**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY**

**KUMASI**

**SCHOOL OF GRADUATE STUDIES**

**COLLEGE OF HEALTH SCIENCES – SCHOOL OF MEDICAL SCIENCES**

**DEPEARTMENT OF CILNICAL MICROBIOLOGY**



# INSECTICIDE SUSCEPTIBILTY, CHARACTERIZATION OF BREEDING SITES AND COMMUNITY PERCEPTIONS ON MALARIA CONTROL INTERVENTIONS ON KNUST CAMPUS

**BY**

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**BSc. ZOOLOGY**

**JUNE 2014**.

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# INSECTICIDE SUSCEPTIBILITY, CHARACTERIZATION OF BREEDING SITES AND COMMUNITY PERCEPTIONS ON MALARIA VECTOR CONTROL INTERVENTIONS ON KNUST CAMPUS

**A THESIS SUBMITTED FOR THE AWARD OF**

**A DEGREE OF MASTER OF SCIENCE IN CLINICAL MICROBIOLOGY**

**BY**

**REBECCA PWALIA**

**B.SC. (HONS) ZOOLOGY**

**JUNE 2014**

## DECLARATION

I hereby declare that this thesis is an account of my own work towards the award of an MSc. degree and that to the best of my knowledge, contains no material previously published by another person or material accepted for the award of any other degree by the University except where due acknowledgement has been made in the text.



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DATE……..

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DATE……..

(HEAD OF DEPARTMENT)

## DEDICATION

I dedicate this work to my family especially, my parents, siblings, for all their support and contributions towards my education.

**ACKNOWLEDGEMENTS**

I would like to thank God for bringing me this far, to the completion of my second degree. I would also like to thank my supervisor Mrs. Ruth C. Brenya for all the guidance and corrections towards the completion of this work and also for her motherly supervision, and also Dr Maxwell Appawu of Noguchi Memorial Institute for Medical Research, for all his help in order for me to be able to carry out this work, Dr Sam Dadzie for all the corrections and editing of this work also of Noguchi Memorial Institute for Medical Research.

I also want to thank the staff of Parasitology Department, Noguchi Memorial Institute for Medical Research especially, Mr. J.H. N. Osei, Miss Sellasse Pi-Bansah and Mr. Sekyi Kojo Yirenkyi for all the guidance you gave me to be able to carry out this work

I thank also Mr. Sylvester Coleman and his team at ABT and Associates for all the things I learnt throughout my stay with them, you really gave me hope that I could do this work and I am truly grateful.

Finally I would like to thank all my colleagues for all their help and encouragement whenever I needed it, God richly bless you all.

## ABSTRACT

This study was carried out to find out the susceptibility status of *Anopheles* sp to the various chemical interventions used by inhabitants of KNUST campus and environs for mosquito control. To determine the association between breeding sites and susceptibility status obtained and also find out their knowledge and perception on ITN use. Seven *Anopheles* larval breeding sites were identified from larval surveys. *Anopheles* larvae were reared to adulthood and tested for (0.05%) deltamethrin, (0.1%) fenitrothion, (4%) DDT and (1%) bendiocarb to determine levels of resistance using the WHO tube assay method. Questionnaires were administered to determine the chemical control methods used by inhabitants within and in the immediate surroundings of the campus, and also their knowledge and perception on ITN use. A total of 2,510 adult female mosquitoes morphologically identified as *Anopheles gambiae s.l.* (98.8%) and *Anopheles funestus* (1.2%). These were exposed and were found to be highly resistant to the four classes of insecticides tested with mortalities of 15-54% for deltamethrin, 10-50% for bendiocarb, 7.5-38.75% for DDT and 5-42.5% for fenitrothion. Overall knockdown was 21-60% for deltamethrin, 11.25-36.25% for fenitrothion, 12.5-26.25% for DDT and10-55% for bendiocarb across all breeding sites. There was no association between susceptibility status and physical parameters of breeding sites. Inhabitants use ITNs, aerosol sprays, mosquito coils and mosquito repellents, impregnated curtains and screens on windows. Most of them had some knowledge about ITNs but a few did not use them due to reasons based on the nature of their rooms, allergies and socioeconomic reasons. The study shows the need for continous monitoring of susceptibility status of insecticides due to the high levels of resistance observed especially in cultivation areas, to slow its spread and restore vector susceptibility.

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## ABBREVIATIONS

| | |
|---|---|
| **ACT** | Artemisinin based Combination Therapy |
| **AIDS** | Acquired Immune Deficiency Syndrome |
| **BP** | Biological Programme |
| **CCP** | Calendar Control Programme |
| **CDC** | Centres for Disease Control and Prevention |
| **DDT** | Dichlorodiphenyltrichloroethane |
| **DFID** | Department for International Development, UK |
| **DNA** | Deoxyribonucleic Acid |
| **FY** | Fiscal Year |
| **GMT** | Greenwich Meridian Time |
| **IPTp** | Intermittent Preventive Treatment of Infants |
| **IRAC** | Insecticide Resistance Action Committee |
| **IRM** | Insecticide Resistance Management |
| **IRS** | Indoor Residual Spraying |
| **ITN** | Insecticide-Treated Net |
| **KAP** | Knowledge, Attitudes and Perception |
| ***Kdr*** | Knocked-down resistance gene |
| **KMA** | Kumasi Metropolitan Assembly |
| **KNUST** | Kwame Nkrumah University of Science and Technology |
| **LLIN** | Long-Lasting Insecticidal Net |

| | |
|---|---|
| **NPK** | Nitrate Phosphate Potassium |
| **PCR** | Polymerase Chain Reaction |
| **PMI** | President's Malaria Initiative |
| **RBM** | Roll Back Malaria |
| **RNA** | Ribonucleic Acid |
| **TDR** | Tropical Diseases Research |
| **TICP** | Targeted Intermittent Control Programme |
| **UNICEF** | United Nation's Children Education Fund |
| **WCE** | Wildlife Conservation EXPO |
| **WHO** | World Health Organization |
| **WHOPES** | World Health Organization Pesticide Evaluation Scheme |

# CHAPTER ONE

## 1. INTRODUCTION

### 1.1 Background

Despite concerted health efforts to control malaria worldwide, malaria is still a major health problem throughout the world. It is estimated that 3.3 billion people were at risk of malaria in 2010, with populations living in sub-Saharan Africa having the highest risk of acquiring malaria. An estimated 655,000 deaths were recorded globally in 2010 of which 86% were children less than 5 years of age. The disparity in region specific mortality is huge with 91% of all deaths recorded in the Africa region (WHO, 2011c). The control of malaria vectors with insecticides remains an essential component in the fight to eliminate or eventually eradicate malaria. Malaria vector control is intended to protect individuals from infective mosquito bites and thereby reducing the intensity of local malaria transmission.(WHO, 2009b).

Vector control is seen as an important component of the prevention and management of vector- borne diseases, as, for some diseases, the vector is the only feasible target for control. (Takken *et al.*, 1990). When well planned and well targeted, vector control can reduce or interrupt transmission, illness and save lives as this has been shown repeatedly and convincingly in areas where malaria has been eliminated. In recent years there has been renewed interest in malaria vector control as an effort to help reduce the malaria burden in most African countries who suffer the brunt of the disease. Insecticide-treated nets (ITN) and indoor residual spraying (IRS) have proven to be the two most powerful and most broadly applied vector control interventions over the years (WHO, 2012). To meet the challenge of reducing the global malaria burden, several donors have committed funds for the rapid scale

up of a package of proven malaria prevention and treatment measures, which include the prevention of malaria infection and illness through the use of ITNs and IRS (PMI, 2009).

Twelve insecticides from four classes namely organochlorines, organophosphates, carbamates and pyrethroids are recommended for IRS (Najera, 2002) and (Kelly-Hope *et al.*, 2008), but pyrethroids are the only class approved for treating bed nets. Since the mid 1950's resistance to all four classes of insecticides in *Anopheles* species in different parts of Africa has been reported (Awolola *et al.*, 2002). Also recently resistance to pyrethroids has been reported with cross resistance to DDT (Dichlorodiphenyltrichloroethane) first in Cote d'Ivoire (Elissa *et al.*, 1993) and has now spread throughout West Africa. Pyrethroid-DDT cross resistance brings a major challenge for control of malaria since pyrethroids are the only group of insecticides recommended for treating bed nets and DDT recommended in IRS (WHO, 2006a).

In Africa, spread of resistance has been reported as a result of the insecticide use in public health for mosquito control and at the same time in agriculture for pesticide control (Awolola, *et al.*, 2002; Yawson *et al.*, 2002). Levels of resistance to insecticides have also been shown to differ even in very small geographical scales during different seasons. In Ghana the National Malaria Control Program (NMCP) intends to embark on a rapid scale up of IRS and ITN's countrywide as part of the strategies aimed at achieving the millennium development goals. Several sectors for example Ghana Health Service and research institutions like Noguchi Memorial Institute for Medical Research, have also adapted to the vector control in reducing malaria burden and also to control mosquito nuisance.

## 1.2 Justification

In an attempt to control malaria on the KNUST campus, a task force was established in 2003 to map out a strategy for a design of an effective intervention. A study on entomological parameters of local mosquito vectors was conducted. The objectives of the study were to determine the vector species present on KNUST campus, their roles in malaria transmission, map out areas of high malaria risk using GIS and seek the perception of inhabitants on malaria on the KNUST campus. The vector species found were *Anopheles gambiae* Giles complex, *Anopheles funestus* Giles complex and *Anopheles zeamanni* Grunberg (Coleman, 2008). *An gambiae* was the main vector species with a sporozoite index of 1.01% to 0.57% and average entomological inoculation rate (EIR) of 0.059%. Of four study sites, faculty area was classified as the area with highest malaria risk with respect to entomological parameters measured. Interviews showed respondents had high malaria knowledge with 94.7% of respondents relating malaria to mosquito bites. Some respondents also thought eating too much oil and long exposure to sunshine caused malaria. High knowledge however did not result in correct attitude and practises. The study provided the needed baseline to initiate a vector control programme.

The application of insecticides as indoor residual sprays (IRS) or through insecticide treated mosquito nets (ITNs) or larviciding are currently the most important means of controlling malaria vectors. It is therefore important that before a rationale decision is made to use any of such interventions, the insecticide susceptibility status of local vector populations identified must be established. The World Health Organization Pesticide Evaluation Scheme (WHOPES) currently recommends insecticide active ingredients representing four chemical classes, namely organochlorines, organophosphates, carbamates and pyrethroids, for adult mosquito control through IRS or ITN.

In the last decade, the emergence of resistance in populations of *Anopheles* sp to common classes of insecticides used in public health has been reported in many African countries including Kenya (Vulule *et al.*, 1999), Cote d'Ivoire, (Elissa, *et al.*, 1993) Benin (Corbel *et al.*, 2004) and many other countries such as Niger, Burkina Faso, Mali, Nigeria, South Africa, and Cameroon. According to the recent World Malaria report for 2011, resistance to pyrethroid insecticides has been detected in 27 African countries and 41 countries worldwide. This class pyrethroid insecticide is most commonly used in 77 percent of Indoor Residual Spraying (IRS) programs and the only class approved to be used in producing long-lasting insecticide- treated nets (LLINs), (WHO, 2011c).

Insecticide resistance in disease vectors due to selection pressure from agrochemicals has also been reported from Central America (Brogdon *et al.*, 1988), Africa (Diabate *et al.*, 2002) and in South Asia (Sharma, 1996). Knowing that the key insecticides for mosquito control are all drawn from molecules developed primarily for agricultural use and are reformulated to deliver mosquito control effects, it is most important to find out how these insecticides would perform against local vectors by conferring resistance since resistance to insecticides could be contributed by these farmlands due to the use of pesticides (Klinkenberg *et al*., 2008). Again there is the need to document the effects of the proliferation and use of ITN's and other control options such as commercially sold aerosol insecticides sprays, mosquito coils and repellents on vector susceptibility.

Although several studies on resistance of *Anopheles gambiae s.l.* have been conducted in Ghana (Achonduh *et al.*, 2008; Anto *et al.*, 2009; Hunt *et al.*, 2011; Kudom *et al.*, 2011) just to mention a few, the need to monitor the changing trends of resistance is still very important. Despite the alarming rate of pyrethroid resistance reported a lot of ITNs are still being distributed for free and most of chemical control methods for protection from mosquitoes are

also formulated from pyrethroids. There have also been a lot of studies on water parameters and characteristics of water bodies that contain *Anopheles* larvae (Afrane *et al.*, 2012; Gimnig *et al*., 2001; Kudom *et al*., 2011;) and furthermore a lot of studies on vector control interventions but most of these studies do not find out the community perception on these interventions. Information from this study will provide the baseline insecticide susceptibility status of mosquito so as to develop appropriate resistance management on KNUST campus. The study will also find out if the various chemical control interventions used by inhabitants within the study contribute to the status of susceptibility obtained. Finally their knowledge and perception on the use of ITNs.

## 1.3 OBJECTIVES

The main objective of this study was to determine the insecticide susceptibility status of mosquitoes on the KNUST Campus and its surroundings, find out if there is an association between susceptibility status and breeding sites in the study areas, also determine knowledge and perception of inhabitants on the use of ITNs within the study area.

The specific objectives were to determine:

- The susceptibility status of *Anopheles* species in KNUST and its surroundings to the 4 classes of WHOPES approved insecticides for public health use;
- The association of breeding sites in the study area with the susceptibility status of *Anopheles* species in KNUST and its surroundings;
- The kind of chemical based vector control interventions used by inhabitants within the study area and;
- The knowledge and perception of inhabitants within the study areas on the use of ITNs.

# CHAPTER TWO

## 2. LITERATURE REVIEW

### 2.1 Definitions of different types of resistance

The term **insecticide resistance** is used to refer to the situation where local vectors are no longer killed by a standard dose of insecticide and are said to be no longer susceptible to the insecticide or avoid any form of contact with the insecticide. The emergence of resistance in local vectors in a population is said to be a problem of evolution (WHO, 2012). There are different ways of looking at resistance mechanisms in mosquitoes and a few are considered below;

Molecular genotyping of resistance is the method of identifying the underlying genes that express the inherited resistance trait (IRAC, 2011) identifying this gene provides evidence of the evolutionary process behind it. Depending on the type of resistance mechanism, this will provide understanding of both the degree of resistance expressed in the insects with that resistance gene and how often such insects occur in the population (WHO, 2011a). Phenotypic resistance is basically the expression of genetic cause of resistance which is seen by the vectors ability to survive and resist effects of the insecticide. This kind of resistance is measured in a susceptibility test of vector mortality when subjected to a standard dose of insecticide. WHO defines phenotypic resistance as "development of an ability, in resistant strains of insects, to tolerate doses of a toxic substance, which could be lethal to the majority of individuals in a normal population of the same species" (WHO, 1957).

Phenotypic resistance gives information about resistance in the vector; in the case of resistance leading to control failure evidence of resistance is linked directly to failure of

control programmes in the field. This kind of resistance is defined as the "selection of heritable characteristics in insect populations that results in repeated failure of an insecticide product to provide intended level of control when used as recommended" (IRAC, 2011). This form of resistance is common in agriculture. Malaria control programmes should not wait for control failures to occur before putting strategies in place to manage insecticide resistance since there is no accepted level of control failure in public health and waiting could result in delay in control till it's too late (WHO, 2012).



### 2.1.1 Types of resistance mechanisms

There are two main forms of resistance mechanisms which are target site and metabolic

### 2.1.2 Target site resistance

Target site resistance occurs when the site of action of an insecticide for instance, the nervous system is changed in resistant strains and as a result the insecticide no longer binds effectively and the insect escapes unaffected. Examples are target site resistance in organophosphates and carbamates through the neurotransmitter acetylcholinesterase in the nerve cell synapses and this confers resistance known as *Ace-1* resistance. Mutation in amino acid sequences in voltage gated sodium channels of membranes of nerve cells resulting in the reduction of sensitivity to the channels in binding to DDT and pyrethroid insecticides confers resistance known as *kdr* or knock down resistance (WHO, 2013). Reduction in susceptibility to pyrethroids caused by *kdr* mutations has been confirmed in *Anopheles gambiae* in West, East and Central Africa (IRAC, 2011).

### 2.1.3 Metabolic resistance

Metabolic resistance involves all the enzyme systems that the insect uses to get rid of all foreign substances within its body. This kind of resistance occurs when the activities of these enzymes prevent the insecticide from reaching its target site of action. The three enzyme systems are esterases, mono-oxygenases and glutathione S-transferases. Resistance mutations such as knockdown resistance *kdr* mutations can affect acetylcholinesterase which is the main target for organophosphates and carbamates, voltage gated sodium channels for pyrethroids and DDT. (IRAC, 2011) and (PMI, 2007). Metabolic resistance is important for all four classes of insecticides but different enzymes have different effects on different classes of insecticides. For example metabolic and target site resistance can occur in the same mosquito but they have different abilities to reduce insecticide based vector control interventions with metabolic resistance being much stronger and a cause of worry (WHO, 2012).

### 2.1.4 Other types of resistance

#### 2.1.4.1 Behavioural resistance

Another form of resistance is behavioural resistance which is known as a change in the insect's behaviour which protects it from effects of the insecticide. Many publications have proven this fact and described it as changes in the vector's feeding or resting behaviour to minimize the lethal effects of the insecticide (IRAC, 2011). However, in most cases there is not enough data to determine whether these changes are adaptive or genetic since genetic traits could have major implications for types of vector control interventions needed. Not all behavioural traits are negative as they could lead to mosquitoes feeding on non-human animals, an initial mistake can be made where reduction in vector species could be attributed

to behavioural resistance (WHO, 2012). Behavioural resistance is said to be an important factor causing avoidance of lethal doses of insecticides by the vector (IRAC, 2011).

#### 2.1.4.2 Cuticular resistance

Cuticular resistance is known as the reduced uptake of insecticide due to changes in the insect cuticle that prevent or slow the absorption or penetration of insecticides. Studies on this form of resistance are said to be very limited. Only one study has suggested a correlation between cuticle thickness and resistance to pyrethroids in *Anopheles funetus* (Wood *et al.*, 2010). Behavioral and cuticular resistances are rare forms of resistance and are seen by experts to be a lesser threat than chemical resistance. They however suggest that behavioural resistance could be of importance and further research should be conducted to understand its significance.

#### 2.1.4.3 Cross resistance

This form of resistance occurs when a resistance mechanism that enables insects to overcome the effects of one type of insecticide, also confers resistance to other compounds within the same class and may also occur between different chemical classes depending on the mechanism. This form of resistant is very common in vector populations for example DDT and pyrethroids are both unrelated chemically but they both act on the voltage gated sodium channel. Use of DDT in the past has resulted in several species of insects developing resistance to DDT due to *kdr* mutation at the target site (IRAC 2011). Where these mutations have remained in populations, the insects have developed some resistance to pyrethroids as

well as to DDT. Cross resistance in organophosphates and carbamates can also occur from changes in acetylcholinesterase (IRAC, 2011).

#### 2.1.4.4 Multiple resistance

This form of resistance is very common and occurs when several different resistance mechanisms occur simultaneously in resistant insects. Combination of the different resistance mechanisms may provide resistance to multiple classes of products. It is a common phenomenon for the contribution of resistance mechanisms to change over time as selection processes evolve (IRAC, 2011).

## 2.2 Malaria Vector Control

Vector control is a very important aspect of controlling malaria and remains the best strategy. It relies exclusively on LLINs and IRS. Vector control is the largest category for spending in expense by donors in malaria control. For example 39% of global expenditure by the Global Fund to fight malaria, AIDS and tuberculosis in 2009 and 59% of expenditures by the United States President's Malaria Initiative (PMI) were dedicated solely to insecticide treated nets (ITNs) and IRS in 2010 (WHO, 2010a). LLINs and IRS are the main methods used in malaria vector control programme because of their relatively low cost, high efficacy and also because their manufacture and distribution can be rapidly scaled up (WHO 2012). Other interventions like environmental management and larviciding are also very useful but only under certain conditions depending on the type of vector targeted and local situation. In Africa about 81% of malaria cases occur, 50% of households owned at least one ITN in the mid-2010 whilst 3% owned one in 2000. In the same way, the number of people protected by IRS in the WHO

African region was estimated to be 11% in 2010 and less than 5% in 2005 (WHO, 2011b). Outside Africa vector control has been upgraded, about 60 million ITNs were distributed outside Africa between 2008 and September 2011, with 40 million distributed in six countries including, 8 million in Indonesia, 14 million in India, 6 million in Afghanistan and 3 million each in Philippines, Pakistan and Papua New Guinea. IRS coverage in the western Pacific region increased to less than 1% of the population at risk in 2008 and to 5% in 2010. This was due to greater coverage of IRS in China which is now comparable to coverage in South-east Asia (WHO, 2011b).



Efforts in controlling malaria in Ghana started in the 1950's and the main aim for this was to control malaria to insignificant levels. The country has made some achievements since then. The main strategies were through the use of ACTs, ITNs and IRS with the support of its development partners; PMI, the Global fund, United Kingdom Department for International development (DFID),WHO, UNICEF and the World Bank. From 2003 to 2008 the free distribution of ITNs, IPTp uptake and treatment with ACTs increased significantly. There have however been significant differences in regional coverage of these interventions and show the need for much better interventions in order for Ghana to achieve the RBM and PMI targets of the nation (PMI, 2011).

### 2.2.1 Insecticides recommended for vector control

Only four classes of insecticides are recommended for use in LLINs and IRS. These are organochlorines, organophosphates, pyrethroids and carbamates. All the four classes can be used but pyrethroids are the only class currently used in LLINs. Available formulations and

prices show that pyrethroids perform better than the other classes in terms of efficacy, safety, durability and cost (WHO 2012). Pyrethroids were estimated to account for 75% IRS coverage in 2009, while DDT which was the second most used insecticide for malaria vector control. Organophosphates and carbamates represented only small percentages of global use (WHO, 2011a). Recent data on worldwide insecticide use on vector control from 2009 has shown that, their use in IRS might have changed due to increasing insecticide resistance and also WHO consultation on this topic in 2010 (WHO, 2011b).



## 2.3 Attributes of the four classes of insecticides used for IRS and LLINs.

### 2.3.1 Pyrethroids

These are used for IRS and LLINs. They are available as α-cypermethrin, bifenthrin, cyfluthrin, deltamethrin, permethrin, λ-cyalohathrin and etofenprox (WHO, 2006b). These chemicals have been the preferred choice of chemicals in public health for the past decades because of their rapid knock down effects, relatively low toxicity to humans, relative longevity of 3-6 months when used for IRS and low cost. These are the only insecticides currently recommended by WHO for use in LLINs (WHO, 2006). Pyrethroids have many modes of action on a mosquito vector, "they open sodium channels leading to continous nerve excitation, paralysis, and death of the vector" (Brown, 2005). They also have an "irritating effect causing an excito-repellency response resulting in hyperactivity, rapid knock-down, feeding inhibition, shorter landing times and undirected flight" (WHO, 2012). All these actions reduce the ability of the vector to bite.

### 2.3.2 Organochlorines

These are used in IRS as DDT, which was the most widely used insecticide in the eradication campaigns of the 1950's (Curtis, 1996). At the Stockholm convention of persistent use of organic pollutants in 2001, the use of DDT was banned for all application except in disease control because of its harmful environmental effects when used in agriculture. The number of equally effective, efficient, alternative insecticides for public health use was limited at that time, therefore DDT use was permitted until a locally safe, effective and low cost alternative was available for a sustainable transition from DDT. Similar to pyrethroids, DDT has been popular due to its rapid knock-down effect, relative longevity of 6-12 months when used for IRS and low cost. The two insecticides have different chemical structures but similar modes of action.

### 2.3.3 Organophosphates

These are made up of a wide range of chemicals but those recommended for use as IRS in vector control are fenitrothion, malathion and pirimiphos-methyl. These insecticides are highly effective but do not induce excito-repellency response from the vector and their current formulations have a short residual activity of 2-3 months when used in IRS. This is shorter than that of pyrethroids and DDT. Also organophosphates used currently in malaria control are more expensive than other insecticides. They act on mosquito vectors by inhibiting cholinesterase, preventing the breakdown of the neurotransmitter acetylcholine, leading to neuromuscular overstimulation and death of the vector (Brown, 2005).

### 2.3.4 Carbamates

Carbamates used for IRS control are bendiocarp (WHO, 2009a), propoxur and carbosulfan (WHO, 2013). This compound is also highly effective and does not induce excito-repellency response just like organophosphates. It has a short residual activity of 2-6 months when used for IRS and more expensive than pyrethroids and DDT. It's mode of action is the same as that of organochlorines.



## 2.4 The spread of resistance

In the African region, there are several areas of critical concern due to the widespread of resistance to pyrethroids and the other classes of other insecticides. These areas have a high incidence of malaria and reduced vector control effectiveness could have serious consequences. Countries in West and Central Africa have detected very high levels of resistance especially in Burkina Faso, Benin, Cameroon, Cote d'Ivoire and Ghana. They all have widespread resistance to pyrethroids and DDT, Cote d'Ivoire has also reported resistance to carbamates and organophosphates (WHO 2012). In Ethiopia, resistance to all the four classes of insecticides including widespread resistance to DDT and frequent reports of resistance to pyrethroids. In East Africa, places with widespread resistance to pyrethroids and DDT are Uganda and its borders with Kenya and Tanzania. Also in South Africa and Mozambique reports of a broad spectrum of resistance has been detected over the past decade. High frequency of metabolic resistance to pyrethroids has also been reported in Malawi and Zambia (WHO, 2012).

In the South-east Asia region, India has widespread resistance to DDT and patches of resistance to pyrethroids and the organophosphate malathion. Indonesia and Myanmar have also reported resistance to pyrethroids. Myanmar and Indonesia have reported resistance to pyrethroids, in Myanmar, resistance to DDT and organophosphates has been confirmed (WHO, 2012). In the region of the America's resistance to pyrethroids, organophosphates and carbamates have been reported. In Colombia resistance spread in the mid-2000 was prevented in several localities by changing the insecticides and thereby removing selection pressure. Despite all these efforts resistance still persists in other localities. Resistance has also been reported in Ecuador, Bolivia, Honduras and Peru (Pan American Health Organisation, 2011).

In the Western Pacific Region, resistance to DDT and pyrethroids in malaria vectors of local importance has been reported in the coastal regions of Vietnam. Also there have been reports of resistance to DDT in Malaysia and Cambodia and resistance to pyrethroids in China. The Eastern Mediterranean region, resistance is reported in several countries including Afghanistan, the Islamic Republic of Iran and Oman. There is DDT resistance also in Yemen. There have been reports of resistance to three of the four classes of insecticides in Afghanistan but this data is yet to be confirmed. Somalia and Sudan have reports of resistance to all four classes of insecticides, with frequent reports of resistance to pyrethroids and widespread resistance to DDT (WHO, 2012). In the European region, resistance to all four classes of insecticides has been reported in Turkey, DDT in Azerbaijan and to carbamates and organophosphates in Uzbekistan. The situation is very disturbing and may have been underestimated because many countries have not carried out routine susceptibility tests (WHO, 2012).

### 2.4.1 Types of insecticides affected by resistance

Though there has been confirmed resistance in all four classes of insecticides, the most recent reports are for pyrethroids and this is a cause of worry because they are the only insecticides currently used on LLINs and also one of the cheapest, long lasting insecticide used for IRS. The WHO 2012 report on the Global plan for insecticide resistant management in malaria vectors states that, more countries are reporting resistance to all four classes of insecticides with different mechanisms of resistance affecting different classes. This will strongly restrict options for managing insecticide resistance in the short term. Metabolic and target site resistance are found throughout the world but different resistance mechanisms are found in different species. For instance only metabolic resistance has been found in *Anopheles funestus sensu stricto (s.s)* whilst both metabolic and target site resistance have been found in *Anopheles gambiae s.s.* (WHO 2012). Furthermore resistance can vary by form for example in *Anopheles gambiae* resistance has been found to be higher in S forms than in M forms. In Burkina Faso when *Anopheles gambiae* M and S forms were tested at four different sites, the S form had a greater probability of surviving DDT or pyrethroid. This means if the two forms had evolved separately, it is natural that evolutionary process will vary.

Cross resistance can restrict the choice of use of other insecticides. This is normally seen in insecticide classes that have the same mode of action in killing vectors. For instance, if there is a modification in a target site vector caused by a resistance gene, it is likely to affect any other insecticides that attack the same target site, thereby conferring cross resistance. In the same way, a change in an enzyme that affects susceptibility to one insecticide may result in cross resistance to another (WHO, 2012).

## 2.5 The role of both public health and agriculture on the use of insecticides in contributing to the evolution of resistance in malaria vectors.

The use of insecticides for public health in selecting of resistance in malaria vector has been evident since the 1940's. However, in some instances, there has been good evidence that agricultural use of pyrethroids, especially on rice and cotton crops, was the main factor causing resistance in malaria vector mosquitoes. Research by (Georghiou *et al.*, 1973) shows that when cotton was a major crop in El Salvador, seasonal fluctuations in resistance in malaria vectors was observed to follow the timing of the cotton spraying. (Lines, 1988) also reports that, there has been cases where agricultural insecticides have been suspected as the cause of insecticide resistance but further investigations put into the matter showed that the resistance was as a result of anti-malarial spraying for example malathion resistance in Sudan and Sri Lanka.

Pyrethroids have been used widely in agriculture in Africa especially in irrigated rice for many decades and also in areas of intensive agriculture in West Africa. Agricultural insecticides may have contributed to the appearance of knockdown resistance in malaria vectors. It is however known that, in the last five to ten years, through intensifying malaria control, resistance genes have been seen to spread throughout the region, reaching high frequencies, even in areas where there is very little agricultural insecticide use. This evidence therefore shows that, in Africa, agriculture has been an important cause for the first appearance of resistance in some localities but massive scaling up of LLINs and IRS for malaria control has been the main factor driving the recent increases in the geographic distribution and frequency of insecticide resistance genes in malaria vectors. The continous use of the same insecticides in agriculture and public health will inevitably increase

resistance (WHO, 2012), effective management of insecticide resistance will therefore require activities both in public health and agriculture and sharing of information and data.

## 2.6 Resistance monitoring

According to the WHO Global plan for monitoring insecticide resistance 2012, insecticide resistance should be monitored carefully so as to understand the current threat and evolution of insecticide resistance among malaria vectors. Till recent times however, monitoring of resistance has been limited in most malaria endemic countries. Monitoring can be undertaken through three main methods and each testing method provides different type of information. These methods complement each other and each choice depends on information needed and ability to operate.

### 2.6.1 Insecticide resistance bioassays

#### 2.6.1.1 Susceptibility tests

In this test, vectors are exposed to fixed insecticide concentrations and the level of vector mortality is recorded afterwards. The results are then expressed as the percentage of vectors knocked down, alive or dead. Susceptibility tests require samples of at least 100 mosquitoes per test site (WHO, 1998b; WHO, 2013). These tests are used generally for routine monitoring since they can be used in the field. They provide standard data which can be easily interpreted. WHO bioassay papers or CDC bottle bioassays can be used, but the results obtained from the two methods cannot be compared. To be able to observe the changing patterns in resistance, countries and academic institutions must use the same method consistently over time. Also according to the WHO, limitations of this method are that,

though susceptibility tests are able to identify the existence of resistance if it is at a detectable level, it does not establish the mechanism involved. It also cannot identify resistance if frequency is low. There has also been reports of countries reporting shortages in supply of testing materials and the problem of switching between WHO and CDC tests making their results difficult to compare and in some cases limited their testing (WHO, 2012).



#### 2.6.1.2 Biochemical assays

Resistance can also be monitored through biochemical assays. According to the World Malaria Report 2010 (WHO, 2010b), "Biochemical assays detect the presence of a particular resistance mechanism or an increase in enzyme activity''. They require fresh mosquitoes but a lesser number of them as compared to bioassays. Also unlike bioassays, biochemical assays can detect some specific resistance mechanisms and indicate an increase in metabolic enzyme activity. These assays are used in together with synergist and molecular assays. Limitations of this method are that, it is more difficult to use in the field and needs sophisticated equipment, interpretation of its results requires strong technical skills (WHO, 1998a). Furthermore, the correlation between chemical reactions in these tests and increased ability to metabolize insecticides has not yet been well defined.

#### 2.6.1.3 Molecular testing

Molecular testing is another method of monitoring resistance, the WHO 2012 report states that "the tests are used on the actual gene therefore allowing detailed and direct analysis of resistance genes''. The test is done straightforward with polymerase chain reaction (PCR) techniques (WHO, 1998a) either with DNA or with more elaborate microarray tests with

RNA. More advanced molecular methods can give complex genetic information including whether mutation is unique or has spread (WHO, 1998a). These are said to be the most accurate method of measuring resistance frequency in vector populations but these tests must however be correlated with susceptibility testing. Limitations of this method are that, it needs sophisticated equipment and entomological capacity. It can be used to identify target site resistance and a few others identified metabolic mechanisms. Therefore, susceptibility tests must be used to complement molecular results since; the absence of an indentified genotypic resistance does not necessarily mean that resistance does not exist (WHO, 2012).



### 2.6.2 Resistance monitoring in endemic countries

Monitoring of insecticide resistance is currently inadequate in many countries. Some countries have a comprehensive monitoring system but malaria endemic countries where vector based-control interventions are used do not monitor levels of insecticide resistance as comprehensively as required. For instance, either they do not cover enough sites or do not have efficient system for reporting or analyzing data. Also, insecticide resistance is hardly monitored consistently over a period of time. In many instances, monitoring is conducted at the last minute or only in response to signs of insecticide resistance rather than as part of routine surveillance, this has resulted in limited data (WHO, 2012).

Another problem is with the methods of testing resistance. Many of the current tests are hardly comprehensive; tests are performed for a single class of insecticides instead of all the classes used potentially for vector control. Molecular and biochemical tests are rarely done

even when it is needed from bioassay results. Other methods for testing and analyses that are important for decision making are rarely performed.

According to the Global report by the WHO 2012, these problems are due to the fact that firstly, routine monitoring of insecticide resistance is hardly ever built into vector control programmes and resistance monitoring has not been a necessity for receiving funds for vector control programmes, even funds meant for vector control are used for other activities. Secondly, though there has been significant capacity building within regions which has improved insecticide resistance monitoring in some countries, some still have limited local entomological, epidemiological, statistical and information technological capacity. The available capacities are often in research institutions rather than national malaria or vector control programmes. Laboratory equipment is often not available or of very poor quality and ability to collect mosquitoes appropriately is often limited. Clear and standard methods for selecting sites for monitoring insecticide resistance have not been provided to help countries classify and group affected sites. This has made routine monitoring difficult and many countries have to rely on research institutions for intermittent data collections (WHO, 2012).

National and local decision making bodies for managing insecticide resistance is limited in many countries. This is mainly due to unavailability of data. Data is often collected by research and academic institutions and this information is not shared until publication of findings which can take several years before the national malaria control programme can access this information on insecticide resistance in the country. The limited data does not help in prompt policy making for resistance management strategies. Also many countries need better capability and external support for analyzing data and applying the WHO

guidelines for decision making in IRM. Some countries even do not have national malaria control programmes resulting in even more limited resources. Furthermore, there has been no clear mandate for creation of data management system for monitoring insecticide resistance and databases have been created with inadequate coordination among several stakeholders for instance standard methods and indicators are not available (WHO, 2012).

### 2.6.3 Approaches to managing resistance

The IRM approach is meant to maintain the effectiveness of vector control despite the threat of resistance and this is through indoor residual spraying, but methods for IRM are still limited and need more improvement. Management of resistance is not a new concept, IRM approaches were used in agriculture and some public health situations during the past century (WHO, 2011b), many approaches have been used or proposed for managing insecticide resistance in vector control, and these are, rotation, combination interventions, mosaic spraying, mixture of insecticides and integrated vector management.

#### 2.6.3.1 Rotation

With this approach, two or more insecticides with different modes of action are rotated from year to year. The assumption is that, if resistance to each type of insecticide is rare those multiple resistances will be extremely rare. Rotation allows any resistance developed to the first insecticide to reduce over time when the second insecticide class is introduced. The time required for rotation must be short to prevent significant levels of resistance to develop to any one rotation partner. Annual rotation is said to be possible in vector control programmes,

whilst in agriculture, rotation of different insecticides with different modes of action is practiced (IRAC, 2011).

#### 2.6.3.2 Combination interventions

Here two or more insecticide based vector control methods are used in a house, for example, pyrethroids on nets and an insecticide of a different class on walls, so that, this same insecticide the insect is likely but not guaranteed, to come into contact with the second insecticide if it survived the first exposure (WHO, 2012).

#### 2.6.3.3 Mosaic spraying

Here one compound is used in a geographical area and a different one in neighbouring areas, the two belonging to different insecticide classes (WHO, 2012). ‘‘a spatially separated application of different compounds against the same insect constitutes a mosaic approach to resistance management’’ (IRAC, 2011). This method can be achieved in vector control programmes for instance by using two insecticides in different houses within the same village. This increases the probability of insects within one generation to come into contact with both insecticides, and reduces the rate of resistance selection, if multiple resistance within the vector population was extremely rare. Mosquito bed nets formed from panels and treated with different insecticides gives a similar mosaic effect to treating houses with different compounds but on a much finer scale (IRAC, 2011).

#### 2.6.3.4 Mixture of insecticides

The final method is the use of mixtures, two or more compounds of different insecticide classes are mixed to make a single formulation, so that, the mosquito is guaranteed to come into contact with the mixtures at the same time. Mixtures are currently not available for malaria vector control, but will be the future of IRM if available (WHO, 2012). Mixture are not used due to cost, logistics, safety and limited number of recommended compounds available, however with the invention of new vector control insecticides, this process may be viable (IRAC, 2011).



#### 2.6.3.5 Integrated vector management

This is the rational decision process for the optimal use of resources for vector control. In some situations, non-insecticidal tools like, non-insecticide based larviciding and environmental management can be used to reduce the overall mosquito population and limit the number and size of breeding sites without selecting for resistance (WHO, 2011b). Integrated vector management, without the use of chemical control, can also be considered as a means of IRM. Also, synergists, which can enhance the potency of an insecticide and could be used in mixtures, should continue to be investigated and tested vigorously to test their usefulness in IRM. For IRS, three of the four mentioned interventions excluding mixtures are available; mixtures are not available on the market but could be developed in the short term. For LLINs, IRM strategies are more limited, combinations of IRS and LLINs are the only currently available options. Individual nets with panels treated with other forms of insecticides could be developed, but, pyrethroids are the only insecticide class currently used in LLINs, another insecticide class other than pyrethroids will have to become available for use on nets, this is currently under study (WHO, 2012).

Although the options for IRS and LLINs are limited, they may retain an effect despite increased resistance to pyrethroids. Firstly, the nets provide a physical barrier against biting mosquitoes as long as they are intact (WHO, 2011b), also in most vector species, resistance to pyrethroids does not reduce the effect of the insecticide completely. It has also been observed that the irritating effect of pyrethroids "hyperexcitatory response" may reduce mosquito blood-feeding or encourage diversion to other hosts by some vector species that do not feed only on human hosts. This can however differ with species and geographical location.



### 2.6.4 Impact of resistance management on resistant populations

IRM can have different effects on resistant vector populations, first of all it reduces the proportion of resistance or delays the emergence of resistance by removing selection pressure, and this method is based on the assumption that "owing to the fitness cost resistance genes will recede from a vector population if selection pressure is removed". This approach involves reducing the selection pressure through for instance, rotations of different classes of insecticides and mosaic applications through spatial reduction of use. These strategies are an attempt to encourage or preserve susceptibility (WHO, 2012).

Furthermore through continous killing of resistant vectors is another approach. This method is based on the assumption that, "if vectors exposed simultaneously to multiple insecticides are not killed by the insecticide to which they are resistant; they will be killed by the alternative insecticide" (WHO, 2012) There are examples in tools used currently, like combination strategies, and potential tools in future like, the use of mixtures. These

approaches are attempting to manage resistance by killing or reducing the proportion of carriers by simultaneous use of alternative insecticides of different classes.

## 2.7 *Anopheles* mosquitoes

There are about 3,500 different species of mosquitoes grouped into 41 genera. Malaria in humans is transmitted only by female mosquitoes of the genus *Anopheles*. There are 430 *Anopheles* species; only 30-40 are vectors in nature. Anophelines are found all over the world except in Antarctica, malaria is transmitted by different *Anopheles* species depending on the environment and region. Those that transmit malaria are not found only in malaria endemic areas but also in areas where there has been eradication. These areas are therefore constantly at risk of re-introduction of the disease (CDC, 2010).

### 2.7.1 Life stages of mosquitoes

Anophelines like all mosquitoes have four life stages namely egg, larvae, pupae and adult stage. The first three stages of its life are aquatic lasting 5-14 days depending on the type of species and temperature. The female at the adult stage is the main vector of malaria. The adult female can live up to a month or longer in captivity but do not live for more than 1-2 weeks in nature (CDC, 2010).

### 2.7.1.1 Eggs

Female adults lay 200-300 eggs in one gonotrophic cycle. The eggs are laid singly and directly on water. *Culex* and *Culiseta* species lay their eggs stuck together in rafts of about 100 eggs; *Anopheles* and *Aedes* species do not lay their eggs in rafts but lay them singly. The eggs are not resistant to drying and can hatch within 2-3 days, but can take 2-3 weeks in cold climates. This is shown in figure 2.1 below:



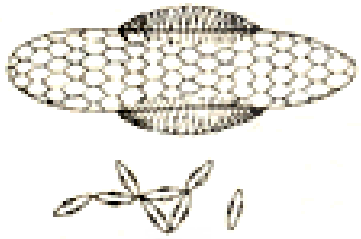

**Figure 2.1 *Anopheles* egg with lateral floats and single laid eggs (source:** www.cdc.gov)

### 2.7.1.2 Larvae

The larvae of mosquitoes have a well developed head and a mouth with brushes for feeding, a large thorax and a segmented abdomen. Larvae of other species have respiratory siphons for breathing under water, *Anopheles* larvae do not have this siphon therefore they lie parallel to the water surface to be able to breathe. They breathe through spiracles found on the 8$^{th}$ abdominal segment and therefore come to the surface most of the time. This is shown in figure 2.2 below.

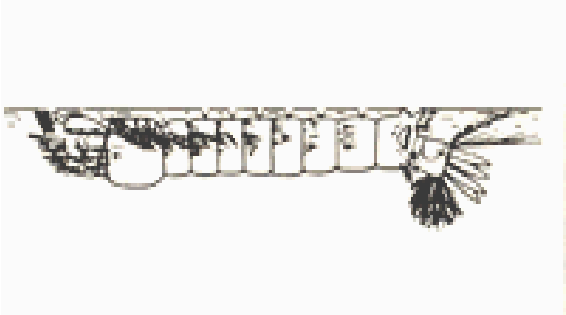

**Figure 2.2 Position of *Anopheles* larvae on water surface. (Source: www.cdc.gov)**

Larvae feed on algae, bacteria and other microorganisms on the water surface. They dive below the water surface when disturbed and move by jerky movements of the entire body or through propulsion with their mouth brushes. They grow by molting 4 times and each stage is called an instar after which they metamorphose into pupae. At the end of each instar, the larvae molt and shed their exoskeleton to allow for further development. Larvae can be found in different habitats but most species like clean unpolluted water. *Anopheles* larvae can be found in fresh or salt water marshes, mangrove swamps, rice fields, and grassy ditches, the edge of streams and rivers and small temporary rain pools. Other species prefer habitats with vegetation whist some others do not like habitats with vegetation. Some breed in open sunlit pools whilst others are found only in shaded breeding sites in the forest. Some also breed in tree holes or leaf axils of some plants (CDC, 2010).

### 2.7.1.3 Pupae

Mosquito pupae are comma-shaped from the side view. The head and thorax are fused in a cephalothorax with the abdomen curved around beneath. The pupae also frequently come to the surface to breath and they do this with a pair of respiratory trumpets on the cephalothorax. A few days after being a pupa, the dorsal surface of the cephalothorax splits open and the

adult mosquito emerges. Development from egg to adult stage differs among species and is strongly influenced by ambient temperature. Some mosquitoes can develop from egg to adults in just 5 days but may take 10-14 days in tropical conditions (CDC, 2010), this is shown in figure 2.3 below.



**Figure 2.3 An *Anopheles* pupa**

**2.7.1.4 Adults**

Adult anophelines like other mosquitoes have slender bodies divided into three segments; head, thorax and abdomen. The head is specialized for obtaining sensory information and feeding. The head also has the eyes and a pair of long numerously segmented antennae. The antennae are used for detecting host odours as well as the odours of breeding sites where females lay eggs. On the head are long forward- projecting proboscis used for feeding and two sensory palps. The thorax is specialized for movement, it has three pairs of legs and a pair of wings attached to it. The abdomen is specialized for digestion of food and egg development. This body segment expands when a female mosquito takes a blood meal. The blood is digested after a while and serves as a source of protein for the production of eggs which gradually fills the abdomen. *Anopheles* mosquitoes can be identified from other mosquitoes by their palps which are as long as the proboscis and the presence of small blocks of white and black scales on the wings. They can also be identified by their typical resting position being, both male and female *Anopheles* mosquitoes rest with their abdomen sticking

up in the air whilst other mosquitoes rest with their abdomens parallel to the surface which they are resting. Adult mosquitoes mate a few days after emerging from the pupal stage. (CDC, 2010), this is shown in figure 2.4 below:

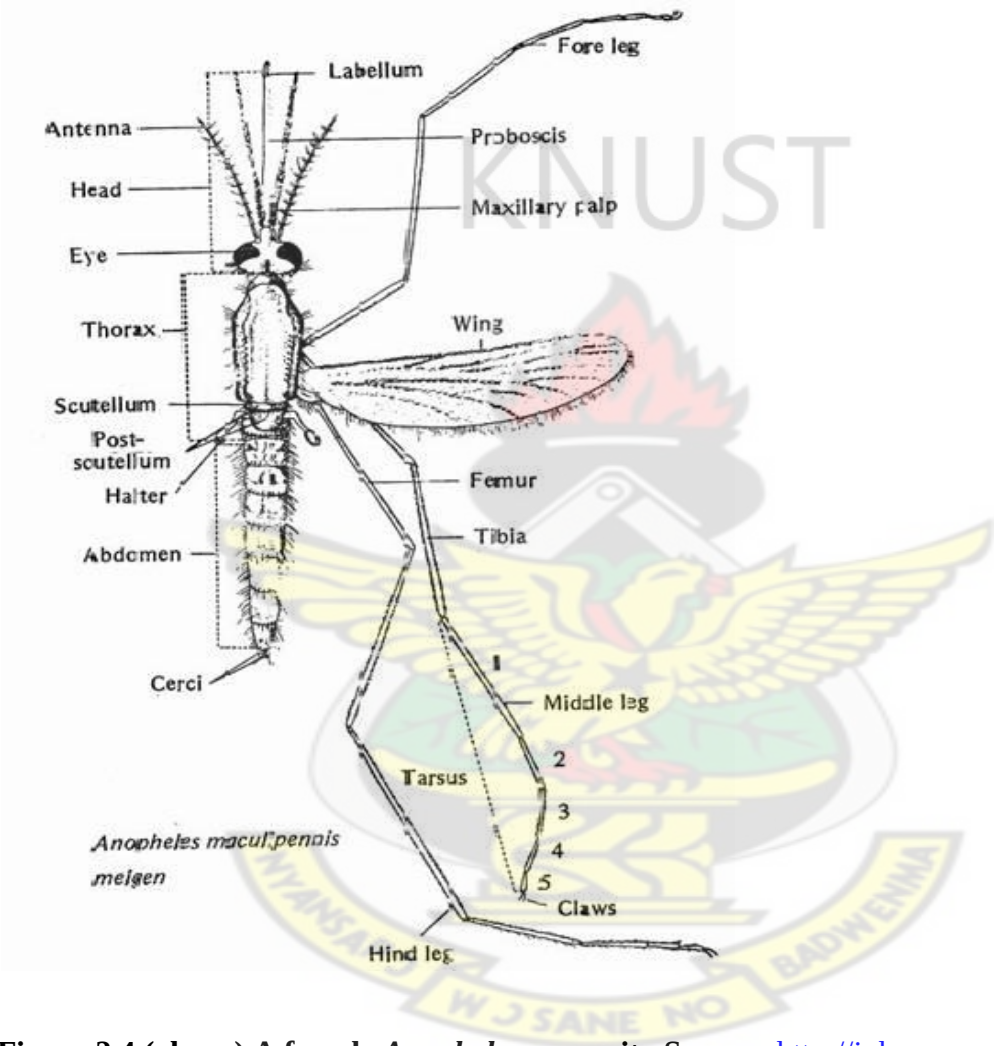


**Figure 2.4 (above) A female *Anopheles* mosquito Source:** http://jpkc.sysu.edu.cn

Adult males live for about a week feeding on nectar and other sources of sugar. Females also feed on sugar sources to obtain energy but needs a blood meal for the development of eggs. After obtaining a full blood meal, the female rests for a few days for the blood to be digested and eggs to develop. This process depending on the temperature but can take 2-3 days in tropical conditions. The female lays the eggs after they are developed and continues looking

for hosts, this cycle continues till she dies. The female can live up to a month or longer in captivity but does not live longer than 3 weeks in nature. Their chances of survival depend on temperature, humidity and also their ability to obtain a blood meal whilst avoiding host defense mechanisms. (CDC, 2010), this is shown in Figure 2.5 below.

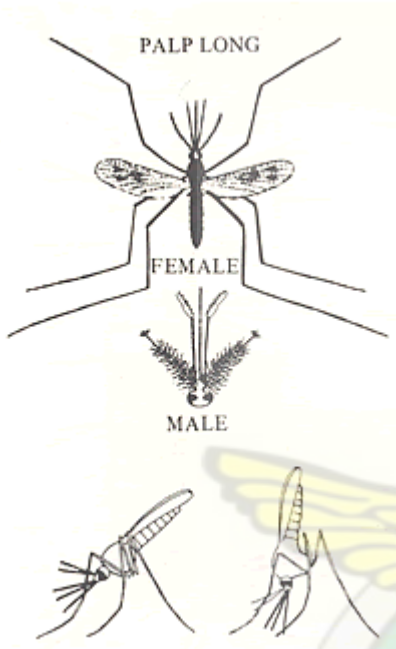


**Figure 2.5 The mouthparts of both male and female mosquitoes above and below showing the resting positions of *Anopheles* species. (Source**: www.cdc.gov)

### 2.7.1.5 Life cycle of the malaria parasite

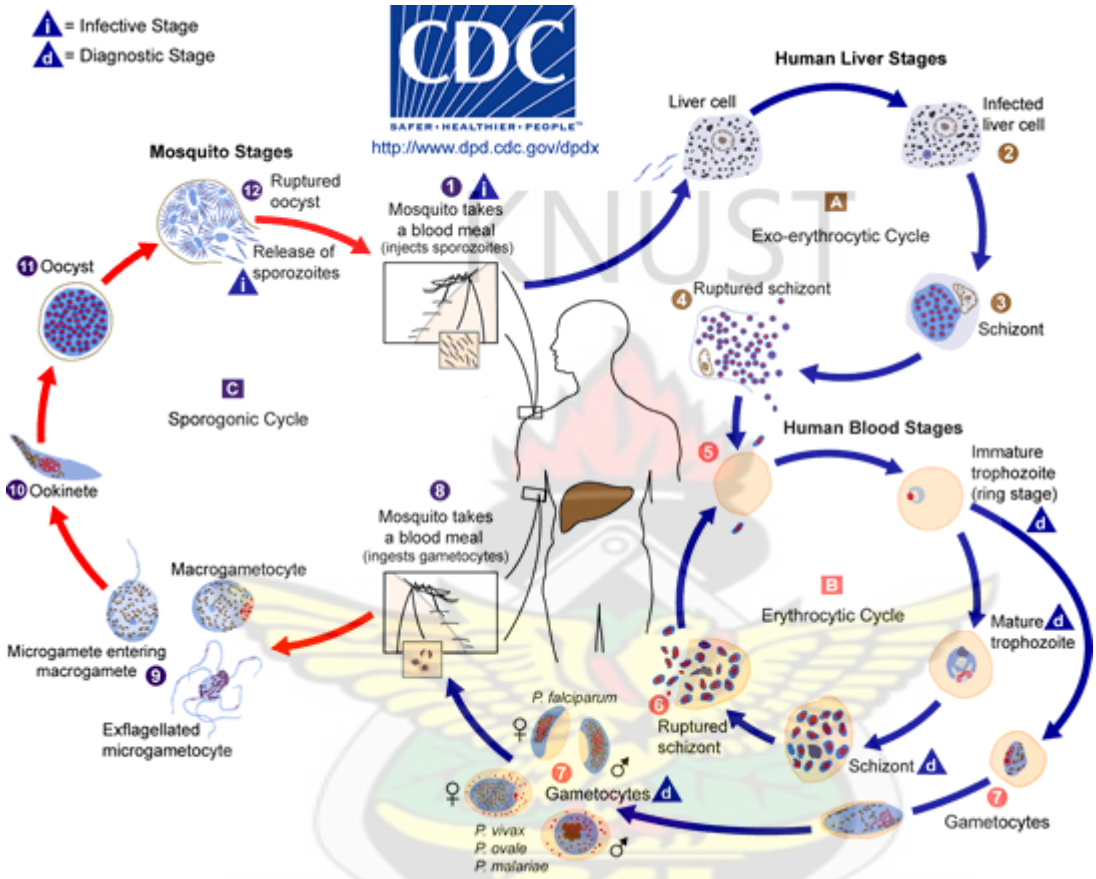


**Figure 2.5 The life cycle of the malaria parasite**. (Source: www.cdc.gov)

When an infected female *Anopheles* mosquito takes in blood from a human it injects sporozoites, the infectious form of the parasite, through it's saliva into the person's blood stream. The sporozoites invade the liver and this is called the exoerythrocytic stage of the cycle. Depending of the type of *Plasmodium* species, each sporozoite develops into schizont

which ruptures to release merozoites which then invade the blood stream; this is called the erythrocytic stage.

Further more in *Plasmodium vivax* and *P. ovale*, the schizonts develop into hypnozoites which are dormant forms of the parasite in the liver cells. If these become active again the hypnozoites develop into schizonts and cause relapses in infected persons.

The merozoites invade the blood or red blood cells and develops into the ring stage trophozoite, and here they undergo asexual reproduction again and develop into schizonts containing numerous merozoites. The schizonts rupture and release merozoites which reinfect other red blood cells. Some of the merozoites that invade the red blood cells do not develop asexually into schizonts but rather into male and female gametocytes which are micro and macro gametocytes respectively, which circulates in the infected person's bloodstream. When a female *Anopheles* mosquito takes a blood meal from this infected person it ingests the gametocytes, which multiply in the gut of the mosquito (Sporogonic cycle). The microgametes penetrate the macrogametes to form a zygote which develop into elongated motile oocysts. Oocysts rupture and release sporozoites that migrate to the salivary gland of the mosquito. The cycle begins again when the mosquito bites it's next victim (Department of Health and Human Services, 2007).

### 2.7.2 Characteristics of *Anopheles* breeding sites

A study was carried out by (Afrane *et al.*, 2012) to find out the abundance and productivity of mosquitoes in an irrigated vegetable farm in Kumasi. These farms are hotspots for breeding of malaria vectors and could lead to high risk of malaria transmission. Breeding sites were

dugout wells, furrows and footprints. The study showed that irrigated vegetable farms contribute to adult mosquito populations and malaria since larvae mosquito abundance and larval survival was high there. Breeding sites are most of the time small water bodies that are scattered, directly under sunlit, turbid temporary and close to human communities (Gimnig *et al.*, 2001). Research by (Kudom, *et al.*, 2011) to characterize mosquito larval habitats in Sekondi-Takoradi and also determine the susceptibility of *Anopheles gambiae s.l* to four classes of insecticides, revealed that most of the larval habitats were anthropogenic as a result of human behaviour and organic polluted water was inhabited by *An. gambiae s. l.* larvae and *Culex quinquefasciatus* larvae (Sattler *et al.*, 2005). Research by Kudom *et al.*, (2011) also found out that *An. gambiae* had developed strong resistance to pyrethroids and DDT which was reported to be susceptible a decade ago in southwestern part of Ghana. They implicated the extensive use of insecticides in households to be a cause of this.

### 2.7.3 *Anopheles gambiae* complex

The *Anopheles gambiae* complex is a group of seven morphologically indistinguishable species of mosquitoes in the genus *Anopheles*. The complex was discovered in the 1960's and is made up of the most important vectors of malaria in sub-Saharan Africa including the very dangerous parasite *Plasmodium falciparum*. The species complex includes: *Anopheles arabiensis*, *Anopheles bwambae*, *Anopheles merus*, *Anopheles melas*, *Anopheles quadriannulatus* and *Anopheles gambiae sensu stricto* and the species complexes of *An. culicifacies* and *An. funestus* (WHO, 2013). Apart from being morphologically indistinguishable, members of the *Anopheles gambiae* complex also have different behavioural traits, for example *Anopheles gambiae sensu stricto* is anthropophilic meaning it takes it's blood meal from humans whilst *Anopheles quadriannulatus* is considered to be

zoophilic meaning, it takes it's blood meal from animals (Besansky *et al.*, 1994; Wilkins *et al.*, 2006).

The river Gambia, one of the great rivers of West Africa flowing North-West in the Tambacounda province of Senegal, westward into the Atlantic Ocean at the city of Banjul, Gambia greatly affects the ecology of neighbouring areas and provides many breeding opportunities for anopheline malaria vectors (Caputo *et al.*, 2008). Detailed surveys on the presence and prevalence of malaria vector species in this region belonging to the *Anopheles gambiae* complex started more than 25 years ago when Bryan and collaborators analyzed the distribution of the three sympatric members of the complex; *Anopheles gambiae* s.s and *Anopheles arabiensis* which are the fresh water species and *Anopheles melas* which is the salt water species in the Gambia and surrounding areas in Senegal. The *Anopheles gambiae sensu stricto* meaning *Anopheles gambiae* in the strict sense has been discovered recently to be in a state of diverging into two species; the Mopti M strain and the Savannah S strain, since 2007 though, both forms are said to be of the same species (Yakob, 2011). The M forms breed in irrigated rice fields whilst the S forms are found mostly in rainwater collections (WHO, 2013)

Studies by Caputo *et al.*, (2008) shows that, during the rainy season *Anopheles gambiae* are widely distributed throughout the Gambia and Senegalese area while *An. melas* reach up to 150km inland and increase in frequency at the beginning of the rainy season in July or the early dry season in Nov-Dec, when brackish environments become more common. A recent study confirmed these findings showing that, *Anopheles melas* is subject to exposure to large fluctuations in its density because of competition with the fresh water species *An. gambiae s.s*

larvae in breeding sites having low salt concentration below 30% sea water (Bogh *et al.*, 2003). The freshwater species was recorded in the eastern inland part of Gambia in the northern neighbouring Senegalese region of Saloum (Bryan *et al.*, 1982) and (Fontenille *et al.*, 1997). Bogh *et al;* (2003) suggested that the main breeding habitat for *An. arabiensis* in the area was in rain fed-rice fields along the edge of alluvial soils.

The Kisumu strain of *An. gambiae s.s* is a reference strain which is susceptible to all insecticides. It was originally isolated from the Kisumu region of western Kenya early in the 1950's and has been maintained in the laboratory since (Djogbenou *et al.*, 2010; Shute, 1956).

### 2.8 Studies on susceptibility tests

These studies provide data on resistance status of local mosquito vector populations which can be used in formulation of vector control programmes.

#### 2.8.1 Asia and Middle East

In an effort to control mosquitoes invading tsunami affected areas in Thailand, a study was conducted by (Narumon *et al.*, 2006) to determine the insecticide susceptibility status of field larvae and mosquitoes of *Anopheles sundaicus* and *Culex sitiens* under laboratory conditions. Larval bioassays were conducted using WHO standard methods. Three larvicides temephos, malathion and a plant extract called ethanolic extract of the South East Asian long pepper *Piper retrofractum* Vahl were used in the experiment. Results showed that *Cx. sitiens* was

more susceptible to temephos than malathion and the plant extract. *Cx. quinquefasciatus* showed greater tolerance to any tested larvicide than *Cx. sitiens*. Adult bioassay tests using WHO test kits and diagnostic doses of 5% malathion, 0.75% permethrin, 0.05% deltamethrin and 4% DDT were conducted. Results showed that *An. sundaicus* and *Cx. sitiens* were susceptible to all tested insecticides. The LT 50 (Lethal time at 50% concentration of insecticide) of 5% malathion ranged between 25.7 to 26.0 minutes for *Cx. sitiens* and 44.7 minutes for *An. sundaicus*. *Cx. quinquefasciatus* showed susceptibility to malathion with LT50 of 19.7minutes. It showed resistance to both pyrethroid insecticides, with LT50 of 33.1minutes of 0.075% permethrin and 19.6 for 0.05% deltamethrin, it showed low percentage mortality after 24 hour post-exposure of 38% and 42% respectively. They concluded that every tested larvicide could be used for controlling *Cx. sitiens*, even in brackish water, pyrethroid insecticides for adult *Cx. sitiens* and *An. sundaicus*, and malathion for all three species.

Bansal and Singh carried out a study in north-western Rajasthan to determine the relative susceptibility of some common mosquito vector larvae (*Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*) to synthetic insecticidal compounds. They found out that anophelines were more susceptible than the other two culicines to four organophosphates ( malathion, fenetrothion, fenethion, temephos) and three synthetic pyrethroid compounds (alphamethrin, deltamethrin and fanvalerate) tested. The results also showed that *Ae. Aegypti* were most susceptible followed by *Cx. quinquefasciatus* and *An. stephensi* to all three pyrethroids tested. Among the three pyrethoroids tested, alphamethrin was found to be the most toxic, followed by deltamethrin whilst fanvalerate was the least toxic. The study provided useful information about planning the use of these insecticides for the use of control of different vector species in this area (Bansal, 2007).

### 2.8.2 Africa

A study carried out by (Kamau *et al.*, 2007) to investigate the resistance status in *Anopheles gambiae sensu lato* and *Anopheles funestus* mosquitoes from western Kenya to the four classes of insecticides approved for IRS by the WHO, *Anopheles gambiae* Kisumu strains were also included in the bioassays. They observed over 98% mortality for tests with all insecticides for both *Anopheles gambiae s.l* and *Anopheles funestus.* Knock down rates were not significantly different between *An. gambiae s.l* and the Kisumu strain control. 50% and 95% knock down times were either slightly lower than that of the Kisumu strain or higher by factors of less than 1.6. Based on the conventional criteria where susceptibility is defined by mortality rates >98% 24hours after exposure, no evidence of resistance was found meaning that, the vector control measures employing any of the insecticides tested will not be hampered. It showed the need for continous monitoring of insecticide resistance status and the impact of any observed resistance on the efficacy of vector control programmes employing insecticides apparent.

### 2.8.3 West Africa

Research was carried out by (Oyewole *et al.*, 2011) involving breeding of Anopheline mosquito larvae carried from six ecological zones in Nigeria, between 2002 and 2004. Larvae were reared to adulthood in an insectary and susceptibility tests were carried out on adult non-blood fed mosquitoes emerging after 2-3 days using WHO standard procedures, diagnostic kits and test papers (WHO, 1998b). They found out that mosquitoes sampled from all zones were susceptible to the diagnostic doses of insecticides tested, although a significant level of resistance was observed in forest-savanna mosaic and guinea savanna; however there was no significant change in knock down effects of insecticides in all the zones.

Furthermore studies carried out by (Betson *et al.*, 2009) to find out the status of insecticide susceptibility in *Anopheles gambiae s.l* from six surveillance sites in the Gambia, through collection of *Anopheles* larvae from Birikama, Essau, Farafenni, Mansakonko, Kuntaur and Basse established by the National malaria control programme and the UK national research laboratories in the Gambia. These mosquitoes were reared to adulthood and identified using morphological keys and species specific polymerase chain reactions (PCR). Two to three day old adult females were tested for susceptibility to permethrin, deltamethrin and DDT using WHO standard protocols, insecticide susceptibility test papers and test kits. They found out that, all mosquitoes tested belonged to *Anopheles gambiae* complex and mosquitoes from two of the six sites Brikama and Basse were fully susceptible to all three insecticides tested. However *Anopheles gambiae* resistance to DDT was found in mosquitoes from Essau where 24 hours post mortality exposure was less than 80% but 88% for permethrin and 95% for deltamethrin. The study provided baseline information for monitoring resistance in the Gambia and highlighted the need for routine resistance surveillance as an intergral part of the proposed nationwide IRS intervention using DDT.

In 2008 a network was established with financial support from WHO/TDR to find out the extent of insecticide resistance in malaria vectors in five African countries. The study was carried out by (Ranson *et al.*, 2009), here the results of bioassays on *Anopheles gambiae sensu lato* from two rounds of monitoring from 12 important sites in three partner countries were reported. They found out that resistance was very heterogenous even over relatively very short distances. Also in some sites large differences in mortality rates were observed during the course of the malaria transmission season. Using WHO diagnostic doses, all populations from Chad, Burkina Faso and two of the four populations from Sudan were classified as being resistant to deltamethrin or permethrin. Very high frequencies of DDT

resistance were found in urban areas of Burkina Faso and Sudan and in a cotton growing district in Chad. They found resistance to be present in areas where *Anopheles gambiae s.s* and *Anopheles arabiensis* were found simultaneously in both species although higher in *Anopheles gambiae s.s. Anopheles gambiae s.l.* remained largely susceptible to the organophosphate fenitrothion and the carbamate bendiocarb in the majority of the sentinel sites with the exception of two sites in Burkina Faso. In the cotton-growing region of Soumousso in Burkina Faso, vector populations were resistant to all four classes of insecticides available for malaria control. Possible factors influencing the frequency of resistance in these sites were discussed and the results of this study highlighted the importance of standardized longitudinal insecticide resistance monitoring and the urgent need for studies to monitor the impact of this resistance on malaria control activities.

Pyrethroid insecticides carbamates and organophosphates are the classes of insecticides commonly used in Benin. WHO recommends pyrethroids as the only class to be used for impregnation of mosquito nets, unfortunately high resistance levels of *Anopheles gambiae s.l* threatens the success of ITNs. (Yadouleton *et al.*, 2011) carried out a study which focused on the investigation of agricultural practices in cotton growing areas and their direct effect on larval populations of *Anopheles gambiae* in surrounding breeding sites. They collected agro-social data where farmers were subjected to semi structured questionnaires based on strategies used for protecting crops. They also carried out bioassay tests to assess the susceptibility of malaria vectors to various insecticides. Molecular analyses were used to characterize resistance genes and molecular forms of *An. gambiae*. Also insecticide residues in soil samples from breeding sites were investigated to find out the important factors that prevent the normal growth of mosquito larvae by exposing susceptible and resistant laboratory strains.

They found out that, there was a common use of local fertilizer NPK at 200 kg/ha and urea at 50kg/ha following insecticide treatment, in both the Calendar Control Programme (CCP) and the Targeted Intermittent Control Programme (TICP). In the Biological Programme no chemicals are involved and farmers use organic and natural fertilizers including animal excreta. Susceptibility tests showed a high resistance to DDT and mean mortality of *An. gambiae* collected from farms practicing CCP, TICP and BP were 33%, 42% and 65% respectively. *An. gambiae* populations collected from areas practicing CCP and TICP showed resistance to permethrin with mortality of 50% and 58% respectively. evidence of cross resistance to pyrethroids and DDT has also been found in Benin by (Corbel *et al.*, 2007). Whilst bioassay results of *An. gambiae* in areas practicing BP gave a high level of susceptibility to permethrin and an average mortality of 94%. Molecular analyses identified *Anopheles gambiae s.s* and *An. arabiensis* with a higher predominance of *An. gambiae* s.s at 94%. M and S forms were also identified with S forms having a higher frequency of 96%. The presence of inhibiting factors in the soil samples under insecticide treatment were found to negatively affect and delay the development of *An. gambiae* larval populations. The study showed that *kdr* gene had spread widely by target site resistance mechanism in *An. gambiae* mainly in CCP and TICP area where pyrethroids are used extensively. They also found out that the way to reduce the negative effect of pesticides used in cotton crop protection was through application of BP like programmes which do not appear to select for vector resistance. The study also served as a source of scientific evidence of the spread of resistance due to massive use of agricultural insecticides and can contribute to management of pesticide usage on cotton crops therefore reducing selection pressure of insecticides on *An. gambiae* populations.

Further studies on an update of resistance status of *Anopheles gambiae s.s* to conventional insecticides at a previous WHOPES field site in "Yaokoffikro" was conducted six years after political crisis in Cote d'Ivoire by (Koffi *et al.*, 2012), for the evaluation of insecticides against highly resistant mosquitoes. Breeding sites of the mosquitoes and selection pressure was maintained by local farmers until the war broke out in September 2006. Six years after the crisis, bioassays and biochemical analyses were conducted in order to update themselves of the resistance status of *An. gambiae s.s* populations and detect other methods of resistance that may have evolved. Larvae of *An. gambiae s.s* from Yaokoffikro were collected and reared to adults and resistance status of the population was assessed using WHO bioassay test kits for adult mosquitoes with seven insecticides namely, two pyrethroids, a pseudo-pyrethroid, an organochloride, two carbamates and an organophosphate. Biochemical assays were also carried out to determine resistant *kdr* genes and alleles.

They found out that, high pyrethroid, DDT and carbamate resistance was confirmed in *An. gambiae s.s* from Yaokoffikro. Mortality rates for pyrethroids and etofenprox were less than 70%, DDT 12% and less than 22% with the carbamates. They also observed tolerance with fenitrothion with mortality of 95% after 24 hours. Molecular analyses also showed the first sign of increased enzyme activity in *An. gambiae* from Yaokoffikro, which could have serious implications in detoxification of insecticides and that, their individual roles in resistance would have to be investigated with additional tools. They concluded that, the insecticide resistance profile at Yaokoffikro was multifocal and that the site presented a unique opportunity to determine its impact on the protective efficacy of insecticidal products and also new tools to manage these complex mechanisms. It called for innovative research on the local vector behaviour, its biology and genetics that drive resistance.

### 2.8.4 Ghana

Furthermore baseline entomological data obtained during surveys carried out in four mining operations in Ghana (Obuasi, Tarkwa/Damang, Ahafo and Akyem), West Africa by Hunt *et al* in 2011, identified majority of samples as *Anopheles gambiae* S forms and a few M forms identified from Tarkwa. They found Plasmodium infection rates ranging from 4.5 to 8.6% in *An. gambiae* and 1.81 to 8.06% in *An. funestus.* They also found high survival rates on standard WHO bioassays tests recorded for all insecticide classes except for organophosphates that showed reasonable mortality of less than 90% at all four locations. The data highlighted the complexity of the situation prevailing in southern Ghana and the challenges facing the national malaria control programme in that region. The study showed the need for vector control programmes in Ghana to carefully consider resistance profiles of the local mosquito populations in order to base their resistance management strategies on sound scientific data.

A study was carried out by (Achonduh, *et al.*, 2008) to determine the susceptibility status of *Anopheles gambiae s.l.* (Diptera: culicidae) from cabbage growing areas associated with pyrethroid and organophosphate use in Accra. *Anopheles gambiae s.l.* were collected from two cabbage growing areas in Accra and resistance levels were assessed with 0.05% deltamethrin, 0.75% permethrin, 5% malathion and 4% DDT using standard WHO susceptibility kits. Comparing resistance profiles from the areas assessed with the Kisumu susceptible strain, Korle-bu and Airport strains were reported to be highly resistant to DDT with levels of over 9 folds for permethrin and over 2.5 folds for deltamethrin, however both wild and susceptible strains showed full susceptibility to malathion. They proposed that,

resistance in *Anopheles gambiae* in these breeding sites contaminated with agricultural insecticides may have occurred over time due to continous exposure to sub-lethal doses.

Insecticide resistance profiles for malaria vectors in the Kassena-Nankana district was carried out by (Anto, *et al.*, 2009). Indoor resting *Anopheles* mosquitoes were collected, blood fed and gravid females were made to oviposit and eggs that hatched were reared into larvae. 1-3 day old adults were then tested against 0.75% permethrin, 0.05% deltamethrin, cyfluthrin 0.15%, lambdacyhalothrin 0.1% and 4% DDT based on WHO standard procedures. Resistance to pyrethroids and DDT was assessed by genotyping the knocked-down resistance gene in the area. A total of 9,749 1-3 day old F1 females were exposed to the insecticides, permethrin 0.05% had the least knockdown effect whilst cyfluthrin 0.15% had the highest knockdown effect for the pyrethroids. There was no difference in susceptibility observed between *Anopheles gambiae* 93.3% (95% CI: 92.5-94.1) and *Anopheles funestus* 94.5% (95% CI: 93.7-95.3) when exposed to pyrethroids. In the same way, there was no difference in susceptibility between the two vector species *An. gambiae* 79.1% (95% CI: 76.6–81.8) and *An. funestus* 83.5% (95% CI: 80.2–86.4) when exposed to DDT. Susceptibility to insecticides overall was between 80 and 98% suggesting that there was some resistance except for cyfluthrin 0.15%. They found out that, the main vectors of malaria in the Kassena-Nankana district *An. gambiae* and *An. funestus* were susceptible to the insecticides being used in the treatment of bed nets in the malaria control programme. They however suggested the need to monitor pyrethroid efficacy as it was not very high.

Research was carried out to determine the distribution species of *Anopheles gambiae* and their pyrethroid insecticide resistance knockdown status in the Kumasi metropolis (Agyepong *et al.*, 2012), by PCR. This work revealed seventy six out of hundred larvae collected to be

identified as *Anopheles gambiae s. s*. and twenty six out of fifty samples possessed the *Kdr* gene, and the remaining twenty being susceptible.

**2.9 knowledge, attitude and perception on the use of insecticide treated nets.**

The use of ITN is an important strategy for the roll back malaria to reduce human and vector contact with mosquitoes to reduce morbidity and mortality as a result of malaria in millions of people and children under the age of five in Africa (RBM *et al.*, 2005). Research has shown that the use of ITN has reduced the incidence of malaria by 48% to 50% (Lengeler, 2004) and also education on ITN use (Rhee *et al.*, 2005) Previously the use of ITN was very low in Africa but due to the free distribution of nets more people now have access to it (Bernard *et al*., 2009), but research has shown that ownership of the nets does not necessarily mean that the net will be used due to factors like difficulty in fixing the nets due to the design of houses, feeling of suffocation due to hot temperatures especially in the dry season, socioeconomic factors like wealth, access to health care, gender and education also affect the use or non use of ITN (Toé *et al.*, 2009).

In order to ensure effective use of ITN in communities there is the need for policy planners to find out the reasons for use and non use of ITN. Malaria control programmes must therefore base their intervention methods on what they find out about the "doers" those who use ITN and " non-doers" (De La Cruz *et al.*, 2006). A study was carried out by De La Cruz et al., (2006) to find out the characteristics of women that affect the use of bed nets for their children under the age of five in Ghana. They found out that, place of residence, availability of food and caregivers' knowledge on symptoms, causes and groups prone to malaria were closely associated with bed net use. Also, most of respondents knew that mosquitoes cause

malaria and 90% of doers and 77% of non doers believed that bed net use protected one from malaria (Adongo *et al.*, 2005; NetMark, 2004) This study showed that great knowledge about malaria does not translate into greater use of ITN due to different cultural beliefs about the disease in communities; therefore integrating these beliefs into traditional health education may help improve the effectiveness of public health efforts.

# CHAPTER THREE

## 3. MATERIALS AND METHODS

### 3.1 Study area

The area for this study was the Kwame Nkrumah University of Science and Technology campus located in the Kumasi Metropolitan area, of the Ashanti Region of Ghana and its surrounding communities. The Ashanti Region is located in the central part of the middle belt of Ghana and lies between longitudes $0^0$ 9'W and $2^0$ 15'W, and latitudes $5^0$ 30'N and $7^0$ 27'N. The region occupies a land area of 24,389 $km^2$ which is 10.2% of the total land area of Ghana (Coleman, 2009).

Kumasi is the capital of the Ashanti Region and is located in the transitional forest zone and it is about 270 Km north of the national capital Accra. It lays between latitudes $6.35^0$-$6.40^0$ and longitude $1.30^0$-$1.35^0$. The Kumasi metropolis lies within the plateau of the South-West physical region ranging from 250-300 metres above sea level. The topography is undulating and has many rivers and streams like Subin, Wiwi, Sisai, Owabi, Aboabo and others. The geology of the metropolitan area is dominated by middle pre-cambian rock. (KMA, 2006).

The metropolis is within the wet sub-equatorial type with average minimum temperature of $21.5^0$C and maximum average temperature of $30.7^0$C. Average humidity is about 84.18% at 0900 GMT and 60% at 1500 GMT. It has a maximum rainfall of 214.3mm in June and 165.2mm in September. The city is within the moist semi-deciduous South-East ecological zone, it has rich soil which promotes agriculture in the area (KMA, 2012). It has a population of 1,468,609 according to the Geonames geographical database (WCE, 2012).

### 3.1.1 Description of sites for mosquito sampling

Sites selected for larval surveys were mostly in the faculty and residential areas. They were mainly shallow, directly exposed to sunlight with different water qualities, others were a dugout well and furrows on vegetable farms and a pool of water leaking from a pipe connected to a dugout well. Study sites were surveyed on foot for larval breeding sites; samples were taken from different sites like conduits on furrows from farmlands on the campus. Temperature of the locations where samples were taken was measured with a thermometer and geographical locations were also determined with a global positioning system (GPS).

### 3.2. Mosquito sampling

Potential breeding sites of mosquitoes were surveyed from November 2012 to June 2013. Larvae were collected with a ladle. *Anopheles* larvae were identified by their parallel position on the water surface and were kept in 1000 ml loosely capped plastic containers and brought to the insectary to be reared to adulthood.

### 3.2.1 Mosquito processing

Larvae were sorted out for identification of only anophelines to be reared to adulthood. Once in the insectary, larvae were poured into plastic bowls of 2cm depth and each bowl was labeled to denote the breeding site and date of collection. The larval bowls were kept at temperatures of 27-30$^0$C and 80 ± 10% relative humidity and also fed with about 100mg fish meal every day. Larvae were closely monitored and those that pupated were collected into plastic cups with Pasteur pipettes and placed in labeled cages for emergence into adults. All

adults were fed on 10% sugar solution imbibed on cotton wool, 2-5 days old non blood fed female adult mosquitoes were then collected and used for susceptibility tests.

### 3.2.2 Susceptibility tests

Resistance among anophelines was determined using the standard WHO tube Assay. Adult female mosquitoes were used because they have lower control mortalities and survive better. The tests were carried out indoors in a building free from insecticide contamination and excess temperature, humidity, light and wind (WHO, 1981a, 1981b).

### 3.2.3 Insecticide impregnated papers

Insecticide impregnated papers from the four classes of WHO approved insecticides for public health use were used for the tests. These were 0.05% deltamethrin, 4% DDT, 0.1% bendiocarp and 1% fenitrothion. Non blood fed female mosquitoes of 2-5 days old were used for the tests. 80-100 mosquitoes were tested for each insecticide impregnated paper, with four replicates each consisting of 20-25 mosquitoes and a control with paper impregnated with oil.

### 3.2.4 Test procedure

80-100 female mosquitoes were collected with an aspirator; 20-25 for each test tube were first collected into the holding tubes before being transferred into exposure tubes.

Into each of the exposure tubes, a sheet of impregnated paper was rolled into a cylinder to line the walls of the tube and held in place with a spring wire clip made of copper. Mosquitoes were then introduced into the exposure tubes by attaching it to holding tube by

the vacant screw in slide, the slide was pulled out beyond its filling point so that, no part of it blocked the tube openings. Mosquitoes were then blown gently into the exposure tubes and then the slide was closed and the holding tube detached and set aside. The exposure tubes were then left standing upright with the screen end up for 5, 10, 15, 20, 30, 40, 50, 60 and 80 minutes. At the end of the exposure period, the mosquitoes were transferred into the holding tube by opening the vacant screw and blowing the mosquitoes gently into the holding tubes. The slide was then closed and the exposure tube was detached. A wad of cotton soaked with 10% sugar solution was placed on the screen of the holding tubes for the mosquitoes to feed.

The holding tube was then left for 24 hours in a quiet place with temperature not more than $30^0$C, the tubes were protected from ants by placing the platform into a pan of water. Mortality counts were then made after 24 hours. Mosquitoes that were dead were removed by gently detaching the slide and removing the tube aside. Results were then recorded (WHO, 1981b).

### 3.3 Breeding sites

All breeding sites of the samples collected were characterized by the following criteria, presence or absence of farmlands or vegetation, presence of predators like dragonflies, water quality including pH and electrical conductivity, turbidity, temperature, whether the site is shaded or sunlit, and finally whether the site was temporary or permanent.(Klinkenberg *et al.*, 2008) The results were then documented to obtain the association of breeding sites in the study area with the susceptibility status of *Anopheles* species in KNUST and its surroundings.

### 3.4 Insecticide usage

600 questionnaires were administered randomly to inhabitants within the study area to determine socio-economic characteristics such as age, sex, occupation and educational level. It also sought to find out the types of chemical based control interventions being used by inhabitants within the study area. Furthermore the knowledge and perception on the use of ITNs and finally, the risk of exposure to mosquito bites by finding out the time of going to sleep and time of awakening of respondents. The total population on KNUST campus is about 39,000, a sample of 600 respondents were chosen looking at the period of time for the study.

### 3.5 Statistical analyses

Results were analyzed to obtain the LT50 value using knockdown time regression through probit analysis (Finney, 1971). Mortalities in control that were between 5% and 20% were corrected with Abbot's formula: $\frac{\%\text{ mortality} - \%\text{ control mortality}}{100 - \%\text{ control mortality}} \times 100$

The resistant status of mosquito samples was determined according to the WHO criteria (WHO 1998b). Following the WHO, 2013 standard procedures;

- 98-100% mortality means the population is susceptible,
- Mortality less than 98% means resistance is suspected and further investigation must be carried out.
- Mortality between 90-97% means resistant genes are present in the population and must be confirmed.

- Mortality less than 90% confirms the presence of resistance genes and additional bioassays may not be necessary, however mechanisms and distribution of resistance must be investigated.

Association of breeding sites with susceptibility was analysed by Bivariate correlation and the questionnaires were analysed using SPSS 16.0 for Windows (SPSS Inc, USA), resistance profiles of the different breeding sites were compared through one way ANOVA, and multiple comparisons were made by Tukey post-hoc tests. Data that failed the Levene test of homogeneity of variances were analysed with a Welch ANOVA and multiple comparisons made by Games-Howell tests. All statistical analysis was done with SPSS 16.0.

# CHAPTER FOUR

## 4. Results

### 4.1 Larval surveys

Larval surveys were carried out on foot and most of *Anopheles* breeding sites were found at the faculty area where there are a lot of agricultural activities with numerous vegetable farms and construction sites leading to deforestation. The rest of the samples were from the lecturer's residences. *Anopheles* larvae were mostly found in shallow water which was clean most of the time and directly under sunlight. Breeding sites were mostly furrows between raised beds on vegetable farms, runoff water at the edge of a dugout well and rain pools from construction sites and also on farms. This is shown in figure 4.1 and 4.2 below.

**Figure 4.1 A furrow between raised beds which served as a breeding site for *Anopheles* larvae**

**Figure 4.2 Water at the edge of a dugout well which served as an *Anopheles* breeding site**

Table 4.1 below shows the characterization of Anopheles breeding sites that were found Predators encountered were mostly tadpoles, frogs, dragonflies and spiders (Afrane *et al*., 2012)

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**Table 4.1 Characteristics of breeding sites in KNUST, Kumasi.**

| Breeding site | Habitat of breeding site | Type of breeding site | Species found | Predators found |
|---|---|---|---|---|
| **SMS 1** | Furrows on vegetable farms | Permanent | *Anopheles gambiae s.l.*, *Culex* | Tadpoles, Dragonflies |
| **COE 1, LR1, LR2.** | Rain pool on a vegetable farm | Temporary | *Anopheles gambiae s.l.*, *Culex* | Tadpoles, Frogs |
| **COE 2** | Rain pool on a construction site | Temporary | *Anopheles gambiae* | None |
| **SMS 2** | Edges of a dugout well | Temporary | *Anopheles gambiae s.l.* and *Culex* | Tadpoles, Spiders |
| **SMS 3** | Pool of water from a pipe connected to a dugout well | Temporary | *Anopheles gambiae s.l.* | Tadpoles |

**SMS = School of Medical Sciences, COE = College of Engineering, LR = Lecturer's Residence**

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### 4.2 Physiochemical parameters of water samples

Physiochemical properties of water collected from the breeding sites measured gave values that varied across all seven breeding sites. pH values ranged from 4.88 to 5.86 (mean=5.1962), conductivity,73.8-209.5µS/cm (mean=126.23µS/cm), turbidity 4.7-25.9 NTU(mean=12.94NTU) and temperature 29-31$^0$C (mean=30$^0$C).. There were significant differences in conductivity at SMS 2 (106.5µS/cm), SMS 3(210µS/cm), LR 2(105.6µS/cm), $p < 0.0005$ and at LR 1(75.2µS/cm) $p = 0.005$) compared to conductivity at SMS 1(81µS/cm). There were significant differences also between conductivity at COE 2 (192.9µS/cm), $p = 0.002$, LR 1 (75.2µS/cm), $P<0.0005$ compared to conductivity at SMS 2(106.5µS/cm). Furthermore there were significant differences in conductivity at LR 1 (75.2µS/cm), LR 2 (105.6µS/cm), $p<0.0005$ and COE 2 (192.2µS/cm), $p = 0.029$ compared to conductivity at SMS 3(216µS/cm). Finally there were significant differences in conductivity at LR 1 (75.2µS/cm), LR 2(105.6µS/cm) $p = 0.001$ compared to COE 2(192.2µS/cm) and LR 2 (105.6µS/cm) $p <0.0005$ compared to LR1 (75.2µS/cm).

There were significant differences in the pH readings, ($F (6, 14) = 819.92$, $p < 0.0005$. Differences between pH of SMS 2 (5.03), SMS 3 (4.92), COE 1 (5.72), COE 2 (4.89), LR 1(5.60) and LR2 (5.03), $p < 0.0005$ compared to SMS 1 and also significant differences in pH at LR1 (5.60), $p < 0.0005$ compared to LR 2 (5.03).

There were significant differences in turbidity of breeding sites ($F (6, 6) = 0.000137$, $p < 0.0005$. Significant differences were observed in turbidity at SMS 2 (8.2NTU), SMS 3 (10.3NTU), COE 2 (26.0NTU), LR 1 (8.2NTU) and LR 2 (25.4NTU), $p < 0.0005$ compared

to turbidity at SMS 1 (7.3NTU). Furthermore there were differences in turbidity at SMS 1 (7.3NTU), SMS 3 (10.3NTU), COE 2 (26.0NTU) and LR 2 (25.4NTU), $p < 0.0005$ compared to turbidity at SMS 2 (8.2NTU). Finally there were significant differences in turbidity at COE 2 (26.0NTU), $p = 0.009$, LR 2 (25.4NTU), $p = 0.009$ compared to COE 1 (5.2NTU) and also significant differences in turbidity at LR 2(25.4NTU), $p < 0.0005$ compared to turbidity at LR 1(8.2NTU).



There were significant differences in temperature readings, ($F (6, 14) = 5.667$, $p = 0.004$). Multiple comparisons revealed significant differences in temperature at SMS 2 ($31^0$C), $p = 0.011$, SMS 3 ($31^0$C), $p = 0.040$, LR1 and LR 2 ($31^0$C), $p = 0.011$ compared to temperature at SMS 1($29^0$C) as shown in Table 4.2 below.

**Table 4.2 Physical parameters measured at breeding sites**

| Site | Geo Location | pH (pH units) | Conductivity (µS/cm) | Turbidity (NTU) | Temperature ($^0$C) |
|---|---|---|---|---|---|
| **SMS 1** | N06$^0$40'13.5' W001$^0$34'05.0' | 5.19 | 81 | 7.3 | 29 |
| **SMS 2** | N06$^0$40'14.3' W001$^0$34'03.0' | 5.03 | 106.5 | 8.2 | 31 |
| **SMS 3** | N06$^0$40'12.3' W001$^0$34'07.2' | 4.92 | 210 | 10.3 | 31 |
| **COE 1** | N06$^0$40'14.2' W001$^0$33'59.0 | 5.72 | 112.6 | 5.2 | 30 |
| **COE 2** | N06$^0$40'22.3' W001$^0$33'50.8' | 4.89 | 192.9 | 26.0 | 30 |
| **LR1** | N06$^0$40'03.3' W001$^0$34'40.5' | 5.60 | 75.2 | 8.2 | 31 |
| **LR2** | N06$^0$40'12.7' W001$^0$34'39.1' | 5.03 | 105.6 | 25.4 | 31 |

**SMS=School of Medical Sciences, COE=College of Engineering, LR= Lecturer's Residence**

### 4.3 Insecticide susceptibility tests

A total of 2,510 female *Anopheles* mosquitoes were exposed to insecticides out of which 2, 480, (98.8%) were identified as *Anopheles gambiae s. l.* and 30, (1.2%) were identified as *Anopheles funestus* according to the morphological keys of (Gilles, 1987).

Insecticides tested were 0.05% deltamethrin, 1% fenitrothion, 4% DDT and 0.1% bendiocarb. Very high resistance was observed at all the breeding sites. For 0.05% deltamethrin mortalities ranged from 15% to 54%. There were significant differences in mortalities after exposure to deltamethrin across all the breeding sites ($F(6, 21) = 5.464$, $p = 0.02$) determined by ANOVA. There was a significant difference in mortality at COE 2 (54±5.19%, $p = 0.007$) compared to mortality at SMS 2 (20±1.83%), there was also a significant difference in mortalities at LR1 (15±2.8%, $p = 0.006$) and LR2 (23.75±2.63%, $P = 0.015$) compared to mortality at COE 2 (54±5.19%). There was no significant difference between mortalities at the other breeding sites, $p > 0.005$. LT50 results show that deltamethrin was most effective at COE 2 with a value of 70.72 minutes as shown in Table 4.3 below.

**Table 4.3 Mortality rates and LT50 of mosquitoes exposed to 0.05% deltamethrin for 80 minutes**

| Site | Number exposed | Number of replicates | Mean mortality(%)±SD | LT50 (min) | 95% C.I (LT50) |
|---|---|---|---|---|---|
| **SMS 1** | 80 | 4 | 51.25±1.50 | 79.65 | 71.69-91.09 |
| **SMS 2** | 80 | 4 | 20±1.83 | 165.65 | 107.88-549.13 |
| **SMS 3** | 100 | 4 | 42±4.36 | 115.17 | 93.67-159.04 |
| **COE 1** | 80 | 4 | 42.5±2.65 | 103.11 | 73.89-221.85 |
| **COE 2** | 100 | 4 | 54±5.19 | 70.72 | 63.15-81.57 |
| **LR 1** | 80 | 4 | 15±2.87 | 153.98 | 116.71-252.08 |
| **LR 2** | 100 | 4 | 23.75±2.63 | 125.24 | 87.04-298.62 |

**SMS=School of Medical Sciences, COE=College of Engineering, LR=Lecturer's Residence, SD= Standard Deviation, C. I =Confidence Interval, LT50=Lethal Time at which 50% of mosquitoes were killed.**

For 4% DDT mortalities ranged from 7.5 to 38.75%. ANOVA tests showed there were significant differences between mortalities ($F(6, 9) = 15.106$, $p < 0.0005$). There were significant differences between mortality at COE 1 (35±1.50%, $p = 0.003$), COE 2 (28.75±0.96%, $p = 0.008$) and SMS 3 (34±1%, $P = 0.001$) compared to mortality at SMS 1 (75±1.00%). There was also significant differences in mortality of LR1 (22.5±1.29%, $p = 0.046$) compared to mortality at COE 1 and also significant difference in the mortality at LR 1 (22.5±1%, $p = 0.027$) compared to mortality at SMS 3. There was no significant difference in mortalities at the other breeding sites, $p>0.05$. LT50 value shows that DDT was most effective at SMS 3 with an LT50 value of 70.71 minutes as shown in Table 4.4.

**Table 4.4 Mortality rates and LT50 of mosquitoes exposed to 4% DDT for 80 minutes**

| Site | Number exposed | Number of replicates | Mean mortality(%)±SD | LT50 (min) | 95% C.I (LT50) |
|---|---|---|---|---|---|
| **SMS 1** | 80 | 4 | 7.5±1.00 | 240.93 | 148.98-1110.11 |
| **SMS 2** | 80 | 4 | 23.75±3.86 | 169.25 | 101.84-1621.74 |
| **SMS 3** | 100 | 4 | 34±1.00 | 70.71 | 53.13-125.97 |
| **COE 1** | 100 | 4 | 35±1.50 | 152.96 | 99.31-590.36 |
| **COE 2** | 80 | 4 | 28.75±0.96 | 155.98 | 106.47-398.78 |
| **LR 1** | 80 | 4 | 22.5±1.29 | 136.21 | 100.09-252.38 |
| **LR 2** | 80 | 4 | 28.75±3.30 | 126.34 | 93.46-230.69 |

For fenitrothion, mortalities ranged from 5% to 42.5%. ANOVA tests showed there was a significant difference between mean mortalities ($F(6, 9) = 7.533$, $p = 0.004$). Multiple comparison revealed that mortality was significant at SMS 2 (23±1.26%, $p = 0.037$) and at SMS 3 (35±1.50%, $p = 0.003$) compared to mortality at SMS 1 (5±1.41%). There was no significant difference in mortalities at the other breeding sites $p>0.05$. LT50 value shows that fenitrothion was most effective at LR 2 with a value of 81.70 minutes as shown in Table 4.5.

**Table 4.5 Mortality rates and LT50 for mosquitoes exposed to 1% fenitrothion for 80 minutes**

| Site | Number exposed | Number of replicates | Mean mortality(%)±SD | LT50 (min) | 95% C.I (LT50) |
|---|---|---|---|---|---|
| **SMS 1** | 80 | 4 | 5±1.41 | 232.62 | 144.71-1028.57 |
| **SMS 2** | 90 | 4 | 23±1.26 | 115.20 | 87.84-190.57 |
| **SMS 3** | 100 | 4 | 35±1.50 | 233.10 | 134.11-8644.60 |
| **COE 1** | 100 | 4 | 19±2.36 | 190.73 | 113.0-3639.48 |
| **COE 2** | 80 | 4 | 30±2.16 | 88.29 | 56.27-2539.25 |
| **LR 1** | 80 | 4 | 25±2.94 | 114.26 | 86.47-194.41 |
| **LR 2** | 80 | 4 | 42.5±3.69 | 81.70 | 68.69-105.49 |

For 0.1% bendiocarb mortalities ranged from 10% to 45%. There were significant differences in mortalities across all breeding sites after exposure to bendiocarb ($F(6, 21) = 10.052$, $P < 0.0005$. There was a significant difference in mortality at COE 1 (10±1.15%, $p < 0.0005$), SMS 3 (26±3.32%, $p = 0.023$), LR 1(16±2.16%, $p = 0.001$, LR 2 (25±0.96%, $p = 0.017$) compared with mortality at SMS 1 (50±4.20%). There was also significant difference between mortality at SMS 1 (50±4.20, $p < 0.0005$), SMS 2 (45±0.50%, $p < 0.0005$) and COE 2 (31±1.17%, $p = 0.032$) compared to mortality at COE 1 (10±1.15%). Finally there was a significant difference between LR 1 (16±2.16%, $p = 0.004$) compared to mortality at SMS 2 (45±0.50), there was no significant difference in mortalities of the other breeding sites $p > 0.05$. LT50 value shows that bendiocarb was most effective at SMS 1 with a value of 52.77 minutes as shown below.

**Table 4.6 Mortality rates and LT50 of mosquitoes exposed to 0.1% bendiocarb**

| Site | Number exposed | Number of replicates | Mean mortality(%)±SD | LT50 (min) | 95% C.I (LT50) |
|---|---|---|---|---|---|
| **SMS 1** | 100 | 4 | 50±4.20 | 52.77 | 42.23-71.18 |
| **SMS 2** | 100 | 4 | 45±0.50 | 83.26 | 62.09-155.51 |
| **SMS 3** | 100 | 4 | 26±3.32 | 125.74 | 99.72-183.96 |
| **COE 1** | 80 | 4 | 10±1.15 | 228.53 | 145.96-806.0 |
| **COE 2** | 100 | 4 | 31±1.71 | 124.63 | 98.57-183.66 |
| **LR 1** | 100 | 4 | 16±2.16 | 250.17 | 151.98-1325.44 |
| **LR 2** | 100 | 4 | 25±0.96 | 104.19 | 74.70-229.14 |

Knockdown effect of all four insecticides at site SMS 1 showed bendiocarb having the greatest knockdown effect at 55%, followed by deltamethrin at 48.75%, DDT at 12.5% and fenitrothion 11.25%. This is shown in Figure 4.3 below.

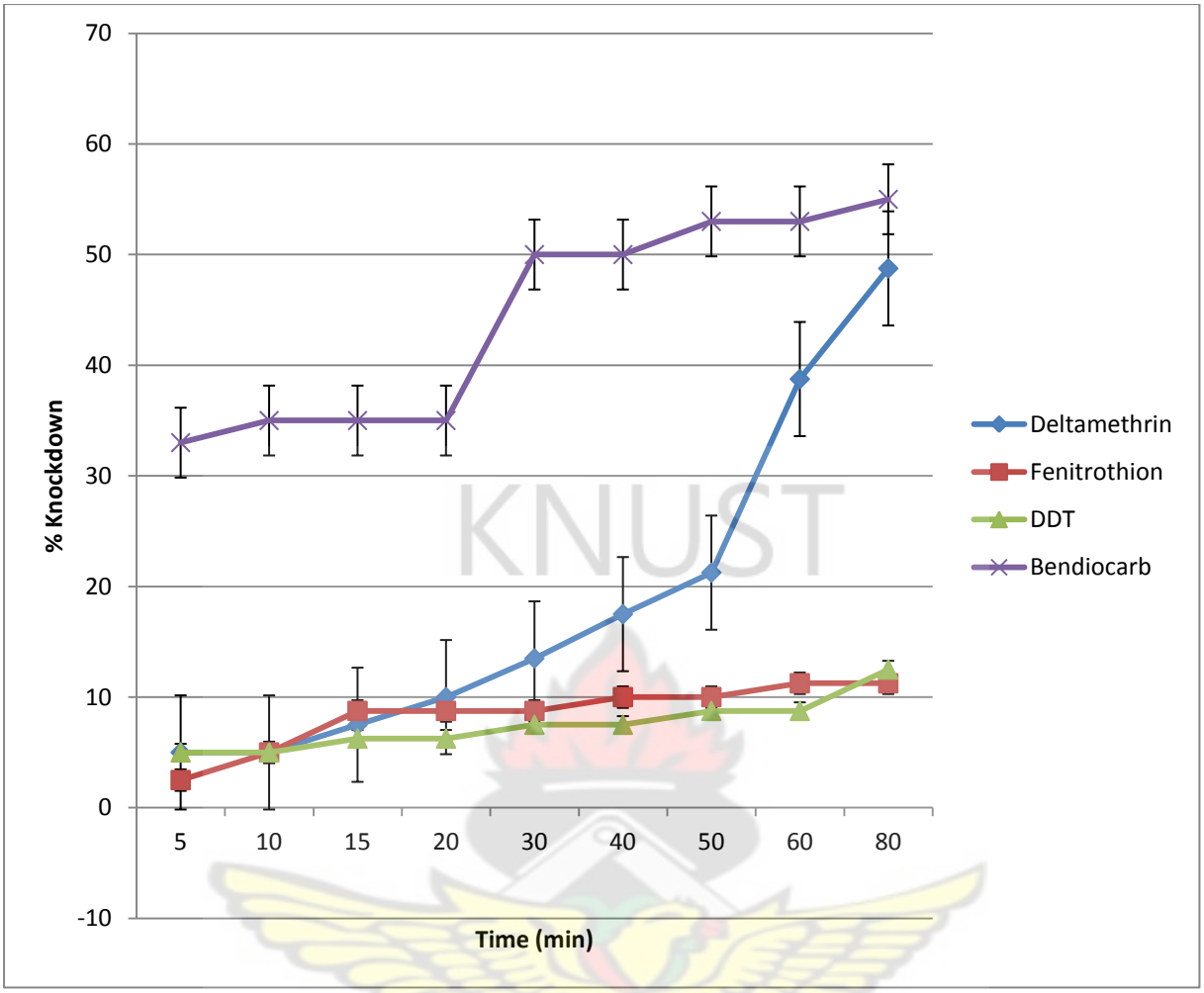


**Figure 4.3 Knockdown effects of all four classes of insecticides at SMS 1.**

Knockdown effect at site SMS 2 also showed bendiocarb having the highest knockdown effect at 47%, fenitrothion 30%, deltamethrin 21.25% and DDT at 18.75%. This is shown below in Figure 4.4.

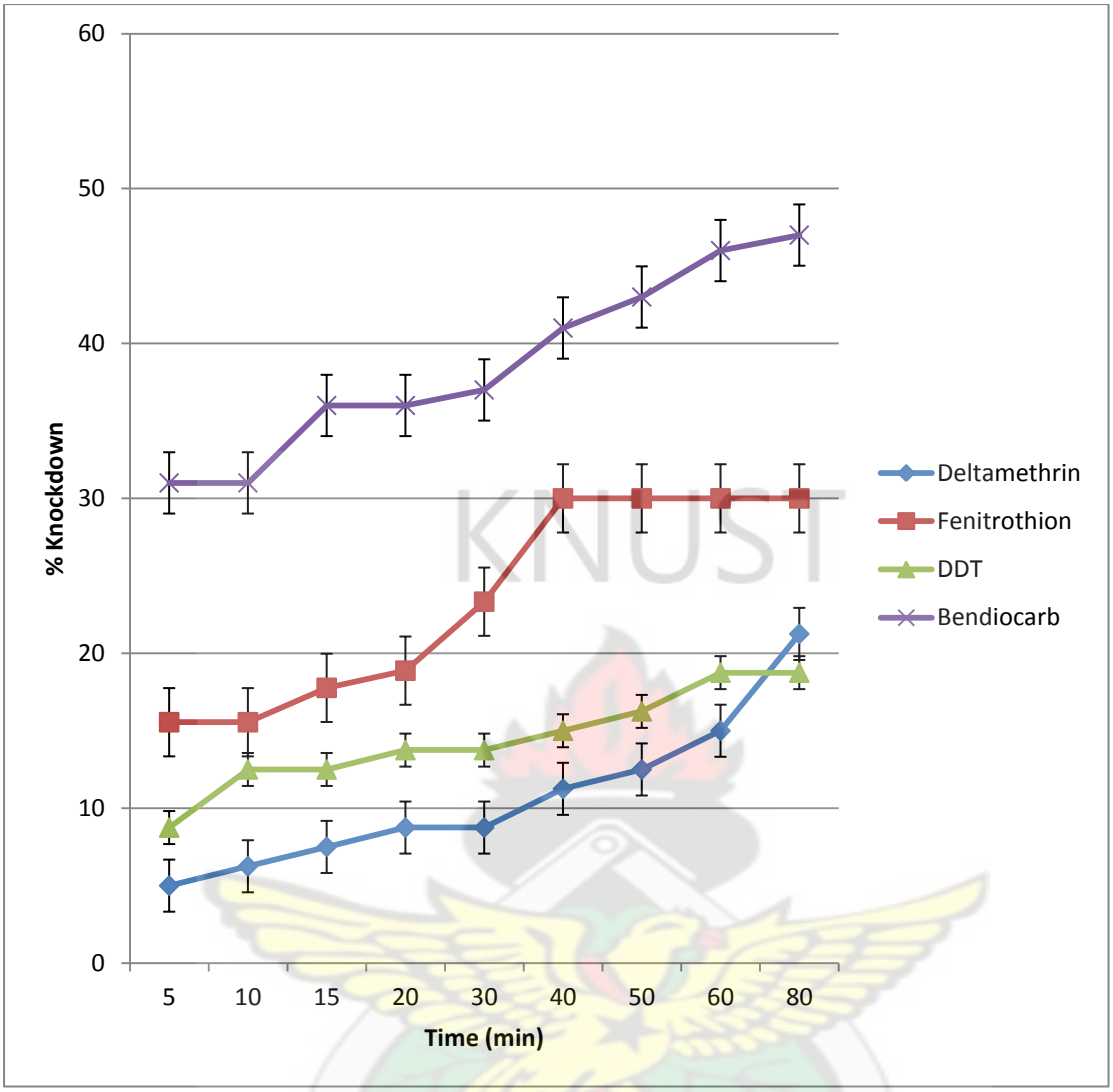


**Figure 4.4 Knockdown effects of all four classes of insecticides at SMS 2.**

Knockdown effect at SMS 3 had DDT having the greatest effect at 48%, deltamethrin 32%, and fenitrothion and bendiocarb both at 28% respectively. This is shown in Figure 4.5 below.

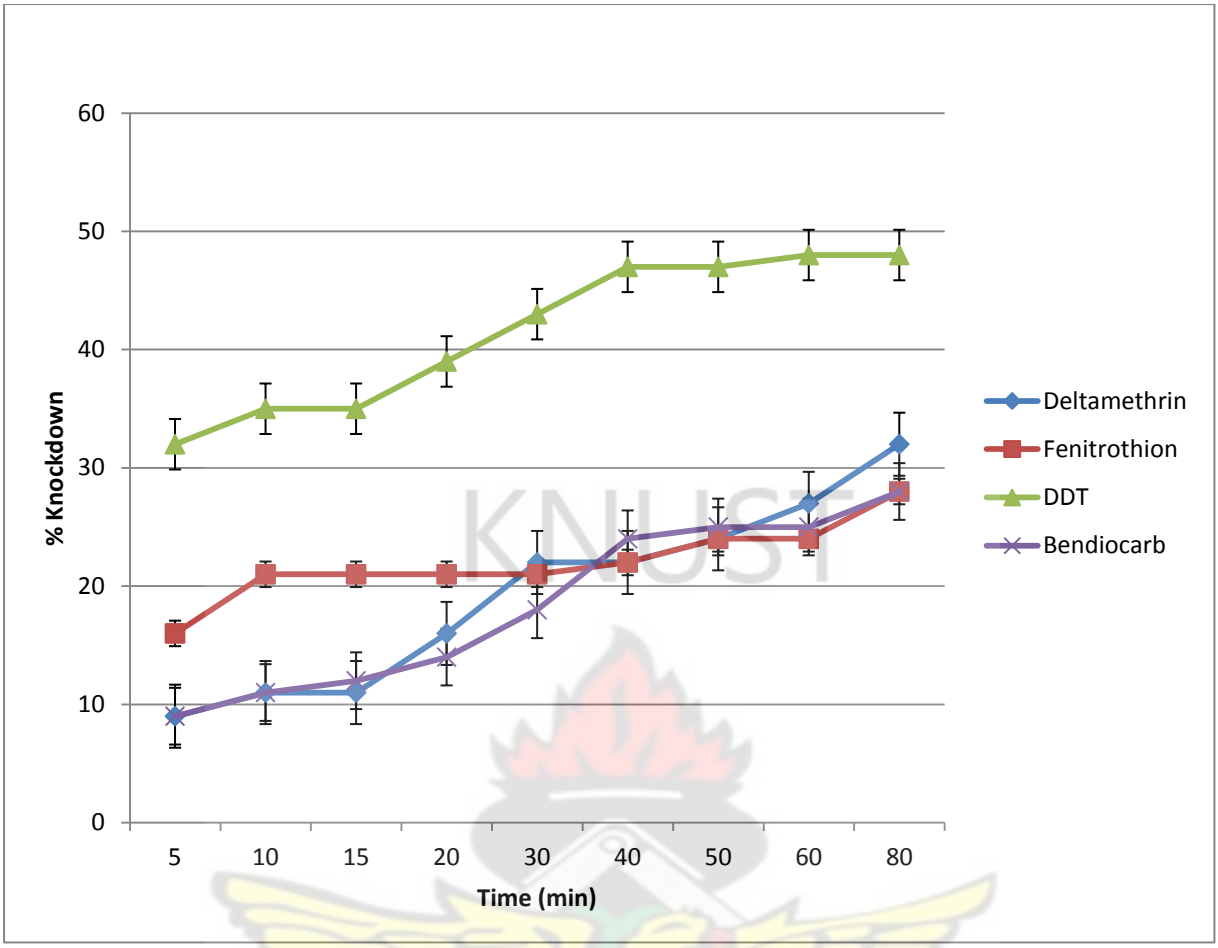


**Figure 4.5 Knockdown effects of all four classes of insecticides at SMS 3.**

COE 1 had deltamethrin having the highest knockdown effect at 52.5%, DDT at 33%, fenitrothion at 32% and bendiocarb at 10%. This is shown in Figure 4.6 below.

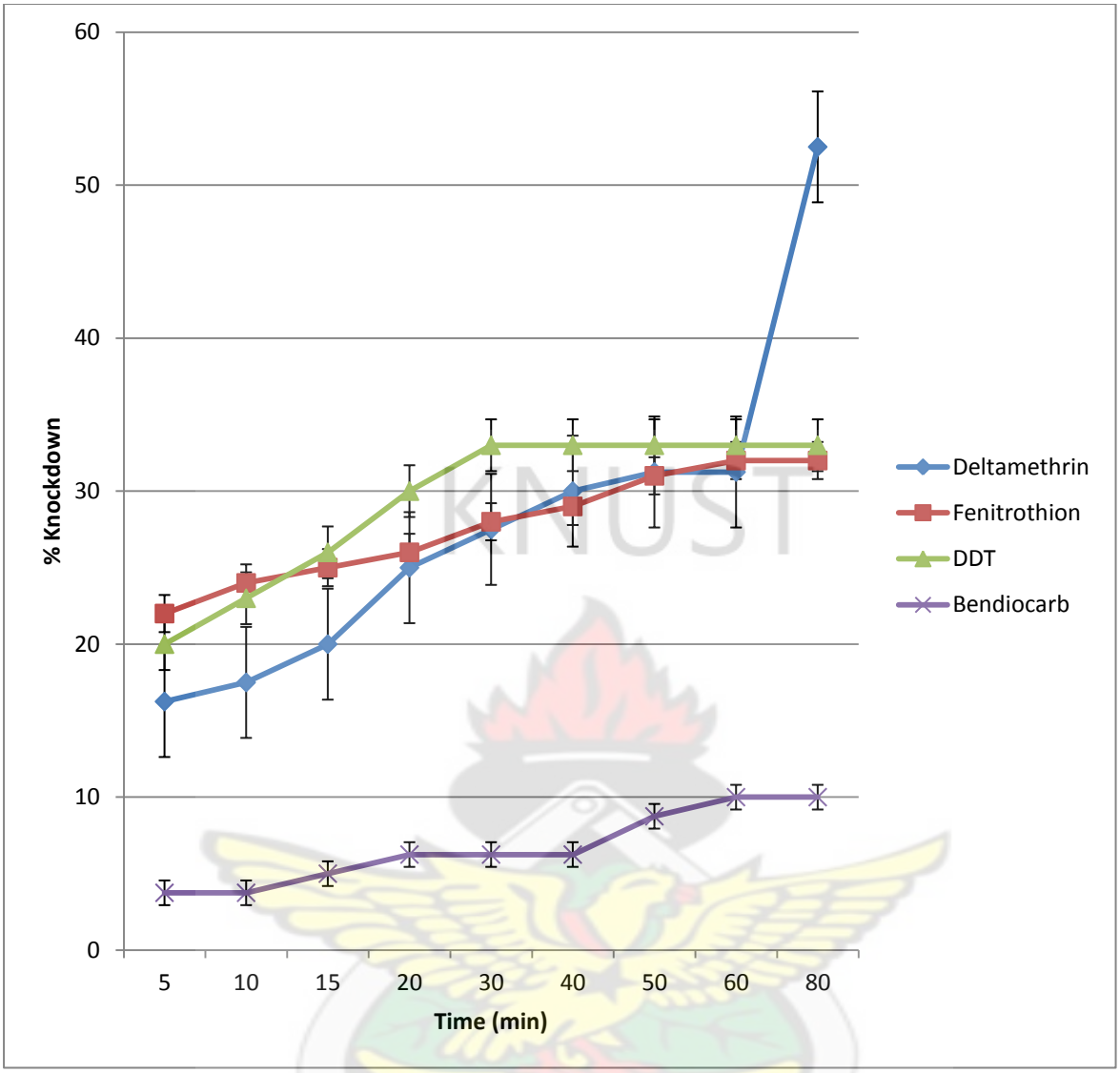


**Figure 4.6 Knockdown effects of all four classes of insecticides at COE 1.**

Knockdown at COE 2 shown below in Figure 4.7, revealed deltamethrin having the greatest knockdown effect at 60%, fenitrothion at 36.25%, bendiocarb 31% and DDT at 25%.

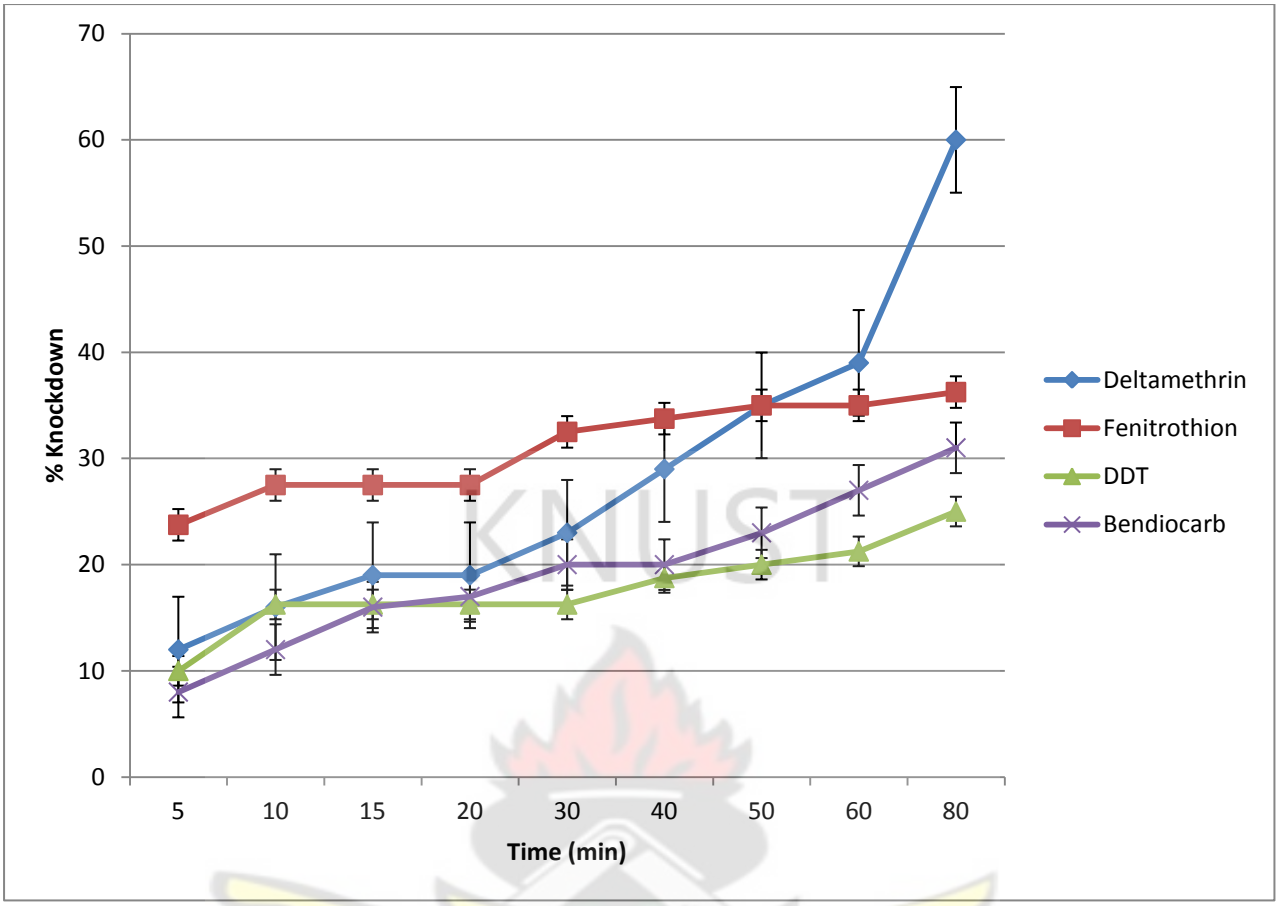


**Figure 4.7 Knockdown effects of all four classes of insecticides at COE 2.**

LR 1 in Figure 4.8 below had fenitrothion showing the highest knockdown effect at 30%, DDT at 22.5%, deltamethrin 21% and bendiocarb at 20%.

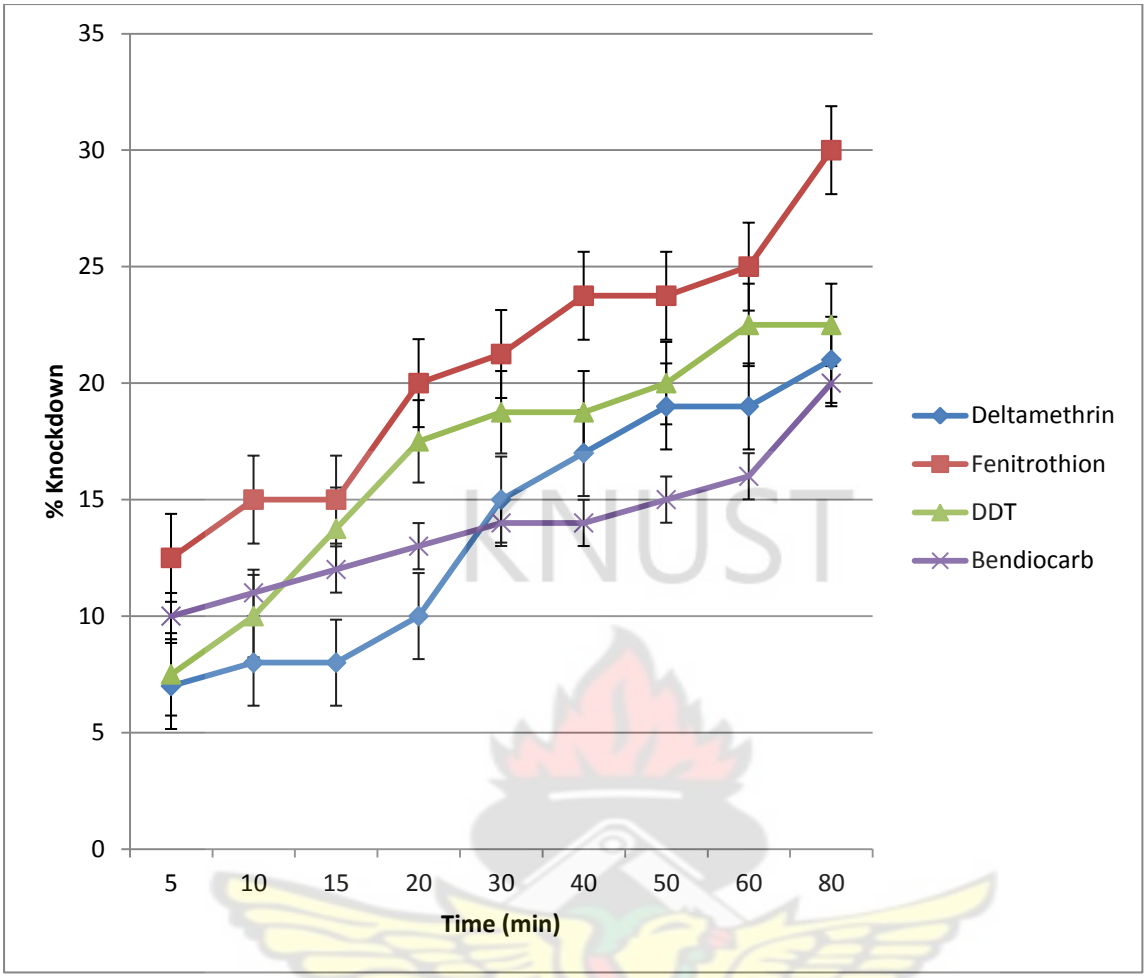


**Figure 4.8 Knockdown effects of all four classes of insecticides at LR 1.**

LR 2 showed bendiocarb having the greatest knockdown effect at 44%, fenitrothion at 35%, DDT at 26.5% and deltamethrin at 22.5%. This is shown in Figure 4.9 below.

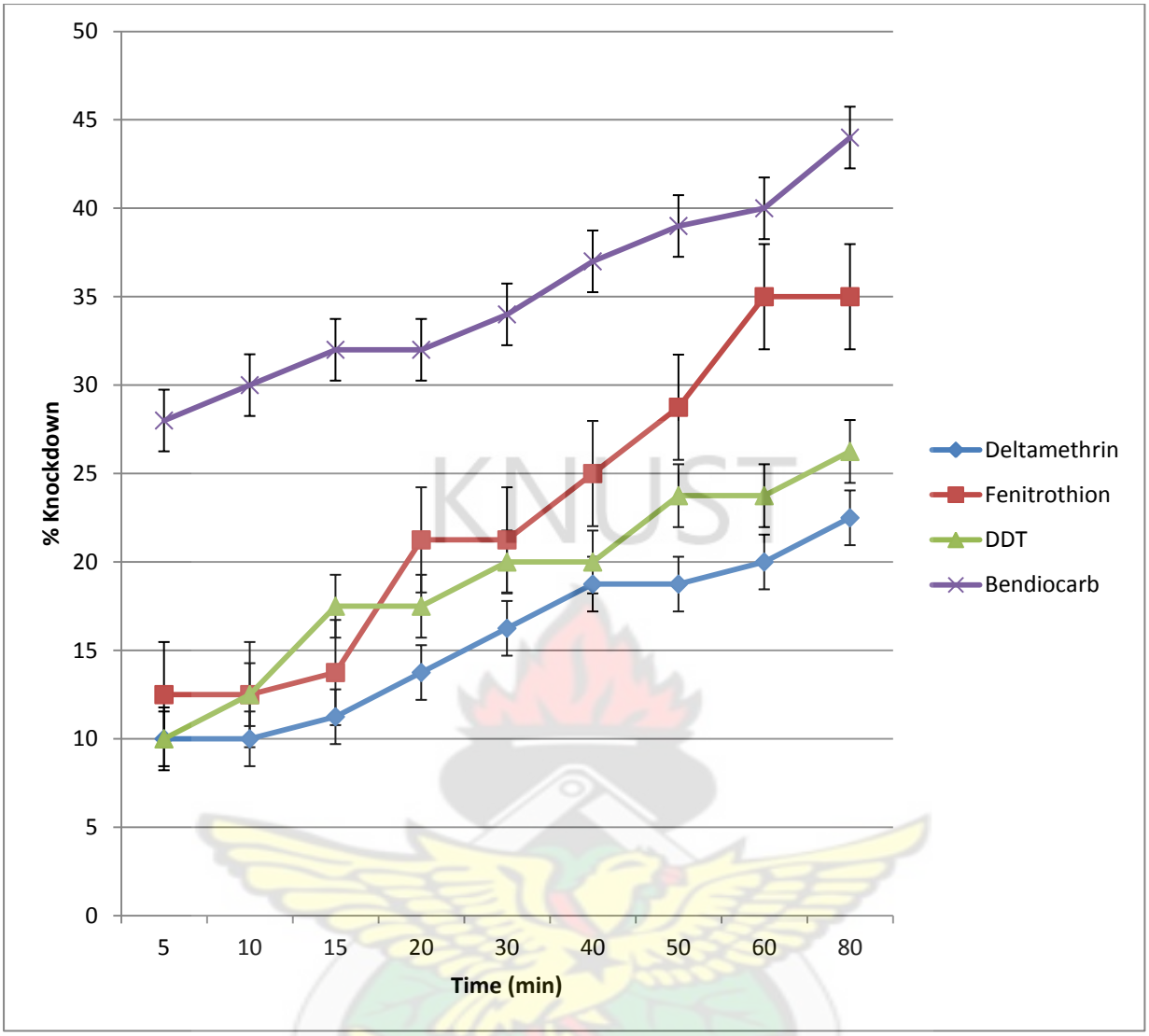


**Figure 4.9 Knockdown effects of all four classes of insecticides at LR 2.**

**Table 4.7 Overall knockdown effects of insecticides on *Anopheles* mosquitoes on KNUST campus, Kumasi.**

| % Knockdown(Kd) after exposure to insecticides for 80 minutes | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Insecticide** | **SMS 1** | **SMS 2** | **SMS 3** | **COE 1** | **COE 2** | **LR 1** | **LR2** |
| **Deltamethrin** | 48.75 | 21.25 | 32 | 52.5 | 60 | 21 | 22.5 |
| **Fenitrothion** | 11.25 | 30 | 28 | 32 | 36.25 | 30 | 35 |
| **DDT** | 12.5 | 18.75 | 48 | 33 | 25 | 22.5 | 26.25 |
| **Bendiocarb** | 55 | 47 | 28 | 10 | 31 | 20 | 44 |

There was no significant difference in knockdown effects across all breeding sites, $p > 0.05$.

## 4.4 Association between susceptibility status and physical characteristics of breeding sites

Bivariate correlation analysis of pH, conductivity, turbidity and temperature measured and susceptibility status of insecticides showed no significant association, $p > 0.05$.

**Table 4.8 Correlation between susceptibility status of insecticides and physical parameters**.

| | **pH** | **Conductivity** | **Turbidity** | **Temperature** |
|---|---|---|---|---|
| **Deltamethrin** | 0.140 | -0.135 | -0.226 | -0.762 |
| **Fenitrothion** | -0.700 | 0.283 | 0.715 | 0.485 |
| **DDT** | -0.253 | 0.261 | 0.584 | 0.623 |
| **Bendiocarb** | -0.139 | 0.277 | -0.148 | -0.600 |

**Level of significance = 0.05 (2 tailed)**

### 4.5 Knowledge, attitudes and perceptions on ITN use

#### 4.5.1 Demographic data

600 questionnaires were administered randomly to respondents on campus and the immediate surroundings. The age group of respondents ranged from 18 to 61 years. It comprised 321 (53.5%) females and 279 (46.5) males. Majority of respondents had tertiary education 396 (66.0%), followed by senior high school education 85 (14.2%), basic education 64 (10.7%) and 55 (9.2%) never attended school. Furthermore, majority of respondents were students 391 (65.25%), 4 (0.7%) lecturers, 6 (1.0%) non teaching staff and 199 (33.2%) mostly traders and self employed people as shown in Table 4.9.

**Table 4.9 Demographics of respondents**

| Character | Frequency | % respondents |
|---|---|---|
| **Gender** | | |
| Females | 321 | 53.5 |
| Males | 279 | 46.5 |
| **Level of education** | | |
| Basic | 64 | 10.7 |
| High school | 85 | 14.2 |
| Tertiary | 396 | 66.0 |
| Never attended school | 55 | 9.2 |
| **Occupation** | | |
| Student | 391 | 65.2 |
| Lecturer | 4 | 0.7 |
| Non teaching staff | 6 | 1.0 |
| Others | 199 | 33.2 |



## 4.6 Places of residence of respondents

In accessing the places of residence of respondents, 154 (25.7%) respondents resided at the halls of residence, 293 (48.8%) resided at Ayeduase, 4 (0.7%) lecturer's residence, 6 (1.0%) junior staff residence, 113 (18.8%) at Kotei and 28 (4.67%) residing at Bomso and Tech junction 2 (0.3%), shown in Table 4.10.

**Table 4.10 Places of residence of respondents**

| Place of residence | Frequency | % respondents |
|---|---|---|
| Halls of residence | 154 | 25.7 |
| Lecturer's residence | 4 | 0.7 |
| Junior staff residence | 6 | 1.0 |
| Ayeduase | 293 | 48.8 |
| Kotei | 113 | 18.8 |
| Bomso | 28 | 4.7 |
| Tech junction | 2 | 0.3 |
| **Total** | **600** | **100** |



## 4.7 Chemical control interventions used

Figure 4.10 below shows the various chemical control interventions used by respondents, 199 (33.2%) used ITN, 75 (12.5%) use repellents 56 (9.3%) use coils 134 (22.3%) use aerosol sprays, 2 (0.3%) use impregnated curtains and 136 (22.5%) use none of the above mentioned control methods. 598 (99.7%) use screens on windows.

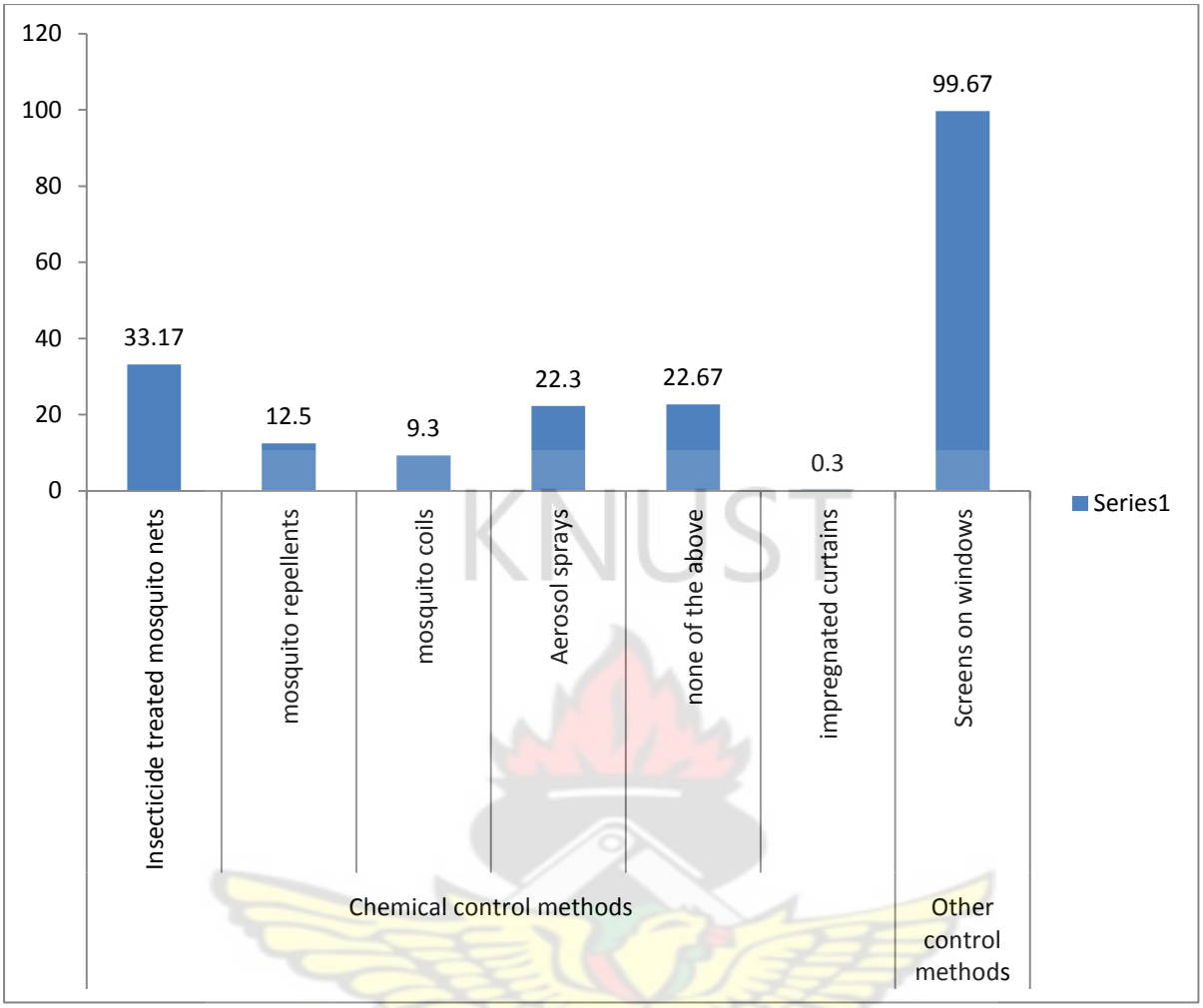


**Figure 4.10 The various chemical control methods used by respondents**

For the specific control methods used apart from the nets, those who preferred using aerosol spray used the following, 105 (17.5%) preferred Raid mosquito spray, 21 (3.5%) preferred Sasso spray, Heaven insecticide spray 5 (0.8%), Oro 3 (0.5%) and 466 (77.7%) do not use aerosol spray so the question did not apply to those respondents. The results are shown in Table 4.11 below.

**Table 4.11 Types of aerosol sprays used by respondents**

| Type of spray | Frequency | % respondents |
|---|---|---|
| Raid insecticide spray | 105 | 17.5 |
| Sasso mosquito spray | 21 | 3.5 |
| Oro mosquito spray | 3 | 0.5 |
| Heaven insecticide spray | 5 | 0.8 |
| Not applicable | 466 | 77.7 |
| **Total** | **600** | **100** |



Some respondents also preferred mosquito coils and the types of coils preferred are as follows, 6 (1%) like Raid mosquito coil, 37 (6.2%) use the Heaven black mosquito coil, 1 (0.2%) uses Jumbo coil, 12 (2.0%) use Holy black mosquito coil and 544 (90.7%) do not use coil. This is shown in Table 4.12.

**Table 4.12 Types of mosquito coils preferred by respondents**

| Type of coil | Frequency | % respondents |
|---|---|---|
| Raid mosquito coil | 6 | 1.0 |
| Heaven black coil | 37 | 6.2 |
| Jumbo coil | 1 | 0.2 |
| Holy black coil | 12 | 2.0 |
| Not applicable | 544 | 90.7 |
| **Total** | **600** | **100** |



Some respondents also preferred to use mosquito repellents and these are Off repellent 6 (1.0%), Medisoft mosquito repellent 39 (6.5%), Odomos 19 (3.2%), Sasso repellent 11 (1.8%) and 525 (87.5%) do not use repellents but prefer the other methods. This is shown in Table 4.13 below.

**Table 4.13 Types of repellents used by respondents.**

| Type of repellent | Frequency | % respondents |
|---|---|---|
| Off | 6 | 1.0 |
| Medisoft mosquito repellent | 39 | 6.5 |
| Odomos | 19 | 3.2 |
| Sasso repellent | 11 | 1.8 |
| Not applicable | 525 | 87.5 |
| **Total** | **600** | **100** |

### 4.7.1 Frequency of use of chemical control interventions

Respondents who use aerosol sprays, coils and repellents, 235 (39.2%) do not use them very often, 28 (4.7%) use them every night and 1(0.2%) gave no answer. For those who use ITN, 164 (27.3%) sleep in the nets every night and 35 (5.8%) do not sleep under the net every night. This is shown in Table 4.14 and 4.15 respectively.



**Table 4.14 Frequency of use of coils, aerosol sprays and repellents**

| Response | Frequency | % respondents |
|---|---|---|
| Every night | 28 | 4.7 |
| Not very often | 235 | 39.2 |
| No answer | 1 | 0.2 |
| Not applicable | 336 | 56.0 |
| **Total** | **600** | **100** |

**Table 4.15 Frequency of use of ITN**

| Response | Frequency | % respondents |
|---|---|---|
| Every night | 164 | 27.3 |
| Not very often | 35 | 5.8 |
| Not applicable | 401 | 66.8 |
| **Total** | **600** | **100** |

### 4.8 Use and non-use of ITN

Reasons given by respondents for non use of ITNs were as follows, 113 (18.8%) said it felt too hot to sleep in the bed net at night so they do not use it, 65 (10.8%) said it was uncomfortable to sleep in the bed net, 70 (11.7%) gave no reason for not using the net, 41 (6.8%) said they had no place to hang the net, 24 (4.0%) said they were allergic to the chemical in the net 1 (0.2%) said he had outgrown the use of the net and that he used it in childhood only, 6 (1.0%) said it made their rooms look clumsy, 17 (2.8%) said they had no mosquitoes in their rooms so they have no need for the bed net, 9 (1.5%) said the net was expensive and could not afford it, 39 (6.5%) said they did not see any necessity to sleep in the net and 14 (2.3%) preferred the aerosol spray or coil instead, 201 use ITN and therefore this question did not apply to those respondents. This is shown below in Table 4.16.

**Table 4.16 Reasons for non use of ITN**

| Reason | Frequency | % respondents |
|---|---|---|
| Heat | 113 | 18.8 |
| Uncomfortable | 65 | 10.8 |
| No place to hang | 41 | 6.8 |
| Allergies | 24 | 4.0 |
| Have outgrown the use | 1 | 0.2 |
| Makes their rooms look clumsy | 6 | 1.0 |
| No mosquitoes in their rooms | 17 | 2.8 |
| Not necessary | 39 | 6.5 |
| Prefer aerosol spray or coil | 14 | 2.3 |
| No reason | 70 | 11.7 |
| Not applicable | 201 | 33.5 |
| **Total** | **600** | **100** |



### 4.8.1 How ITNs were obtained

For those who use the ITN 78 (13%) purchased the bed nets themselves, majority 101 (16.8%) got theirs from the free distribution by the National Malaria Control Programme, 20 (3.3%) purchased one themselves and also got some from the free distribution, 401 (66.8%) do not use nets and therefore the question did not apply to them. This is shown in Table 4.17 below.

**Table 4.17 How respondents obtained their ITNs**

| Reason | Frequency | % respondents |
|---|---|---|
| Purchased | 78 | 13.0 |
| Given by free distribution | 101 | 16.8 |
| Purchased and also given by free distribution | 20 | 3.3 |
| Not applicable | 401 | 66.8 |
| **Total** | **600** | **100** |



### 4.8.2 Respondents' perception if ITN use was necessary

Respondents were interviewed to find out if ITN use was necessary, majority 535 (89.2%) said yes and 65 (10.8%) said no as shown in Figure 4.11 below.

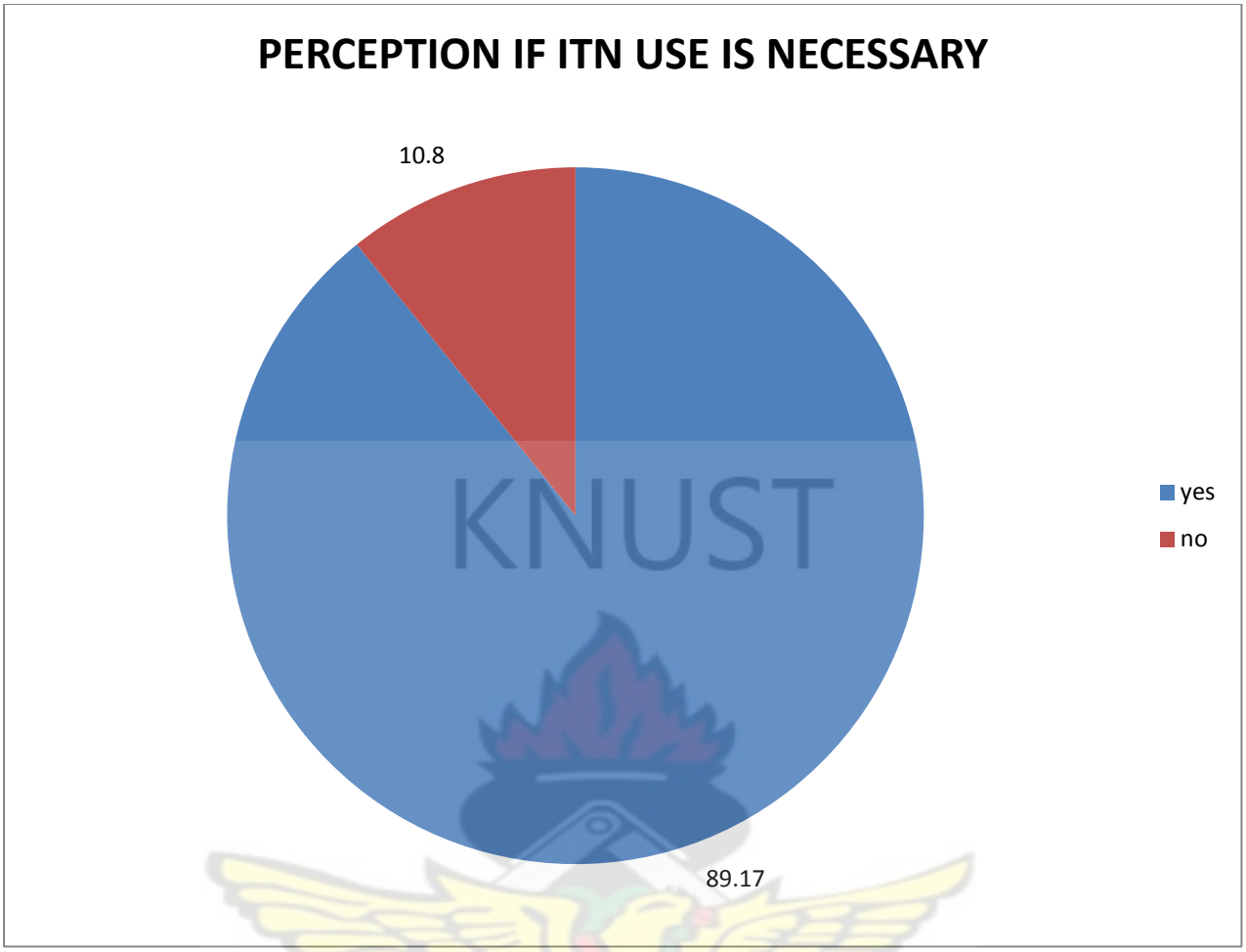


**Figure 4.11 Respondents perception if ITN use is necessary.**

### 4.8.3 Respondents reasons why ITN use is necessary

Respondents reasons why ITN use is necessary are as follows, 136 (22.7%) said to prevent malaria, 232 (38.8%) said to prevent mosquito bites, 115 (19.2%) said to prevent mosquito bites and malaria, 13 (2.2%) said it was a cheaper option, 20 (3.3%) said it was a more effective method for mosquito control, 25 (4.2%) gave no reason, 59 (9.8%) said the use of ITN was not necessary so the question did not apply to them. This is shown in Table 4.18 below.

**Table 4.18 Reasons why ITN use is necessary**

| Reason | Frequency | % respondents |
|---|---|---|
| To prevent malaria | 136 | 22.7 |
| To prevent mosquito bites | 232 | 38.7 |
| To prevent mosquito bites and malaria | 115 | 19.2 |
| It is a cheaper option | 13 | 2.2 |
| A more effective method | 20 | 3.2 |
| No reason | 25 | 4.2 |
| Not applicable | 59 | 9.8 |
| **Total** | **600** | **100** |



### 4.8.4 Respondents reasons why ITN use is not necessary

For the respondents who said ITN use was unnecessary as shown in Table 4.19, 31 (5.25%) said aerosol spray or coil gave them enough protection from mosquitoes, 11 (1.8%) said keeping their surroundings clean will destroy breeding sites and therefore give them no need for the bed net, 12 (2.0%) said it felt uncomfortable sleeping in the net, 5 (0.8%) gave no reason and 541 (90.2%) did not have this question apply to them because they agreed it was necessary to use ITN.

**Table 4.19 Respondents reasons why ITN use was unnecessary**

| Reason | Frequency | % respondents |
|---|---|---|
| Aerosol spray or coil provide enough protection | 31 | 5.2 |
| Clean surroundings destroys breeding sites therefore no need for ITN | 11 | 1.8 |
| uncomfortable | 12 | 2.0 |
| No reason | 5 | 0.8 |
| Not applicable | 541 | 90.2 |
| **Total** | **600** | **100** |



### 4.8.5 Risk of exposure to mosquito bites

In order to assess the risk of respondents being bitten by mosquitoes at night or at dawn or dusk respondents time of going to bed and time of awakening was inquired. The time of rising given by respondents were as follows, 33 (5.5%) wake up between 3 to 4am, 120 (20.0%) wake up between 4 to 5am, 99 (16.5%) wake up between 5 and 6am, 235 (39.2%) wake up between 6 and 7am, 106 (17.7%) wake up between 7-8am and 7 (0.2%) wake up from 9am to 11am. Time of going to sleep given by respondents were as follows 59 (9.8%) got to sleep between 8 and 9pm, 219 (36.5%) got to sleep between 9 and 10pm and 322 (53.7) go to sleep after 10pm. This is shown in Figure 4.12 below.

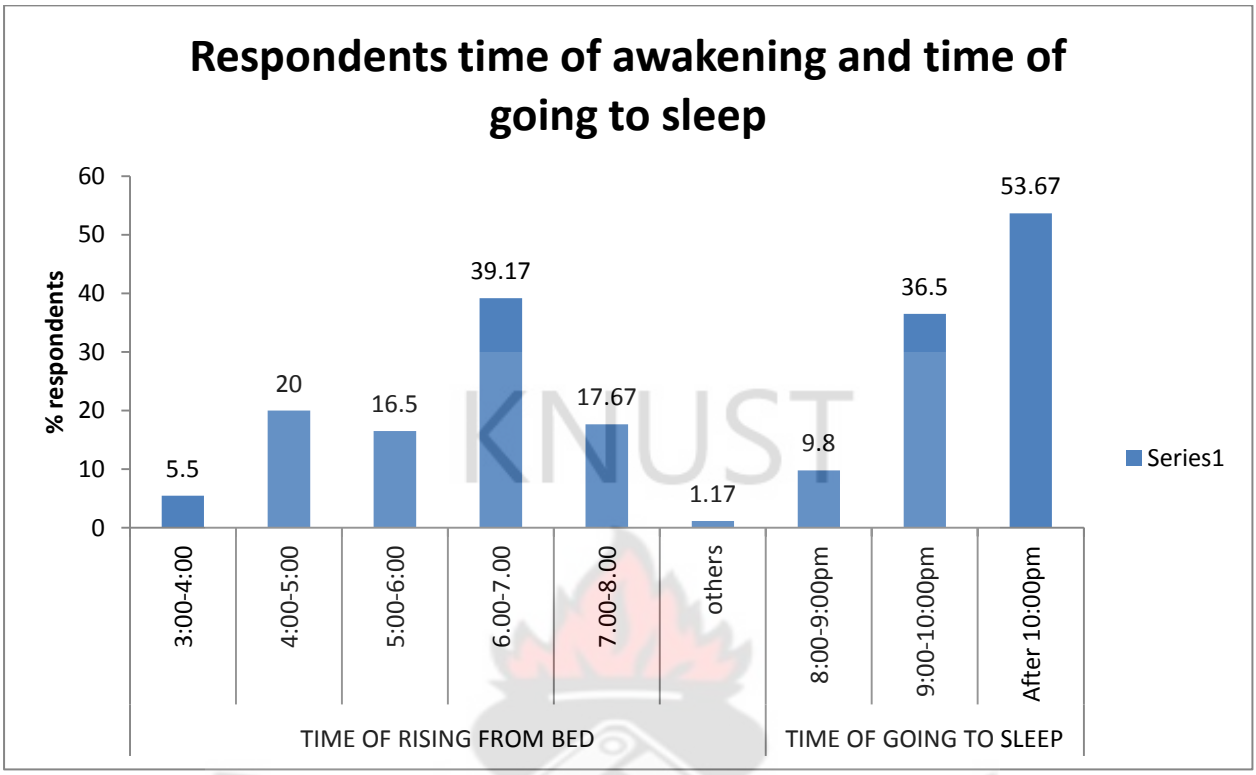


**Figure 4.12 Sleeping behaviour of respondents**

# CHAPTER FIVE

## 5. Discussion

The study showed that, all *Anopheles* breeding sites were man-made, all of the larval breeding sites were found at places that had been manipulated by man. This has been reported in other studies by Klinkenberg *et al*., (2008) and Kudom *et al.,* (2011). The most common species encountered was the *Anopheles gambiae s. l.* which was common in most of the breeding sites. This has also been reported in a research by Hunt *et al*., (2011), Afrane *et al*., (2012) in Ghana who also reported majority of species identified to be *Anopheles gambiae s.l.* Majority of breeding sites were found within the faculty area are as compared to the residential area. This may be due to the presence of a lot of farmlands which are close to water bodies like dugout wells and wetlands and also construction sites exposed to sunlight which create favourable breeding sites for anophelines.

Some of the breeding sites had both *Anopheles* larvae occurring together with larvae of culicines especially those with pH values of 5.0 and above, this is also reported by research done by (Sattler *et al*., 2005) that a pH value of less than 7.3 was normally associated with high culicine larvae. *Anopheles* larvae are known to be found in different types of breeding sites. They are normally small water bodies diversely scattered with different levels of turbidity. Most often directly under sunlight, temporary and found close to human communities as reported by (Gimnig *et al*., 2011). In this study physiochemical parameters varied in the different types of breeding sites identified and there were significant differences observed in all physical parameters measured. Differences may be due the different habitats where breeding sites occurred.

Susceptibility test results showed resistance to all four classes of insecticides across all the seven breeding sites with less than 60% mortalities after exposure to all four classes of insecticides. Field populations of *Anopheles gambiae* and *Anopheles funestus* showed resistance to deltamethrin, bendiocarb and even higher resistance to fenitrothion and DDT. The high level of resistance is supported by the increased median lethal time of deaths LT50 values recorded from all breeding sites. This may be due to the fact that most of the breeding sites were located on farmlands except COE 2 which was on a construction site. Data from the study suggests that pyrethroids are losing their efficacy for mosquito control in this study area and this is a major cause for concern since pyrethroids are used extensively in public health for mosquito control and also in agriculture (Awolola *et al*., 2002; Yawson *et al*., 2002) and the only class used for treating insecticide treated nets. This could also be as a result of extensive use of pyrethroids as pesticides in agriculture as reported by Kudom *et al*., (2011), Achonduh *et al*., (2008) and Yadouleton *et al.*, (2012). Interviews with farmers at breeding sites on vegetable farms showed that most of the pesticides they used were made from different formulations of pyrethroids especially lamdacyalohathrin. These pesticides may have been washed away by rains and collect into small water bodies that are potential breeding sites for mosquitoes therefore exposing them to the chemical at an early stage. This could later result in resistance to insecticides later in their adult stages due to the selection pressure.

Resistance to pyrethroids and DDT in this study may suggest the presence of target site resistance known as *Kdr* or knockdown resistance gene which is caused by a mutation in the voltage gated sodium channels of the vector as a result of continous exposure to pyrethroids or DDT as has been shown by Agyepong *et al.*, (2012) who found 26 out of 50 samples of mosquitoes possessing the *kdr* gene in the Kumasi metropolis. This may also suggest the

existence of cross resistance between DDT and pyrethroids as reported by Corbel *et al*., (2007); Koffi *et al*., (2012) in Benin and Ivory Coast respectively. Even though the use of DDT is not common, extensive use of pyrethroids in agriculture especially on the vegetable farms may be a cause of this. High resistance to fenitrothion and bendiocarb may also suggest the presence of metabolic resistance mechanism *Ace-1* on KNUST campus. Further molecular analysis is required to confirm this assertion.



There was no association between the insecticide susceptibility status of insecticides tested and physical characteristics of breeding sites. This suggests the need for further investigation into other chemical parameters of breeding sites to be able to find out if any degree of association exists. This was reported in research conducted by (Kudom *et al*., 2011), who found no correlation between physiochemical properties and occurrence of mosquito larvae and therefore in this case no effect on vector susceptibility.

### 5.1 Knowledge and perception on ITN use and risk of exposure to mosquito bites

In order to assess the various chemical control interventions used by inhabitants, it was realized that a lot of respondents preferred the use of bed nets as was reported by de la Cruz *et al*., (2006) who found that 90.7% of respondents used ITNs (doers) to prevent malaria whilst 76.6% of respondents do not use ITNs (non-doers) to prevent malaria. Inhabitants do not use the nets very often especially in the dry season when the weather is hot and rather use the spray or coil. Others also preferred the spray or coil because they had allergic reactions to ITNs; these include burning sensations in the eye and rashes all over their bodies. For the other control methods most respondents had screens on their windows and a few used

impregnated curtains. This may be due to the fact that the study area was a university community and inhabitants have a fair knowledge about the malaria vector. For those who preferred aerosol sprays Raid insecticide spray was the most commonly used, Heaven black mosquito coil was the most commonly used coil and Medisoft mosquito repellent was the most used among respondents. These according to respondents were the best on the market for protection from mosquito bites. Furthermore majority of respondents who used coils, aerosol sprays and repellents did not use them very often; they only use them when they realize they have a lot of mosquitoes in their rooms or when they cannot use bed nets especially when the weather is hot. Majority of those who used the ITN slept in it every night to avoid any form of contact with mosquitoes to prevent malaria.

Respondents had various perceptions about why they would use an ITN and why they would not use one. Reasons for not using one were mainly due to discomforts experienced due to the nature of their rooms being either too small or having no place to hang or heat in the night. This result was comparable to research done by Toe *et al*., (2009) who reported that technical difficulties related to fixing ITNs and the nature of the building in which they reside. Some have allergic reactions like burning sensations in the eyes, rashes on the skin and itchy eyes. Other reasons given were that: it made their rooms look disorganized; others were careful not to allow mosquitoes into their rooms and therefore did not need an ITN; others said they preferred the coil or spray because they felt imprisoned sleeping under a bed net; some also said the use of bed nets was only necessary in childhood and had therefore outgrown its use and finally some said the net was too expensive to buy and they could not afford it.

Due to free distribution of nets by the national malaria control programme, most respondents obtained their bed nets for free as reported by (Bernard *et al.*, 2009), that free distribution has greatly increased ownership of ITNs. A few also purchased it themselves and some also received theirs from the free distribution programme. This may be due to widespread exposure to education on malaria control on television, radio and other sources. There is now wide spread knowledge on what the ITN is. The widespread education on malaria control may also be the reason why majority of respondents thought use of ITN was necessary, there were some who had even never attended school before but knew what the bed net was and that it protected one from mosquito bites.

Respondent's reasons why they thought ITN use was necessary were mainly to prevent mosquito bites, this is comparable to research carried out by de la Cruz *et al.*, (2006) Adongo *et al.*, (2005) Netmark, (2004) who reported most respondents acknowledging that use of ITNs prevent malaria but Adongo *et al.*, (2005) reported that respondents agreed that ITNs reduced the nuisance of mosquitoes but do not prevent malaria.

Knowledge about malaria and how to control mosquitoes with insecticides was quite high at the study area and this may be due to the fact that it is a university community and also because knowledge may have been passed on to inhabitants in the immediate surroundings through interactions with students, public health programmes, education and awareness through the media. This is also reported in research conducted by (Rhee *et al.*, 2005), who realized that households that received net impregnation services as well as educational intervention used ITNs more than those that did not receive any education.

Reasons why some respondents thought ITN use was unnecessary were mainly associated with discomforts associated with sleeping in the nets. Majority therefore preferred the use of spray or coil. Others also due to knowledge about the vector said keeping their surroundings clean by cutting bushes, draining all stagnant water will destroy breeding sites and therefore give them no need for ITNs which is also reported by Toe *et al.*, (2009). Some also gave no reason because they just felt they do not have any reason but just did not feel like using one.



To assess the risk of exposure to mosquito bites between time of sleep and time of awakening by respondents was inquired. Most respondents wake up between 5 and 6am and may be at risk to bites by mosquitoes that bite at dusk. Traders and other self employed respondents who wake up even much earlier between 3 and 5am to go to the market may even be more at risk to mosquito bites. Respondents go to sleep after 10pm and this is because most of respondents were students but the others also go to sleep between 8 and 10pm therefore will escape contact with the malaria vector if they use any of the chemical control methods. Students may be at risk especially during exams period when they stay up to study all night which may expose them to vector bites from 10pm to dawn.

# CHAPTER SIX

## 6. Conclusions

The susceptibility status of *Anopheles gambiae s. l.* on KNUST campus determined from this study to the four classes of insecticides tested is resistant with the highest resistance observed in fenitrothion and DDT. There was no significant association between the susceptibility status of *Anopheles* mosquitoes as compared to the physical parameters of breeding sites measured.



Knowledge on the use of ITN was high among inhabitants on KNUST campus and immediate surroundings were high due to widespread awareness and education through the media and also free ongoing distribution of ITNs. Majority of respondents also knew that the bed nets protect them from mosquito bites and malaria. Chemical control methods used by inhabitants were ITNs, Aerosol sprays, mosquito repellents and mosquito coils. Most of them also used screens on windows, a few use impregnated curtains and then some also did not use any method of control because they kept their surroundings clean and did not see the need for use of any control method. Most respondents go to sleep after 10pm and may escape mosquito bites if they use any of the mentioned control methods but the traders who wake up between 3am to 5am may be at risk of being bitten and also students who study throughout the night during exams periods will be at risk of being bitten if they do not protect themselves.

## 6.1 Recommendations

The findings show that there is the need for more continous monitoring of resistance status here on KNUST campus because of resistance detected especially due to the pyrethroids. Most of the mosquito control measures used by inhabitants are made up of different formulations of pyrethroids except the repellents; this together with the extensive use of pyrethroids in pesticides on the farms on campus may have contributed to the high levels of resistance.



- I recommend further molecular studies to detect the presence of *Kdr* gene and other mechanisms of resistance within the population.
- I also recommend that pesticides used on vegetable farms should be rotated to remove the selection pressure so development of resistance will be slowed so that susceptibility of mosquitoes to insecticides may be restored.

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# APPENDICES

## APPENDIX 1

Questionnaire

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY



**This is a questionnaire designed to find out the various chemical control interventions used by inhabitants within the KNUST campus and surroundings for the control of mosquitoes. This will help provide information for a project to determine the presence of resistance in local mosquito vectors to insecticides. Please know that any information given will be kept confidential and used only for academic purposes. Thank you for answering this questionnaire**

**Please respond appropriately and tick the corresponding box**

**SECTION A**

**Personal information**

1. Sex : Female ☐ Male ☐
2. Age:

Educational background: 1. Basic ☐ 2. High school ☐ 3. Tertiary ☐

4. Never attended school ☐

Occupation: 1. Student ☐ 2. Lecturer ☐ 3. Non teaching staff ☐

Please specify any other occupation not mentioned…….

**Location**

1. Halls of residence ☐ 2. Ayeduase ☐ 3.Lecturer's residence ☐

4. Junior staff residence ☐

Please specify any other area not mentioned……..

**SECTION B**

**Chemical control methods used**

**For methods of application tick the appropriate method of application**

1. Indoor Residual spraying ☐ 2. Insecticide Treated mosquito nets ☐

3. Mosquito repellents ☐ please specify the type of repellent.....

4. Mosquito coils ☐ please specify the type of mosquito coil…….

5. Aerosol sprays ☐ please specify the type of spray ……..

6. Larviciding ☐ 7. None of the above ☐

**OTHER CONTROL METHODS**

1. Screens on windows ☐ 2. Impregnated curtains ☐

Other…….

Do you use any other form of chemical control? Yes ☐ No ☐

If yes please specify……

Formulation……

Frequency of usage…….

How long have you used the control method mentioned above…….

**SECTION C: If you use an Insecticide treated net (ITN) answer all the questions in this section except question 3, but if you do not use an ITN answer only questions 3, 6, 7 and 8.**

**USE OF INSECTICIDE TREATED NETS**

1. Who uses the nets……

2. How often is the net used…….

3. If insecticide treated nets are not used, please state the reason …….

……

4. How did you get the insecticide treated net, please specify……..

……..

5. How long has the net been used, please specify……..

6. What time do you wake up in the morning?

1. 3:00-4:00am ☐ 2. 4:00-5:00am ☐ 3. 5:00-6:00am ☐ 4. 6:00-7:00 ☐

5. 7:00-8:00 ☐ 6. Others ☐

7. What time do you go to sleep at night?

1. 7:00-8:00pm ☐ 2. 8:00-9:00pm ☐ 3. 9:00-10:00pm ☐ 4. After 10:00pm ☐

8. Do you think it is necessary to have a mosquito net at home?

Yes ☐ No ☐

Please state your reason for either choice…….

……

……

……

……