

CHAPTER ONE

1.0 INTRODUCTION

Malaria is a leading global public health problem. It is also one of the main causes of mortality and morbidity in the world (WHO, 2018). At the end of 2016, malaria was endemic in 91 countries compared to 108 in year 2000 (WHO, 2017). Despite this remarkable progress, malaria continues to have a devastating impact on people's health and livelihoods. Updated estimates indicate that there were 219 million cases of malaria incidence in 2017 globally, leading to 435,000 deaths (WHO, 2018). In 2017, World Health Organisation (WHO) African Region accounted for 93% of the deaths and fifteen countries accounted for 80% of the 435,000 deaths. Fourteen of these countries are found in sub-Saharan Africa except with Nigeria alone accounting for 25% of the malaria case (WHO, 2018).

The tropical African climate that favours the existence of most vector-borne diseases in the region has been attributed to the high intensity and prevalence of malaria in sub-saharan Africa (Githeko *et al.*, 2000). Malaria causes widespread premature death, financial hardship, holds back economic growth and compromises standard of living (Pluess *et al.*, 2010).

The life threatening parasitic disease is caused by *Plasmodium spp.* which is transmitted through the bites of infected mosquitoes of the genus *Anopheles*. There are four types of human malaria parasites; *P. falciparum*, *P. ovale*, *P. vivax* and *P. malariae*. Most (99%) malaria infections in sub-saharan Africa are caused by *P. falciparum* (Cox, 1982) while *P. vivax* accounts for majority of the incidence of the disease in other regions of the world (WHO, 2017).

In Africa, the main malaria vector that aids the transmission of the disease belongs to the complexes, *Anopheles gambiae sensu lato* (Giles) and *Anopheles funestus* (Giles) (Kelly-Hope *et al.*, 2009). From the sibling species of *An. gambiae sensu lato*, the two most important and are

widely distributed in sub-saharan Africa are *An. gambiae sensu stricto* and *An. arabiensis* (Coetzee *et al.*, 2000). Also, until the year 2011, the sibling species *An. gambiae* s.s is assumed to exist as two genetic variants; M and S forms (Wondji *et al.*, 2005) but the two siblings have now been discretely considered as *An. gambiae* s.s and *An. coluzzi* for the S and M forms respectively (Coetzee *et al.*, 2012). Both M and S forms have been reported in Nigeria by Awolola *et al.*, (2005) and Oduola *et al.*, (2010) and no studies have established a clear geographical or temporal pattern of the two forms. However, previous studies have reported that the S form were more predominant than the M form and the M/S hybrid form is very rare in West Africa (Constantini *et al.*, 2009, della Torres *et al.*, 2005)

For decades, malaria has consistently been one of the most devastating diseases of sub-Saharan African. This has made malaria control policies one of the most important cardinal points of health programs in all countries of sub-Saharan Africa. The malaria control policies have been built on two fundamental strategies; chemotherapeutic control and vector control. Vector control of malaria has proved to be very efficient in addressing the problem of malaria (WHO, 2017). This is because it prevents the incidence of the disease by eliminating the vectors. This approach usually involves elimination of both larvae and adults of the vectors using various methods including environmental modification as well as biological and chemical control measures. Of these three types of vector control measures, the chemical control is the most adopted considering it widespread, ease of use, availability of materials and effectiveness against the vector.

Chemical control of mosquito primarily entails the use of insecticides against the larvae and adults of mosquitoes. Two main strategies i.e Insecticide Treated Net (ITNs) and Indoor Residual Spray (IRS) (Pluess *et al.*, 2010) adopted have resulted in unprecedented progress in

malaria vector control efforts in most sub-Saharan African countries (WHO, 2017). IRS and ITNs are widely used in Africa but IRS has the advantage of been able to make use of a wider range of insecticides unlike ITNs, for which pyrethroids are the only class of insecticide currently used (WHO, 2012a).

Insecticide Treated Nets (ITNs) is a mosquito net that repels, incapacitates or kills mosquitoes that come into contact with it. This strategy imposes both chemical and physical barriers against insects. There are two main types of ITNs; the conventional nets and Long Lasting Insecticide Nets (LLINs) (Hill *et al.*, 2006; WHO, 2006). Indoor Residual Spraying (IRS) is a strategy in which residual insecticides are applied on the surface of walls and ceilings of houses (WHO, 2006). This strategy functions when the mosquitoes, after feeding on blood, rest on these treated surfaces and remain long enough to absorb lethal doses of the insecticide (Silva *et al.*, 2014).

The initial success of insecticide based strategies informed the optimism that trailed the possibility of eliminating malaria. These strategies are however, highly threatened today, due to the emergence of vector populations resistant to insecticides (Silva *et al.*, 2014). Insecticide resistance is the ability of an insect to evade the harmful effects of toxicants by the development of certain strategies (Silva *et al.*, 2014). It offers the insect an escape route from toxicants. The development of insecticide resistance by vectors constitute a major impediment that has slowed down and has the potential to completely hinder the control of vectors by the use of chemicals. Contrary to the successes achieved by the use of chemical strategies earlier in some part of Asia, Russia, Europe and Latin America (Roberts *et al.*, 2004), the development of insecticide resistance by mosquitoes had been a major setback to malaria abatement in all regions. Resistance is so widespread that it has been reported for the four classes of WHO recommended insecticides all across the world (WHO, 2017). Resistance of mosquitoes to all the recommended

classes of insecticides have been reported in different regions of Africa (Diabate *et al.*, 2002; Corbel *et al.*, 2007; Djouaka *et al.*, 2008) as well as in different part of Nigeria (Awolola *et al.*, 2002, 2005, 2007; Oduola *et al.*, 2012, 2018).

The geometric spread of insecticide resistance has been attributed to increase in the volume of insecticides released into the environment. Consequently, this leads to selection for resistance which will continue to increase with increase in the use of insecticides unless effective management strategies are implemented (Jones *et al.*, 2013). Thus, WHO (2012) in its Global Plan for Insecticide Resistance Management (GPIRM) proposed 4 strategies that can be employed in effective insecticide resistance management (IRM). Out of the four IRM strategies proposed by WHO, simulation models show that mixtures could greatly reduce selection for resistance when compared to other strategies (Denholm and Rowland, 1992). Yakob *et al.* (2011) also suggests synergism arising from interaction of insecticides can also lead to some desirable impacts. Furthermore, unlike the 3 other strategies which are only relevant in either areas of susceptibility or area of resistance, mixtures are highly relevant in helping to conserve susceptible species as well as overpowering resistant species. Therefore, mixtures are relevant in both areas of susceptibility and area of resistance (Ngufor, 2015). At present, these mixtures are not readily available but could be developed in the short term (WHO, 2012). Considering, the enormity of insecticide resistance and the negative impact on the highly desirable fate of reducing the burden of malaria, a workable plan involving the use of insecticide mixtures for better management of malaria vectors to avert resistance development becomes essential and imminent.

1.1 Justification of study

The spread of resistance to insecticides is already alarming as it has been recorded in at least 68 out of 80 endemic countries that provided data for 2010 -2017 (WHO, 2018). Resistance in malaria vectors to 2 or 3 of the 4 insecticide classes have been documented in 57 of these countries found in all WHO regions except Europe (WHO, 2018). Consequently, WHO projected that increase in resistance leading to pyrethroids failure at the current level of malaria vector control coverage will result to an ~15% increase in death of children under 5 years of age. Secondly, if insecticide resistance leads to increase in malaria incidence, a corresponding increase in the cost of diagnosis, treatment and case management will be witnessed. This will have a general effect on the GDP of nations as funds that are to be used in some other developmental project will be diverted to address increase in the incidence of the disease. Thirdly, increase in malaria case incidence will increase the pressure on the use of antimalarial drugs. Consequently, the pressure on antimalarial drugs may also speed up the development of drug resistance. This poses a great threat for the future of malaria elimination programmes. Furthermore, inadequate and delayed management of insecticide resistance may not only lead to the failure of IRS method in the control of malaria vector, but will lead to the emergence of resistance by numerous other vectors to all the available insecticides in use. Therefore, this dictates the need to address this challenge by trying one of the IRM strategies to avert this imminent challenge. This also becomes desirable and apt for a better and more effective vector control.

1.2 Significance of the study

Considering the fact that one of the most important methods employed in malaria vector control; chemical method of control, has been compromised due to the development of resistance by

vectors to the chemicals, there is a need to devise and try out strategies that would help manage the challenge so as not to jeopardize this method in totality. Use of mixtures has been proposed as one possible strategy useful in managing insecticide resistance to chemicals and it has been previously employed in agriculture and has turned out desirable outcomes (WHO, 2012a). Moreso, extended range of insecticides available for IRS has important benefits for insecticide resistance management and the long-term sustainability of malaria vector control. It allows for combination and development of safer, more residual insecticides which are paramount to the future of malaria vector control (Rowland *et al.*, 2013). It also allows for reformulation of insecticides from the currently used and approved set of insecticides, thereby offering one of the most pragmatic solutions to insecticide resistance in malaria vector control (Hemingway *et al.*, 2006).

While the availability of mixtures may also produce an alternative to pyrethroids for LLINs, Curtis *et al.* (1998) had earlier noted that insecticide mixtures may also help delay the development and/or spread of resistance.

1.3 Aim of the study

This study is aimed at;

- Exploring the possibility of enhancing malaria vector control using insecticide mixtures with reference to its potency and efficiency in reducing incidence of resistance development.

1.4 Objectives of the study

The specific objectives this study seeks to achieve are to;

- evaluate the knowledge, attitude and practice (KAP) of Lagos state residents to malaria vector control methods.

- establish a baseline for assessing the use of mixture on resistant populations of *Anopheles* by determining the susceptibility status of *Anopheles* in Lagos state to the four classes of insecticide (Pyrethroid, Carbamate, Organochlorine and Organophosphate) commonly used in vector control of mosquitoes.
- determine the susceptibility status of *Anopheles spp* to mixtures of insecticides.
- determine the resistance mechanisms adopted by resistant population of *Anopheles* mosquitoes in the study area.
- compare the activities of metabolic enzymes involved in insecticide resistance in individual insecticides and insecticide mixtures.

CHAPTER TWO

2.0 LITERATURE REVIEW

Malaria is one of the leading public health problems of the world especially in tropical and subtropical regions including Nigeria (WHO, 2018). It is caused by the pathogen; *Plasmodium* and prominent species prevalent to Africa are *P. malariae*, *P. ovale*, *P. vivax* and *P. falciparum*. *P. falciparum* and *P. vivax* are the primary cause of malaria in humans (Gething *et al.*, 2012). Until recent years, focus has been only on *P. falciparum* because it causes many deaths especially in sub-saharan Africa where *P. vivax* is rarer, however the prevalence of *P. vivax* in some parts of the world's most densely populated and impoverished regions, coupled with its now proven association with severe and fatal outcomes has made it a focal point in recent years (Howes *et al.*, 2016). In all, four species of *Plasmodium* are capable of infecting humans and they are transmitted by more than 60 of the 450 known species of anopheline mosquitoes (Neafsey *et al.*, 2015).

The vectors of the disease pathogen are mosquitoes which are organism belonging to the family Culicidae. The family is made up of 41 recognised genera incorporating about 3500 species, with most of them capable of transmitting disease pathogens known to infect human and some domestic animals for centuries (Foster and Walker, 2019). Human malaria is transmitted only by mosquitoes in the genus *Anopheles*, but not all species within the genus are efficient malaria vector (Neafsey *et al.*, 2015). *Anopheles gambiae* is the most important malaria vector in sub-saharan Africa (Holt, 2002).

2.1 Biology of *Anopheles*

2.1.1 Taxonomic lineage

Mosquitoes are metazoans with taxonomic lineage as follows;

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: Culicidae

The *Anopheline* species belong to the Sub-family: *Anophelinae* (Gillies and Coetzee, 1987).

2.1.2 Morphological identification of *Anopheles* mosquito

Typical of every dipteran, the body of *Anopheles* mosquito is made up of three major segments; the head, thorax and abdomen. There are usually hairs all around the body or sometimes they possess tufts of hairs in some species. There is a pair of antenna attached to head. The shafts of the antennae have hairs at the end and on the sides. The upper surface of the head has several hairs; the position and shape of these hairs are important as a means of identification (Gillies, 1967). These hairs are also important in species identification (Service, 1977). There is usually a pair of palp on both sides of the antenna and a distinguishing feature of the anopheline is that the length of their palp is usually of same length as the proboscis (Gilles and Coetzee, 1987). Also, another distinguishing feature of adult anopheline from other genera is the positioning of the stomach when attached to a surface. While the culicines position their stomach parallel to the surface they are attached to, the anopheline abdomen is positioned at 45° to the surface. (Gilles, 1988). Also, the larva stages are distinguishable with their parallel position on the surface of water (Gilles and Coetzee, 1987).

The wings of *Anopheles* mosquitoes have several veins made of costa, sub-costa and six other veins. There are several dark and pale ends on the veins and these dark and pale ends are used in identification of the different species of *Anopheles* (Gilles and Coetzee, 1987). The six veins after the costa and sub-costa are given numbers so as to aid the process of morphological

identification of the different species and the colouration and arrangement of these veins are specific to species. The leg is divided into three major parts; femur, tibia and tarsus. There are five tarsi on the leg and the colouration of the tarsi are also important features in the identification of different species (Gilles and Coetzee, 1987).

2.1.3 Reproductive biology of *Anopheles* mosquito

Like all mosquitoes, the development of *Anopheles* mosquito is in four stages: egg, larva, pupa and adult. The egg, larva and pupa stages are aquatic and span between 6-14 days. The development is depending on species type and other abiotic factors such as pH and temperature (Gilles and de Mellion, 1968). In the insectarium, under optimum conditions, adult anopheline live up to 4 weeks but the average life span is lesser under natural field conditions. Adult females lay eggs in aquatic and moist environment and about 50-200 eggs are laid at a time. Eggs usually do not survive desiccation and hatch within 2-5 days under normal conditions but may be delayed due to other factors such as temperature. The eggs hatch into the larva and the larva passes through four stages before developing into the pupa. The four stages are distinguishable with their sizes. The pupa of mosquitoes has a distinguishing comma shape. After a few days as a pupa, the dorsal surface of the cephalothorax splits and the adult mosquito emerges (Gillies and de Mellion, 1968).

2.1.4 Mosquito habitat and behaviour

Mosquito breeding habitats vary from water bodies such as fresh water swamps, marshes, rice fields and burrowed pits, to smaller collections of smaller man-made water such as ditch, fish ponds and drains (Sattler *et al.*, 2005). Mosquito larvae are usually not found in large uninterrupted water bodies with fishes and predators. Majority of the *Anopheles* spp in Africa breed in temporary and unpolluted water (Service, 1977). However, some species have been seen

to breed in water bodies polluted with organic matter in Dar-es-Salaam, Tanzania (Sattler *et al.*, 2005). Breeding preference of *Anopheles* mosquito is also known to be related to species as *An. funestus* and *An. arabiensis* are known to prefer some sets of breeding sites. Different species prefers different water bodies such as dams, cow footprints, vehicle ruts, clean gutters, gardens, fish ponds, construction sites, water reservoirs and drains (Klinkenberg *et al.*, 2005). *An. gambiae* s.l have also been reported to breed in water bodies polluted with organic matters (Sibomsana, 2002). Also, distribution and population of *An. gambiae* have also been reported to be impacted by urbanisation and level of salinity of breeding habitats (Akogbeto, 1995). Physical and chemical parameters of breeding sites also impact on *Anopheles* larvae development (Robert *et al.*, 1998). The diversity in breeding site characteristics, available toxins and pesticides has been attributed to the development of adaptive response in the form of genetic mutation and production of detoxification enzymes (Strode *et al.*, 2006).

Furthermore, *Anopheles* species feeds on both human and animals such as cattle. Most of the species are not exclusively attached to either humans or animals (Keating *et al.*, 2004), however, *An. gambiae* and *An. funestus*, have strong affinity for human blood while *An. quadriannulatus* is attracted to animals. This makes them the most efficient malaria vectors in the world (Coetzee *et al.*, 2000).

2.2 *Anopheles* species as disease vectors

Species of *Anopheles* mosquitos possess different ability in carrying pathogens. Also, strains of same species have been documented to possess different capacity in the transmission of pathogens (Charlwood *et al.*, 2002). Of all the sibling species of *A. gambiae* complex, *A. gambiae* s.s. (M & S) are known to be strong carriers of diseases pathogens. It should be noted that, *A. gambiae* complex was formerly composed of seven sibling species: *A. gambiae* s.s. (M &

S), *A. arabiensis*, *A. melas*, *A. merus*, *Anopheles quadriannulatus* species A, *A. quadriannulatus* species B and *Anopheles bwambiae*. They are morphologically indistinguishable; however, they can be classified according to fixed and polymorphic chromosomal inversions (Slotman *et al.*, 2005). However, in 2012, the M form of *A. gambiae* s.s and *A. quadriannulatus* species B was reclassified as *Anopheles coluzzi* and *Anopheles amharicus* respectively (Coetzee *et al.*, 2013).

2.3 Distribution of malaria vectors in Africa

Malaria is characterized by its biological diversity which is conditioned by the vector species that are involved in transmission (including their distribution, behaviour and vectorial capacity (WHO, 2005). It is also conditioned, among others, by seasonality of transmission, pathogenicity of parasites and species and by immune response of human hosts. A regional data base on the geographical distributions of vectors belonging to the *Anopheles gambiae* complex has been developed (Besansky *et al.*, 2004). The four main vector species *An. gambiae* s.s., *An. arabiensis*, *An. quadriannulatus*, *An. bwambiae*, *An. melas* and *An. merus* belonging to the *An. gambiae* complex have quite different distribution patterns. *Anopheles gambiae* s.s., globally the most important vector, is widely distributed in lowlands throughout inter-tropical Africa (Coetzee, 2004). *Anopheles arabiensis* has a wide distribution but is found predominantly in fringes and highlands: Southern and Eastern Africa, highlights, sahelian areas of Western and Central Africa (Petraca *et al.*, 1990). In these areas, it is commonly associated with *Anopheles funestus* and, to a lower extent, to *Anopheles gambiae* s.s. These areas are characterized by very seasonal transmission, most commonly of low intensity and by occurrence of outbreaks whose frequency and intensity are closely related to climatic conditions (WHO, 2005). *Anopheles melas* in the western Africa and *An. merus* in the East are mostly found in coastal areas where they can be locally important vectors, especially when associated with *An. gambiae* s.s. (WHO, 2005).

Anopheles gambiae s.s. is the primary vector in West Africa (Awolola *et al.*, 2002; Corbel *et al.*, 2007; Yadouleton *et al.*, 2009). This can be attributed, in part, to its relatively long life, strong anthropophily (the tendency to target humans for blood meal) and endophily, the tendency to enter and rest inside houses. Adult *An. gambiae* rest during the day inside the human habitats and emerge to feed at night. Their larvae tend to develop in temporary bodies of water, such as bodies of water, such as those found near agricultural areas or even stagnant water (Besansky *et al.*, 2004).

2.4 Mosquito vector control

In an attempt to alleviate the malaria scourge, different control methods have been employed and one very prominent and effective method is the control of the vector of the disease. The disease is primarily prevented through two main vector control interventions; Insecticide Treated Net (ITNs) and Indoor Residual Spray (IRS) (Pluess *et al.*, 2010) and these have resulted in unprecedented progress in malaria vector control efforts in most sub Saharan African countries (WHO, 2016). The success recorded as regards the decrease in incidence rate of malaria can be attributed to the spread of malaria control interventions. A study in South Africa which was aimed at promoting “insecticide use” in spraying indoor surface recorded significant positive impacts on mosquito control (Khairy *et al.*, 2017). Similarly, studies (Robert and Carnevale, 1991; D’Alessandro *et al.*, 1995; Cham *et al.*, 1996; Neville *et al.*, 1996, Binka *et al.*, 1996; Akogbeto *et al.*, 1996; Doannio, 2003) have reported the positive impacts of interventions targeted at the adult vectors; insecticide-treated mosquito net (ITN) and indoor residual spraying (IRS). In sub-Saharan Africa, the proportion of household with sufficient nets remains inadequate at 43% but household ownership of at least one ITN increased from 50% in 2010 to 80% in 2016 (WHO, 2017). However, fewer people at risk of malaria are protected by IRS with

a global decline of 5.8% in 2010 to 2.9% in 2016 (WHO, 2017). IRS and ITNs are widely used in Africa but IRS has the advantage of been able to make use of a wider range of insecticides unlike ITNs, for which pyrethroids are the only class of insecticide currently used (WHO, 2012). Insecticide Treated Nets (ITNs) is a mosquito net that repels, incapacitates or kills mosquitoes that come into contact with it. There are two main types of ITN; the conventional nets and Long Lasting Insecticide Nets (LLINs) (WHO, 2006; Hill *et al.*, 2006). Indoor Residual Spraying (IRS) involve spraying of inner surfaces of houses with some selected insecticides (WHO, 2006). The first insecticide employed in IRS was DDT (Mellanby, 1992). In 1995, WHO proposed the global eradication of malaria based on spraying of DDT inside houses. It was able to achieve success on a global scale but issues of environmental concern and development of insecticide resistance led to abortion of its use (Govere *et al.*, 2002). In spite of that, after the “Stockholm Convention on Persistent Organic Pollutants” in 2007, DDT was allowed in certain areas with re-high malaria burden (WHO, 2007).

Currently, malaria vector control using insecticides basically depend on Organochlorine (DDT) and 3 other classes of insecticides; Organophosphates, Pyrethroids and Carbamates. Organophosphates have the advantage of being biodegradable and non-cumulative in nature. However, they are chemically instable and highly toxic to vertebrates (Morales-Rojas and Moss, 2002). Also, carbamates which are derived from the methylcarbamic acid (Fukuto, 1990) are used as insecticides. They have low environmental persistence and are less toxic to living organisms than organochlorines. Pyrethroids are synthetic insecticides naturally found in leaves of *Chrysanthemum cinerifolius*. There is the type 1 Pyrethroid like permethrin, and type 2 like deltamethrin (Davies *et al.*, 2007). They are biodegradable, non-cumulative insecticides that rarely cause acute intoxication in birds and mammals (Palchick, 1996).

Unlike 50 years ago when DDT was the only insecticide on hand for IRS, there are now 12 WHO recommended insecticides (dichlorodiphenyltrichloroethane (DDT) wettable powder (WP); Bifenthrin (WP); malathion (WP); fenitrothion (WP); pirimiphos-methyl (WP) and emulsifiable concentrate (EC); bendiocarb (WP); propoxur (WP); alpha-cypermethrin (WP) & suspension concentrate (SC); cyfluthrin (WP); deltamethrin (WP); etofenprox (WP); lambda-cyhalothrin capsule suspension (CS) and (WP)) within the 4 chemical classes recommended for IRS ([WHOPES, 2007](#)).

The initial success of achieved with use of insecticide caused the optimistic sensation that the elimination of malaria is possible, however, these strategies are threatened today due to the development of insecticide resistance (Silva *et al.*, 2014). Contrary to the success achieved some years back in some part of Asia, Russia, Europe and Latin America where mosquito was eliminated or greatly reduced ([Roberts *et al.*, 2004](#)), the development of insecticide resistance by mosquito to the different insecticides has been a major setback to the progress previously witnessed. The selection for resistance will continue to increase with increase in the use of insecticides unless effective management strategies are implemented (Jones *et al.*, 2013).

2.5 Insecticide Resistance

Insecticide resistance is the ability of an insect to evade the harmful effects of toxicants by the development of certain strategies. It offers the insect an escape route from toxicants. It is an enormous challenge that has greatly hindered vector control activities in all regions of the world. Insecticide resistance is also defined as the acquired ability of an insect population to tolerate doses of insecticides which can kill the majority of individuals in a normal population of the same species (Dennehy & Dunley, 2009).

Insecticide resistance is mainly attributable to 4 mechanisms; decreased penetration, behavioral reaction, target site insensitivity and metabolic resistance. While decreased penetration and behavioral reaction contributes little to resistance in many cases (Ahmad and McCaffery, 1999), enhanced metabolic detoxification system comprising of esterases, glutathione enzyme complex, and cytochrome P450 monooxygenase (Hemingway *et al.*, 2004) and decreased target site sensitivity through mutations such as changes in the sodium channel that leads to so-called ‘knockdown resistance’, are the dominant forces which shape resistance to insecticide in mosquitoes (Hemingway and Ranson, 2000).

2.5.1 Target-Site Resistance

Target-site resistance occurs when there are conformational changes in the molecule that the insecticide normally attacks (typically within the nervous system), such that the insecticide no longer binds effectively to it and the resistant insect is therefore unaffected or less affected by the insecticide (Silva *et al.*, 2014). Two types of mutations have been reported for target site resistance in *An. gambiae*; knock-down resistance (*kdr*) mutation, which impact on the voltage-gated sodium channels (NaV) which is the target for both pyrethroids and DDT and also acetylcholinesterase mutation, which is the molecular target of organophosphates and carbamates (GPIRM, 2012).

2.5.1.1 Knockdown Mutation

Knock-down resistance mutation has been associated with pyrethroids and organochlorines. These neurotoxins share a similar mode of action by targeting NaV, which is present in cells of the central and peripheral nervous systems by changing the kinetics of propagation of nerve impulses (Scheleier and Peterson, 2011). Normally, action potentials are generated across the membrane in order to conduct electrical information throughout the nervous system. In an

inactive state, when the membrane is at the resting potential, the sodium channel is closed. However, upon channel activation, the membrane becomes depolarised. This causes the sodium channel to open by generating transient sodium current. After approximately 1 min, inactivation occurs due to a conformational change in the sodium channel that blocks the passage of ions across the membrane. When the membrane potential returns to the resting level, the channel closes (Vais *et al.*, 2001). Pyrethroids and DDT impact on the gating kinetics of voltage sensitive sodium channels (Lund and Narahashi, 1983) by slowing both the activation and inactivation of the channel. The resultant impact on the gating kinetics of NaV is termed knockdown effect which leads to loss of coordination and paralysis, often accompanied by spasms and tremors (Scheleler and Peterson, 2011).

2.5.1.2 Acetylcholinesterase Mutation

Biochemical assays in several mosquito species have identified insecticide resistance due to insensitive acetylcholinesterase (AChE), the target site of Organophosphate and carbamate insecticides. AChE has a key role in the nervous system, terminating nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine. The insecticides inhibit enzyme activity by covalently phosphorylating or carbamylating the serine residue within the active site gorge (Corbett, 1974). Insensitive AChE in *An. gambiae* has been extensively described by N'Guessan *et al.*, 2003.

2.5.2 Metabolic Resistance

2.5.2.1 Esterases

Esterases aid insecticides resistance through 2 major mechanisms; Quantitative changes and qualitative changes. The 2 mechanisms are not mutually exclusive; both have been found in *C. tarsalis* (Ziegler *et al.*, 1987).

2.5.2.1.1 Quantitative changes

Over-production of non-specific esterases as an evolutionary response to organophosphorus and carbamate insecticide selection pressure has been documented in numerous arthropod species including mosquitoes, cattle ticks, aphids and cockroaches. The most widely studied mosquito species demonstrating this resistance mechanism are members of the *Culex* genus (Hemingway and Karunaratne, 1998). The predominant cause of this excessive enzyme synthesis is amplification of the gene(s) within the genome (Vaughan and Hemingway, 1995), although up-regulated transcription without an underlying gene amplification event has been reported (Rooker *et al.*, 1996).

2.5.2.1.1 Qualitative changes

In some mosquito species with insecticide resistance, elevated carboxylesterase activity involves rapid hydrolysis of the insecticide rather than increased sequestration. This mechanism is almost always found in association with malathion resistance and gives a much narrower cross-resistance spectrum (sometimes malathion-specific) than the amplified esterase-based mechanism. Malathion carboxylesterase resistance has been found in *An. culicifacies*, *An. stephensi* and *An. arabiensis* (Herath *et al.*, 1987; Hemingway, 1983).

2.5.2.1 Monooxygenases

Cytochrome P450-dependent monooxygenases are an important and diverse family of hydrophobic, heme-containing enzymes involved in the metabolism of numerous endogenous and exogenous compounds. There are many reports demonstrating elevated P450 monooxygenase activities in insecticide-resistant mosquitoes, frequently in conjunction with altered activities of other enzymes (Hemingway *et al.*, 2004). For example, Vulule *et al.* (1999)

demonstrated elevated oxidase and esterase levels in permethrin-resistant *An. gambiae* from Kenya, suggesting co-regulation. Brogdon *et al.* (1999) have reported oxidase-based and esterase-based resistance mechanisms alone and in combination in permethrin-resistant *An. albimanus* from Guatemala. However, the identification of the particular P450 gene(s) associated with resistance has been far from easy in any insect (Hemingway *et al.*, 2004).

2.5.2.1 Glutathione S-transferases

Glutathione S-transferases (GSTs) are found ubiquitously in aerobic organisms. Their central role in detoxification and drug resistance pathways in mammals has now been established but additional functions are continually being attributed to this complex enzyme family (Ranson and Hemingway, 2004). There are two distantly related classes of GSTs, classified according to their location within the cell (Silva *et al.*, 2014): microsomal or cytosolic. At least six classes of insect GSTs have been identified in *An. gambiae* (Ranson *et al.*, 2002). The primary function of GSTs is generally considered to be the detoxification of both endogenous and xenobiotic compounds either directly or by catalysing the secondary metabolism of a vast array of compounds oxidised by the cytochrome P450 family. Elevated GST activity has been implicated in resistance to at least four classes of insecticides. Higher enzyme activity is usually due to an increase in the amount of one or more GST enzymes, either as a result of gene amplification or more commonly through increases in transcriptional rate, rather than qualitative changes in individual enzymes (Ranson and Hemingway, 2004).

Furthermore, the molecular mechanisms involved in insecticide resistance in general and the functioning of up-regulated P450 genes, GSTs, and esterases in particular are not yet totally clear (Mouhamadou *et al.*, 2014), however, increased expression of these enzymes have been correlated with insecticide resistance (Li *et al.*, 2007). Management of insecticide resistance is a

big challenge in insect management, however, insecticide resistance management will be possible if the underlying mechanisms employed in conferring resistance are better understood (Muthusamy *et al.*, 2013).

2.6 Insecticide resistance management

Considering the enormity of the challenges posed by insecticides resistance to malaria vector control, WHO developed some insecticide resistance management (IRM) strategies to address the problem in its Global Plan for Insecticide Resistance Management (GPIRM) in malaria vectors launched in 2012. The IRM strategies are intended to maintain the effectiveness of vector control despite the threat of resistance. These IRM strategies are not novel because they have been used in the past to address some agricultural and public health challenges (WHO, 2011). The proposed strategies are; rotation of insecticides, combination of interventions, mosaic spraying and use of mixtures (WHO, 2012).

- Rotation of insecticides: Two or preferably more insecticides with different modes of action are rotated from one year to the next.

- Combination of interventions: Two or more insecticide based vector control interventions are used in a house (e.g. pyrethroids on nets and another insecticide of a different class on the walls). This will ensure that the same insect is likely but not guaranteed to come in contact with the second insecticide if it survives exposure to the first insecticide.

- Mosaic spraying: One compound is used for malaria vector control in a geographic area and different compounds are used in neighbouring areas, with the insecticides belonging to different insecticide classes.

- Mixtures: Two or more compounds of different insecticide classes are mixed to make a single product or formulation. This ensures that the mosquito is guaranteed to come in contact

with the two classes at the same time. In addition, synergists which can considerably enhance the potency of an insecticide could be used in combinations.

CHAPTER THREE

3.0 MATERIALS AND METHODS

The study was carried out in two phases; in phase one, I assessed community knowledge, attitude and practices to malaria vector control in Lagos Nigeria and in phase two, I investigated the susceptibility status of *Anopheles* population in Lagos state to the Permethrin, DDT, Propoxur, Pirimiphos-methyl and combinations of these insecticides. I also assessed and compared the activities of metabolic enzymes in *Anopheles* population induced with individual insecticides and combinations of the insecticides. In respect of the study, ethical clearance with Approval Number, UERC/ASN/2016/244 was obtained from the University of Ilorin Ethical Review Committee (UERC), Ilorin, Nigeria.

3.1 Study Area

Lagos state is one of the six states which constitute south-west zone in Nigeria (Figure 1). Although, it is the smallest state in Nigeria, it is the commercial nerve centre and former capital. It is located along the west coast of Africa between longitude 2°42'E and 3°22'E and Latitude 6°22'N and 6°42'N. The state has a total area of about 3,577.28 square kilometers including water bodies and it is the north and east by Ogun State, bounded to the south and west by the Coast of the Atlantic Ocean and the Republic of Benin, respectively. It is the second most populous state with a population of 9.01 million in 2006, which accounted for 6.44% of the total population of the country in the 2006 National Census (NBS, 2007).

Politically, Lagos State has 20 Local Government Areas (LGAs) and 37 Local Council Development Areas (LCDAs) categorised into three senatorial districts; Lagos East, Lagos West and Lagos Central. Lagos Mainland LGA was randomly selected from the 5 LGAs in Lagos Central district (comprising of Lagos Mainland, Lagos Island, Eti-Osa, Apapa, Surulere), Kosofe

LGA was randomly selected from the 5 LGAs in Lagos East (Kosofe, Epe, Ikorodu, Shomolu and Ibeju Lekki) and Ojo LGA was randomly selected from the 10 LGAs in Lagos West division (Ojo, Ifako Ijaye, Ikeja, Ajeromi Ifelodun, Mushin, Badagry, Amuwo Odofin, Oshodi Isolo, Alimosho and Agege). The choice of the 3 LGAs were informed by the resistance profile of *Anopheles* from previous researches (Awolola *et al.*, 2002; 2005; 2007; 2018; Oduola *et al.*, 2010; 2012). Lagos Mainland LGA is an urban LGA with a total area of 19.62 square kilometres. It is found at the central part of Lagos. The indigenous dwellers are the Otto-Awori but now inhabited by almost all other ethnic groups. Kosofe LGA is located at the northern part of Lagos State and bounded by three other local government areas. It covers a total area of 84.4 square kilometers. The indigenous dwellers of Kosofe were mainly the Awori whose major occupations then were mat weaving, farming and fishing. Ojo LGA has a total area of 182 square kilometres with a sizeable proportion of about 19 square kilometres covered by water. It is found in the Eastern part of the state.

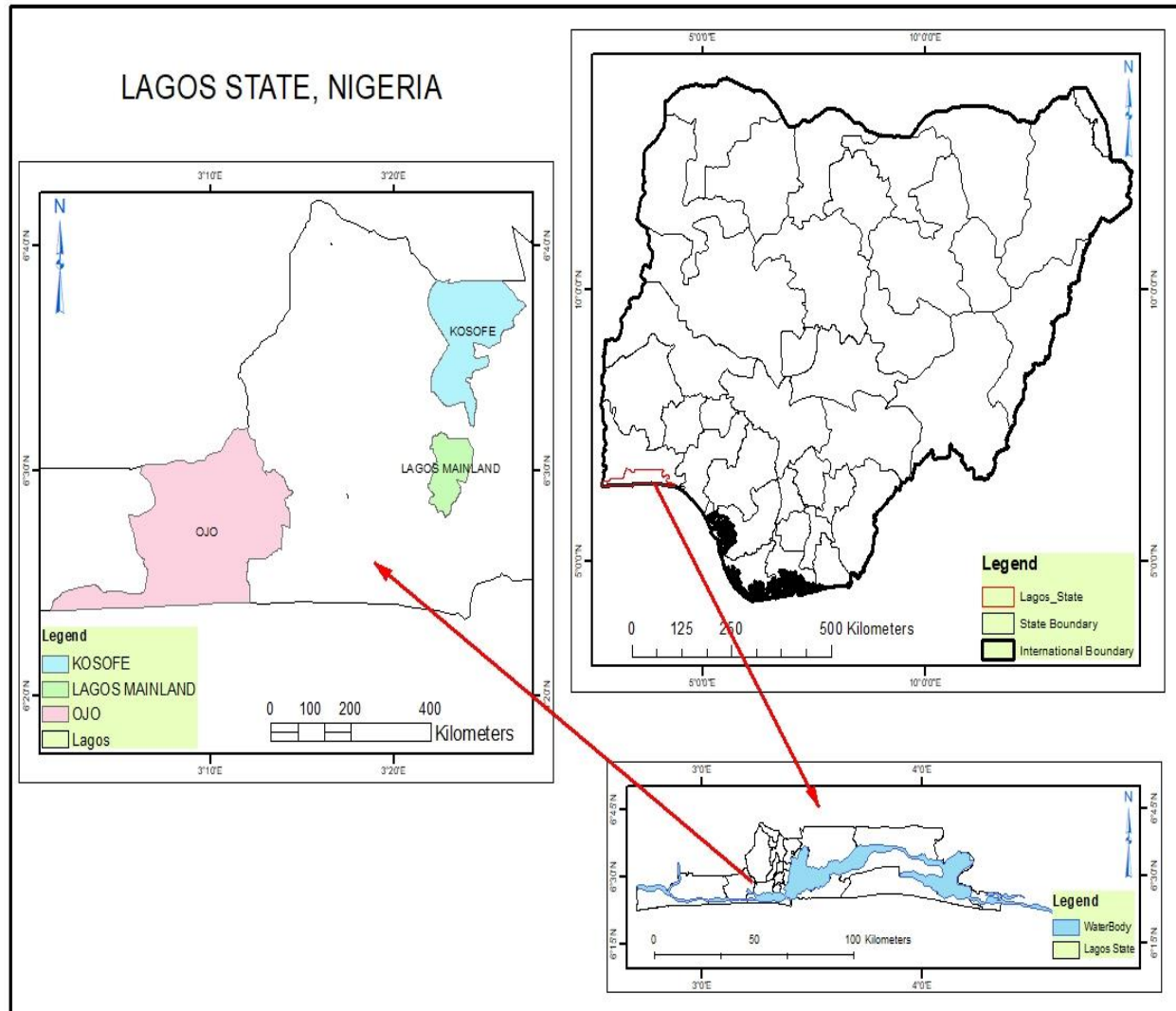


Figure 1: Map of study area

3.2 Knowledge, Attitude and Practices to Malaria Vector Control Methods

3.2.1 Research design

The study was a cross sectional survey of the knowledge, attitude and practices to malaria vector control methods among residents of Lagos state, South-West, Nigeria. The study was conducted between May and August 2016 in Lagos state, South-West, Nigeria.

3.2.2 Sampling Procedures

A multi-stage sampling technique involving a combination of sampling procedures was used to select a representative sample. Cluster sampling technique was first used to divide Lagos along the 3 senatorial zones in the state. After which one Local Government Area (LGA) from each of the 3 senatorial districts was selected based on previous reports of insecticide resistance in *Anopheles spp.* Four (4) communities were randomly chosen from each of the LGAs. Individuals from different households from the four communities were then randomly selected for the interview. Permission was sought from local authorities where necessary and the questionnaires were administered in the form of interview by house-to-house visitation in the communities. The sample size of 384 was calculated as the minimum sample size (Lemershow *et al.*, 1990), however, 520 questionnaires were administered. The sample size was divided across the LGAs in accordance with the human population (Table 1). The questionnaire was validated by experts for content validity and a pretest was done to test the reliability of the questionnaire before the study.

3.2.3 Inclusion and exclusion criteria

Inclusion criteria are limited to respondents aged 18 years and above and residing in the study areas.

Table 1: Projected 2015 human population distribution in LGAs for questionnaire allocation

Local Government Area	Growth Rate for Lagos State *	2006 Human Population in LGAs	Projected 2015 Population	% Population distribution of LGAs	Number of Questionnaire required	Number of Questionnaire Allocated
Kosofe	3.2%	682, 772	935, 562	42.60	164	220
Lagos Mainland		326, 700	447, 657	19.56	75	100
Ojo		609, 173	834, 714	37.84	145	200

*Population growth rate according to the Lagos state government
Population statistics according to the National Population Commission (NPC, 2006)

3.2.4 Data Collection on Malaria Vector Control Knowledge, Attitude and practices

Data was collected using a structured interview-led questionnaire. Data was collected by the researchers and two other trained data collectors. The structured questionnaire (Appendix 1) was designed to capture;

- socio-demographic characteristics of respondents
- knowledge of malaria vector control methods
- practice to malaria vector control methods
- attitude to malaria vector control methods
- insecticide in use
- Status of malaria vector control programmes in Lagos state.

The socio-demographic section assessed age, marital status, level of education and number of household members. Knowledge of malaria control section examined; awareness in respect of methods employed in mosquito prevention and control, preference and reason(s) for the methods and government/NGOs community mosquito control programmes. Questions on attitude and practices to IRS and ITNs include time span of usage, frequency of use, timing of use and attitude to irritation from insecticide. A pretest was done to test the reliability of the questionnaire. The questionnaire was validated by experts for content validity.

3.3 Insecticide Susceptibility Profile of *Anopheles spp* in Lagos to the four classes of insecticide commonly used in vector control of mosquitoes

The susceptibility status of *Anopheles* mosquitoes in Lagos state to the four classes of insecticide commonly used in vector control of mosquitoes was investigated to provide the baseline for the assessment of the insecticide mixtures. *Anopheles* larvae were harvested from the study site and

reared in the insectary at NIMR to adult. The adult mosquitoes were then exposed to the various individual insecticides. The following insecticides; DDT, Permethrin, Propoxur and Pirimiphos-methyl were selected as representative members of Organochlorines, Pyrethroids, Carbamates and Organophosphates respectively.

3.3.1 Field Larval Sample Collections

Mosquito larvae were collected from natural breeding sites (ponds, pools, streams, surface water, ditches, brick pits) in each LGA. Anopheles larvae were identified by morphology and their horizontal position on water surface. Larvae were scooped into a small transparent container and conveyed to the insectary of the Nigerian Institute of Medical Research (NIMR).

3.3.2 Mosquito larvae rearing in Insectary

Once in the insectary of Nigerian Institute of Medical Research (NIMR), the water containing pre-adult mosquitoes was poured into plastic trays (5cm x 27cm x 36cm) to a depth of approximately 2cm and each tray was covered with nets labeled to reflect the site and date of collection following the procedure of Das *et al.* (2007). The trays containing mosquito larvae were kept at 25-28°C and 76±5% relative humidity. The immature larvae were fed with 200mg of ground fish-food and yeast powder once every morning. The development of the larvae was monitored regularly and the emerged adults were collected with the aid of aspirator into well labeled cages reflecting the LGA and date of emergence. Emerged mosquitoes were fed 10% sugar solution imbibed in cotton wool. The adult cages were kept on shelves and positioned away from direct contact with the wall to prevent ant infection which would be attracted by the sugar. Only 2-5 days old female adult mosquitoes were picked from the cages and used for further bioassays.



Plate 1: Adult mosquitoes emerging from transparent containers containing larvae collected from field



Plate 2: 2 – 5 days old Adult mosquitoes in cage at NIMR

3.3.3 Morphological identification of female *Anopheles* spp.

Male mosquitoes were separated from the female mosquitoes using the morphological keys described as Gillies and Coetzee (1987). Males were distinguished by their large club-shaped palps and bushy antenna while, in adult females, the mouthparts were pointed, the palps were the same length as the proboscis and the antenna did not appear as bushy as those of males.

3.3.4 Insecticide Susceptibility tests

The insecticide susceptibility test was carried out in line with WHO standard procedures (1998). Mosquitoes were exposed to insecticide papers prepared at University Sains Malaysia. Insecticide papers impregnated with WHO recommended discriminating concentration of 0.05% permethrin, 4% DDT, 0.1% propoxur and 0.25% pirimiphos-methyl were used for the study. Four batches of 25 unfed female mosquitoes, aged 2–5 days, were exposed to insecticide treated papers for 60 min at $27 \pm 1^\circ\text{C}$ and 80% relative humidity. The setup was monitored at different time intervals (10, 15, 20, 30, 40, 50, 60 minutes) and the number “knocked-down” was recorded. Unfed *An. gambiae* ss (Kisumu strains) female adults of 1–3days post-emergence was used as reference to validate the quality of treated papers. After one hour of exposure, mosquitoes were transferred into holding tubes and provided with cotton wool saturated with a 10% honey solution. Two batches of 25 unfed female mosquitoes (from same population) exposed to untreated papers were used as control. Mortalities was recorded after 24 hours and the susceptibility status of mosquito samples was determined according to WHO criteria (WHO, 1998); i.e.

- Mortality rate is $> 98\%$; population was considered fully susceptible.
- Mortality rates ranged between 90 - 98%; resistance suspected in the population.
- Mortality rates $< 90\%$; population was considered resistant to the tested insecticide.

The percentage mortality was calculated using the formula shown below:

$$\text{Percentage mortality} = \frac{\text{Total number of dead mosquito after 24 hours}}{\text{Total number of exposed mosquito}} \times 100\%$$



Plate 3: WHO susceptibility test showing test experiment and control

3.3.5 Mosquito Handling, Morphological Identification and Storage

The resistant and susceptible mosquitoes were now harvested and separated into different cages and the resistant ones were demobilised by placing the cage in the freezer for about 10mins. All the mosquitoes used for the test were examined under the microscope (Olympus SZ40) and identified to species level using taxonomic keys adopted by Gilles and Coetzee (1987). They were stored in appropriately labelled Eppendorf tubes (Eppendorf International, Germany) containing silica gel and kept in the laboratory for molecular analysis of species and resistance genes involved in insecticide resistance.

3.4 Insecticide Susceptibility Profile of *Anopheles spp* in Lagos to Insecticide Mixtures

The procedures for determining susceptibility profile of *An. gambiae* to insecticide mixtures is same as the procedures for determining susceptibility profile of *An. gambiae* to individual insecticides. The insecticide classes were mixed in the following format; Pyrethroid + Carbamate (Permethrin + Propoxur), Pyrethroid + Organophosphate (Permethrin + Pirimiphos-methyl), Carbamate + Organochlorine (Propoxur + DDT) and Organophosphate + Organochlorine (Pirimiphos-methyl + DDT). The other possible combinations of insecticides were left out due to the presence of mutational cross resistance between insecticides class.

3.5 Molecular Analysis of Sibling Species and Resistant Genes

Polymerase chain reaction (PCR) was used in identification of sibling species of *An. gambiae* s.l and restriction fragment length polymorphism (RFLP) was employed in distinguishing *An. coluzzi* and *An. gambiae* s.s. Genomic DNA of resistant mosquitoes were extracted and used for analysis of resistant genes. The extracted DNA were stored under -20°C.

3.5.1 DNA Extraction

Three hundred and seventy-five (375) resistant and thirty (30) susceptible samples were randomly selected from the whole population of mosquitoes. The legs of the samples were carefully moved with a sterile forceps and deposited in a 1.5ml eppendorf tube. The following steps as described by Scott *et al.*, (1993) were followed for DNA extraction.

1. Homogenising aliquot in 100ml of grinding buffer
2. Centrifuging for 20 secs at 14,000 rpm
3. Incubating at 65⁰C for 1 hour
4. Adding 13µl of 8M potassium acetate solution
5. Placing on ice for 30 minutes
6. Centrifuging at 14,000 rpm for 15 minutes
7. Transferring supernatant to empty eppendorf tube and add 200µl ice cold 100% ethanol
8. Placing at room temperature for 5 minutes.
9. Spinning at 14,000 rpm for 20 minutes
10. Discarding supernatant and wash firstly with 200µl ice cold 70% ethanol, then 100% ethanol
11. Re-suspending DNA in 25ml sterile TE-buffer.

Grinding buffer is produced from 0.1M NaCl, 0.2M sucrose, 0.1 Tris-HCl, 0.05M EDTA, pH 9.1 and 0.5% SDS while the sterile TE-buffer is produced from 10mM Tris-HCl (pH 8.0) and 1mM EDTA.

3.5.2 Molecular Identification of Sibling Species of *An. Gambiae* s.l Using Standard Polymerase Chain Reaction (PCR).

Molecular identification of the sub-species of *An. gambiae* s.l was carried out using polymerase chain reaction (PCR) techniques proposed by Scott *et al.* (1993). One microlitre of the extracted

DNA was taken using a micropipette and transferred into separate tubes each of 12.5ul PCR-mix containing 1x GeneAmp PCR buffer I (New England Biolabs) 0.5ul MgCl₂, 1.25ul dNTPs, 4.9ul of water, 0.1ul of *Taq* and 1.0ul each of *Anopheles gambiae* s.s, *Anopheles arabiensis*, *Anopheles melas*, *Anopheles quadriannulatus* and Universal primers (New England Biolabs). Magnesium Chloride served to stabilize the template DNA and prevent complete denaturation of product at each cycle. The *taq* polymerase (Promega, France) served to extend the primers.

The primers used in this PCR had the following sequences: 5'CTGGTTTGGCGGCACGT-3', 5'AAGTGTCTTCTCCATCCTA-3', 5'GTGTGCCCCTTCCTCGATGT-3', 5'CAGACCAAGATGGTTAGTAT-3' and 5'-GTGACCAACCCACTCCCTTGA-3' corresponding to *Anopheles gambiae* s.s, *An. arabiensis*, *Anopheles quadriannulatus*, universal and *An. melas* respectively. All the primers share similar annealing temperatures and had no complementarity to each other to prevent the formation of primer complexes.

The PCR was carried out in a thermocycler (Primus 96 PCR-system, MWG Genomic technology) with an initial denaturation at 94°C for 5 minutes followed by 30 cycles of 95°C for 30 seconds, and 72°C for 30seconds, followed by one cycle of 72°C for 5 minutes.

The amplified DNA was separated on a 2% (0.5xTBE) agarose gel run in 0.5XTBE buffer. The agarose gel (UltraPure agarose) was brought to room temperature and mixed with 1.5% ethidium bromide (GelRed). The ethidium bromide which is a radioactive compound, was used to fix the nucleotides making them fluoresce in the presence of UV light. Some wells were made on the gel using electrophoresis comb. When the gel solidified, the comb was removed and the wells left opened. The amplicons from the thermocycler were then loaded in the wells before the gel was cast in the electrophoresis tank. Fragment sizes were estimated using a 1kb ladder marker (Inyitrogen) after this stage, the electrophoresis tank was closed. The migration lasted between

45 minutes and 1 hour, and the gel was carefully removed and the banding pattern of each amplified sample was checked and photographed under ultraviolet light illumination for further interpretations.

3.5.3 Identification of *An. coluzzi* and *An. gambiae* s.s Using Restriction Fragment Length Polymorphism (RFLP) assays

After amplification, 0.3 ul of *Hha* 1 in 1xNEBuffer 4 (New England Biolabs) were directly added to the PCR reactions and digestions was carried out at 37°C for four hours, as described by Fanello *et al.* (2002). Digested fragments were run through an ethidium bromide 2% agarose gel and photographed under light illumination. At this stage, *An. gambiae* s.s was sub-grouped into *An. gambiae* s.s and *An. coluzzi* forms using the protocol described by Fanello *et al.* (2002). The positive control used was made available by the molecular Entomology and Vector Research Unit of the Nigeria Institute of Medical Research (NIMR), Lagos.

3.6 Insecticide Resistance Mechanism Assays

3.6.1 Identification of target-site resistance mechanism

Two target-site resistance mutation were analysed in the study; knockdown resistance mutation known to confer resistance to organochlorine (DDT) and pyrethroids (Permethrin) and acetylcholinesterase resistance mutation that confers resistance to organophosphate (Pirimiphos-methyl) and carbamates (Propoxur).

3.6.1.1 Analysis of *knockdown* resistance mutation using Allele-Specific Polymerase Chain Reaction (AS-PCR)

Genomic DNA that was extracted from the field samples, was used for allele-specific PCR to determine the phenotypes for insensitive *kdr*. The following primers were used 5'-ATAGATTCCCCGACCATG-3', 5'-AGACAAGGATGATGATGAAC-3' and 5'-CTGTAGTGATGATAGGAAATTA-3' corresponding to Agd1, Agd2, Agd3, Agd4 (from

Biomers.net) respectively were used to detect the west *kdr* (L1014F) mutations associated with DDT and pyrethroid resistance according to the method described by Martinez-Torres *et al.* (1998). The PCR reaction condition was one minute at 94°C, 2 minutes at 72°C for forty cycles with a final extension step at 72°C for 10 minutes. Amplified fragments were analyzed on 1.5% agarose gel and were visualized by ethidium bromide staining under ultraviolet light.

3.6.1.2 Analysis of *ace-1* resistance mutation Using Allele-Specific Polymerase Chain Reaction (AS-PCR)

The restriction fragment length polymorphism PCR described by Weill *et al.* (2004) was used to detect *ace-1* resistance mutation in the acetylcholinesterase gene in mosquitoes exposed to pirimiphos-methyl and propoxur. Genomic DNA was PCR amplified with the primers EX3A_{gdir} 5'GATCGTGGACACCGTGTTTCG3' and EX3A_{grev} 5'GGATGGCCCGCTGGAACAG'. The PCR reaction condition included an initial denaturation step at 94°C for 5 minutes followed by thirty-five cycles of 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for five minutes. The *Alu1* restriction enzyme (New England Biolabs) digest was performed to determine if the survivors had the *ace-1* mutation associated with carbamate resistance. Specimens' representative of the wild-type sample (SS), heterozygous resistant (RS), and homozygous resistant (RR) genotypes were included as controls.

3.6.2 Metabolic/Biochemical Resistance Assay

The specimens were stored in refrigerator at -20°C after exposure before the start of the assays. For the four assays, individual mosquitoes were homogenised in 200µl of distilled water on ice, then the homogenate was spin at 14K for 30secs in a microfuge (or maximum speed on a fixed speed microfuge). The microfuge step is essential only for the Glutathione-S-transferase assay, as particulate matter in the crude homogenate has a significant impact on the absorption of light

at 340 nm. The homogenate was kept on ice to avoid enzyme activity loss and the whole body of the specimen was fully crushed.

All reagents used are basic grade chemicals. Phosphate buffer is made from a mixture of NaH_2PO_4 + Na_2HPO_4 . The molecular weight of the monobasic phosphate is 120 and the dibasic phosphate is 142.

3.6.2.1 Esterase Assay

The method followed in the procedure are;

1. 2 x 20 μl replicates of homogenate were placed in separate wells in a microtitre plate.
2. 200 μl of working solution of β -NA was added to the replicates. The setup was then left at room temperature for 15 mins.
3. 50 μl of fast blue stain solution was then added.
4. The setup was left for 5mins before reading the results to allow colour transformation.
5. Result was read in the spectrophotometer at 570nm.

The optical density (OD) read from the spectrophotometer was analysed and interpreted using the following steps;

- 1.) Standard curve was set up for β -naphthyl acetate by varying the concentrations (0.0ml, 0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml) in the working naphthyl acetate solutions (i.e. the various concentrations of the working naphthyl acetate solutions in 220 μl 0.02M phosphate buffer (pH 7.2) mixed with 50 μl of stain solution).
- 2.) Absorbance values obtained from the mosquito samples was converted to nmoles β -naphthol using the standard curves. The values in units of nmoles β -naphthol/min/mg protein were then calculated as follows:

3.6.2.2 Glutathione-S-transferase Assay

The method followed in the procedure are;

1. 2 x 10 μ l replicates of homogenate was place in separate wells of a microtitre plate.
2. 200 μ l of the GSH/CDNB working solution was added.
3. Two wells 10 μ l distilled water + 200 μ l of the GSH/CDNB working solution.
4. The experiment was left at room temperature for 20mins and then read at 340nm as an end point.

The OD read from the spectrophotometer was analysed and interpreted using the following steps;

In calculating the activity in mMole/min/mg protein, the absorption value was converted to concentration with the formular; $A = Ecl$

where

A = absorbance

E = extinction coefficient

c = concentration

l = path length

For the assay, the extinction coefficient of the product of the reaction (3-(2-chloro-4-nitrophenyl)-glutathione) has been determined at 340nm to be 4.39 mM⁻¹. The path length is a function of well volume and with a constant assay volume of 210 μ l, the path length (i.e. depth of solution in the curvette used for the spectrophotometer) = 0.7cm.

3.6.2.3 Monooxygenase titration Assay

1. 2 x 20 μ l replicates of homogenate was place in separate wells of a microtitre plate.
2. 80 μ l of 0.625 M potassium phosphate buffer (PH 7.2) was added to each replicate.

3. 5ml methanol solution of tetramethyl benzidine was mixed with 15ml of 0.25 M sodium acetate buffer (pH 5.0).
4. 200µl of the above mixture was added to each replicate.
5. Then 25µl of 3% hydrogen peroxide was added to each replicate.
6. The mixture was left for 2 hours at room temperature before reading absorbance at 650nm.
7. Controls were run with 20µl of buffer in place of the insect homogenate.

3.6.2.4 Protein Assay

Protein content of the mosquitoes was determined in accordance with the protocol of Biuret test.

3.7 Statistical Analysis and Data Interpretation

For the KAP study, data collected were cleaned, coded and entered into the computer for analysis using Statistical Package for Social Sciences (IBM SPSS) software version 21.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to determine the relative frequencies, percentages, mean and range. Chi-square was used to determine the influence of LGA of residence, level of education and type of dwelling on respondents' knowledge, attitude and practices on malaria control using LLIN and IRS.

For the WHO susceptibility/resistance assay, *Knockdown time* (KDT₅₀ and KDT₉₅) was determined with SPSS version 22.0. Resistance reduction was calculated by dividing the KDT₅₀ obtained for the individual insecticide by the KDT₅₀ obtained for the mixtures of the same insecticide (Chouaïbou *et al.*, 2014).

The correlation between mortality to DDT and permethrin was analysed using Pearson correlation. The conformity of *kdr* frequency with Hardy-Weinberg equilibrium was analysed using Chi-Square (X^2) in line with the work of Wittke-Thompson *et al.*, (2005). Paired T-Test

was employed to compare and determine the significant difference in mean activities of enzymes.

CHAPTER FOUR

4.0 RESULTS

4.1 Knowledge, Attitude and Practices to Malaria Vector Control Methods

4.1.1 Demographic characteristics of population

Results from analysis of the questionnaires are presented in tables (2 – 10) below. A total of 502 respondents were interviewed. Two hundred and twenty-one (44.0%) of these were from Kosofe, 82 (16.3%) from Ojo and 199 (39.6%) from Lagos Mainland LGAs. The mean age of the respondents was 37.53 ± 12.34 (range; 18 – 64) and median is 34 years. Majority (58.0%) of the respondents were married. More than half (56.5%) of the respondents had education beyond the secondary level while those with no formal education were very few (5.6%). The mean number of inhabitants in a household was 4.35 ± 1.94 (Range; 1 – 12) and the median is 4. Majority (40.0%) of the respondents lived in a single room, while a very few (1.0%) lived in a duplex. Summary of the socio-demographic characteristics of the respondents is presented in Table 2.

Table 2: Baseline characteristics of study participants

Characteristics		N = 502 (%)
Local Government Area		
	Kosofe	221 (44.0)
	Ojo	82 (16.3)
	Lagos Mainland	199 (39.6)
Age		
Mean ± SD (Range)		37.53 ± 12.34 (18 – 64)
	Age group	
	Missing	88 (17.5)
	18 – 30	135 (26.9)
	31 – 40	135 (26.9)
	41 – 50	78 (15.5)
	51 – 60	39 (7.8)
	>60	27 (5.4)
Marital Status		
	Never married	154 (30.7)
	Married	291 (58.0)
	Divorced	13 (2.6)
	Separated	20 (4.0)
	Widowed	24 (4.8)
Level of Education		
	No formal education	28 (5.6)
	Primary	38 (7.6)
	Secondary	153 (30.5)
	Post-secondary	283 (56.5)
Occupation		
	Unemployed	70 (13.9)
	Housewife	9 (1.8)
	Farming	9 (1.8)
	Artisan	97 (19.3)
	Student	48 (9.6)
	Formally employed in public sector (e.g civil servant)	75 (14.9)
	Fishing	6 (1.2)
	Formally employed in private sector (e.g banking)	85 (16.9)
	Trading	91 (18.1)
	Retired	3 (0.6)
	Others	9 (1.8)
Number of Children		
	0	153 (30.5)

1	66 (13.1)
2	128 (25.5)
3	104 (20.7)
4	45 (9.0)
5	6 (1.2)

Number in Household

Mean $\pm$ SD (Range) 4.35 $\pm$ 1.94 (1 – 12)

1	32 (6.4)
2	63 (12.5)
3	62 (12.4)
4	142 (28.3)
5	61 (12.2)
6	75 (14.9)
7	37 (7.4)
8	17 (3.4)
9	11 (2.2)
10	1 (0.2)
12	1 (0.2)

Number of Children Under 5 years

0	307 (61.2)
1	130 (25.9)
2	52 (10.4)
3	12 (2.4)
4	1 (0.2)

Number of Pregnant Women

Missing	2 (0.4)
0	452 (90.0)
1	48 (9.6)

Type of Dwelling Structure

Single family house	62 (12.4)
Duplex	5 (1.0)
Two/three bedrooms flat	88 (17.5)
Mini flat	56 (11.2)
Room and parlour	90 (17.9)
Single room	201 (40.0)

4.1.2 Knowledge and practices to mosquito control

The results of the knowledge and practices related to mosquito control presented in Table 3 shows that majority of the participants use combination of several methods to control mosquito. The most common method employed include: use of insecticides (390; 77.7%), sleeping under LLINs (242; 48.2%), window screening with ordinary nets (202; 40.2%), sleeping with windows closed (154; 30.7%) and covering of bodies with clothes (124; 24.7%). The relationship between LGAs of residence and the various methods of mosquito control was insignificant for all the methods ($p>0.05$). Level of education was significantly related to the methods of mosquito control ($p<0.05$) except for "Use of Insecticides" where $p = 0.064$. Also, type of dwelling was significantly related to all the methods of control ($p<0.05$) (Table 4). Numerous reasons informed respondents preferences for various methods; effectiveness (218; 43.4%) was however the most prominent. A good number of respondents (58; 11.6%) were also concerned about the negative effects of insecticide use while few (19; 3.8%) were concerned about the heat associated with use of nets (Table 3). Table 4 shows that LGAs has no significant relationship with the reason for respondents' preference for the methods ($p>0.05$) while education and dwelling structure had a strong relationship with respondent's reasons for preference ($p<0.05$). A large number (438; 87.3%) of respondents were oblivious of any community interventions related to mosquito control programmes by government organisations, NGOs or other bodies (Table 3) and this was insignificantly related to LGAs, level of education and type of dwelling ($p>0.05$).

Table 3: Knowledge and practices to mosquito control

		N (%)	
Methods employed for protection against mosquito		Yes (N=502)	No (N=502)
	Sleeping under ordinary net	74 (14.7)	428 (85.3)
	Sleeping under LLINs	242 (48.2)	260 (51.8)
	Sleeping with windows closed	154 (30.7)	348 (69.3)
	Screening of windows with net	202 (40.2)	300 (59.8)
	Use of insecticides	390 (77.7)	112 (22.3)
	Burning of coil/grass as repellants	112 (22.3)	390 (77.7)
	Cutting bushes and grasses around the home	54 (10.8)	448 (89.2)
	Draining stagnant water	83 (16.5)	419 (83.5)
	Clearing gutters	92 (18.3)	410 (81.7)
	Covering the body with clothes	124 (24.7)	378 (75.3)
	Rub repellent cream on the body	33 (6.6)	469 (93.4)
Reason for method's preference		N = 502	
	Because of the negative health impact of insecticides	58 (11.6)	
	Because it prevents contact with mosquito	85 (16.9)	
	Because it is very effective	218 (43.4)	
	Because it is cheap	8 (1.6)	
	Because it is easy to use and not time wasting	49 (9.8)	
	Because it was recommended	8 (1.6)	
	Because it is readily available	28 (5.6)	
	I can't say	25 (5.0)	
	Because it does not cause heat like nets	19 (3.8)	
	Because I have no alternative	4 (0.8)	
Government organizations, NGO or other bodies interventions in community mosquito control programme		N = 502	
	Yes	64 (12.7)	
	No	438 (87.3)	

Table 4: Relationship between, LGA, level of education, type of dwelling on knowledge and practices on mosquito control

	Local Government Area	Level of education	Type of dwelling
Methods employed for protection against mosquito			
Sleeping under ordinary net	0.985	0.028*	0.000*
Sleeping under LLINs	0.841	0.003*	0.004*
Screening of windows with net	0.221	0.000*	0.006*
Use of insecticides	0.470	0.064	0.000*
Reason for method's preference	1.000	0.000*	0.005*
Government organizations, NGO or other bodies interventions in community mosquito control programme	0.157	0.117	0.190

*Level of significance < 0.05

4.1.3 Knowledge and attitude related to LLIN usage

Table 5 presents the result of respondents' knowledge and attitude related to LLINs. Out of the 502 respondents, 242 (48.2%) use LLINs for mosquito control. Half (49.6%) of the 242 respondents obtained it freely from the hospital/clinic. Others obtained theirs from pharmacy/chemist shop (25.5%), market (9.9%), retail shops (4.6%), souvenir at workshop/meeting (1.7%), gift from family and relations (5.0%) and through free distribution in the locality (3.7%). Almost all the respondents (93.0%) that used LLIN attested to its effectiveness 225 (93.0%). Majority of the LLINs used were less than 3 years old; with 92 (38.0%) less than a year, 73 (30.2%) between 1-2 years and 54 (22.3%) between 2-3 years. The mean usage time of the LLINs was 1 year and 7 months with a median of 2 years. The perception of LLIN effectiveness and source were not significantly related to both LGAs and education of respondents ($p>0.05$) but significantly correlated with dwelling structure ($p<0.05$). Similarly, the time span of usage of treated mosquito net is significantly related to education and dwelling structure ($p<0.05$) (Table 6).

Table 5: Knowledge and attitude related to LLIN

	N (%)
LLIN as a mosquito control tool	N= 502
Yes	242 (48.2)
No	260 (51.8)
LLIN effectiveness	N = 242
Yes	225 (93.0)
No	17 (7.0)
Source of LLIN	N = 242
Free distribution in my area	9 (3.7)
Gift from family/relations	12 (5.0)
Souvenir at workshop/meeting	4 (1.7)
Retail shop in my neighbourhood	11 (4.6)
Market	24 (9.9)
Free distribution in hospital	120 (49.6)
Pharmacy/chemist shop	62 (25.5)
Time span of treated mosquito net (year)	N = 242
Mean	1 year 7months
<1	92 (38.0)
>1-2	73 (30.2)
>2-3	54 (22.3)
>3-4	6 (2.5)
>4	17 (7.0)

Table 6: Relationship between LGA, level of education, type of dwelling on knowledge and attitude related to LLIN

	Local Government Area	Level of Education	Type of Dwelling
LLIN effectiveness	0.296	0.310	0.002*
Source of LLIN	0.942	0.055	0.000*
Time span of treated mosquito net (year)	0.965	0.000*	0.000*

*Level of significance < 0.05

4.1.4 Knowledge, attitude and practices to insecticide

4.1.4.1 Level of insecticide use

Three hundred and ninety (77.7%) respondents out of 502 use insecticides. Of the 112 that do not use insecticides, 60 (53.6%) have used insecticides before while others (52; 46.4%) have never been in a household where insecticides were used before. Of the 52 respondents who never used insecticide before as presented in Table 7, 51.9% attributed the non-usage to the negative health implications of insecticides.

Table 7: Level of insecticide use

	N (%)
Insecticide use in mosquito control	N = 502
Yes	390 (77.7)
No	112 (22.3)
Not using insecticides at present but used before	N = 112
Yes	60 (53.6)
No	52 (46.4)
Reason for not using insecticides	N = 52
Because it causes irritation	7 (13.5)
I can't say	11 (21.2)
Because of its negative health implication	27 (51.9)
We just don't like it	1 (1.9)
Because it is not efficient like nets	5 (9.6)
Because of the unpleasant smell	1 (1.9)

4.1.4.2 Choice and perception of insecticide brand

In Table 8, most (170; 37.8%) of the respondents use insecticide against mosquito 1 or 2 days in a week, (146; 32.4%) use it 3 to 5 times in a week, (89; 19.8%) use insecticide everyday and (45; 10.0%) mentioned using it only once in a month. A large proportion (73.6%) of the respondents mentioned they apply the insecticide only in the evening and a few (7.7%) use it only in the morning. There are also those who apply it both in the morning/evening (14.9%) and morning/afternoon (3.8%). Majority (44.9%) allow a short break of an hour to two hours before entry into treated apartment for the offensive irritation to diffuse while 89 (19.9%), 136 (30.2%) and 27 (6.0%) mentioned allowing the irritation to diffuse for less than an hour, 2-4 and >4 hours respectively. A good number of the respondents have changed insecticides such as; Raid (28.1%), Baygon (15.1%), Mobil (10.0%), Mortein (8.4%) etc due to health implications and safety (26.9%), availability (25.6%) and reduction in the effectiveness of the insecticides (37.6%). The perception of the respondents showed that, 133 (28.4%) believed IRS is effective, cheap and safer, 44 (9.4%) had the opinion that it is highly detrimental to health.

Table 8: Choice and perception of insecticide brand

		N (%)
First choice brand for insecticide use		N = 450
	Baygon	35 (7.8)
	Brandless	1 (0.2)
	DD force	31 (6.9)
	Go 90	50 (11.1)
	Killit	2 (0.4)
	Mobil	20 (4.4)
	Morayor	1 (0.2)
	Morning fresh	2 (0.4)
	Mortein	35 (7.8)
	Mosquito coil	11 (2.4)
	Otapiapia	85 (18.9)
	Raid	25 (5.6)
	Rambo	26 (5.8)
	Sniper	114 (25.3)
	Tetmosol Spray	1 (0.2)
	Total	11 (2.4)
Effectiveness of choice brand of insecticide		N = 450
	Very effective	287 (63.8)
	Somewhat effective	143 (31.8)
	Not effective	19 (4.2)
	Not effective at all	1 (0.2)
Knowledge of insecticide brand contents		N = 450
	Yes	29 (6.4)
	No	421 (93.6)
Influence behind choice of insecticide brand		N = 450
	Yes	No
	Affordability	159 (35.3) 291 (64.7)
	Availability	236 (52.4) 214 (47.6)
	Ability of continuous protection	97 (21.6) 353 (78.4)
	Efficacy/Effectiveness	329 (73.1) 121 (26.9)
	It kills all insects	179 (39.8) 271 (60.2)
	Safety/Lack of side effects	49 (10.9) 401 (89.1)
	Pleasant fragrance	56 (12.4) 394 (87.6)
Source of Insecticide		N = 450

	Yes	No
Gift from family / relations	12 (2.7)	438 (97.3)
Friends/neighbours	39 (8.7)	411 (91.3)
Souvenir at workshop/meeting	13 (2.9)	437 (97.1)
Retail shop in my neighbourhood	192 (42.7)	258 (57.3)
Market	239 (53.1)	211 (46.9)
School	0 (0.0)	450 (100.0)
Pharmacy	96 (21.3)	354 (78.7)
Chemist shop	248 (55.1)	202 (44.9)
Street hawkers	75 (16.7)	375 (83.3)
Others	3 (0.7)	447 (99.3)

4.1.4.3 Practices and attitude to insecticide use

Table 9 presents the results of practices and attitude to insecticide use. The choice of insecticides by respondents is highly varied but majority (25.3%) opt for “Sniper” as their first choice of insecticide. Other insecticides used by the respondents include; Otapiapia (18.9%), Go 90 (11.1%), DD force (6.9%) etc and 95.6% says the insecticide are effective. While effectiveness, availability and affordability are the major factors influencing choice of brand, majority (93.6%) of the respondents don’t have an idea of the content of the insecticide brand they prefer. The insecticides are mostly sourced from markets, chemist shops, pharmacy, retail shops and hawkers.

Table 9: Practices and attitude to insecticide use

	N (%)
Frequency of insecticide use at home in a week	N= 450
Sometimes (1-2days out of 7days)	170 (37.8)
Often (3-5 days out of 7 days)	146 (32.4)
Everyday (7 days out of 7 days)	89 (19.8)
Others	45 (10.0)
Timing of insecticide use	N= 450
Morning	35 (7.7)
Evening	331 (73.6)
Both morning and afternoon	17 (3.8)
Both morning and evening	67 (14.9)
Time allowed for insecticide offensive irritation to diffuse before returning to room (Hours)	N= 450
0 - 1	89 (19.9)
>1-2	202 (44.9)
>2-3	69 (15.3)
>3-4	67 (14.9)
>4	27 (6.0)
Ever continuously used, then STOP any other brand(s) of insecticide(s) for mosquito control	N= 450
Yes	242 (53.8)
No	208 (46.2)
Name of insecticide brand continuously used then stopped	N= 299
ABB Spray	3 (1.0)
Baygon	45 (15.1)
Flit	1 (0.3)
Go 90	21 (7.0)
Mobil	30 (10.0)
Morning fresh	3 (1.0)
Mortein	25 (8.4)
Mosquito coil	19 (6.4)
Otapiapia	27 (9.0)
Raid	84 (28.1)
Sniper	12 (4.0)
Tablet insecticide	6 (2.0)

	Without brand	23 (7.7)
Reason for stopping use of an insecticide		N= 242
	Because it causes irritation	6 (2.5)
	I can't say	1 (0.4)
	Because of its negative health implication and safety	65 (26.9)
	Because its no longer affordable	8 (3.3)
	Because its no longer effective	91 (37.6)
	Because its no more available	62 (25.6)
	Because of the unpleasant smell	9 (3.7)
Control any other vector/pest/insect in the house		N= 502
	Yes	368 (73.3)
	No	134 (26.7)
Means used in controlling other vector/pest/insect in the house		N= 368
	Any available insecticide	16 (4.4)
	Baygon	8 (2.2)
	Flit	3 (0.8)
	Fumigation	17 (4.6)
	Go 90	5 (1.4)
	Green Leaf	4 (1.1)
	Kerosine	6 (1.6)
	Mobil	5 (1.4)
	Mortein	10 (2.7)
	Otapiapia	27 (7.3)
	Paper rat gum	122 (33.2)
	Pesticide powder	3 (0.8)
	Pestox	4 (1.1)
	Piff paff	2 (0.5)
	Raid	35 (9.5)
	Rainbow	2 (0.5)
	Rat poison	50 (13.6)
	Rat trap	5 (1.4)
	Sniper	42 (11.4)
	Zinc Sulphide	2 (0.5)
General perception about IRS		N= 468
	It is effective, cheap and safer	133 (28.4)
	It is highly detrimental to health	44 (9.4)
	It is good for mosquito protection	68 (14.5)
	It is good for controlling all insects/vectors/pests	35 (7.5)
	It is effective but detrimental to health	101 (21.6)

It is not effective and also detrimental to health	9 (1.9)
It is not effective again	61 (13.1)
Its impact is long lasting than other methods	8 (1.7)
No comment	9 (1.9)

4.1.4.4 Knowledge and attitude to insecticide resistance

Table 10 presents results of knowledge and attitude to insecticide resistance. 327 (72.6%) of the respondents usually notice mosquitoes again < 3hours after insecticide use. 60 (12.0%) still thinks all mosquitoes die after exposure to insecticides and 261 (52.0%) ignore the mosquitoes they notice after application of insecticide.

Table 10: Knowledge and attitude to insecticide resistance

	N (%)
Time when mosquitoes is noticed after insecticide use or hanging of net (Days)	N = 450
0 - 1	147 (32.6)
>1-3	180 (40.0)
>3-5	73 (16.2)
>5	40 (8.9)
I can't say	10 (2.2)
Mosquitoes are not killed by insecticides again	N = 502
Yes	329 (65.5)
No	60 (12.0)
Don't know	113 (22.5)
The quantity of mosquitoes that survived after use of insecticides over the years	N = 502
Increased	134 (26.7)
Decreased	312 (62.2)
Don't know	56 (11.1)
Methods employed against mosquitoes that appear some hours or minutes after insecticide use or hanging of net	N = 502
I make use of my hand to kill them	33 (6.6)
I cover up myself	47 (9.4)
I don't notice there are mosquitoes	52 (10.4)
I spray the room again	65 (13.0)
I take other measure I can think of then	30 (6.0)
I make use of broom soaked in kerosene	6 (1.2)
I ignore the mosquitoes and sleep like that	261 (52.0)
I can't say	7 (1.4)
I spread my net again	2 (0.4)

4.2 Susceptibility profile of *Anopheles gambiae* s.l in Lagos, Nigeria

A total of 2000 mosquitoes were used for the WHO insecticide susceptibility tests. The mosquitoes were all morphologically identified to be female *Anopheles* mosquitoes. Mortality was recorded in some controls and this was adequately catered for with Abbott's formula (Abbott, 1925).

4.2.1 Species characterisation of *Anopheles* spp in Lagos, Nigeria

A total of 405 of mosquito sample comprising of 375 resistant species and 30 susceptible species was assayed for species identification. 189 samples were confirmed to belong to *Anopheles gambiae* sibling species with all the confirmed samples belong to *A. gambiae*. Other sibling species like *A. melas*, *An. merus*, *An. arabiensis* and *An. quadriannulatus* and *An. Amharicus* were not detected (Table 11). Out of the 189 samples belonging to *An. gambiae* s.l, 179 (94.71%) was identified with PCR-RFLP as *An. coluzzi* while only 2 (1.06%) was identified as *An. gambiae* s.s (Table 12). The hybrid form of *An. gambiae* s.s and *An. coluzzi* was not found in the sampled population. The detailed presentation along the 3 study sites is presented in Tables 11 and 12.

Table 11: PCR analysis of *Anopheles gambiae* s.l species characterisation

Study site	No Examined	<i>Anopheles gambiae</i> species identification		
		<i>gambiae</i> (%)	others (%)	NA
Kosofe	129	73 (56.59)	0 (0.00)	56 (43.41)
Ojo	138	68 (49.28)	0 (0.00)	70 (50.72)
Lagos Mainland	138	48 (34.78)	0 (0.00)	90 (65.22)
Total	405	189 (46.67)	0 (0.00)	216 (53.33)

Table 12: PCR-RFLP analysis of *Anopheles gambiae* s.s and *Anopheles coluzzii*

Study site	No Examined	<i>Anopheles gambiae</i> species identification		
		<i>gambiae</i> s.s (%)	<i>coluzzii</i> (%)	NA
Kosofe	73	2 (2.74)	67 (91.78)	4 (5.48)
Ojo	68	0 (0.00)	66 (97.06)	2 (2.94)
Lagos Mainland	48	0 (0.00)	46 (95.83)	2 (4.17)
Total	189	2 (1.06)	179 (94.71)	8 (4.23)

NA = Not amplified

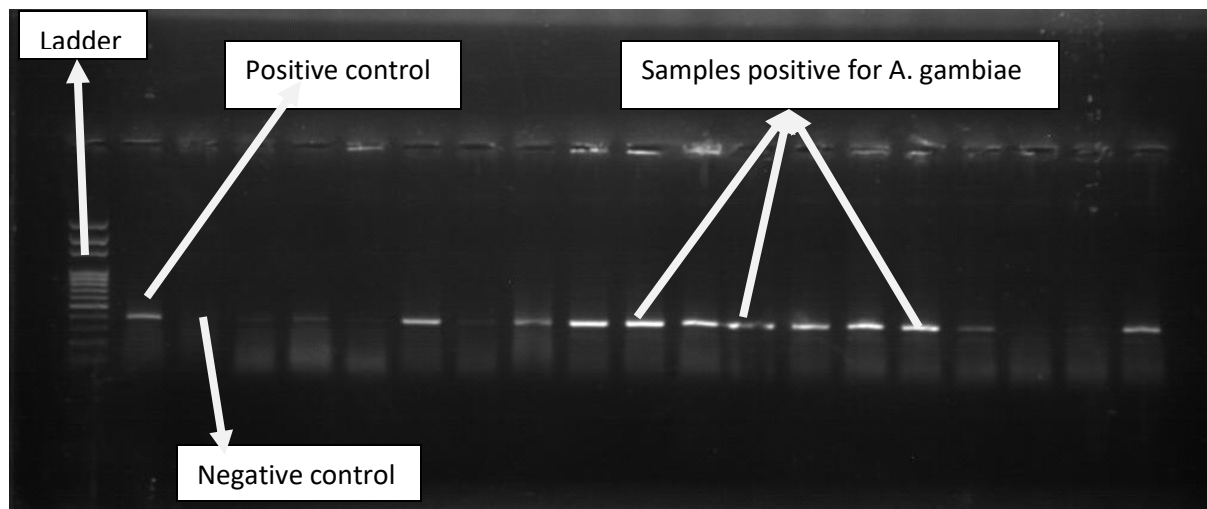


Plate 4: Photomicrograph for species identification showing *Anopheles gambiae*

4.2.2 Susceptibility status and knockdown time of *Anopheles gambiae* s.l. in Lagos to the four classes of insecticide

Anopheles gambiae s.l from the 3 study sites show varying degree of susceptibility to the 4 recommended insecticide classes. The result of the susceptibility tests is presented in Table 13. For Kosofe study site, mortality was lowest (25%) to pirimiphos-methyl. The mortality in DDT was 44% while that of permethrin was 50%. However, the mortality to propoxur was 100%. At Ojo, similar to the situation in Kosofe, mortality in propoxur was 100% but susceptibility to permethrin in this study location was however different from Ojo with a mortality of 96% thereby putting the susceptibility status on suspicion. Mortality to DDT was the lowest at 29% while 32% was the mortality recorded for pirimiphos-methyl after 24 hours. The result of the susceptibility test in Lagos Mainland was close to what was obtained in Kosofe with mortality of 46%, 48%, 38% and 100% for exposure to DDT, permethrin, pirimiphos-methyl and propoxur respectively. Using Pearson's correlation, the mortality rate in DDT and Permethrin across the study sites showed significant correlation ($R^2=0.995$, $p=0.045$). There was no correlation in mortality between pirimiphos-methyl and propoxur across the study sites. The KDT_{50} recorded across the 3 study sites show that pirimiphos-methyl has the lowest KDT_{50} in Ojo (13.92) and Lagos mainland (14.61) while propoxur has the lowest KDT_{50} in Kosofe (10.46). The KDT_{50} obtained for DDT across the 3 study sites was very high (Figure 2-4).

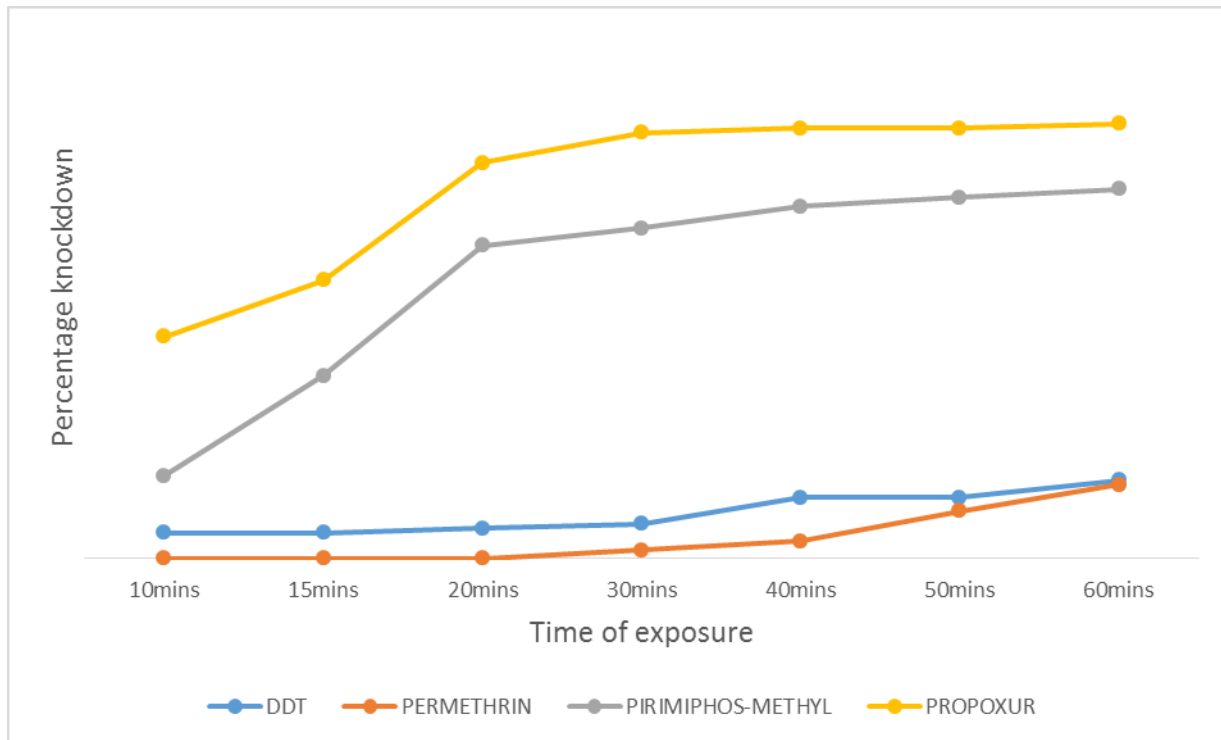


Figure 2: Comparison between percentage knockdown of *Anopheles* spp. exposed to insecticides in Kosofe LGA, Lagos state

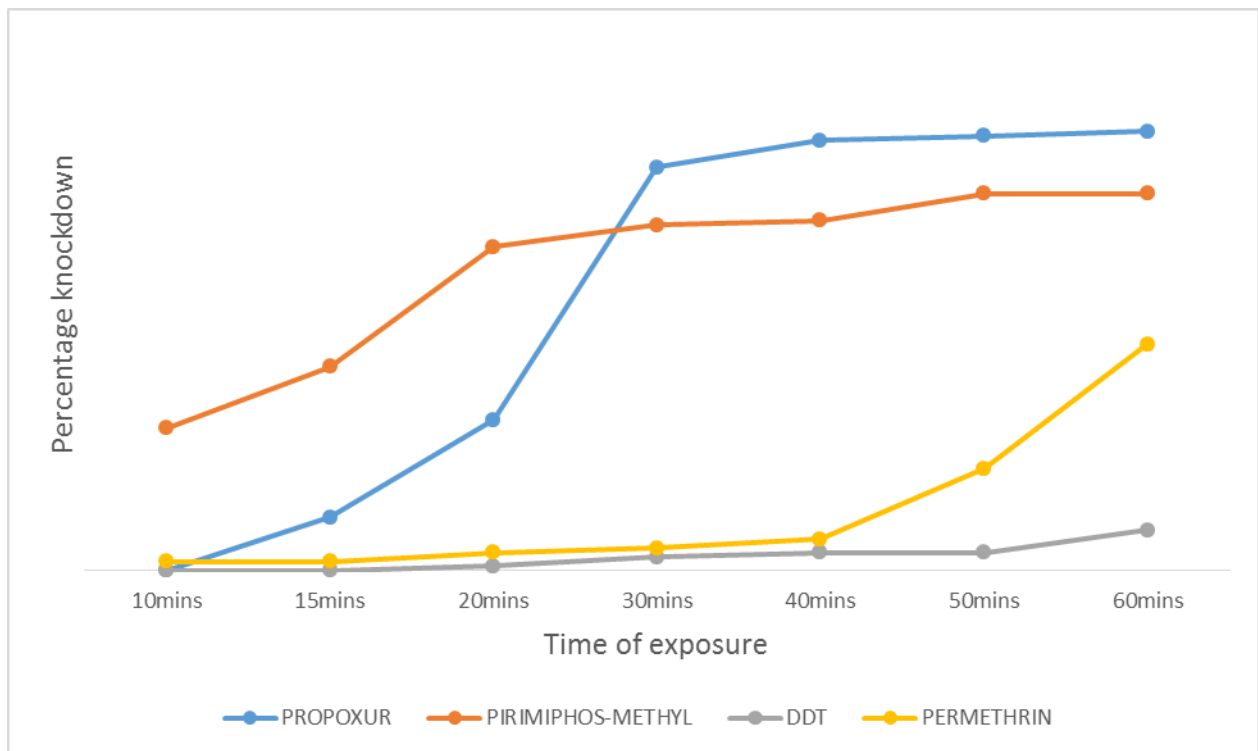


Figure 3: Comparison between percentage knockdown of *Anopheles* spp. exposed to insecticides in Lagos Mainland LGA, Lagos state

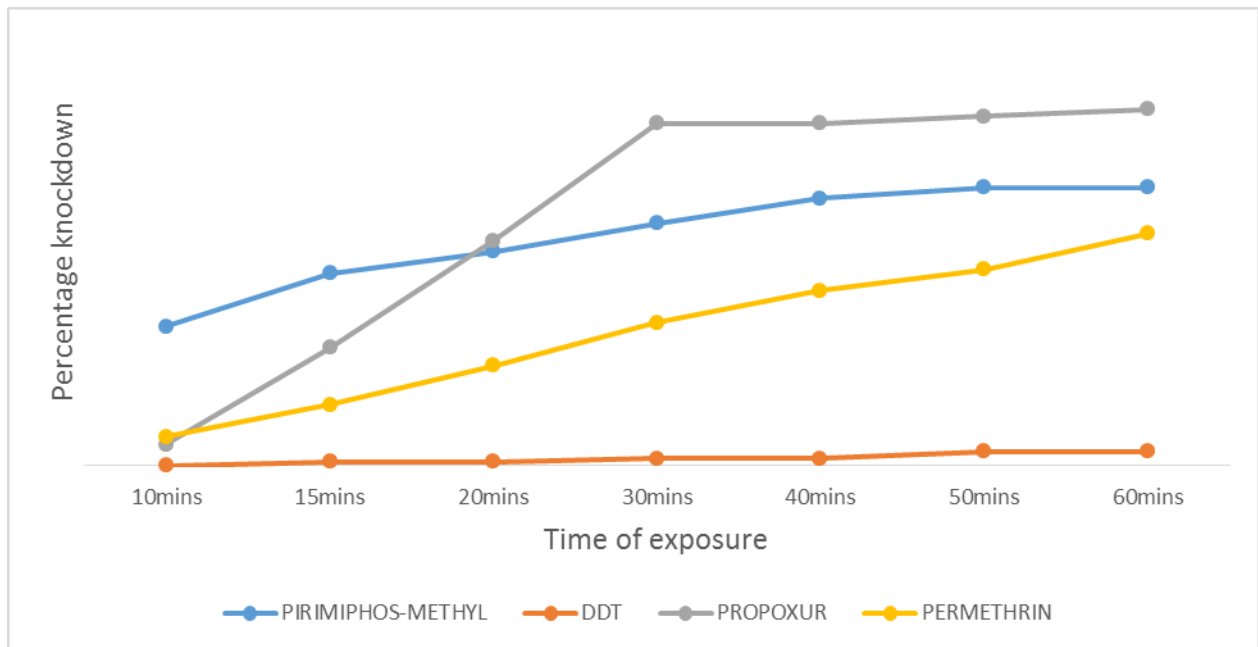


Figure 4: Comparison between percentage knockdown of *Anopheles spp.* exposed to insecticides in Ojo LGA, Lagos state

Table 13: Mortality and knock-down of *Anopheles spp.* in Lagos state, Nigeria exposed to diagnostic doses of the 4 insecticide classes recommended for mosquito control

Insecticide		Study site		
		Kosofe	Ojo	Lagos Mainland
DDT*	% Mortality (24h)	44	29	46
	KDT ₅₀ (min)	724.16	1223.68	2588.07
		(228.058-39710.504)	(252.054-22.574 X 10 ⁸)	(133.942-2226.555)
	KDT ₉₅ (min)	47242.25	22078.61	1447.84
		(3385.074-503294457.442)	(1266.842-63.360 X 10 ¹⁴)	(414.356-95109.381)
	Susceptibility status	Resistant	Resistant	Resistant
Permethrin*	% Mortality (24h)	50	96	48
	KDT ₅₀ (min)	103.48	40.72	78.49
		(81.448-179.220)	(36.394-46.582)	(52.888-1001.677)
	KDT ₉₅ (min)	266.11	234.20	261.72
		(160.592-881.659)	(166.537-383.816)	(110.822-268647.696)
	Susceptibility status	Resistant	Resistance suspected	Resistant
Pirimiphos-methyl	% Mortality (24h)	25	32	38
	KDT ₅₀ (min)	17.35	13.92	14.61
		(11.143-22.614)	(10.350-16.992)	(9.387-18.868)
	KDT ₉₅ (min)	86.00	222.25	98.24
		(55.378-244.809)	(137.157-507.052)	(63.447-249.802)
	Susceptibility status	Resistant	Resistant	Resistant
Propoxur	% Mortality (24h)	100	100	100
	KDT ₅₀ (min)	10.46	17.76	22.06
		(8.032-12.321)	(15.806-19.747)	(19.304-25.035)
	KDT ₉₅ (min)	27.05	34.27	37.44
		(22.500-37.011)	(29.466-43.092)	(31.800-49.458)
	Susceptibility status	Susceptible	Susceptible	Susceptible

*Significant relationship exist between mortality to insecticide across the study sites

4.3 Susceptibility profile of *Anopheles gambiae* s.l population in Lagos, Nigeria to insecticide combinations

4.3.1 Susceptibility status to insecticide combinations

Anopheles gambiae s.l from Kosofe and Lagos Mainland were exposed to insecticide combinations of DDT/Propoxur, DDT/Pirimiphos-methyl, Permethrin/Propoxur and Permethrin/Pirimiphos-methyl. The mortality recorded for DDT/Propoxur at both Kosofe and Lagos Mainland was 99%, however, the KDT₅₀ and KDT₉₅ in Kosofe (23.92 and 53.31) was slightly lower than what was obtained in Lagos mainland (30.98 and 79.80). Mortality recorded on exposure to DDT/Propoxur combination was low at both sites. 43% mortality was recorded at Kosofe while 29% mortality was recorded at Lagos Mainland. A combination of Permethrin / Propoxur achieved 100% mortality at both sites while Permethrin/Pirimiphos-methyl combination recorded 99% mortality at Kosofe and 97% mortality at Lagos Mainland (Table 14). The KDT₅₀ in Kosofe was 23.92, 48.38, 21.29 and 57.58 for DDT/Propoxur, DDT/Pirimiphos-methyl, Permethrin/Propoxur and Permethrin/Pirimiphos-methyl respectively while that of Lagos Mainland was 30.98, 48.28, 21.22 and 54.96 for DDT/Propoxur, DDT/Pirimiphos-methyl, Permethrin/Propoxur and Permethrin/Pirimiphos-methyl (Figure 5 & 6).

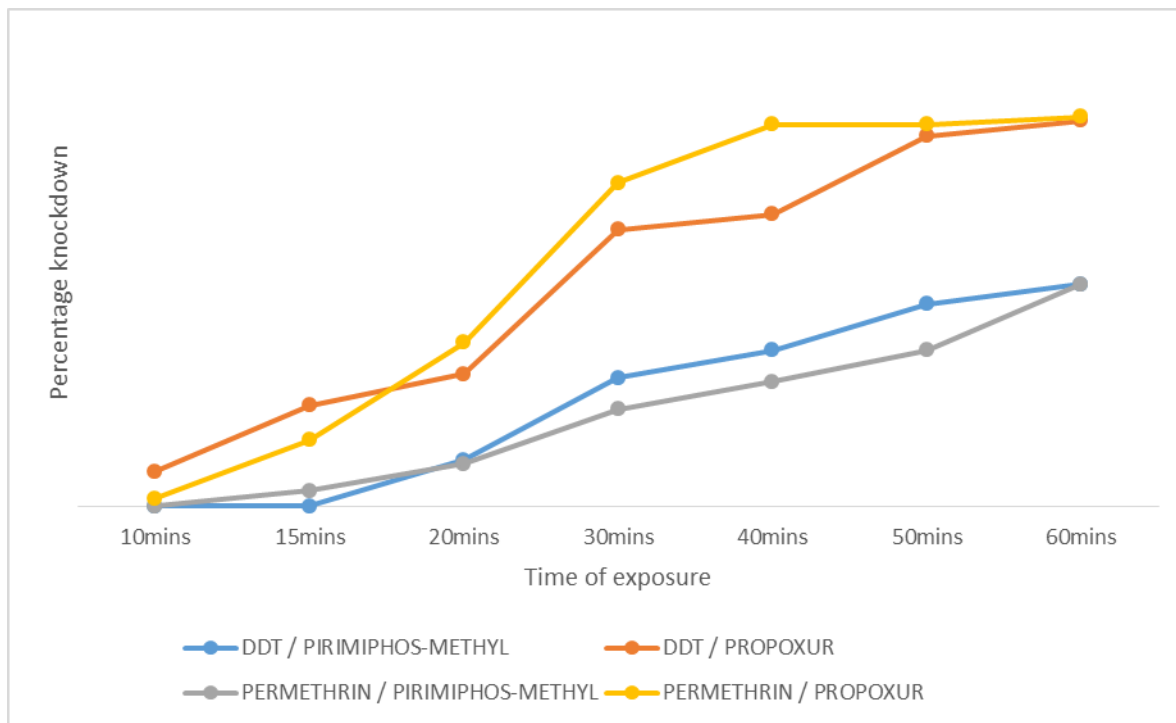


Figure 5: Comparison between percentage knockdown of *Anopheles* spp. exposed to combination of insecticides in Kosofe LGA, Lagos state

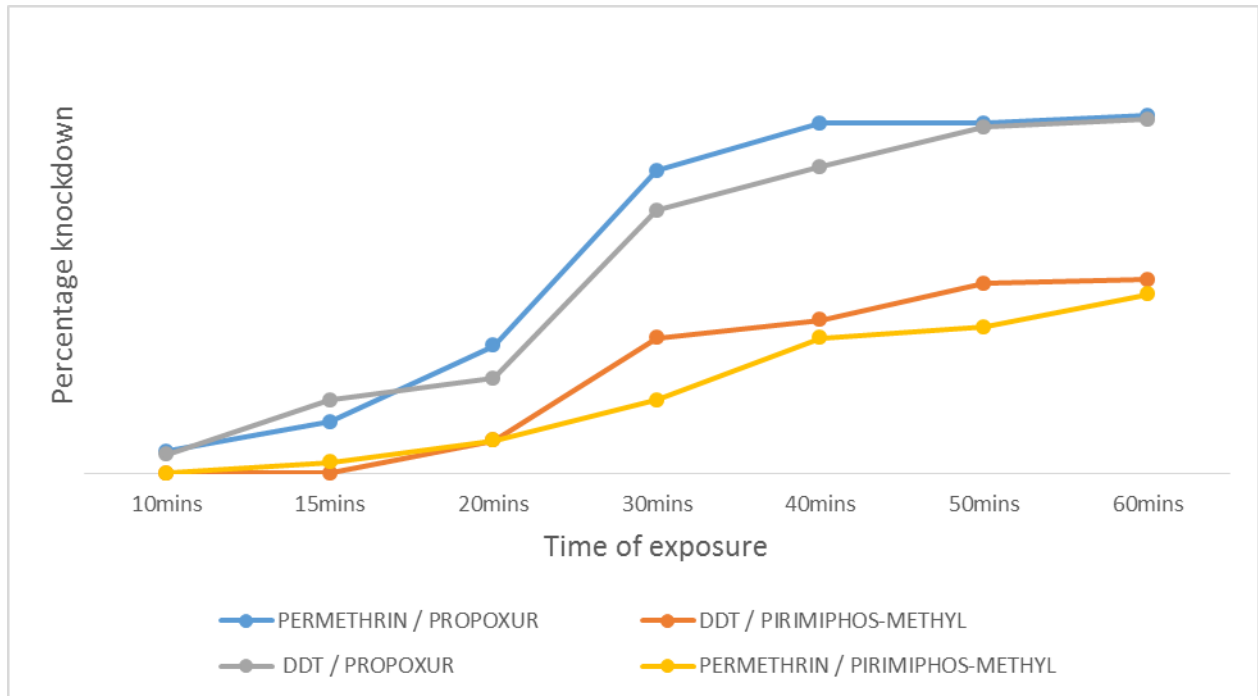


Figure 6: Comparison between percentage knockdown of *Anopheles spp.* exposed to combination of insecticides in Lagos Mainland LGA, Lagos state

Table 14: Mortality and knock-down of *Anopheles gambiae* s.l in Lagos state, Nigeria exposed to insecticide combinations

Insecticide		Study site	
		Kosofe	Lagos Mainland
DDT / Propoxur	% Mortality (24h)	99	99
	KDT ₅₀ (min)	23.92 (22.516-25.361)	30.98 (22.516-25.361)
	KDT ₉₅ (min)	53.31 (48.508-59.791)	79.80 (48.508-59.791)
	Susceptibility status	Susceptible	Susceptible
DDT / Pirimiphos-methyl	% Mortality (24h)	43	29
	KDT ₅₀ (min)	48.38 (39.729-66.915)	48.28 (39.729-66.915)
	KDT ₉₅ (min)	145 (92.966-435.066)	151.27 (92.966-435.066)
	Susceptibility status	Resistant	Resistance
Permethrin / Propoxur	% Mortality (24h)	100	100
	KDT ₅₀ (min)	21.29 (19.464-24.563)	21.22 (19.464-24.563)
	KDT ₉₅ (min)	43.85 (37.428-55.383)	38.30 (37.428-55.383)
	Susceptibility status	Susceptible	Susceptible
Permethrin/Pirimiphos-methyl	% Mortality (24h)	99	97
	KDT ₅₀ (min)	57.58 (51.751-66.208)	54.96 (51.751-66.208)
	KDT ₉₅ (min)	197.09 (149.257-294.939)	190.06 (149.257-294.939)
	Susceptibility status	Susceptible	Resistance suspected

4.3.2 Resistance reduction of insecticide combinations to *Anopheles gambiae* s.l population from Lagos state, Nigeria

The results for the resistance reduction fold of the combinations of insecticide are presented in Table 15 and 16. Table 15 presents the resistance reduction for *Anopheles* mosquitoes from Lagos Mainland. For DDT and its two possible combinations; DDT/Propoxur and DDT/Pirimiphos-methyl, resistance reduction was very high. The resistance reduction was 10.79 and 5.33 when DDT is compared with DDT/Propoxur and DDT/Pirimiphos-methyl respectively. Resistance reduction for permethrin when related with Permethrin / Propoxur and Permethrin / Pirimiphos-methyl is 3.56 and 1.36 respectively. For pirimiphos-methyl, the resistance reduction was very low in its two possible combinations while that of propoxur was 0.92 for DDT / Propoxur and 1.01 for Permethrin / Propoxur. The results of resistance reduction in mosquitoes from Kosofo when exposed to insecticide combinations showed the same trend as that of mosquito samples from Lagos Mainland except for the values which are much higher in DDT and its combinations and very low for propoxur and its combinations (Table 16). Figures 7-14 presents comparisons between knockdown rate of single insecticide and their various possible combinations.

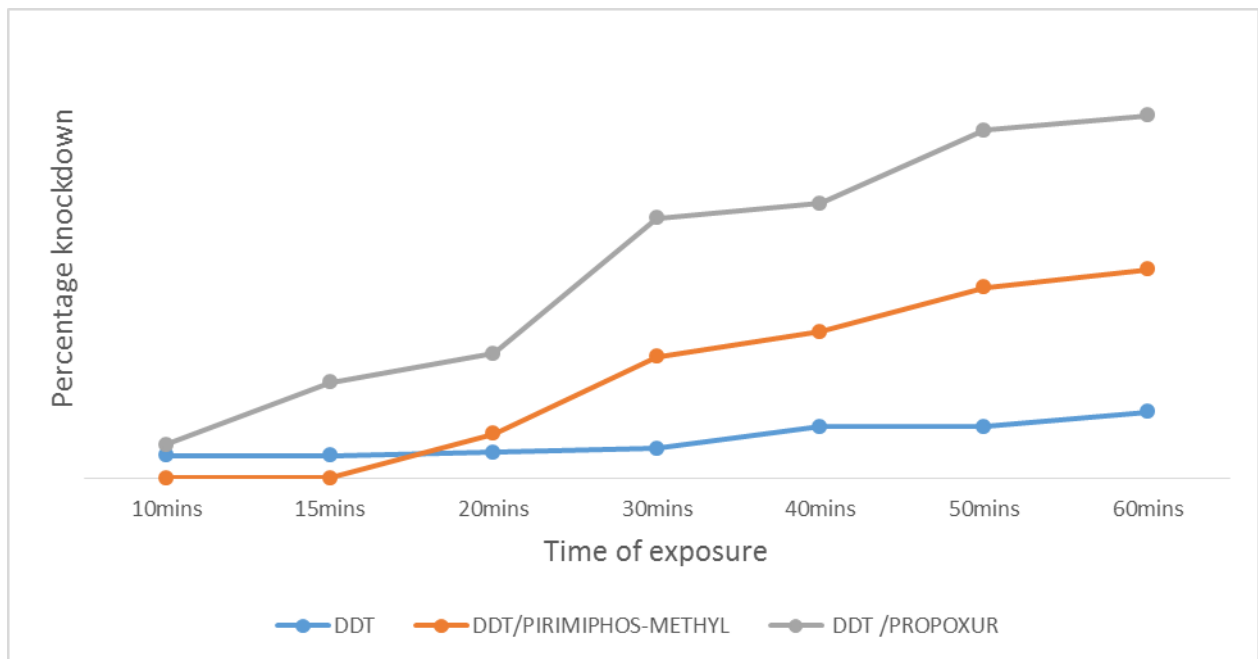


Figure 7: Comparison between percentage knockdown of *Anopheles* spp. exposed to **DDT**, **DDT/PIRIMIPHOS-METHYL** and **DDT/PROPOXUR** in Kosofe LGA, Lagos State

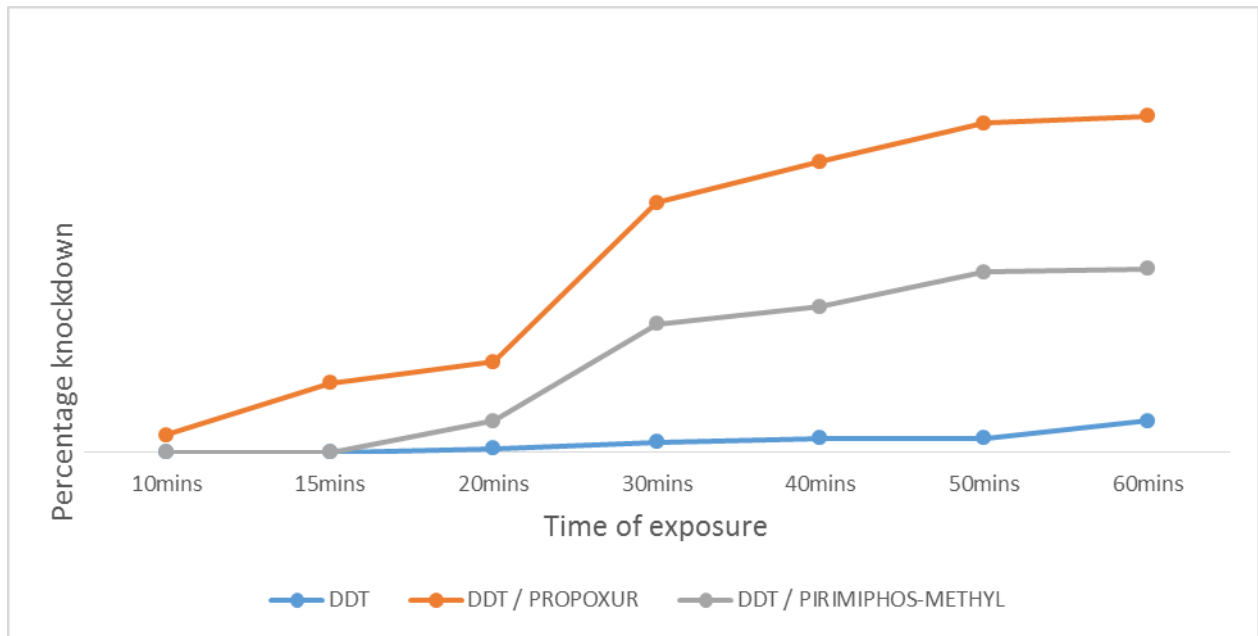


Figure 8: Comparison between percentage knockdown of *Anopheles* spp. exposed to **DDT**, **DDT/PROPOXUR** and **DDT/PIRIMIPHOS-METHYL** in Lagos Mainland LGA, Lagos state

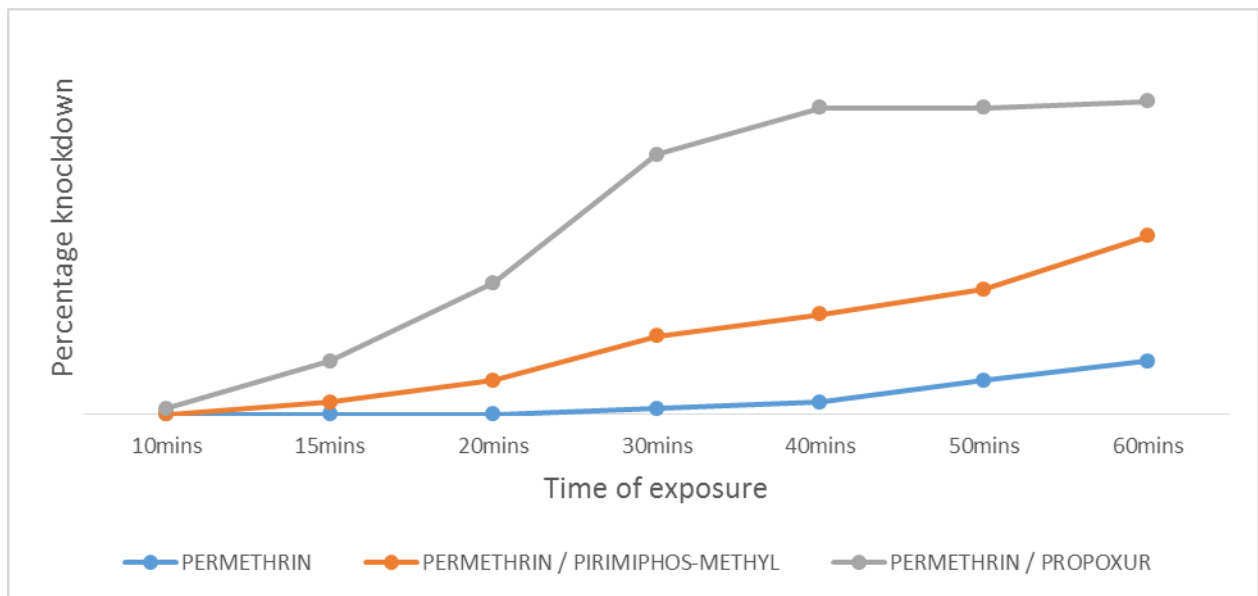


Figure 9: Comparison between percentage knockdown of *Anopheles* spp. exposed to **PERMETHRIN**, **PERMETHRIN/PIRIMIPHOS-METHYL** and **PERMETHRIN/PROPOXUR** in Kosofe LGA, Lagos State

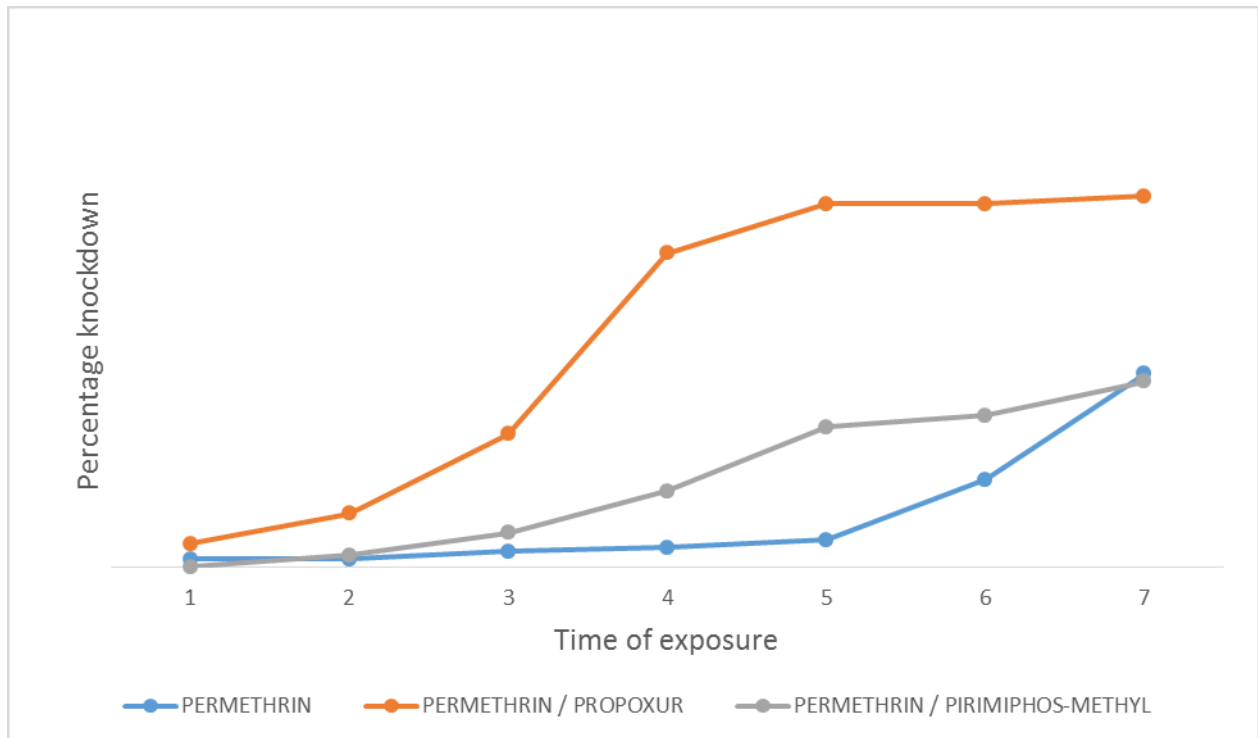


Figure 10: Comparison between percentage knockdown of *Anopheles* spp. exposed to **PERMETHRIN**, **PERMETHRIN / PIRIMIPHOS-METHYL** and **PERMETHRIN / PROPOXUR** in Lagos Mainland LGA, Lagos state

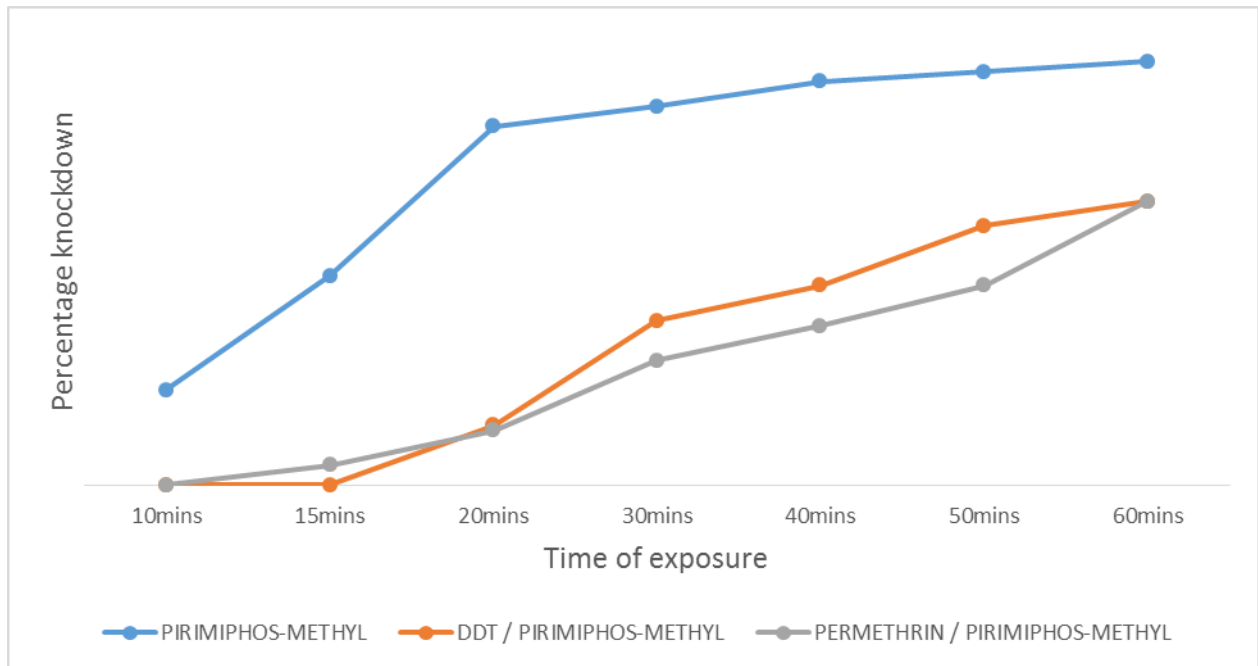


Figure 11: Comparison between percentage knockdown of *Anopheles* spp. exposed to PIRIMIPHOS-METHYL, DDT/PIRIMIPHOS-METHYL and PERMETHRIN/PIRIMIPHOS-METHYL in Kosofe LGA, Lagos State

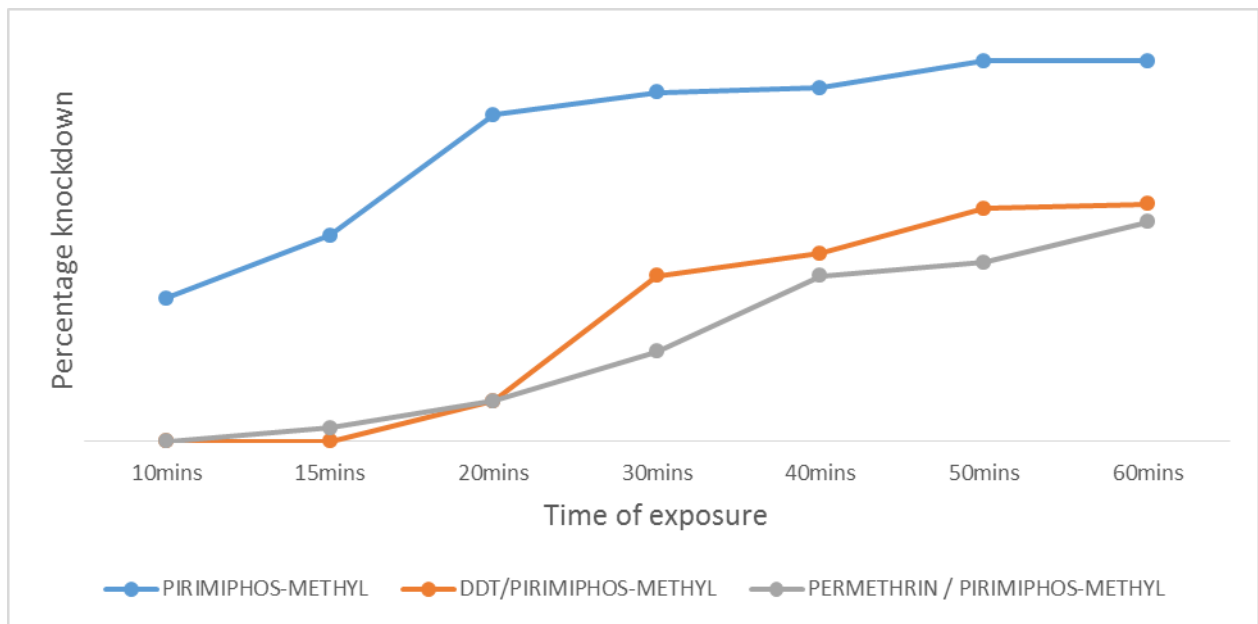


Figure 12: Comparison between percentage knockdown of *Anopheles spp.* exposed to **PIRIMIPHOS-METHYL**, **DDT/PIRIMIPHOS-METHYL**, **PERMETHRIN/PIRIMIPHOS-METHYL** in Lagos Mainland LGA, Lagos state

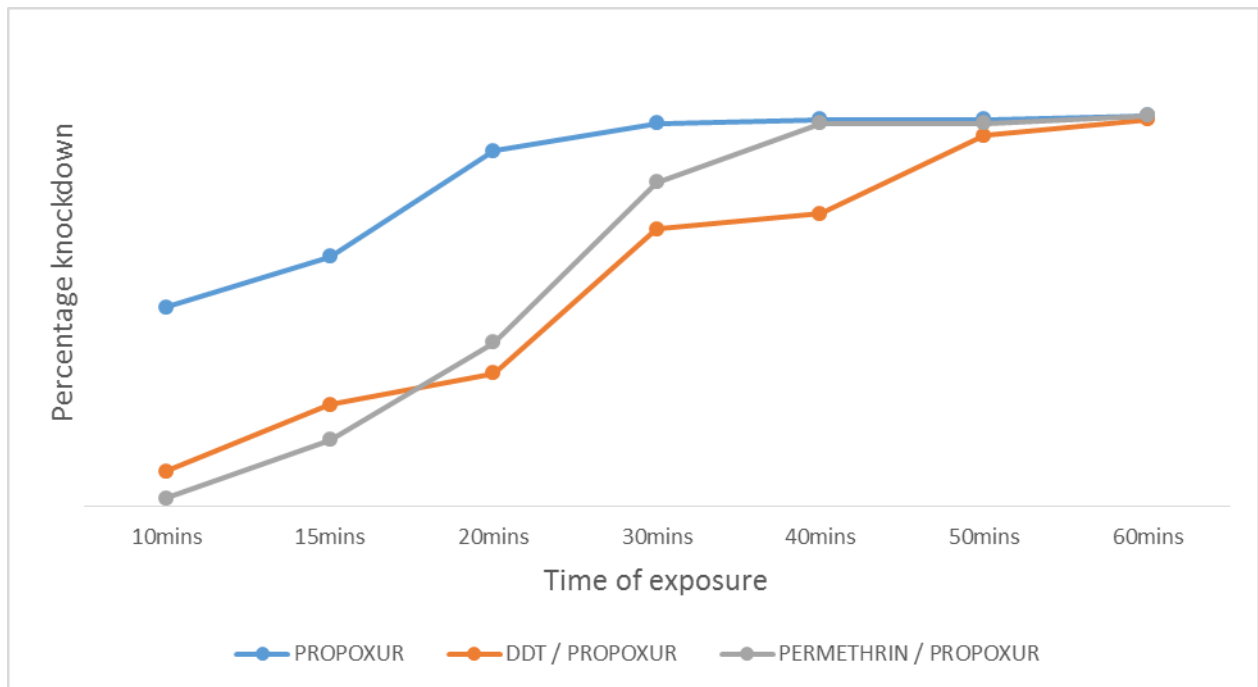


Figure 13: Comparison between percentage knockdown of *Anopheles spp.* exposed to **PROPOXUR, DDT/PROPOXUR and PERMETHRIN/PROPOXUR** in Kosofe LGA, Lagos State

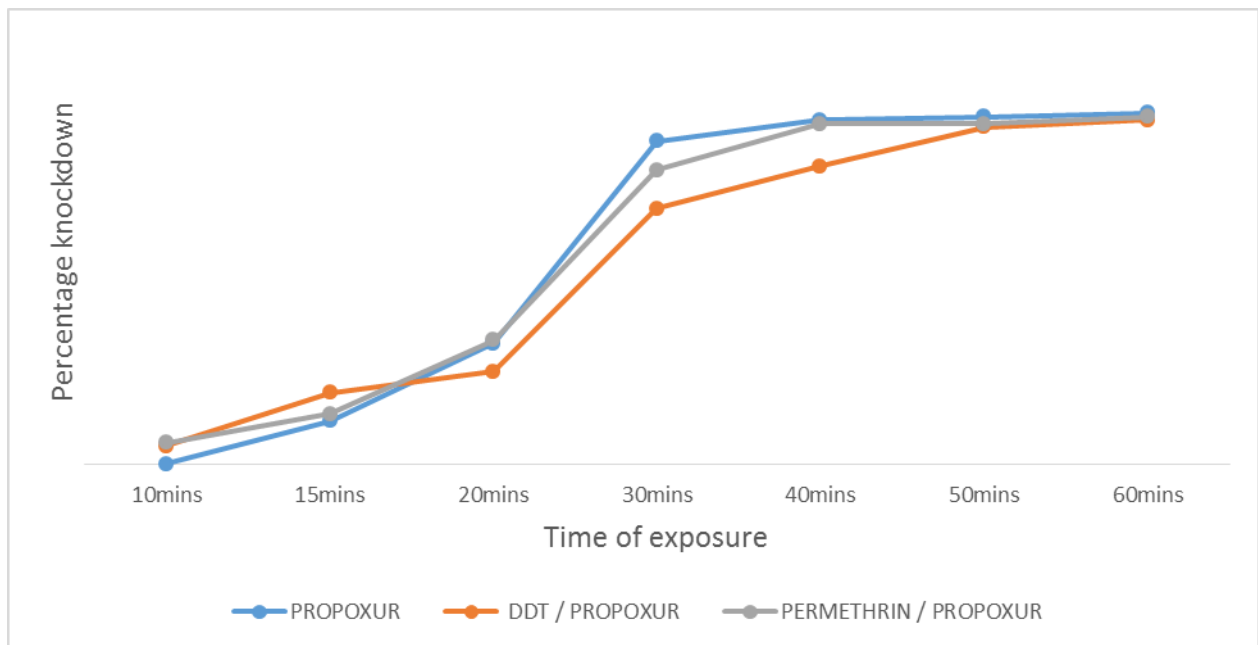


Figure 14: Comparison between percentage knockdown of *Anopheles* spp. exposed to **PROPOXUR**, **PROPOXUR/DDT** and **PERMETHRIN/PROPOXUR** in Lagos Mainland LGA, Lagos state

Table 15: Resistance reduction of insecticide mixtures to *Anopheles spp*, from Lagos Mainland, Lagos state, Nigeria

Study site	Insecticide Combination	KDT ₅₀ (mins)	Insecticide	KDT ₅₀ (mins)	Resistance Reduction
Lagos Mainland	DDT / Propoxur	23.92	DDT	258.07	10.79
			Propoxur	22.06	0.92
	DDT / Pirimiphos-methyl	48.38	DDT	258.07	5.33
			Pirimiphos-Methyl	14.61	0.30
	Permethrin / Propoxur	21.95	Permethrin	78.49	3.56
			Propoxur	22.06	1.01
	Permethrin / Pirimiphos-methyl	57.58	Permethrin	78.49	1.36
			Pirimiphos-methyl	14.61	0.25

Table 16: Resistance reduction of insecticide mixtures to *Anopheles spp.* from Kosofe, Lagos state, Nigeria

Study site	Insecticide Combination	KDT ₅₀ (mins)	Insecticide	KDT ₅₀ (mins)	Resistance Reduction
Kosofe	DDT / Propoxur	30.98	DDT	724.16	23.38
			Propoxur	10.46	0.34
	DDT / Pirimiphos-methyl	48.28	DDT	724.16	14.99
			Pirimiphos-Methyl	17.35	0.36
	Permethrin / Propoxur	21.22	Permethrin	103.48	4.88
			Propoxur	10.46	0.49
	Permethrin / Pirimiphos-methyl	54.96	Permethrin	103.48	1.88
			Pirimiphos-methyl	17.35	0.32

4.4 Resistance mechanisms of resistant *Anopheles gambiae* s.l in Lagos

4.4.1 Target-site mutation

A total of 241 mosquitoes resistant to DDT and permethrin susceptibility test were analysed for *kdr* mutation. The Leu-Phe (L1014F) mutation was detected in the study area in homozygous resistant form but only one heterozygous form was found at Ojo study site. AS-PCR identified 29 mosquitoes as 'RR', 1 mosquito as 'Rr' and 211 mosquitoes as 'rr' (Table 17). The highest *kdr* frequency was recorded at Lagos mainland (16.4%) while the lowest was recorded at Ojo (6.7%). Using Chi-squared P value for Hardy-Weinberg expectations for the *kdr* gene ($X^2 = 231.78$, $df = 1$, $P < 0.05$), there was deviation from Hardy-Weinberg expectations (HWE).

One hundred and thirty-four (134) mosquito samples resistant to pirimiphos-methyl were screened for *ace-1^R* allele, no *ace-1^R* mutation was detected in all the sampled resistant population.

Table 17: Distribution and frequency of *kdr* allele in *Anopheles gambiae* s.l in Lagos

Study site	No Examined	<i>Kdr</i> Mutation			Allele frequency F (<i>kdr</i>) (%)
		RR	Rr	rr	
Kosofe	69	8	0	61	11.6
Ojo	68	4	1	63	6.7
Lagos Mainland	104	17	0	87	16.4
Total	241	29	1	211	12.2

4.4.2 Metabolic enzymes

The activities of 3 enzymes associated with metabolic resistance in *Anopheles gambiae* s.l; Esterase, Glutathione-S-Transferases (GST) and Monooxygenase were determined. Mean activities of enzymes in untreated mosquitoes and insecticide induced population is presented in Table 18.

Table 18: Mean activity of enzymes in untreated mosquitoes and insecticide-induced population

Enzyme/Insecticide	Esterase	GST	Monooxygenase
Untreated	37.982 $\pm$ 2.897	120.250 $\pm$ 13.972	1.345 $\pm$ 0.107
DDT	38.550 $\pm$ 2.568	110.220 $\pm$ 31.686	1.152 $\pm$ 0.251
Permethrin	46.990 $\pm$ 5.423	63.650 $\pm$ 9.861*	2.371 $\pm$ 0.261*
Pirimiphos-methyl	50.153 $\pm$ 10.441	110.912 $\pm$ 22.581	2.065 $\pm$ 0.452
Propoxur	35.877 $\pm$ 5.077	95.033 $\pm$ 10.862	1.393 $\pm$ 0.131
DDT/Propoxur	36.768 $\pm$ 2.609	91.765 $\pm$ 4.959*	1.361 $\pm$ 0.067
DDT/Pirimiphos-methyl	52.008 $\pm$ 5.306*	108.562 $\pm$ 9.658	1.101 $\pm$ 0.291
Permethrin/Propoxur	40.823 $\pm$ 5.296	107.473 $\pm$ 16.734	1.172 $\pm$ 0.176
Permethrin/Pirimiphos-methyl	48.558 $\pm$ 9.833	130.213 $\pm$ 16.642	1.448 $\pm$ 0.297

Result is expressed in Mean $\pm$ standard error of mean

* Significance difference at (P<0.05) when compared with untreated mosquitoes

4.4.2.1 Esterase

Figure 15 presents the standard graph with best fit line ($R^2=0.904$, $y= 0.06 + 0.46*x$) for esterase activity.

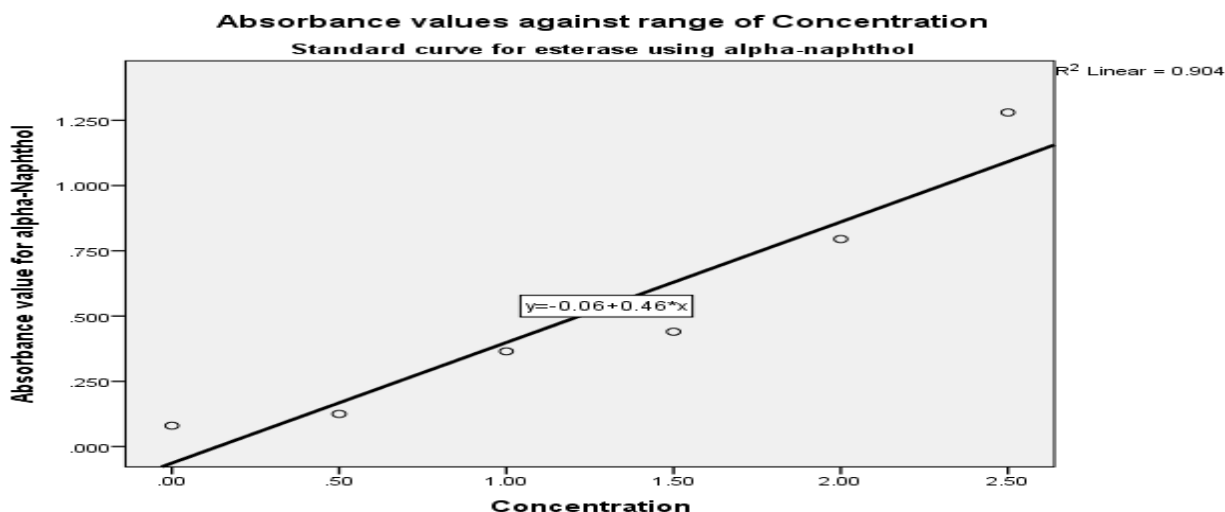


Figure 15: Standard graph of absorbance against concentration for esterase using α -naphthol

Results of esterase activities in mosquitoes resistant to DDT/Pirimiphos-methyl and the two individual insecticide is presented in figure 16. Esterase activities in DDT and Pirimiphos-methyl was not elevated, however, esterase activity in DDT/Pirimiphos-methyl was significant ($p=0.039$) elevated when compared with unexposed samples. Also, mean esterase activity of DDT ($p=0.849$) and DDT/Propoxur ($p=0.676$) induced mosquitoes when compared with unexposed samples were insignificant (figure 17). Esterase activities in permethrin ($p=0.229$), permethrin/propoxur ($p=0.640$) and permethrin/pirimiphos-methyl ($p=0.294$) induced mosquitoes showed no significant difference when compared with unexposed samples (Figures 18 & 19). Activity of esterase in propoxur and propoxur combinations were all insignificant (Figures 17 & 19). Comparison of esterase activities between propoxur induced mosquitoes and unexposed population, propoxur/DDT combination and unexposed population and

propoxur/permethrin and unexposed population gave p values of 0.782, 0.676 and 0.640 respectively.

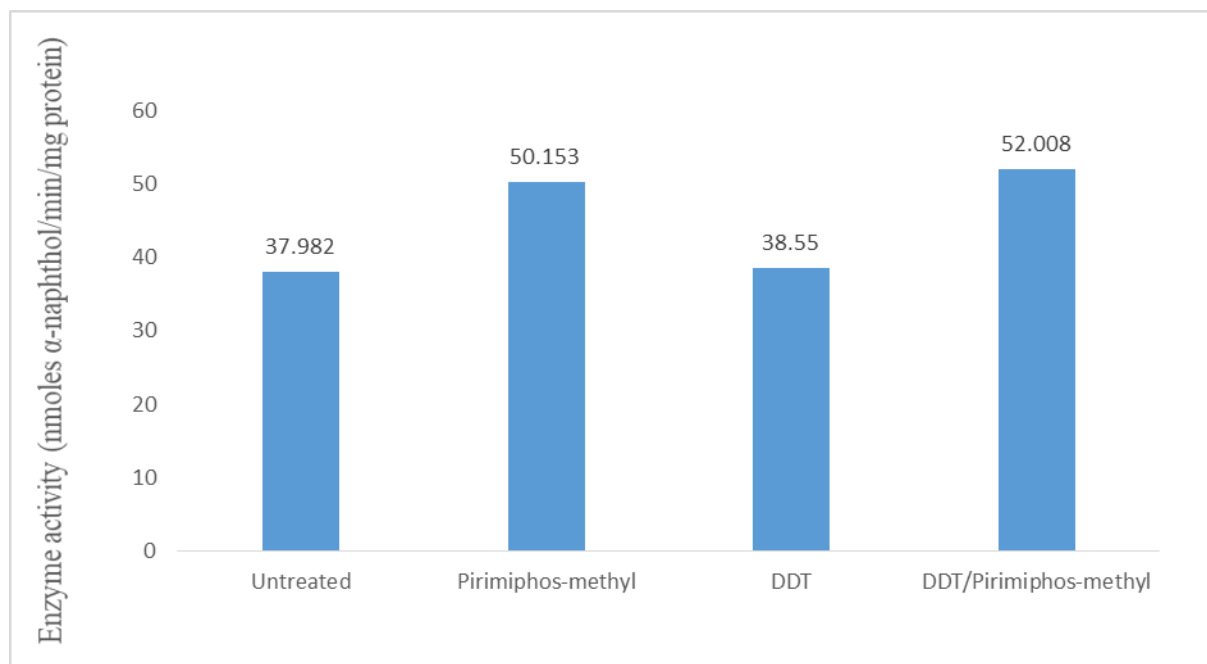


Figure 16: A comparison of esterase activity in DDT/Pirimiphos-methyl, DDT, Pirimiphos-methyl induced mosquitoes and untreated mosquitoes

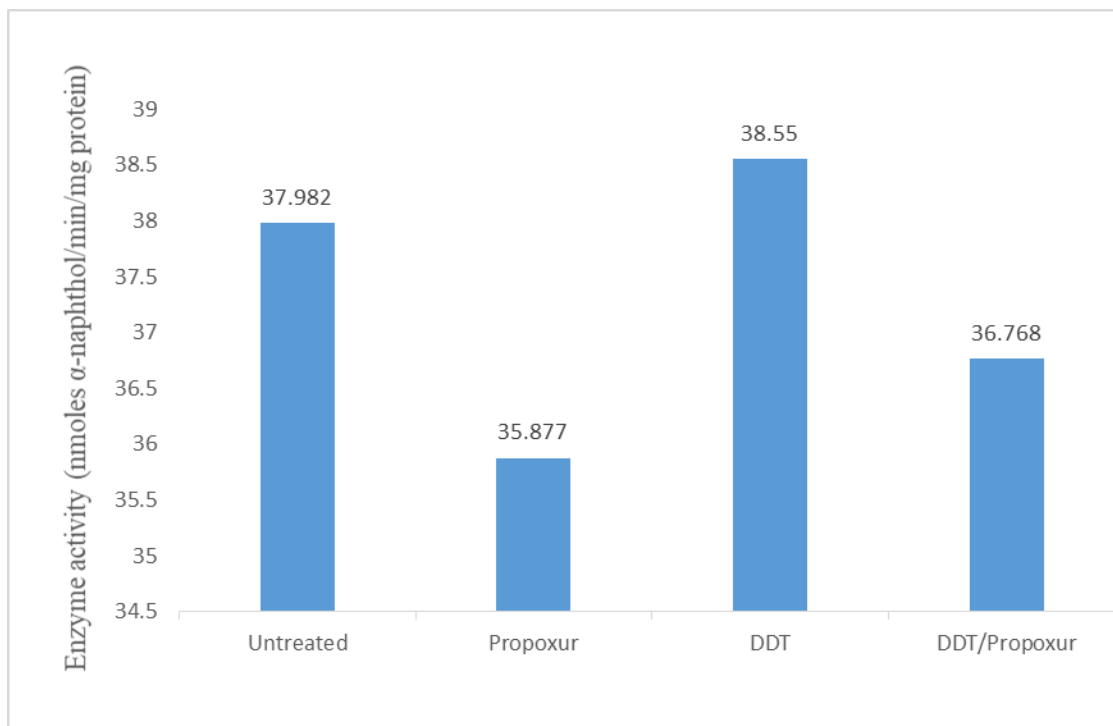


Figure 17: A comparison of esterase activity in DDT/Propoxur, DDT, Propoxur induced mosquitoes and untreated mosquitoes

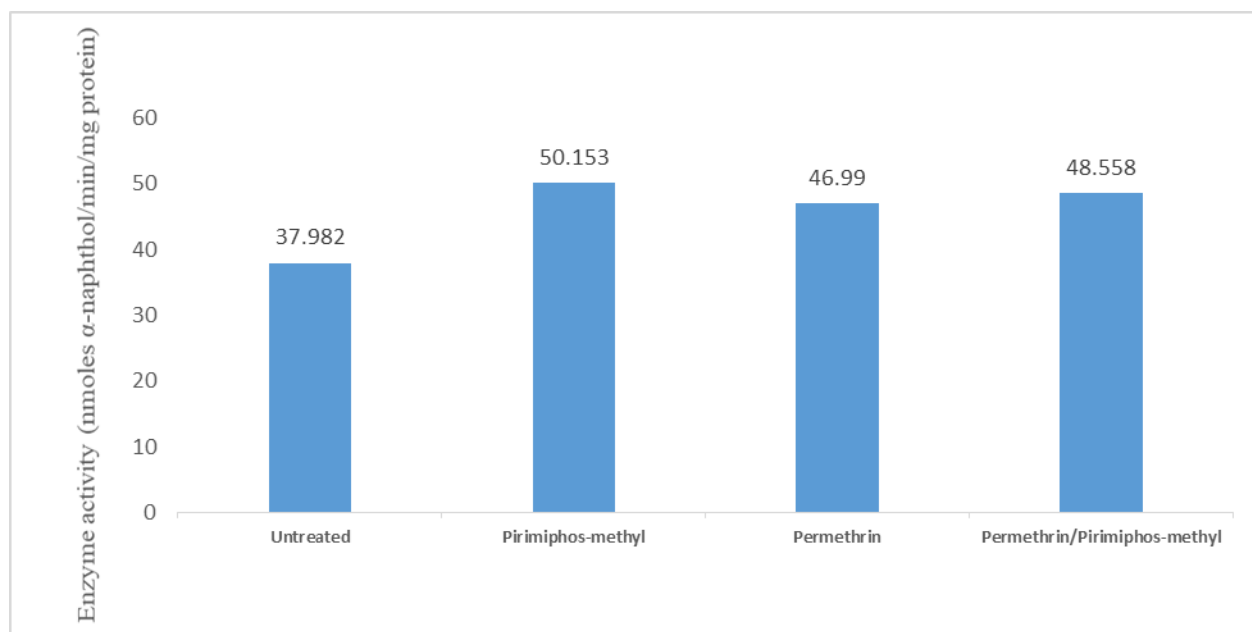


Figure 18: A comparison of esterase activity in Permethrin/Pirimiphos-methyl, Permethrin, Pirimiphos-methyl induced mosquitoes and untreated mosquitoes

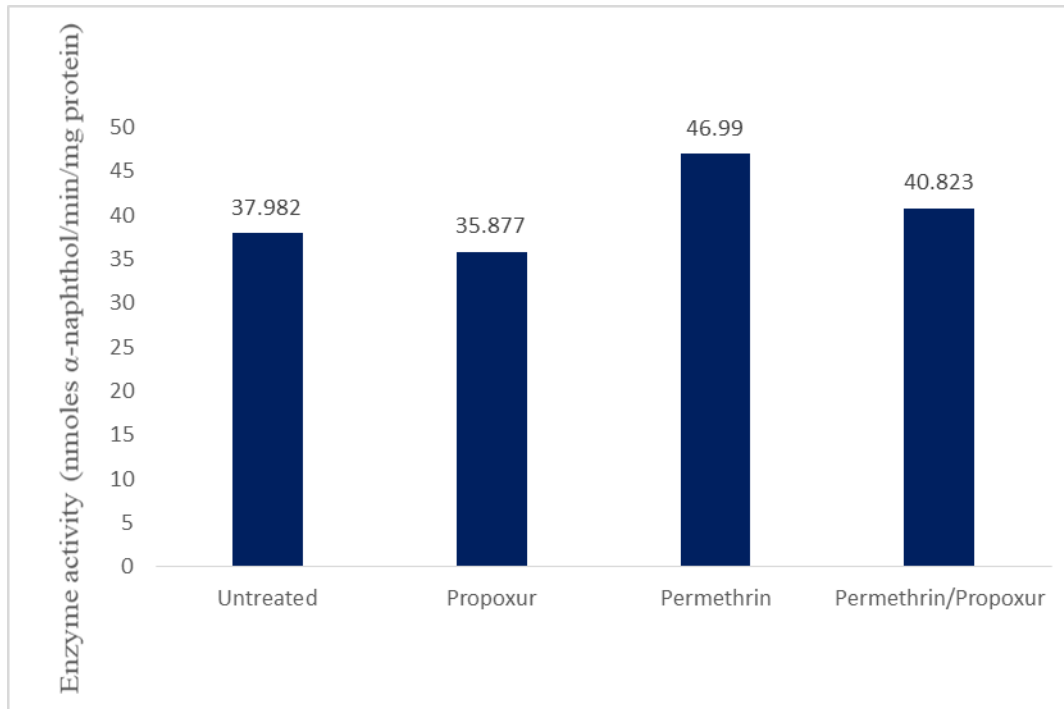


Figure 19: A comparison of esterase activity in Permethrin/Propoxur, Permethrin, Propoxur induced mosquitoes and untreated mosquitoes

4.4.2.2 Gluthathione-S- Transferase (GST)

Results of GST activities in mosquitoes resistant to DDT and DDT combinations revealed that difference in mean GST activity of DDT ($p=0.632$) and DDT/Pirimiphos-methyl ($p=0.608$) induced mosquitoes when compared with unexposed mosquitoes were insignificant (Figure 20), however the downregulation in GST activity in DDT/Propoxur induced mosquitoes when compared with unexposed mosquitoes was significant ($p=0.042$) (Figure 21).

The downregulation in GST activity of permethrin induced mosquitoes when compared with unexposed mosquitoes was highly significant ($p=0.007$) (Figure 22) but showed no significant difference in permethrin/pirimiphos-methyl ($p=0.451$) and permethrin/propoxur ($p=0.552$) induced strains (Figures 23).

Activities of GST in pirimiphos-methyl and pirimiphos-methyl combinations were all insignificant (Figures 20 & 22). Comparison of GST activities between pirimiphos-methyl resistant mosquitoes and unexposed mosquitoes, pirimiphos-methyl/DDT combination and unexposed mosquitoes, pirimiphos-methyl/permethrin and unexposed mosquitoes gave P values of 0.736, 0.608 and 0.451 respectively.

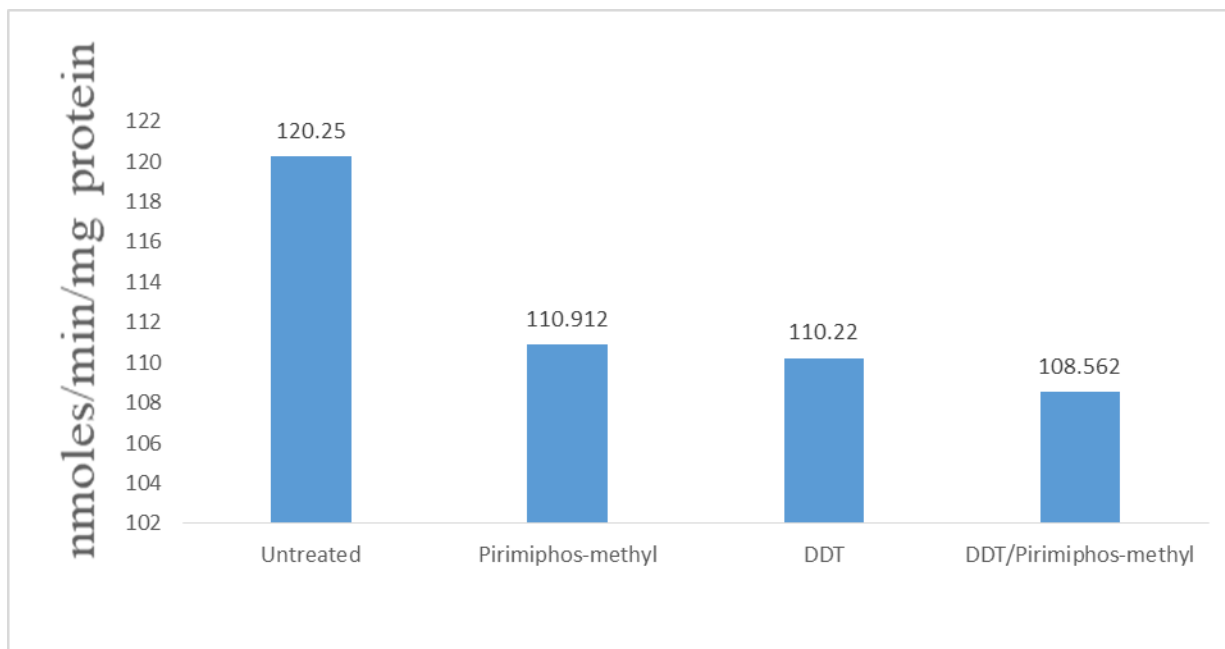


Figure 20: A comparison of GST activity in DDT/Pirimiphos-methyl, DDT, Pirimiphos-methyl induced mosquitoes and untreated mosquitoes

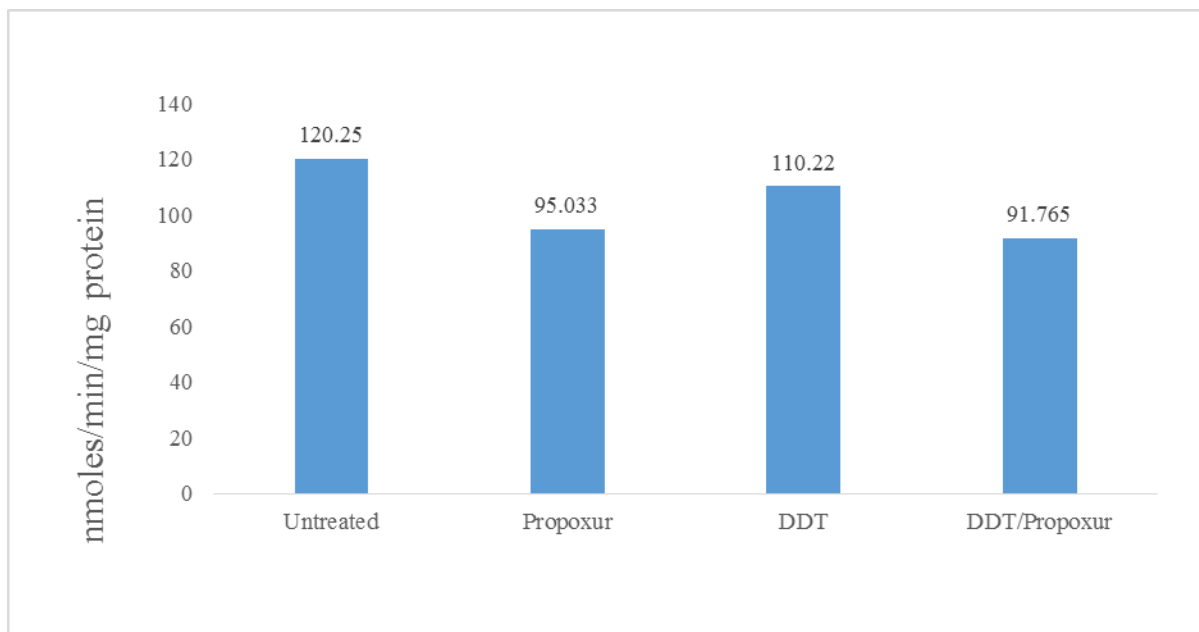


Figure 21: A comparison of GST activity in DDT/Propoxur, DDT, Propoxur induced mosquitoes and untreated mosquitoes

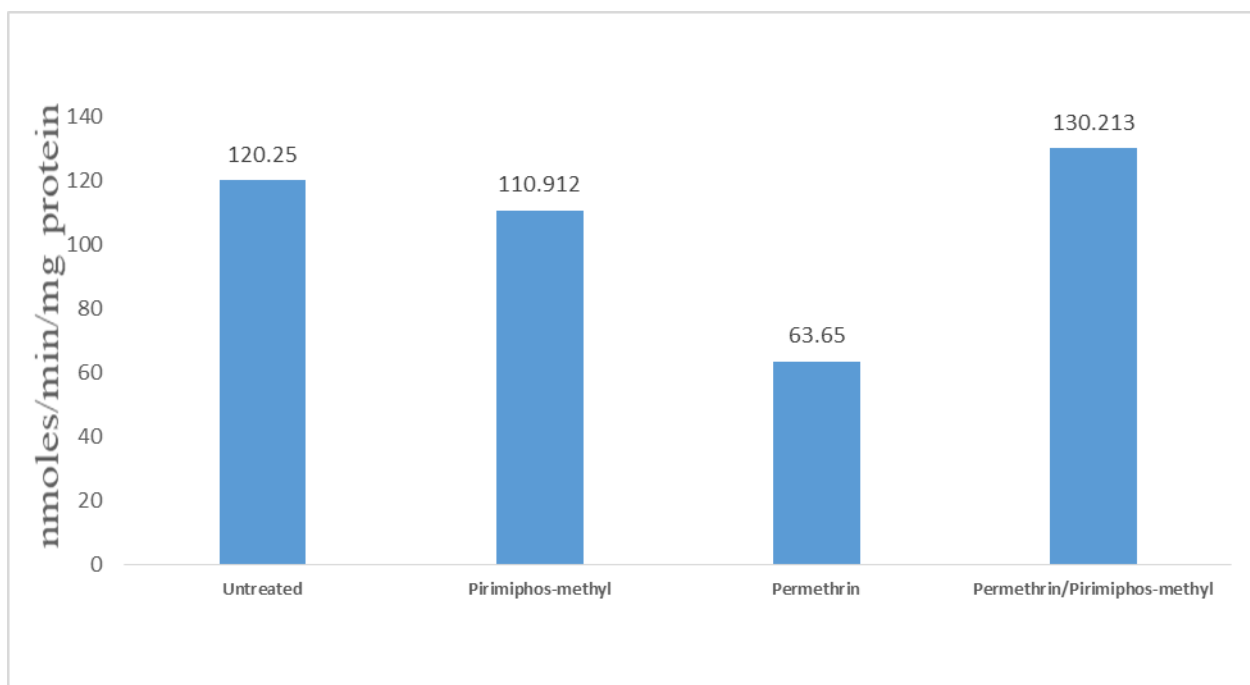


Figure 22: A comparison of GST activity in Permethrin/Pirimiphos-methyl, Permethrin, Pirimiphos-methyl induced mosquitoes and untreated mosquitoes

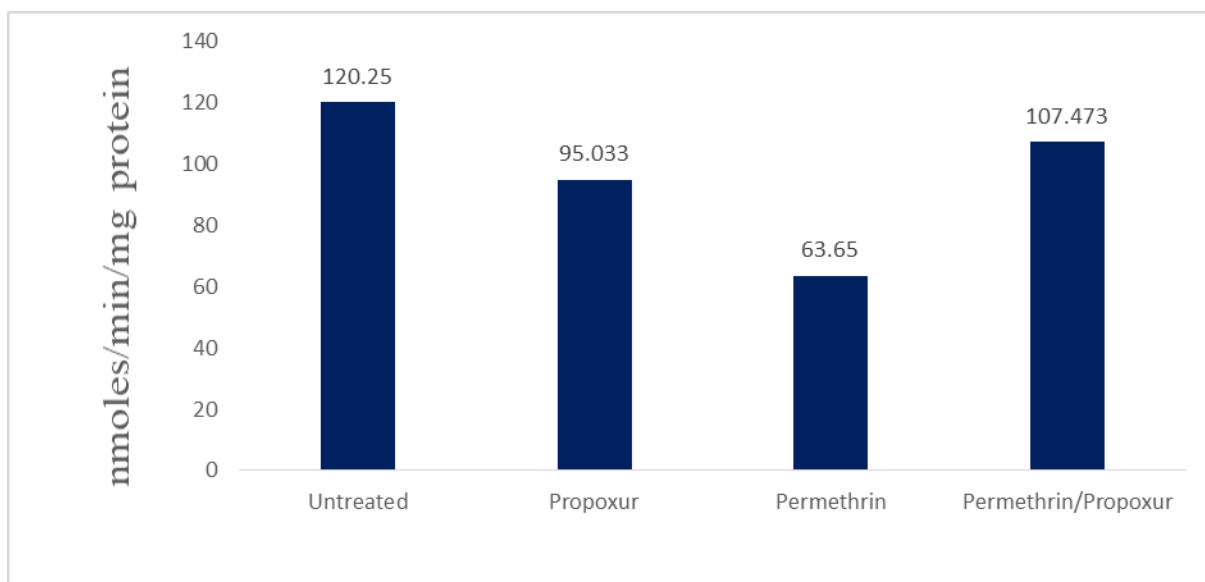


Figure 23: A comparison of GST activity in Permethrin/Propoxur, Permethrin, Propoxur induced mosquitoes and untreated mosquitoes

4.4.2.3 Monooxygenase

Figure 32 presents the standard graph with best fit line ($R^2=0.268$, $y= 0.02 + 0.86*x$) for esterase activity.

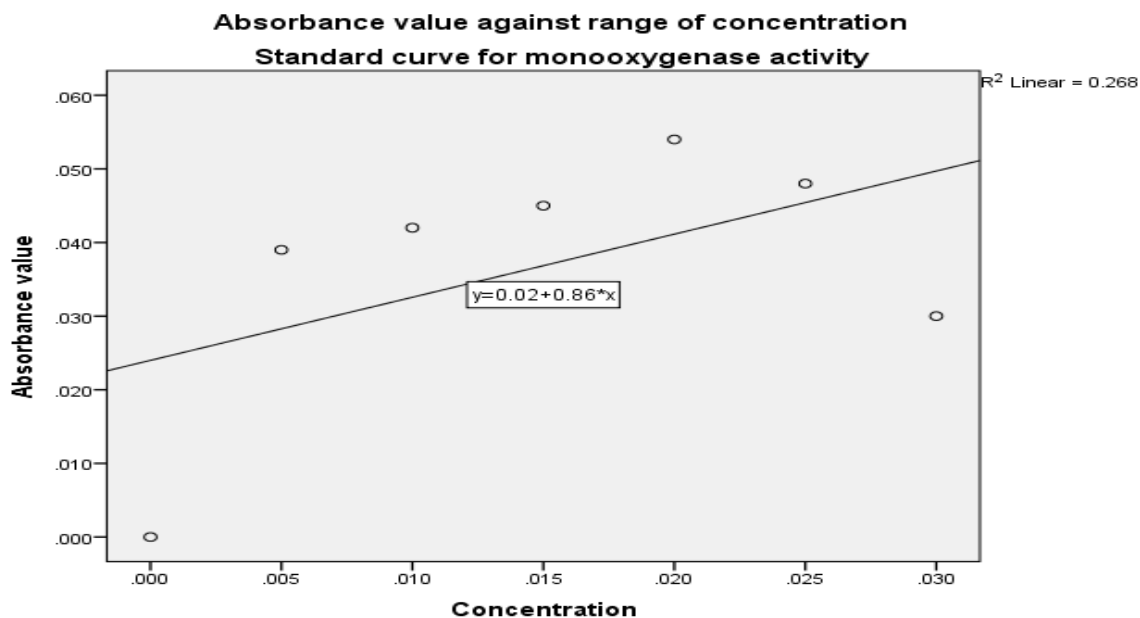


Figure 24: Standard graph of absorbance against concentration for monooxygenase

Results of monooxygenase activities in mosquitoes resistant to DDT and DDT combinations revealed that the difference in mean monooxygenase activity when DDT induced mosquitoes is compared with unexposed mosquitoes ($p=0.538$) (Figure 25), DDT/pirimiphos-methyl is compared with unexposed mosquitoes (0.460) (Figure 25) and DDT/propoxur combination is compared with unexposed mosquitoes ($p=0.920$) are all insignificant.

Similar to the results for GST, there was significant upregulation in monooxygenase activity of permethrin induced mosquitoes when compared with unexposed mosquitoes ($p=0.007$) (Figure 26). In contrast, there was no significant difference when activities in permethrin/pirimiphos-methyl combination induced mosquitoes were compared with unexposed mosquitoes (0.748) (Figure 26) and permethrin/propoxur induced mosquitoes was compared with unexposed mosquitoes (0.441) (Figure 27).

The results for monooxygenase activities for pirimiphos-methyl induced mosquitoes and unexposed mosquitoes shows there is no significant difference in their activities (Figure 26). Activity of monooxygenase in propoxur induced mosquitoes ($P=0.849$) when compared with unexposed mosquitoes was insignificant (Figure 27).

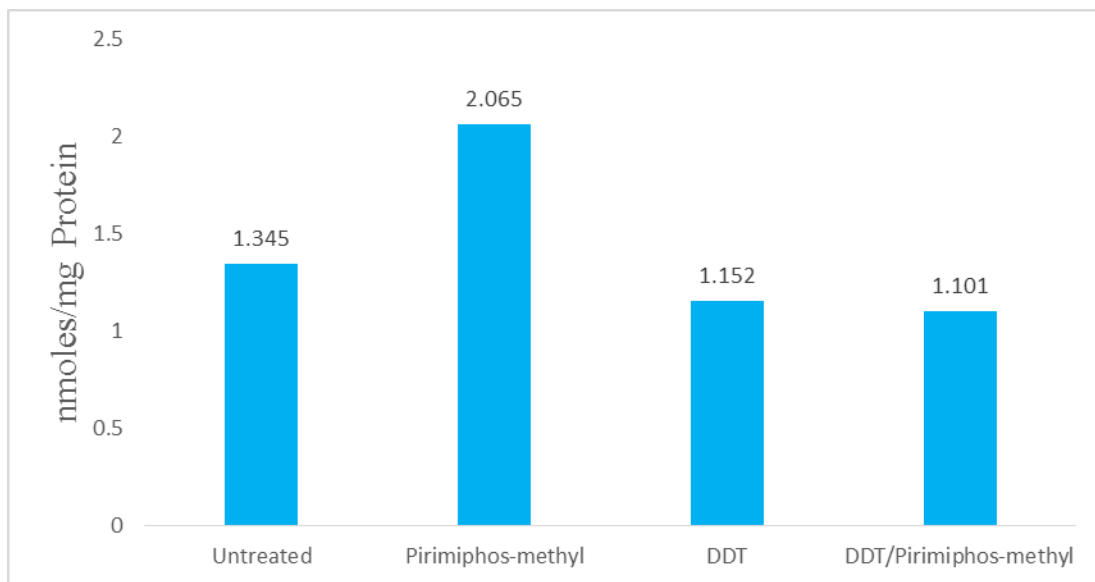


Figure 25: A comparison of monooxygenase activity in DDT/Pirimiphos-methyl, DDT, Pirimiphos-methyl induced mosquitoes and untreated mosquitoes

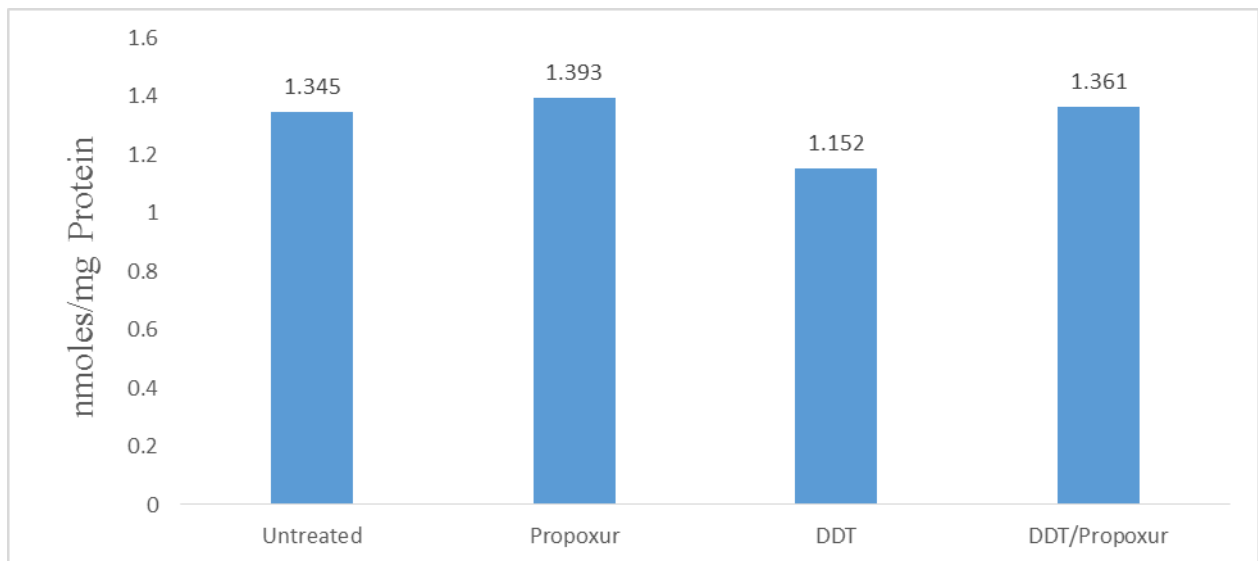


Figure 26: A comparison of monoxygenase activity in DDT/Propoxur, DDT, Propoxur induced mosquitoes and untreated mosquitoes

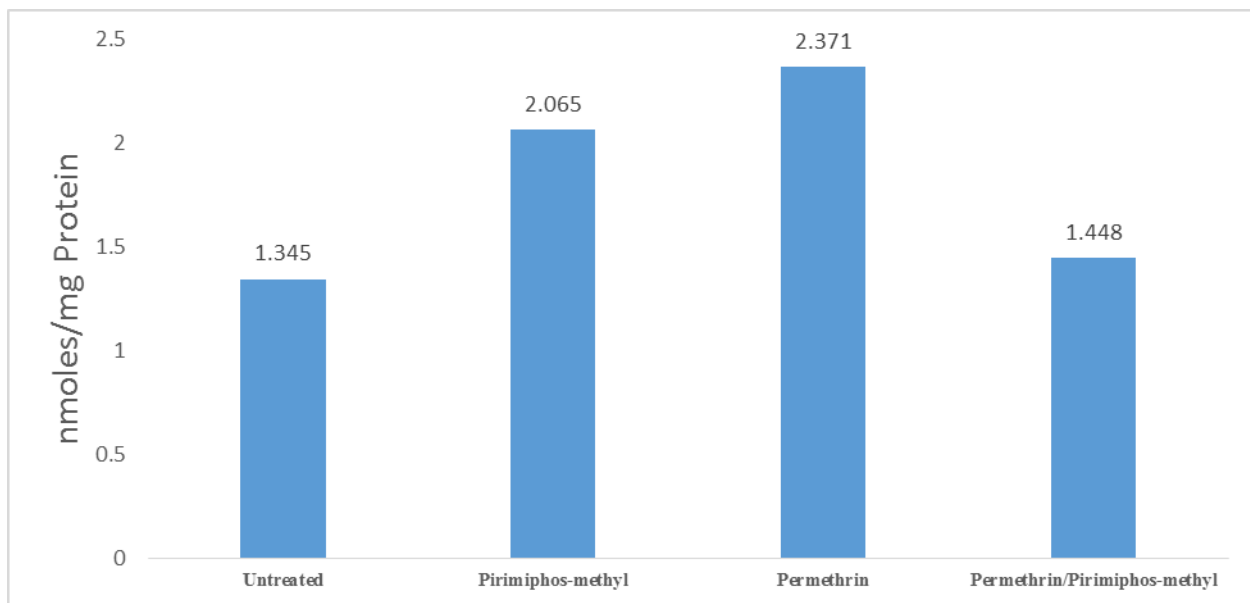


Figure 27: A comparison of monooxygenase activity in Permethrin/Pirimiphos-methyl, Permethrin, Pirimiphos-methyl induced mosquitoes and untreated mosquitoes

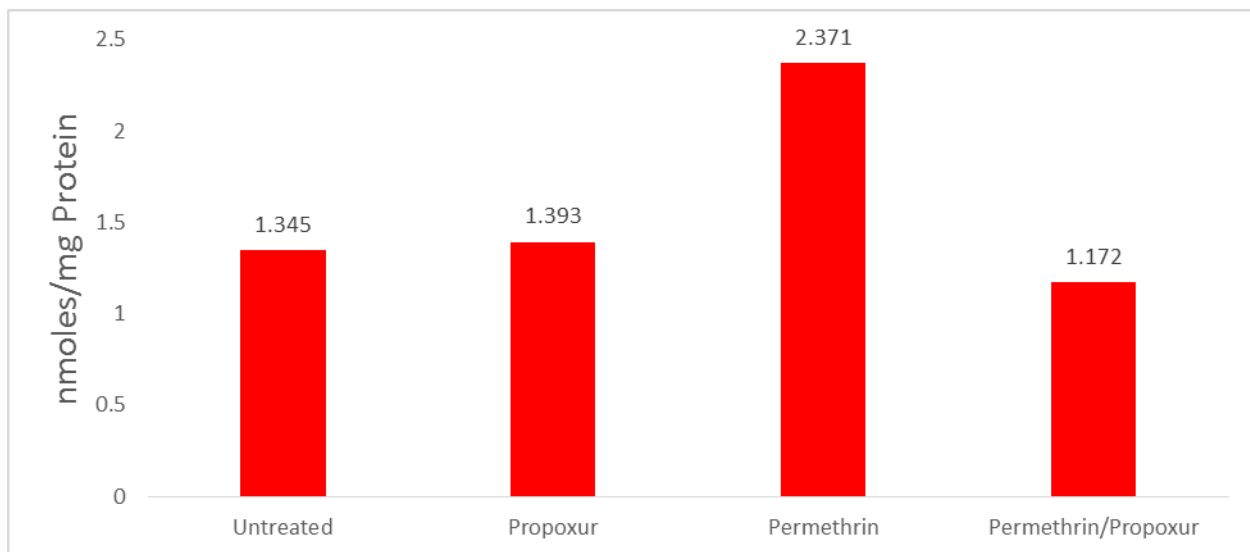


Figure 28: A comparison of monooxygenase activity in Permethrin/Propoxur, Permethrin, Propoxur induced mosquitoes and untreated mosquitoes

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Knowledge, Attitude and Practices to Malaria Vector Control Methods

As in numerous urban settings, results from the KAP study revealed that majority of the respondents in Lagos state were youths with post-primary education (Honwana, 2014). All respondents in the study employ a combination of methods to protect themselves against mosquito. The pattern of response is similar to an earlier study conducted by Obembe *et al.*, (2014) which found use of LLINs, insecticides and mosquito coils to be the 3 major methods employed by residents of Ilorin to combat mosquitoes. In the present study, majority (77%) make use of insecticides in combination with other methods. Also, approximately half of the respondents (48.2%) sleep under LLINs. High level of LLIN use have been recorded in similar studies in Nigeria (Singh *et al.*, 2014), Malawi (Masangwi *et al.*, 2012) and Ethiopia (Abate *et al.*, 2013). Other prominent method employed against mosquito include, sleeping with windows closed (30.7%), screening of windows with net (40.2%), burning of coil/grass as repellants (22.3%) and covering of the body with clothes (24.7%). IRS and burning of coil and local plants have also been documented to be the major precautionary tools in mosquito control in Atacora, Benin republic (Aikpon *et al.*, 2013) and in two rural farming communities in Oyo state, Nigeria (Oladebo *et al.*, 2010). As stated in the WMR (2016), concurrent increase in the use of insecticide and access to LLINs can be attributed to the decrease in the incidence of malaria. Similarly, Bekele *et al.*, (2012) in a study conducted in Ethiopia also established that increase in usage of IRS and ITNs leads to significant reduction in incidence of malaria.

From this survey, the main reason determining the choice of methods among respondents was the “effectiveness” of the preferred method. For respondents that decline use of insecticides,

knowledge of the negative health impact of insecticides was a key factor for non-acceptance. The negative health impact is mainly attributed to the irritation caused to the eyes and nose as well as deposit of residue on house materials. Similarly, the discomfort caused by this offensive irritation affects human with clinical conditions such as asthma. Albeit considered very effective, the production of heat and disruption of airflow by LLINs and ordinary net also influenced choice of some respondents. Earlier, Aina *et al.* (2013) had also noted that the use of LLINs in a community in Lagos state was low due to the heat, which made respondents to prefer screening their windows with net. Similar occurrences have also been reported on low usage of insecticide treated nets in other Nigerian communities (Oguonu *et al.* 2005). A good number of respondents also considered “ease of use” and also make use of any readily available method.

Unlike some other communities in Lagos state like Ibeshe (Aina *et al.* 2013), 87.3% of the respondents in the sampled communities have not benefited in any community vector control programme, pointing to the fact that majority of the available LLINs were not obtained by direct community vector control interventions. Also, insecticides were sprayed individually at homes and not through a coordinated IRS programme by an organised body and this allows for indiscriminate use of insecticides. As personal and indiscriminate use of insecticides have been one of the main factors leading to spread in insecticide resistance, the result from this survey further lend credence to the fact that the increasing insecticide resistance in Lagos state may have been a result of indiscriminate use of insecticides over years.

Of the 242 respondents making use of LLINs, 225 (93.0%) vouched for its effectiveness in mosquito control. The high percentage of respondents attesting to the effectiveness of LLINs in mosquito control is a positive stance, as these individuals can be relied on to help orientate other residents when the need arises. This portends a positive outcome in the scale up of LLIN use, as

little effort would be put into creating awareness on the effectiveness of LLIN. This leaves us at the shore of other challenges facing LLINs usage which include orientating residents on the aspect of heat production or obstruction of air flow as well as organisation of community based intervention that makes LLIN available to majority of the communities through NGOs, government and other relevant bodies.

Majority of those that don't use insecticide opined that the irritation caused by the chemicals have negative health implications. Its health impacts is mostly felt in the eyes and olfactory organs. Practices and attitude to insecticides use revealed that almost all respondents that use insecticides, spray the insecticides in home at least once a week. Majority spray insecticide every 2 days. The frequency of use and volume released to the environment calls for concern as its resultant impact on non-target organisms as well as induction and spread of insecticide resistance cannot be overlooked. The perfect timing for usage was in the evening as revealed in the survey. None of the respondents sprayed insecticide in the afternoon and few (7.7%) sprayed in the morning alone. Majority that spray in the morning do it to avoid coming in contact with the insecticide and to allow enough time for the diffusion of the chemicals. For the respondents that sprayed the insecticide in the evening, most (44.9%) allow the chemical to diffuse for about 1-2 hours before returning to their rooms. A very good number allows for more than 2 hours for diffusion before returning to their rooms.

The general perception to insecticide usage from the survey are; it is effective, cheap and safer than other methods (28.4%), it is effective but detrimental to the health of the population (21.6%), it impacts negatively on the health condition of users (9.4%) and also it is good for controlling other insects and pests in the home (7.5%). Similarly, while some respondents attest to its long lasting impact (1.7%), some other respondents (13.1%) said the insecticides have lost

their potency and not effective again for mosquito control. Control of other pests with insecticides is old, its cost and availability has made it the first choice of control for many pests found in human habitations. Loss of potency and lack of effectiveness is the consequence of development of resistance by mosquitoes. While the populace may not be conversant with the term; insecticide resistance, the literal understanding and consequence of resistance has been experienced by a good percentage of the population.

Results from choice of insecticides showed that majority relied on insecticides that are locally mixed from high grade pesticides such as “Sniper” and “DD Force” which are not solely produced for mosquito control. They are top grade chemicals containing the organophosphate; Dichlorvos. This has been earlier reported in the work of Idowu *et al.* (2016). These chemicals are pesticides that are produced usually for agricultural use but the populace opt for various grades of dilutions of the pesticides because of its effectiveness as against the certified brands such as Raid, Mobil, Mortein etc. The choice of top grade raw pesticides can be easily attributable to the fact that more than 70% of the respondents usually notice resuscitation of mosquitoes after application of certified brands thereby opting for top grade undiluted pesticides that can be diluted by respondents to achieve more mortality and avoid resuscitation of the mosquitoes. While many of the respondents may not actually be conversant with the term “insecticide resistance”, their experience and responses buttresses the various scientific conclusions about spread and magnitude of insecticide resistance (WHO, 2018). This knowledge is to the advantage of IRM as little or no time is needed to convey the concept and reason for any IRM strategy that is adopted as almost all the population are aware and would easily see a reason to address the challenge of insecticide resistance.

More importantly, the relationship between the responses and the LGAs of respondents were determined. Generally, The LGAs of respondents have no significant impact on the knowledge, attitude and practices of residents in the 3 LGAs. This can be attributed to population homogeneity in Lagos. While Kosofe LGA and Lagos Mainland LGA are found in the urban part of Lagos, Ojo LGA is situated in the semi-urban part of Lagos. This is a pointer to the fact that while the degree of urbanisation in various regions of Lagos state differs, the knowledge, attitude and practices of Lagos state population to mosquito control does not differ significantly from one locality to the other. This will invariably aid the application of policies from one end of the state to another end. This particular factor needs to be seriously considered during the application of interventions so as to forestall the failure of policies and interventions within the state.

Generally, the level of education and type of dwelling were not significantly related ($p>0.05$) to responses of respondents on insecticide use. This may be due to the fact that insecticide has over ages been the predominant method employed across all grades of the population for general pest control. This has made it a common practice among the general population in Lagos without reference to their level of education. The fact that it is cheap also has made it a free-for-all method employed by the elites and non-elites. Also, Education has no impact on sources of LLINs as almost all the respondents making use of LLINs sourced it from the hospital. The source of LLINs in Lagos state is generally limited to the free distribution done at government clinics, Primary Health Centres and hospitals.

5.2 Susceptibility profile of *Anopheles gambiae* in Lagos

5.2.1 Species composition of *Anopheles* mosquitoes in Lagos

Out of the 405 samples selected for species identification, 179 (94.71%) was identified with PCR-RFLP as *An. coluzzii* while only 2 (1.06%) was identified as *An. gambiae* s.s and no *An.*

arabiensis was identified. The predominance of *An. coluzzii* in some sites has also been documented in Akron, Ojoo and Gbedjromede in Benin (Djouaka *et al.*, 2008; Yadouleton *et al.*, 2010), South-Western Burkina Faso (Dabire *et al.*, 2008), Northern and coastal savannah regions of Ghana (De Souza *et al.*, 2010) and Lagos (Oduola *et al.*, 2012). The existence of *An. coluzzii* in isolation in some sites can be attributed to habitat segregation and strong assortative mating in nature which leads to hinderance in gene flow (Weill *et al.*, 2000), however, *An. coluzzii* and *An. gambiae* s.s have been recorded to exist in sympatry in some other studies (Awolola *et al.*, 2012; Wondji *et al.*, 2005; Tene *et al.*, 2013; Namountougou *et al.*, 2012). Hybrid form of *An. coluzzii* and *An. gambiae* s.s was not found at all.

5.2.2 Susceptibility status and knockdown time of single insecticides

Results of susceptibility study showed that field population of *Anopheles* mosquitoes collected from 3 LGAs in Lagos state are resistance to DDT, permethrin and pirimiphos-methyl. However, resistance to permethrin in Ojo LGA is not fully ascertains as the mortality rate stands at 96%. Mortality was generally lowest in pirimiphos-methyl then followed by DDT then permethrin. Widespread resistance to DDT (Oduola *et al.*, 2010; Sulaiman *et al.*, 2014; Awolola *et al.*, 2007), permethrin (Djouaka *et al.*, 2008; Awolola *et al.*, 2007; Awolola and Coetzee, 2005) has been reported in numerous other study from different parts of Nigeria. Due to the high rate of mosquito dispersal (Koekomoer *et al.*, 2011), high rate of resistance to DDT and pyrethroid in Nigeria suggest similar trend across Africa and this have been confirmed in numerous studies (Kristan *et al.*, 2003; Thomsen *et al.*, 2014; WHO, 2017; WHO, 2018). As similarities between DDT and pyrethroid chemical structure have earlier raised concerns about cross-resistance between the two classes of insecticide (Malcolm, 1988), similarities in their mode of action by attacking the sodium voltage gated channel have made resistance to both classes possible with

just one mode of action. The significant correlation ($p=0.045$) in mortality between DDT and pyrethroids in the three study sites also lend credence to the similarity in the mode of action as earlier observed in the work of Yewhalaw *et al.*, (2011). Resistance to both DDT and pyrethroids have been attributed to the presence of Knockdown mutation (*kdr*) (Awolola *et al.*, 2009; Kristan *et al.*, 2003; Djouaka *et al.*, 2008) as well as to metabolic resistance mechanism (Awolola *et al.*, 2009). While the *kdr* mutation is known to arise from the substitution of the leucine to phenylalanine on the 1019 gene of the vector (*kdr-w*), another type of the *kdr* known as *kdr-east* (*kdr-e*) exist predominantly in East Africa and result from the substitution of leucine on the 1019 gene to serine. The *kdr-w* predominantly found in West Africa has been reported in all west African countries where resistance to pyrethroids have been reported (WHO, 2018) and its first report in Nigeria was in 2002 (Awolola *et al.*, 2005). It is however worthy of note that *kdr-east* known to have originated from East Africa have also been reported to exist in Senegal (Ndiath *et al.*, 2015), Burkina Faso (Namountougou *et al.*, 2013) and Benin (Djegbe *et al.*, 2011).

As revealed in this study, resistance to pirimiphos-methyl has also been reported (Oduola *et al.*, 2018; Kisinza *et al.*, 2017; Nwankwo *et al.*, 2017) but contrary reports of high mortality rate to pirimiphos-methyl have been documented in Benin (Aikpon *et al.*, 2014; Gnanguenon *et al.*, 2015), Malawi (Mzilahowa *et al.*, 2016), Zambia (Thomsen *et al.*, 2014), Tanzania (Emidi *et al.*, 2017) and Ghana (Riveron *et al.*, 2016; Fuseini *et al.*, 2011).

Exposure of *Anopheles* mosquitoes to 0.1% propoxur from the three study location witnessed 100% mortality, hence establishing its susceptibility. This scenario is however different from the work of Oduola *et al.*, (2012) where resistance to propoxur was established in some areas in south-west Nigeria. However, recent studies by Hassan *et al.*, (2018) in North-Central, Nigeria documented similar result to the result in this finding as 100% mortality was established for 4

study sites in the study. Anogwih *et al.*, (2014) also recorded resistance to propoxur for *Anopheles* mosquitoes collected in Bariga area of Lagos state, Nigeria while mosquitoes from Ikorodu and Lekki area in Lagos state were 100% susceptible to propoxur.

Analysis of KDT₅₀ and KDT₉₅ shows slight variation from mortality. While pirimiphos-methyl recorded low mortality, the KDT₅₀ was very low when compared with DDT that witnessed higher mortality. This suggest that pirimiphos-methyl has the potential to quickly impact negatively by knocking down mosquitoes but the mosquitoes have developed a strategy to address the impact of the insecticide thereby leading to low mortality after 24hrs. Almost all the mosquitoes exposed to propoxur died before 60 mins of exposure.

5.2.3 Susceptibility status and knockdown time of insecticide combinations

The insecticide combinations assessed in this study are DDT/Propoxur, DDT/Pirimiphos-methyl, Permethrin/Propoxur and Permethrin/Pirimiphos-methyl. The other possible combinations; DDT/Permethrin and Pirimiphos-methyl/Propoxur were not assessed because of cross-resistance that exist among the insecticide class the insecticides belong. Susceptibility study for Lagos Mainland and Kosofe recorded that mosquito is susceptible to the 2 combinations having propoxur as a component; DDT/Propoxur and Permethrin/Propoxur. Susceptibility was also recorded for Permethrin/Pirimiphos-methyl in Kosofe while resistance is suspected (96% mortality) in Lagos mainland. Exposure to DDT/Pirimiphos-methyl witnessed low mortality both at Kosofe (43%) and Lagos Mainland (29%). Unsurprisingly, combinations containing propoxur recorded same result obtained when mosquitoes were exposed to propoxur alone, suggesting that the impact felt in the combination may be an impact singly influenced by propoxur. Little researches have been conducted on the impact and viability of insecticide combinations in

malaria control. However, Ngufor (2017) stated that use of a combination of chlorfenapyr and alphacypermethrin provided improved control against pyrethroid resistant malaria vector. Interestingly, resistance was established to permethrin and pirimiphos-methyl when they are used individually but was able to achieve very high mortality when used in combination. This effectiveness of permethrin/pirimiphos-methyl noticed in mosquito population resistant to DDT, pyrethroid and organophosphate can be explained by looking into the synergistic effect that could have aroused through the combination of both insecticides. This calls for further studies and trials to ascertain the underlying mechanism that led to high mortality when both insecticides are combined. Apart from the synergistic effect noticed in the case of Permethrin/Pirimiphos-methyl, insecticide combinations have the benefit of reducing the volume of insecticide put into the environment thereby reducing the selection pressure especially in a situation where resistance has not been established for one of the components, similar to the what is obtained in the case of propoxur in this study.

5.2.4 Resistance reduction in insecticide combinations

The resistance reduction fold showed that insecticide combinations have the ability to increase mortality in a situation where resistance is already established for an insecticide but still susceptible to another as seen in the case of all combinations involving propoxur. Also, resistance reduction as seen in the case of permethrin/pirimiphos-methyl is a positive for the ongoing effort in the fight against insecticide resistance. While resistance was established to the two insecticides when used singly, combination of the insecticides was effective and achieved very good percentage mortality. More studies need to be conducted on this to see the viability of

the combination on *Anopheles* mosquito population that has been established to be resistant to both insecticides individually. Furthermore, apart from increase in mortality in insecticide combinations when compared with the single insecticide on resistant populations of mosquitoes, combining insecticides also have the advantage of reducing the volume individual insecticides that would be released into the environment thereby reducing selection pressure on resistant genes. Consequently, this aids delay in the appearance and spread of resistance.

5.3 Resistance mechanisms in *Anopheles gambiae* s.l in Lagos

5.3.1 Target-site resistance

5.3.1.1 Knockdown mutation (*kdr*)

Knockdown resistance was detected in 30 (24.5%) of the 241 samples assayed for *kdr* mutant gene with 29 homozygous resistant species (*RR*) and 1 heterozygous resistant specie (*Rr*). The lowest allelic frequency was found in Ojo (6.7%) while the highest was found in Lagos mainland (16.4%). *kdr* allele has been found in low frequencies in some studies (Kristan *et al.*, 2003) and higher in other study sites (Djouaka *et al.*, 2008; Oduola *et al.*, 2012; Ibrahim *et al.*, 2014; Awolola *et al.*, 2009). According to the work of Wittke-Thompson *et al.*, (2005), the P value ($P < 0.05$) for the frequency of *kdr* allele found in the study sites shows that the observed field genotypic proportion deviates from Hardy-Weinberg equilibrium (HWE) expectations. This simply means the *kdr* loci (1019) in the population of mosquitoes in the study sites is evolving and the chance of having a change in the gene pool thereby a change in the allelic frequencies of *kdr* gene any another time is likely. Assessing the five basic assumptions of Hardy-Weinberg model (Li, 1967); occurrence of mutation, random mating and occurrence of natural selection within the mosquito population remains vague and cannot be ascribed with certainty to be the underlying condition influencing the departure of the mosquito population from HWE. Further

studies are needed to clarify the impact of these factors on the deviation of the mosquito population from the study sites to HWE. However, the assumption that there should not be gene flow through migration in or out of the population is a non-workable assumption in the population under study considering the open habitat and mobile ability of mosquitoes. This account easily for the deviation from HWE. Hemingway *et al.*, (2004) had earlier stated that dispersal pattern of malaria vectors is important in analysing and predicting spatial and temporal spread of resistance through gene flow and this is also supported by earlier modelling studies by Georgiou and Taylor, (1977) where “mobile ability” of mosquitoes was documented to alter gene pool and influence the evolution and spread of insecticide resistance.

Contrary to the report of Martinez-Torres *et al.*, (1998) and Awolola *et al.*, (2002), the correlation between resistance and *kdr* genotype in resistant species was insignificant. This serves as a pointer to the fact that *kdr* gene is not solely responsible for the large resistance to DDT and Permethrin witnessed in the population. An additional mechanism is responsible for the large resistance as the resistance genotype is not enough to explain the large population of resistant species.

5.3.1.2 Acetylcholinesterase mutation (*Ace-1^R*)

Ace-1^R gene was not found at all in the population resistant to pirimiphos-methyl and propoxur. While there is full susceptibility to propoxur, there is large resistance to pirimiphos-methyl. The large difference in the percentage mortality between pirimiphos-methyl and propoxur confirms the non-involvement of any resistant gene that can instigate cross-resistance in both resistant populations, hence a confirmation for the absence of *ace-1^R* gene. The absence of *ace-1^R* in population of mosquitoes from previous study sites have also been documented (Sangba *et al.*,

2017; Oduola *et al.*, 2012; Alou *et al.*, 2010). The low proportion of resistant genes in some localities has been attributed to the fitness cost of the mutation (Djoungbenou *et al.*, 2008). Further studies are needed to clarify the fitness cost of *ace-1^R* mutation (G119S) so as to provide a valid answer to the absence of *ace-1^R* in some populations of mosquitoes. Lack of a correlation in the frequency of mutant genes and observed resistance level in both pirimiphos-methyl and propoxur calls for probe into other resistance mechanisms that may be responsible for the resistance in pirimiphos-methyl.

The spread of resistance has been a major concern in the fight against malaria vector for decades. It is one of the strongest impediments to the control of malaria case incidence (WHO, 2018). The widespread nature of resistance calls for the implementation of sound insecticide resistance management (IRM) strategy that would help to ameliorate the incidence of resistance as well as help in delaying the onset and spread of resistance. One of the strategies that have been applied previously in agriculture is combination of insecticides (WHO, 2012). According to Bielsa (2008), combination of insecticides aid management of resistance in two ways; (1) It ensures that the prevalent resistance mechanisms in the population can be avoided by one of the components in the combinations (2) It reduces the selection pressure on resistance mechanisms since there would be reduction in the volume of individual components released into the environment. While simulation models have also corroborated the possible positive impact of insecticide combinations (Denholm and Rowland, 1992), the results from the present study where 3 out of the 4 possible combinations achieved higher mortality to be classified into the susceptible range using WHO (1998) criteria present more facts to support this claim. Ngufor (2015) had earlier stated that combination of insecticides has the advantage of been relevant in both areas where susceptibility or resistance is established unlike the three other IRM strategies, however, the

underlying mechanism that make the combinations achieve more mortality in an area where resistance is already established to 3 out of the 4 insecticides needs further studies.

5.3.2 Metabolic enzyme activities

The enzymes assessed in the present study are; Esterase, Gluthathione-S-Transferase (GST) and Monooxygenase.

5.3.2.1 Esterase activities

For esterase, inducement with exposure to the four insecticides separately did not lead to significant upregulation or downregulation of the enzymes when compared with enzyme activity in unexposed mosquitoes. This is contrary to report that esterase is significant in the metabolism of the insecticides (Tsiriniaina *et al.*, 2018; Naeem *et al.*, 2019; Cisse *et al.*, 2015; Badirou *et al.*, 2016). Expectedly, considering the susceptibility to the insecticide combinations, there was also no elevation in the activities of esterase after inducement with the combinations except the esterase activity in the mosquitoes exposed to combination of DDT/pirimiphos-methyl which achieved very low mortality from the 2 sites. The significant elevation of esterase activity may account for the low mortality recorded for exposure to DDT/pirimiphos-methyl combination. Elevation of esterase activities in DDT (Etang *et al.*, 2007) and pirimiphos-methyl (Aikpon *et al.*, 2014) resistant species have been reported separately but this is the first study reporting elevation of esterase activities in mosquito induced by exposure to a combination of DDT/pirimiphos-methyl. As the inability of DDT/pirimiphos-methyl combination to cause high mortality in a DDT and pirimiphos-methyl mosquito resistant populations makes a case for the irrelevance of the combination, the increase in esterase activity corroborates this proposition.

5.3.2.2 Glutathione-S-Transferase activities

Inducement of mosquitoes from the population under study by the 4 insecticides separately did not lead to significant elevation of GST activity except in the permethrin induced population. Non-elevation of GST in mosquito exposed to DDT and organophosphate had earlier been reported by Wanjala and Kweka (2018). GST activity in permethrin induced population was significantly elevated ($p = 0.007$) when compared with the GST activity in unexposed mosquitoes. This is in line with earlier findings (Etang *et al.*, 2007; Vulule *et al.*, 1999; Chabi *et al.*, 2016) where elevated GST activity was reported to have been implicated in pyrethroid resistance. Also, from the 4 combinations, only DDT/propoxur combination induced elevated GST activity when compared with unexposed mosquitoes. The elevated activity of GST in DDT/propoxur exposed population did not however aid resistance to the combination as 99% mortality was recorded after exposure. Considering that the 2 components did not cause elevated GST activity separately, the synergistic or additive action on GST activity of mosquito needs further probe.

5.3.2.3 Monooxygenase activities

Elevation of monooxygenase was not observed in mosquitoes induced with exposure to DDT, pirimiphos-methyl and propoxur. Significant upregulation of monooxygenase was observed in permethrin exposed mosquitoes when compared with unexposed mosquitoes. Earlier studies have found that monooxygenase is relevant in the detoxification of permethrin (Cuamba *et al.*, 2010; Rodriguez *et al.*, 2005) and may have accounted for the incidence of resistance witnessed in the mosquito population under study. While monooxygenase is known to aid the detoxification of insecticides (Cisse *et al.*, 2015; Berge *et al.*, 1998; Rodriguez *et al.*, 2005), there

is no significant upregulation in the activities of monooxygenase in mosquitoes induced with the insecticide combinations. This is the first report on monooxygenase activity in mosquito induced with a combination of recommended insecticides.

The overall result of the metabolic studies revealed that while esterase is not elevated in permethrin induced mosquitoes, the significant upregulation in GST and monooxygenase support the earlier proposition that altered target site is not the only factor responsible for resistance observed in permethrin and this is further supported by earlier studies (Vontas *et al.*, 2001; Vulule *et al.*, 1999). Contrarily, Hemingway *et al.*, (1989) reported target site mutation as the only mode of action influencing resistance to permethrin.

Despite the absence of *ace1^R* gene and non-elevation of three enzymes in both propoxur and pirimiphos-methyl induced mosquitoes, susceptibility was achieved with propoxur while high resistance was observed in pirimiphos-methyl, this is a pointer to the possibility of another resistance strategy involved in resistance to pirimiphos-methyl. This is because cross resistance to the two insecticides has been attributed to the presence of *ace1^R* gene (Corbel *et al.*, 2007) and elevation of detoxifying enzymes (Grafton-Caldwell *et al.*, 2004). This calls for further research on the impact of *ace1^R* gene and the 3 metabolic enzymes in conferring resistance to organophosphates.

5.4 Conclusion

The findings of the study revealed that, while level of education and dwelling may be an important factor taken into consideration in policy making and application of interventions in Lagos state, LGAs have no significant correlation with knowledge, attitude and practices of Lagos state resident with regards to malaria vector control. Respondents made use of two or more methods in combination to combat mosquito. “Use of insecticides” and LLINs remain the

two prominent vector control strategies for communities in Lagos state. Insecticides are used against mosquito almost on a daily basis and this put out large volume of the chemicals to the environment which may affect non-target organisms as well as aid the spread of insecticide resistance. Few respondents have benefitted from community based intervention where LLINs is given out freely. Efforts are still needed to orientate the populace on the positive impact of LLINs and downplay the inconveniences suffered as a result of heat when sleeping under LLINs. Lastly, since the respondents attested to the effectiveness of insecticides and LLIN, awareness to encourage its use won't be a tedious exercise, however, the government needs to move down to the community level to distribute LLINs at no cost to the end users and engage in community based IRS. Also, NGOs and other relevant bodies should be encouraged to follow suit.

Resistance to DDT, pirimiphos-methyl and permethrin is common in Lagos state, Nigeria. However, there are places within Lagos state where resistance to permethrin is under suspicion. All the sites selected for this study within Lagos state witnessed susceptibility of *Anopheles spp.* to propoxur. All the insecticide combinations assessed recorded high mortality except DDT/pirimiphos-methyl combination. Enzymes activity assay showed that esterase activities is not significantly upregulated in all the resistant and insecticide combination induced mosquitoes except mosquitoes induced with a combination of DDT/propoxur, albeit mosquito population were still susceptible to the combination. Upregulation of GST and monooxygenase activities in permethrin resistance population showed that the two enzymes are involved in permethrin resistance. The insecticide combinations achieved very high mortality even in the case of permethrin/pirimiphos-methyl combination that resistance to the individual components was witnessed. The combinations also did not lead to significant upregulation in the detoxification

enzymes. The results from the study make a good case for use of insecticide combinations in mosquito vector control.

5.5 Recommendation

- 1.) Surveys should be conducted to assess community knowledge, attitude and practises before implementation of insecticide resistance management strategies.
- 2.) Relevant bodies should engage in state wide community distribution of LLINs and not restrict to distribution at hospitals or clinics. A well-coordinated orientation about the benefits and challenges of LLINs (especially production of heat) should be done.
- 3.) State wide IRS program covering a large portion of the state should be engaged in, so that households wont engage in individual IRS allowing for indiscriminate use of pesticides. This will help reduce the chemicals put into the environment thereby addressing the issue of selection pressure and resistance development.
- 4.) Baseline assessment of mosquito population dynamics in Lagos state should be conducted at regular intervals.
- 5.) Continuous monitoring of the dynamics of insecticide resistance to all the recommended insecticides should be done.
- 6.) Further studies should be conducted to assess and fully establish the benefits and challenges of insecticide combination usage on a large scale. This would involve experimental huts and field trials of insecticide combinations.
- 7.) A detailed and full gene profiling of insecticide induced *Anopheles spp* must be carried out to fully assess the specific genes responsible for the enzyme activities. Also, this should be done to assess the possibility of another resistant strategy in pirimiphos-methyl that does not confer cross resistance to propoxur.
- 8.) Development of new insecticides should be encouraged and these insecticides should be used in combination. These insecticide combinations should be employed for IRS in

place of single insecticide to help reduce the volume of insecticide put into the environment and also help delay the onset of resistance to the individual insecticides.

- 9.) Government should establish a sound IRM programme that would combine 2 or more of the IRM strategies proposed by WHO. Example; use of insecticide combination rotationally.