for mosquitoes as explained earlier. The swamps in the study area are temporary (seasonal) water bodies and are thus capable of producing a thousand mosquitoes in a short period of time (Liu, et al., 2008). Mosquitoes that have out door biting (exophagy) and outdoor resting (exophily) behaviors, though in small proportions can be sufficient to interfere with mosquito control interventions and elimination of malaria (Govella & Ferguson, 2012). Furthermore, the study revealed that the warm temperatures and high rainfall amounts in the study area have contributed the increased numbers of mosquitoes and malaria prevalence the area. Literature reviewed suggested that these are conducive conditions for mosquito breeding and therefore high malaria prevalence.

4.3 Mosquito Indoor Populations in Relation to Eave Nets

Mosquitoes were caught in the month of July from 45 houses. Out of the 45 houses, 17 had eave nets installed while 28 were without eave nets. A total of 138 mosquitoes were caught, these were *Anopheles gambiae* mosquitoes which is the species the study concentrated on. Other species were also identified and these included *An. funestus An. melas, An. marshali, An. paludis, An. longipalpis* and *An. coustani*, these however were in very small insignificant numbers. The table below compares the number of mosquitoes from houses with eave nets and those without eave nets.

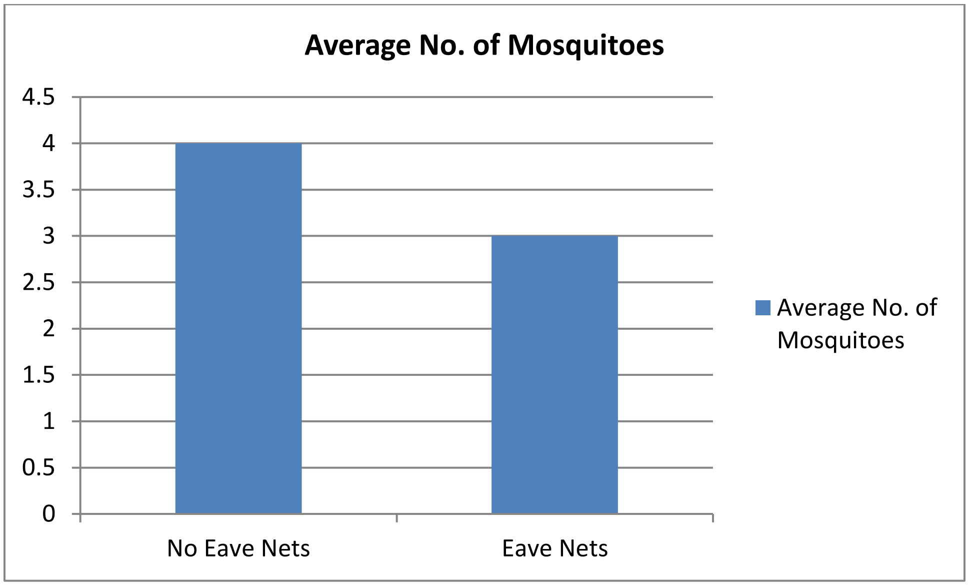


Figure 5: Number of Mosquitoes compared between Houses with Eave nets and those without

Results showed that there were fewer mosquitos caught from houses that had eave nets than from houses without eave nets. Using Poisson distribution, the results showed that the difference in mosquito numbers in houses with eave nets and those without eave nets was significant with P-value = 0.009. The odds ratio suggested that if eave nets are installed the number of mosquitoes inside a house can be reduced by 26%. Table 4 below shows that there were more *Culicine* mosquitoes than *Anopheline* mosquitoes in both types of houses.

Results above show that there was a significant difference in the number of mosquitoes from houses that had eave nets and those without eave nets. 5This may have been due to the closure of the eave with eave nets which prevent entry of mosquitoes especially *Anopheles* mosquitoes which enter houses through eaves (Kirby, et al., 2009). Hence installing eave nets may have prevented their entry route. Literature from similar studies also shows that eave nets reduce mosquito house entry by 47% (Kirby, et al., 2009). The few mosquitoes that were found in houses that had eave nets could have entered the houses through open doors or windows as they made their way up in search of open eaves.

Table 4: Comparison between Culicine and Anopheline Mosquitoes

Mosquito Species	**With Eave Nets**	**Without Eave Nets**
Culicine Males	20	45
Culicine Females	25	46
Anopheles gambiae males	8	6
Anopheles gambiae females	12	19

4.4 Malaria and Presence of Eave Nets

Parasitaemia results against presence of eave nets showed that out of those sleeping in houses that have eave nets 11.7% (=46) tested positive of malaria and 43.1% (n=170) tested negative.

Of those sleeping in houses without eave nets, 9.4% (n=31) tested negative while 35.8% (n=141) tested negative. There was no significant difference in RTD results between individuals sleeping in houses that have eave nets and those sleeping in houses that do not have eave nets (p-value=0.902, 95% CI=-0.0865 to 0.0763). Negative RTD results were high in both houses with eave nets and those without eave nets. This implies that bed nets were reducing malaria prevalence since they were being used by 88% of the individuals.

Malaria prevalence was not different between individuals sleeping in houses with eave nets and those sleeping in houses without eave nets, this could have been due to the community effect of the eave nets, whereby the effect of an intervention spreads around within the community even to where the intervention was not implemented. The insecticides used in the eave nets and bed nets become air borne and surround the community and thereby kill and repel mosquitoes away from the community, hence the community effect. Furthermore, this could have been due the use of bed nets; results revealed that 88% of the respondents were using bed nets. The study showed that net use had a significant effect on prevalence of malaria with p-value=0.0432. The Odds Ratio also suggested that using a bed net reduces the risk of being infected by malaria by 53%. Table 5 below summarizes parameter estimates explaining variables affecting malaria prevalence and mosquito house entry.

Table 5: Parameter estimates by a GLMM explaining variables affecting Parasiteamia prevalence and mosquito house entry

Parasitaemia

Parameter	Estimate	SE	Z	P
Intercept	0.19	0.35	0.52	< **0.01**
Bed net use	-0.67	0.33	-2.02	**0.04**
Age	0.003	0.01	-2.02	**0.01**
Distance to swamp	0.003	0.001	0.34	**0.001**

Mosquitoes

Parameter	Estimate	SE	Z	P
Intercept	1.80	0.22	1.80	0.07
Eave nets	-0.03	0.12	-2.61	**0.01**
Number of residents	0.15	0.04	3.34	**0.001**

P values in bold are statistically significant (P < 0.05)

CHAPTER 5

CONCLUSION

5.1 Conclusion

From the findings of the study it shows that transmission of malaria in an area cannot be affected by the distance between the location of the area and possible mosquito breeding sites such as swampy areas like land that is used for rice cultivation if the residents are sleeping under bed nets every night. Numbers of indoor mosquitoes is not affected by the distance from residential areas to mosquito breeding sites when there are eave nets present. The number of indoor mosquitoes is affected by the presence of eave nets, if a house has eave nets the number of mosquitoes gaining entry into the house is reduced, they can only enter through windows and doors and these are usually mosquitoes that do not transmit malaria such as *Culex* mosquitoes.

Malaria transmission is reduced with the use of bed nets and risk of infection depends on the age of an individual. The use of bed nets reduces malaria transmission through prevention of contact between mosquitoes and human beings. Eave nets have a community effect on malaria transmission, such that if they are installed in certain houses within the community, the rest of the community will be protected after a period of time when the effects have spread through the community. The use of bed nets combined with installation of eave nets can therefore have great results in reducing transmission of malaria.

5.2 Limitations

The study encountered the following limitations

- Some village members refused to have their houses entered into for collection of mosquitoes especially when the PSSC method was used. This was sometimes due to lack of understanding despite thorough explanation and assurance of their safety from the pyrethroid. This however, did not affect the data collected because the number of housed from which mosquitoes were caught was still representative of the population.
- Some members refused to have their blood tested for malaria. This was largely due to lack of medication for those that were found with malaria, hence some people preferred not to be tested at all. Still the data that was collected was not affected by this since those that gave consent to be tested were representative of the main population. Individuals that were found with malaria were referred to a nearby health facility.
- The study involved collection of data twice for comparison, participants became exhausted. Sometimes participants were required to move some of their belongings and even take some of them out of their houses especially during collection of mosquitoes. However, this did not affect the quality of data because mosquitoes were also collected using light traps and this was a less intrusive method, so either way, mosquito collection was done successfully.

5.3 Recommendations

Eave nets should be adopted for household use to reduce number of mosquitoes and therefore preventing malaria

Eave nets should be used together with bed nets for a greater impact on malaria prevention.

Further studies should be conducted to show the difference between interventions when applied during different seasons of the year.

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