# UNIVERSITY OF NAIROBIa

## EVALUATION OF LAMBDA-CYHALOTHRIN (ICON®) INCORPORATED INTO 1, 4-DICHLOROBENZENE (PCB®) FOR THE CONTROL OF LEISHMANIASES AND MALARIA VECTORS


**BY**

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## DECLARATION

I declare that this is my original work and has not been submitted elsewhere for award of a degree.

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## DEDICATION

To my loved ones; my late mum Harriet who planted the seed, natured it but did not wait to see it sprout, my loving husband Dickson Libendi, my son Collins munene and daughter Shantel Makena.

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# ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| **ACTs** | Artemisinin-based Combination Therapies |
| **AIDS** | Acquired Immunodeficiency Syndrome |
| **CBRD** | Centre for Biotechnology Research and Development |
| **CDC** | Centre for Disease Control and prevention |
| **CL** | Cutaneous Leishmaniasis |
| **CNS** | Central Nervous System |
| **CS** | Capsule Suspension |
| **DDT** | Dichlorodiphenyltrichloroethane |
| **DEET** | Diethyl-toluamide |
| **DNA** | Deoxyribonucleic Acid |
| **EIR** | Entomological Inoculation Rate |
| **EPA** | Environmental Protection Agency |
| **FAO** | Food and Agriculture Organization |
| **GPIRM** | Global Plan for Insecticide Resistance Management |
| **HIV** | Human Immunodeficiency Virus |
| **HRM** | High Resolution Melt |
| **ICON** | Lambda-cyhalothrin |
| **PCB** | 1,4 dichlorobenzene |
| **IVM** | Integrated Vector Management |
| **IRS** | Insecticide Residual Sprays |
| **JE** | Japanese Encephalitis |
| **KEMRI** | Kenya Medical Research Institute |

| | |
|---|---|
| **$LC_{50}$** | Lethal Concentration at 50% |
| **$LD_{50}$** | Lethal Dose at 50% |
| **LF** | Lymphatic Filariasis |
| **LLINs** | Long Lasting Insecticidal Nets |
| **MCL** | Mucocutaneous Leishmaniasis |
| **MOA** | Mode of Action |
| **NACOSTI** | National Commission for Science Technology and Innovation |
| **NTD** | Neglected Tropical Diseases |
| **NIB** | National Irrigation Board |
| **PCR** | Polymerase Chain Reaction |
| **p- DCB** | Paracide, 1, 4-dichlorobenzene |
| **PKDL** | Post Kala-azar Dermal Leishmaniasis |
| **PSC** | Pyrethrum Spray Catches |
| **RH** | Relative Humidity |
| **SSG** | Sodium stibogluconate (Pentostam) |
| **USA** | United States of America |
| **VL** | Visceral Leishmaniasis |
| **WHO** | World Health Organization |
| **WNR** | West Nile Virus |
| **WP** | Wettable Powder |

## ABSTRACT

Leishmaniases and human malaria, transmitted by phlebotomine sand flies (Diptera: Psychodidae) and anopheline mosquitoes (Diptera: Anophelinae) respectively, are diseases of public health importance in Kenya. Control of phlebotomine sand flies (Diptera: Psychodidae) and mosquitoes (Diptera: Anophlenae) remains a priority as they pose a significant public health threat in many parts of the world, transmitting the agents of several zoonotic and parasitic diseases to humans. Efficient vectoring role of sand flies and mosquitoes is enhanced by specific biological and behavioural characteristics that may be targeted by control measures to limit pathogen spread or vector abundance. The overall aim of this study was to evaluate the efficacy of 1,4-dichlorobenzene (PCB®), an organic compound with insecticidal and antimicrobial properties incorporated into lambda-cyhalothrin (ICON®), a synthetic pyrethroid, in the control of leishmaniasis and malaria vectors in the laboratory and field settings. In the laboratory, survival and mortality of sand flies and mosquitoes was monitored following exposure to the treatments while in the field the study involved vector collections before treatments and monitoring the number of vectors after treatments using the entry-exit trap. Paired *t* test was used to analyze the average number of sand flies and mosquitoes collected before and after treatment and to compare between groups. There was high mortality of mosquitoes exposed to ICON® / PCB® combination compared to either PCB® or ICON® independently and their mean difference was significantly different (15.17±5.59, P=0.021; 17.66±6.96 P=0.03, respectively). This implies that the combined product was more effective in killing the vectors compared to the individual insecticides. Likewise, the mean number of sand flies that survived following exposure to ICON® /PCB®

combination was less compared to those exposed to PCB® or ICON® (2.17 ±0.91, P=0.03 and 2.5 ±1.06, P=0.03, respectively). There was significant decrease in sand fly densities after treatment with ICON® (P=0.03, t=1.81, SEM±25.99) or PCB® (P=0.02, t=1.81, SEM±11.46322), but the densities of sand fly were not comparable before and after treatment with the combination of ICON® /PCB® or ICON® (P=0.11, t=1.83, SEM±66.30). The decrease of sand fly populations following exposure to ICON®, PCB® or ICON®/PCB® is an indication that all the trial insecticides can be used in their control. Overall, significant reduction of mosquito densities after treatment with ICON® (45.4±18.043, P= 0.02) and ICON®/PCB® (95.7±43.15, P= 0.03) was observed. However, there was no significant difference when mosquitoes were exposed to PCB® (193.3±131.94, P= 0.09) for a period of six months. The differences in mosquito densities seen in collection from the houses which had ICON® and the combination of ICON®/PCB® showed that PCB® alone could not be effective. Lambdacyhalothrin and paradichlorobenzene alone or in combination can be used safely for the effective control of indoor-feeding sand fly and mosquito vectors of human disease.

# CHAPTER ONE: INTRODUCTION

## 1.1 Background information

Phlebotomine sand flies (Diptera: Psychodidae) are widespread in the tropics and subtropics. They transmit *Leishmania*, the protozoan parasites which cause visceral leishmaniasis (VL) and various forms of cutaneous leishmaniasis (CL) infecting more than 350 million people in more than 98 countries (Hesam-Mohammadi *et al*., 2014). Global distribution and estimates of incidences of visceral and cutaneous leishmaniasis indicate that a total 350 million people are at risk, with 1.5-2.0 million new cases and 70,000 deaths reported annually (Alvar *et al.,* 2012). Based on these estimates, approximately 0.2 to 0.4 cases of visceral leishmaniasis and 0.7 to 1.2 million cases of cutaneous leishmaniasis (CL), occur each year. Moreover, 500,000 new cases of visceral leishmaniasis (VL) are reported annually out of which 5,000 have resulted in deaths (WHO, 2011). This global distribution shows that more than 90% of global VL cases occur in six countries, namely, India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil (WHO, 2011). Leishmaniasis is one of the neglected tropical diseases with a complex epidemiology and ecology, and lacks simple easy-to-use tools for case management (Alvar *et al*., 2012).

Phlebotomine sand flies are haematophagous insects that transmit *Leishmania* parasites through the bite of infected females that have previously fed on an infected mammal (Maroli *et al*., 2013). Other than leishmaniasis, phlebotomine sand flies are also vectors of other pathogens such as *Bartonella* and viruses belonging to three different genera: (i) the *Phlebovirus* (family Bunyaviridae) including sand fly fever Silician virus, sand fly

fever, Naples virus, Toscana virus and Punta Toro virus; (ii) the *Vesculovirus* (family Rhabdoviridae) including Chandipura virus and (iii) the *Orbivirus* (family Reoviridae) including Changuinola virus (Mong'are *et al*., 2013). *Phlebotomus martini* and *P. duboscqi* (Diptera: Psychodidae) are known vectors for visceral and cutaneous leishmaniasis caused by *Leishmania donovani* and *Leishmania major* (*Kinetoplastida*: Trypanosomatidae), respectively, in Baringo County, Kenya (Anjili *et al*., 2014). These sand flies live in termite mounds (*P. martini*) and in animal burrows (*P. duboscqi*) where they rest and feed on mammals such as small rodents (Ngumbi *et al*., 1998).

Currently, there is no potent vaccine against leishmaniasis hence control is based on case management and vector control. However, case management is difficult to conduct since it is restricted by several factors such as lack of access to affordable and active drugs, incorrect prescribing and poor treatment compliance (Mong'are *et al*., 2015). It is difficult to eliminate leishmaniasis since there is no potent vaccine or a cost-effective chemoprophylaxis. Primary tools available include elimination of reservoir populations and some form of vector control, including barriers to sand fly feeding and construction of iron corrugated sheet houses. In order to reduce disease risk significantly, a reservoir population should be eliminated inside a 500m radius of a protected area (Tonui, 2006; Ngure *et al*., 2017).

Current leishmaniasis control strategies rely on reducing man-vector contact through application of vector control measures such as wearing proper clothing at night, sleeping under a fine mosquito net, using repellents, zoo-prophylaxis, destroying vector habitats

near homesteads, avoiding playing or standing near vector habitats (termite mounds) late at night and sleeping in corrugated iron sheet houses (Ngure *et al.,* 2017). Synthetic insecticides pose adverse effects to the user and the environment. Concerns are not only raised on the effectiveness of these spraying programs but also on their side effects which are of great importance on health and environment, and their potential for sustainability, which relies on how much the insecticides will cost and also how they will be applied. It has been reported that sand flies have developed resistance to some chemicals, such as DDT and in some instances to Malathion and synthetic pyrethroids (Umakant and Sarman, 2008).

Malaria is a disease that is caused by protozoan parasites which are transmitted to humans through the bite of an infected female *Anopheles* mosquito. Though a life-threatening condition, it is preventable and curable. In 2016, the estimated number of malaria cases was 216 million in 91 countries. This was an increase of 5 million cases and 445,000 deaths recorded in 2015. Africa has a staggering high share of the malaria burden across the globe. For example, 90% of all malaria cases and 91% of all malaria-related deaths recorded in 2016 occurred in Africa (WHO, 2016). Malaria is caused by *Plasmodium* parasites. There are five species of *Plasmodium* are known to infect humans. These are *Plasmodium falciparum, Plasmodium. vivax, Plasmodium malariae, Plasmodium ovale* and *Plasmodium knowlesi.* These parasites are transmitted to humans through the bite of an infected female *Anopheles* mosquito. *Plasmodium falciparum* and *P. vivax* species pose the greatest threat of causing malaria with *former* being the most common parasite in Africa. *Plasmodium falciparum* is responsible for most malaria-

related deaths globally, while *P. vivax* is the dominant malaria parasite in most countries outside of sub-Saharan Africa (WHO, 2018). Most malaria cases and deaths occur in sub-Saharan Africa. However, the WHO regions of South-East Asia, Eastern Mediterranean, Western Pacific, and the Americas are also at risk. In 2016, a total of 91 countries had active malaria transmission taking place with newborn babies, children below the age of five years, expectant mothers and people living with HIV/AIDS being under the biggest threat of getting infected includes; non-immune migrants, mobile populations and travelers are also at great risk of being infected (WHO, 2018)

There are 400 different species of *Anopheles* mosquito, and around 30 are malaria vectors of major importance. Almost all the vector species bite between dusk and dawn. *Anopheles* mosquitoes lay their eggs in water, and hatch into larvae, which eventually change into pupa. The pupa opens up releasing an adult mosquito after a day or two days. Every species of *Anopheles* mosquito always has its best preferred aquatic habitat e.g. small shallow pods of fresh water, puddles and hoof prints that are commonly evident during prolonged rainy seasons in tropical countries.

Vector control is the best method to prevent and reduce malaria transmission. When coverage of vector control interventions in a particular area is high enough, a good measure of protection will be conferred across the community. The two broadly applicable measures for malaria vector control are long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) (WHO, 2018). Vector control strategies should be devised through an integrated vector management (IVM) approach. Such an approach

seeks to improve the efficacy, cost effectiveness, ecological soundness and sustainability of controlling disease vectors. Key considerations for malaria vector control include vector abundance, behavior and susceptibility to the insecticides used in LLINs and IRS (WHO, 2015). In Kenya, the methods that are in place to eliminate malaria show good signs of preventing transmission (WHO, 2017). However, control of malaria has also proven difficult because of insecticide resistance by mosquitoes.

The use of repellents and a user-friendly insecticide with no known resistance would therefore be an appropriate approach in the control of endophilic vectors of leishmaniasis and malaria. Lambda-cyhalothrin (ICON®), a synthetic pyrethroid insecticide in the group of alpha-cyano was cleared for residual spraying indoors by World Health Organization **(**WHO, 2007). It is odourless with no reported resistance in mosquitoes and sand flies. On the other hand, 1, 4-dichlorobenzene (PCB®), a commercially available household disinfectant and deodorant, has not been tested in the control of malaria and leishmaniasis vectors alone or when combined with lambda-cyhalothrin (ICON®) making it a user friendly insect repellant. Previous studies have shown that combining two or more insecticides has a synergistic effect (Killeen *et al*., 2017). This study sought to analyze and evaluate the efficacy of a slow-release mixture of 1, 4-dichlorobenzene and lambda-cyhalothrin in controlling malaria and leishmaniasis vectors in both laboratory and field settings.

### 1.2 Statement of the problem

Current efforts directed at mosquito control rely heavily on insecticide-treated bed-nets, indoor residual spraying with insecticide, and application of chemical larvicides. No new

public health insecticides have been developed for mainstream vector control in disease endemic countries for 30 years and the only two core insecticide-based vector control practices are the use of long-lasting insecticide nets (LLINs) and indoor residual spraying (IRS) (WHO, 2017). Despite diligent application of available strategies, malaria still poses a global health burden, most especially for those living in resource constrained countries in the developing world (Breman *et al*., 2007). Resistance to insecticides is known to evolve rapidly and is already threatening the use of pyrethroids on bed-nets (Shetty *et al*., 2013; Mnzava *et al*., 2015). The arsenal of available insecticides remains small, and judicious use will be required to manage resistance (Kelly-Hope *et al.*, 2008; WHO, 2017). Pyrethroids are the only insecticides currently used operationally on long lasting insecticide nets (LLINs) and are also the dominant insecticide class for indoor residual spraying (IRS). The leishmaniases are classified as neglected diseases and are also emerging and re-emerging diseases in HIV/AIDS infected subjects. Control of phlebotomine vectors of the disease has achieved very little success because of the poor knowledge of breeding and resting sites of the sand flies. Control of sand flies and mosquitoes, the vectors of leishmaniasis and malaria, respectively, has further been complicated by the emergence of insecticide resistance. It is because of these problems that transmission still goes on unabated necessitating the need to develop an alternative control method. One of the approaches to combat insecticide resistance in disease vectors is to explore new insecticides or methods of control that can cut across a wide range of disease vectors (Bellinato *et al*., 2016; Ishak *et al*., 2017). It has been observed that integrated vector management (IVM) can reduce the incidences of transmission of malaria and leishmaniases, and the magnitude of the problem if applied successfully

(Mutero *et al*., 2015). Synergism of control drugs or products has also been found to help in the control of disease vectors (Darriet and Chandre, 2012). In line with the goal of WHO global plan for insecticide resistance management in malaria vectors (GPIRM), to develop new, innovative vector control tools (WHO, 2012), this study sought to test the safety and efficacy of ICON®/PCB® in combination on sand flies and mosquitoes' mortality. The overall goal was to come up with a new safe and cost-effective malaria and leishmaniases vector control tool for use in homes that is easily available, environmentally friendly and easy to formulate.

### 1.3 Justification

The lack of an effective vaccine, prohibitive cost of treatment and side effects and difficulties associated with chemoprophylaxis has served to emphasize the need to use vector control in preventing diseases (Yakob and Walker, 2016). However, control of sand flies remains a difficult problem throughout the world. Although the use of insecticides remains the most effective method of sand fly control, the high cost and resistance to synthetic insecticides have proved to be the major challenges (Kishore *et al.*, 2006). In Kenya, the use of bed nets, permethrin-impregnated wall cloth, repellents and other personal protective measures had proved to be unreliable as early as 1994 (Mutinga *et al.,* 1994). In addition, high toxicity and harmful side effects to both animals and humans have progressively limited the use of synthetic insecticides. With these shortcomings, there is need to identify novel insecticides against the phlebotomine sand flies and mosquitoes with minimal or no deleterious effects to man and animals as well as the environment.

Lambda-cyhalothrin (ICON®) is a synthetic pyrethroid insecticide that has been shown to be effective in killing mosquitoes and phlebotomine sand flies when used in low doses (Elnaiem *et al.,* 1999; Claborn 2010; Choi *et al.,* 2017). It has no smell and has a long residual period of six months (Davies *et al.,* 2000; Choi *et al.,* 2017). It is non-toxic to vertebrates (Elnaiem *et al.,* 1999; Claborn 2010; Asidi *et al.,* 2005) and no resistance has been reported in sand flies and mosquitoes (Asidi *et al.,* 2005; Dhiman and Yadav, 2016). The efficacy of lambda-cyhalothrin in protecting people from bites of sand flies (Kroeger *et al.,* 2002), its ready acceptance by users (Gonzalez *et al.,* 2015), and its cost-effectiveness (Raghavendra *et al.,* 2011) makes it a valuable insecticide for the control of malaria and leishmaniasis vectors. On the other hand, 1, 4-dichlorobenzene (PCB®) is commonly used as a deodorant and pesticide (Kelly, 2009). It is used mainly as a fumigant for the control of moths, molds, and mildews, and as a space deodorant for toilets and refuse containers as well as an intermediate in the production of other chemicals, in the control of tree-boring insects, and in the control of mold in tobacco seeds (ATSDR 2006). Incorporation of lambda-cyhalothrin into 1, 4-dichlorobenzene under slow release characteristics may results in stronger insecticidal and repellence properties against sand flies, mosquitoes and other nuisance household pests and also act as an air freshener in the houses.

In its pure form (99.8%) it can be used as a repellent against snakes, rats, mice, squirrels, bats and insects, as deodorizer for toilets, urinals, as fumigant and air freshener (Chin *et al.,* 2015). When used at low concentrations as moth repellents, 1, 4-dichlorobenzene (in form of crystals, flakes or cakes) are usually placed in closed drawers, closets and plastic

bags where clothes, blankets and other goods are stored. As a deodorizer, 1, 4-dichlorobenzene is normally placed in toilets, bathrooms, basements, pet cages, vehicles or any other location where the nice smell maybe needed. The 1, 4-dichlorobenzene is relatively stable in the environment compared to other volatile organic compounds and its estimated atmospheric shelf-life is 14-31 days (Chin *et al.,* 2015).

### 1.4 Research Questions

1. What is the effect of lambda-cyhalothrin combined with 1, 4-dichlorobenzene on survival and mortality of sand flies and mosquitoes under laboratory conditions?
2. What is the efficacy of lambda-cyhalothrin combined with 1, 4-dichlorobenzene on sand flies and mosquitoes under field conditions?

### 1.5 Null Hypotheses

1. Sand flies and mosquitoes are not equally susceptible to lambda-cyhalothrin or 1, 4-dichlorobenzene or a combination of the two.
2. Sand flies and mosquitoes are not equally repelled by lambda-cyhalothrin or 1, 4-dichlorobenzene or a combination of the two.
3. Feeding behavior and survival of sand flies and mosquitoes is not affected by exposure to lambda-cyhalothrin or 1, 4-dichlorobenzene or a combination of the two.

## 1.6 Objectives

### 1.6.1 General Objective

To evaluate the effects of a combination of lambda-cyhalothrin and 1, 4-dichlorobenzene on survival and fecundity of sand flies and mosquitoes in the laboratory and the potential of this combination to control these vectors in a field setting

### 1.6.2 Specific Objectives

1. To determine the fecundity and survival rates of laboratory-bred mosquitoes and sand flies exposed to lambda-cyhalothrin combined with 1, 4-dichlorobenzene in the laboratory.
2. To identify the species of sand flies and mosquitoes and determine densities before and after intervention using lambda-cyhalothrin combined with 1, 4-dichlorobenzene, in the dry seasons and rainy seasons in Marigat and Mwea sub-Counties.
3. To determine mammalian toxicity levels of lambda-cyhalothrin combined with 1, 4-dichlorobenzene and the period (time) it remains effective in the control of phlebotomine sand flies and mosquitoes.

## 1.7 Significance of the study

No new public health insecticides have been developed for mainstream vector control in disease endemic countries in the last 30 years. Despite diligent application of available strategies, malaria still poses a global health burden, especially for those living in resource constrained countries in the developing world. Resistance to insecticides is

known to evolve rapidly and is already threatening the use of pyrethroids on bed-nets. It is anticipated that the lambda-cyhalothrin cubes developed for vector control which are cost effective, feasible, fragrant friendly and protective, will provide control of leishmaniasis in endemic areas. However, increasing resistance to this insecticide has already been reported in a number of dipteran species. If a synergistic effect of a combination of lambda-cyhalothrin and 1, 4-dichlorobenzene, if proved to occur against mosquitoes and sand flies. This will offer an alternative chemical tool with a reduced risk of resistance development that can be used safely for effective control of these vectors in malaria and leishmaniasis endemic regions in Kenya.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1 Leishmaniasis as a neglected tropical disease

The World Health Organization (WHO) lists leishmaniasis as a 'Neglected Tropical Disease' although it is responsible for high mortality worldwide (WHO, 2012). Alvar *et al.,* (2012) summarizes the reasons leishmaniasis is often forgotten: '*This problem of consignment to critical oblivion results from its complex epidemiology and ecology, lack of simple, easily applied tools for case management and the paucity of current incidence data, and often results in a failure on the part of policy-makers to recognize its importance*'.

Leishmanisis is associated with the poorest in the society who inhabit the arid and semi-arid regions within the tropics worldwide. It receives poor funding and attention from donors when compared to other diseases like malaria and cancer (WHO, 2016). Prevention and control of leishmaniasis requires a combination of intervention strategies because transmission occurs in a complex biological system involving the human host, parasite, sand fly vector and in some cases an animal reservoir host (Claborn, 2010). Vector control helps to reduce or interrupt transmission of disease by reducing the populations of sand flies and mosquitoes (Bastiaens *et al.*, 2012). Control methods include insecticide spray, use of insecticide treated nets, bioinsecticides, environmental management and personal protection among others (WHO, 2012).

In some recent studies, the use of environment friendly and easy biodegradable insecticides has received much attention for the control of medically important

arthropods (Ngumbi *et al*., 2011; Ngure *et al.*, 2017). Vector borne diseases such as malaria and leishmaniases still cause thousands of deaths and morbidity (Bastiens *et al.*, 2012; Alvar *et al.*, 2012). Management of these disease vectors using most synthetic chemicals have failed due to insecticide resistance, vector resurgence and environmental pollution. Consequently, an intensive effort has been made to look for alternative methods of control (Claborn, 2010). The initial attempt to have sand flies controlled by the use of modern insecticides were done with DDT (1, 1-bis (4-chlorophenyl)-2, 2, 2-trichloroethane) in Peru around the Rimac Valley, targeting bartonellosis, in January 1944 (Alexander and Maroli, 2003). Recently African countries that have reverted to DDT use have seen spectacular successes in their malaria control efforts. These include South Africa, Mozambique, Zambia, Madagascar and Swaziland who within two years of starting DDT program reduced their malaria rates by 75 percent or more. There is also a need to carry out efficient and effective vector control programs and to properly monitor the impact (WHO, 2016). It has been noted that residual spraying of houses and animal shelters would have an impact on transmission only if the vector is restricted to the intra- and peri domiciliary area as is the case in India (Bublitz *et al.*, 2016). Residual spraying of two adobe huts with a 5% solution of these insecticides conferred protection against the bites of sand flies (mostly *Lutzomyia verrucarum* Theodor) for about a week.

The WHO (2010), Expert Committee recommended integrated surveillance and control, which has advantages for controlling leishmaniasis, diseases often neglected compared with malaria and Chagas' disease in the same regions. Control of leishmaniases is currently based on chemotherapy to treat infected cases and on vector control to reduce

transmission (Tonui, 2006). Currently, there are no vaccines for leishmaniasis (Murray *et al*., 2005; Modabber, 2010) and the drugs available to manage leishmaniasis are toxic, expensive and are faced with frequent treatment failure (Croft and Yardley, 2002; Croft *et al*., 2006). This then means vector control is critical in minimizing and/or preventing bites from potentially infectious sand flies.

Vector control using insecticides has been recommended by the World Health Organization (WHO, 2010). Depending on the application techniques, timing and the target species, sand flies are known to be highly susceptible to insecticides (Alexander *et al*., 1995a; Alexander and Maroli, 2003; Wilamowski and Pener, 2003). Due to malaria control in some areas, successful leishmaniasis control has been realized as a side effect (Kishore *et al.,* 2006). Residual formulations of DDT have been used expressly to control sand flies (Hertig and Fairchild, 1948; Hertig, 1949) and have demonstrated insecticidal activity against sand flies in Sudan (Hassan *et al*., 2012). The synthetic pyrethroid deltamethrin has been used against sand flies in Bolivia (Le Pont *et al.*, 1989) and Brazil (Bermudez *et al.,* 1991; Marcondes and Nascimento, 1993; Courtenay *et al*., 2009). In other countries where sand fly vectors are endophilic, control of leishmaniasis has traditionally been based on residual insecticide spraying in houses, with significant effectiveness (Alexander *et al.,* 1995a; Vieira and Coelho, 1998). Other studies have tested the efficacy of insecticide impregnated textiles, such as curtains, bed nets or bed covers, with varying degrees of success (Alexander *et al.,* 1995a, 1995b; Basimike and Mutinga, 1995; Kroeger *et al*., 2002; Courtenay *et al*., 2007). Environmental modification, involving the total eradication of rodents, destruction of burrow systems

and spraying of herbicides to kill their food plants, has been demonstrably effective in controlling CL caused by *L. major* in foci in the Asian republics of the former USSR and in Tunisia (Vioukov, 1987; WHO, 1990).

However, natural conditions, as well as resistance from continued exposures and damage to the environment have necessitated the call for new natural chemicals (Viegas-Junior, 2003). Other disadvantages include high toxicity and harmful side effects for both animals and humans, and their potential for environmental pollution which have progressively limited their usage (Rogan and Chen, 2005). In this context, screening of natural products for their effectiveness has received the attention of researchers around the world. Since many diseases transmitted by insects (for example., malaria, dengue, yellow fever, leishmaniasis and Chagas disease) are endemic in developing countries, the search for insecticides and repellents of botanical origin in these countries has been driven by the need to find new products that are effective, but also safer and cheaper than current products (Paula *et al*., 2004).

## 2.2 Insecticides for vector control

### 2.2.1 The 1, 4-dichlorobenzene (PCB®)

The 1, 4-dichlorobenzene, also known as *para*-dichlorobenzene, *p*-DCB, PCB or PCB®, is colorless solid organic compound with a strong smell. The molecular formula is given as $C_6H_4Cl_2$, showing two chlorine atoms on opposing sites of the benzene ring (Fig. 2.1).

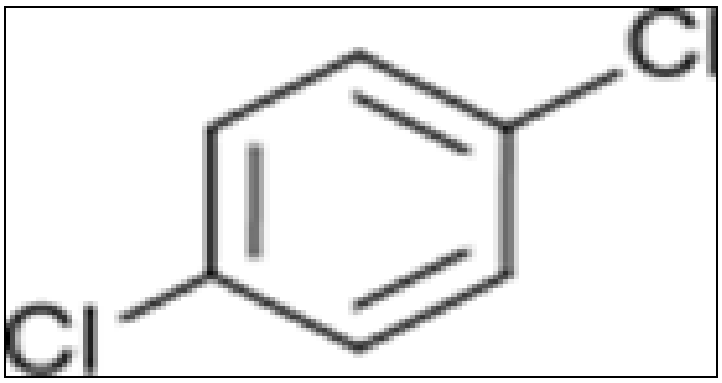


**Figure 2.1:** Chemical structure of 1, 4-dichlorobenzene

The 1, 4-dichlorobenzene (PCB®) has been used as a pesticide and a deodorant, mostly in toilet balls and as an air freshener replacement for the traditional naphthalene. It has also been incorporated in the initial development of polyphenylene-sulphide (p-pheynylene sulphide) polymer (Lopez & Wilkes, 1989). Ferric chloride catalyzes the process in the chlorination of benzene during the production of PCB® as shown in the formula; $C_6H_6 + 2\ Cl_2 \rightarrow C_6H_4Cl_2 + 2\ HCl$

Purification of the compound is achieved by fractional crystallisation, taking advantage of its relatively high boiling point of 174°C and melting point of 53.5 °C. 1, 4-dichlorobenzene is poorly soluble in water (10.5mg/100ml at 20°C) and is not easily broken down by soil organisms (Blair *et al*., 2003). Like many hydrocarbons, 1, 4-dichlorobenzene is lipophilic and would accumulate in the fatty tissues. There is no direct evidence of carcinogenicity from the environmental protection agent (EPA). The 1, 4-dichlorobenzeneis registered by USA-EPA for water use at a concentration of 75µg per litre and also as a pesticide (Pontius, 2003). There is no report of insecticidal resistance against 1, 4-dichlorobenzene in sand flies and mosquitoes in Kenya (Kaburi *et al*., 2018).

### 2.2.2 Lambda-cyhalothrin (ICON®)

Lambda-cyhalothrin is a synthetic pyrethroid insecticide, which is available as an emulsifiable concentrate (EC), wettable powder (WP) or ultra-low volume (ULV) spray liquid, which is commonly used to control a wide range of agricultural pest and disease vectors. It is compatible with most other insecticides and it is commonly mixed with buprofezin, pirimicarb, dimethoate or tetramethrin (Olenyuk *et al*., 2018). The chemical name of lambda-cyhalothrin is A-cyano-3-phenoxybenzyl-3-(2-chloro-3, 3, 3-trifluoro-1-propenyl)-2, 2-dimethylcyclopropanecarboxylate, which is a mixture of isomers of cyhalothrin, with a molecular formula of $C_{23}H_{19}ClF_3NO_3$ (MW=449.86) (Figure 2.2).

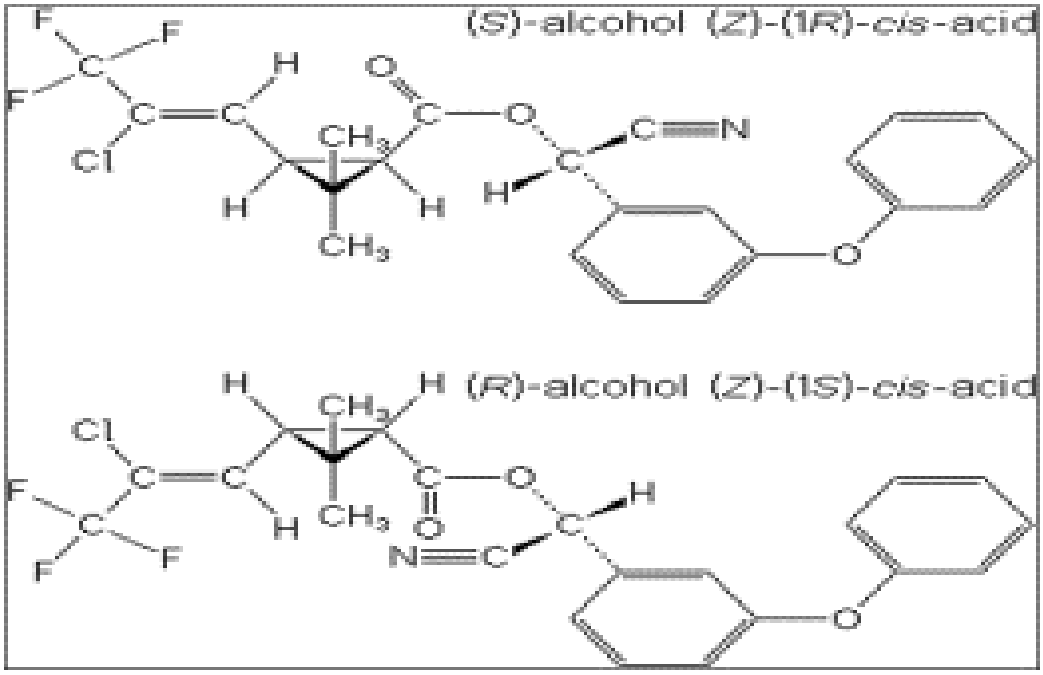


**Figure 2.1:** Chemical structure of lambda-cyhalothrin

Lambda-cyhalothrin has been used successfully in the control of sand flies. For example, Gonzalez *et al.,* (2015) reported a reduction of about 90% in *Lu. longipalpis* Lutz following application of lambda-cyhalothrin at a rate of 20 mg a.i./$m^2$ in animal pens in a

village in the Brazilian Amazon. However, residual treatment in human dwellings and animal shelters depends on the availability of suitable public health infrastructure, including adequate supplies of the insecticide, spraying equipment and trained personnel (Sharma and Singh, 2008). Elsewhere, Maroli and Khoury, (2004) sprayed inside walls and ceilings of houses with 10% wettable powder of lambda-cyhalothrin, at the rate of 25mg a.i. /$m^2$, resulting in a 60% reduction of cutaneous leishmaniasis in Kabul and a reduced risk of cutaneous leishmaniasis in the Peruvian Andes by 54%. Both trials measured protection at the household level, and it remains unclear under what circumstances "blanket spraying" of all houses in the village would have an additional mass effects on sand fly population (Davies *et al.,* 2003).

A research conducted in Peruvian Andes, demonstrated that lambda-cyhalothrin when administered by spray pump at the rate of 25 mg a.i. /$m^2$, proved effective against wild caught *Lu. verrucarum* sand fly species and remained 100% lethal for up to six months (Davis *et al*., 2000). Sharp *et al.,* (1993) compared the efficacy of 75% w/w DDT powder at 2.5g a.i. /$m^2$ and lambda-cyhalothrin at the rate of 30mg a.i. /$m^2$, sprayed in houses against malaria vector, *Anopheles arabiensis,* and discovered that mosquitoes in numbers trapped by exit traps leaving the huts treated with lambda-cyhalothrin were markedly less than those caught leaving DDT sprayed huts demonstrating that lamba-cyhalothrin was more effective than DDT.

Studies on susceptibility of *P. argentipes* and *P. papatasi* (vectors of *L. major* in India and Middle East, respectively) showed that these two species of sand flies are resistant to

DDT and Malathion but they are susceptible to lambda-cyhalothrin (Dinesh *et al*., 2010). Other studies in Venezuela showed that curtains impregnated with 12.5mg a.i. /$m^2$ lambda-cyhalothrin effectively protected populations against bites of sand flies capable of disseminating cutaneous leishmaniasis and significantly reduced sand fly numbers in treated houses (Kroeger *et al*., 2002). With 2.5% EC lambda-cyhalothrin, no resistance or tolerance in *P. papatasi* Scopoli and *P. argentipes* Annandale was reported (Dinesh *et al.,* 2010). Asidi *et al.,* (2005) evaluated nets treated with Chlorpyrifos-methyl 38.8% CS (100 mg a.i./$m^2$) and lambda-cyhalothrin 25% CS (18 mg a.i./$m^2$) in 11 huts in Cote d'Ivore and found that estimated deterrence of *Culex spp*. occurred between 36% with Chlorpyrifos-methyl impregnated net to 58% for lambda-cyhalothrin demonstrating the superiority of the latter.

Lambda-cyhalothrin (ICON®) has been used for control of intra-domiciliary *An. arabiensis,* Patton, in huts in South Africa (Casimiro *et al.,* 2014). Comparison of lambda-cyhalothrin with DDT showed that the percentage survival of blood-fed mosquitoes ranged from a low 55% caught leaving lambda-cyhalothrin- sprayed huts, to 82% of those caught leaving DDT-sprayed huts. Okumu and Moore, (2011) conducted a field trial using lambda-cyhalothrin to control intra domiciliary *An. darlingi* Root and DDT residual sprays inside surfaces in houses. This study showed that the number of people infected with *P. falciparum* was fewer in areas where houses were sprayed with lambda-cyhalothrin than in DDT-sprayed houses indicating that, like in the earlier study by Casimiro *et al*., (2014), lambda-cyhalothrin is more effective than DDT.

Field investigations carried out in Sudan on the protective efficacy of lambda-cyhalothrin-treated bed-nets (10 mg a.i. /$m^2$) against *P. orientalis* showed that this species of sand fly were highly susceptible to lambda-cyhalothrin and that the treated nets provided complete protection from bites of this vector for 12 nights. Moreover, exposure of *P. orientalis* females to nets treated with 10 mg a.i. /$m^2$ for 30 seconds resulted in death of all the flies within one hour. It was observed by Raghavendra *et al.,* (2011) that the efficacy of lambda-cyhalothrin, its acceptance by the local populace, and its cost-effectiveness make it a more useful insecticide for anti-malaria campaigns than DDT. It is for this reason that lambda-cyhalothrin was chosen for this study.

### 2.3 Leishmaniasis

Leishmaniasis is a zoonotic infection caused by protozoan parasites of the genus *Leishmania***.** The infection is transmitted to humans by infected sand flies of the genus *Phlebotomus* and *Lutzomyia* (Piscopo and Mallia, 2006). *Leishmania* parasites enter the immune system cells following the bite of an infected sand fly. The parasites then spread either to the skin, causing disfiguring lesions, or to internal organs, causing lethal infections (Gelanew *et al.,* 2011). The clinical forms of leishmaniasis are cutaneous leishmaniasis, mucocutaneous leishmaniasis and visceral leishmanaisis. Clinical manifestation depends on the parasite species and the host's specific immune responses to *Leishmania* antigens (Roberts, 2005). The worldwide incidence of leishmaniases has been on the increase and this has mainly been attributed to increase of several risk factors that are clearly man-made which include massive migration, deforestation, urbanization, immunosuppression, malnutrition and treatment failure (Zavitsanon *et al*., 2008). These factors may increase human exposure to infected sand flies. It has been shown that

majority of human infections by *Leishmania* parasites remain asymptomatic (Zavitsanon *et al*., 2008). These asymptomatic human subjects are able to clear the infection or they remain asymptomatic carriers for years. Thus, leishmaniasis development depends on several risk factors such as malnutrition, immunosuppression, age, immunological status and genetic factors.

Leishmaniasis is endemic in 98 countries in the world with 350 million people being at risk. An estimated 14 million people are infected, and each year about two million new cases occur (WHO, 2007). The global burden of leishmaniasis has remained stable for some years, causing morbidity and mortality loss of 2.4 million disability adjusted life-years (DALYs) and approximately 70,000 deaths, a significantly high rank among communicable diseases (Ngure *et al.,* 2009). The wild endemic pattern has been replaced by the spread of the disease associated with environmental modifications, disordered human occupation and substandard living conditions. Therefore, the disease is spreading in both rural and urban areas, exceeding old defined geographic limits and becoming a serious public health problem (Amóra *et al*., 2009).

Leishmaniasis is not only associated with poverty but also propagates poverty, because treatment is expensive and either unaffordable or imposes a substantial economic burden, including loss of wages (WHO, 2007). The global prevalence of leishmaniasis has risen in recent times because of an increase in international travel and human alteration of both vector and host habitats. Recent international conflicts have also contributed to an increase in and spread of leishmaniasis in previously unaffected countries (Rosypal *et al*.,

2003). Currently, a cost effective cure for leishmanisis does not exist. The chemotherapeutic agents most commonly used for treating the disease, that is, sodium stibogluconate, *N*-methylglucamine antimoniate, pentamidine, and amphotericin B, are not effective when administered orally. Moreover, they often require long periods of treatment and cause serious side effects, including cardiac and renal toxicity (WHO, 2017). This has prompted the World Health Organization to emphasize the need for development of new drugs in the treatment of leishmaniasis (WHO, 2005).

**2.4 Forms of leishmaniasis**

Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis globally with an annual incidence of 1.5 million cases (Desjeux, 2004). Multiple species produce CL in children and adults, primarily *L. major, Leishmania tropica*, *Leishmania aethiopica*, *Leishmania infantuma, Leishmania chagasi*, *Leishmania mexicana, Leishmania amazonensis Leishmania braziliensis, Leishmania viannia panamensis, Leishmania viannia peruviana* and *Leishmania viannia guyanensis* (Murray *et al*., 2005). Confirmed vectors include *P. duboscqi, P. guggisbergi* and *P. pedifer*. The disease produces skin lesions mainly on the face, arms and legs (Akilov *et al*., 2007). Cutaneous leishmaniasis starts as a papule at the site of a sand fly bite, which then increases in size, crusts and eventually ulcerates (Piscopo and Mallia, 2006). About 90% of infections are concentrated in Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia, and Syria. The disease is of limited importance in Eastern Africa where it occurs in small foci in North Sudan, Kenya, and Ethiopia (Malaria consortium, 2010).

Visceral leishmaniasis (VL) is the most severe form of leishmaniasis and is potentially dangerous if it is not treated (Garg and Dube, 2006). It is largely considered a rural disease, often correlated with malnutrition, poor sanitary conditions, and other factors associated with poverty (David *et al*., 2011). Visceral leishmaniasis coexists with malaria and other debilitating parasitic infections (Hailu *et al.*, 2005). Parasites of the *L. donovani* complex are the typical etiological agents of VL (Tonui, 2006). The main sand fly vector is *P. martini*, which breeds in termite hills, animal burrows, tree holes and house walls (Ngure *et al*., 2009).

*Leishmania tropica* has been reported to produce visceral disease in immune-compromised persons while visceralization by *L. amazonensis* has also been reported (Soliman, 2006). The three main factors that drive the increased incidence of VL are migration, lack of control measures and HIV–VL co-infections (Chappuis *et al*., 2007). The clinical symptoms of visceral leishmaniasis include splenomegally, recurring and irregular fever, anemia, pancytophenia, loss of appetite, weight loss and general body weakness. The disease is a silent killer, killing almost all untreated patients (Hailu *et al.*, 2005). Visceral leishmaniasis symptoms often persist for several weeks to months before patients either seek medical care or die from bacterial co-infections, massive bleeding or severe anemia (Chappuis *et al*., 2007).

Post kala-azar dermal leishmaniasis (PKDL) appears after treatment of visceral leishmaniasis (Ghalib and Modabber, 2007), and it requires prolonged and costly treatment (WHO, 2006). It is a dermatropic form of leishmaniasis characterized by skin

lesions that are macular, maculo-papular or nodular, and usually spread from the peri-oral area to other areas of the body. The symptoms first appear around the mouth; those which do not heal spontaneously become dense and spread over the entire body (Zijlstra *et al.*, 2003).

Mucocutaneous leishmaniasis (MCL) also called espundia, produces disfiguring lesions to the face, destroying the mucous membranes of the nose, mouth and throat (WHO, 2007). It may occur many years after the initial cutaneous ulcer has healed. After an initial skin lesion, that slowly but spontaneously heals, chronic ulcers appear after months or years on the skin, mouth and nose, with destruction of underlying tissue e.g. nasal cartilage. It is mostly related to *Leishmania* species of the new world such as *L. braziliensis*, *L. panamensis* and *L. guyanensis*. The mucosal lesions have also been reported in old-world leishmaniasis caused by *L. donovani*, *L. major* and *L. infantum* in immunosuppressed patients (Babiker *et al*., 2014). Ninety percent of all cases of MCL occur in Bolivia, Brazil and Peru.

**2.5 Global distribution of leishmaniasis**

Leishmaniasis causes substantial clinical, public health and socioeconomic problems in endemic regions in more than 98 countries in the Indian sub-continent, South Western Asia, Southern Europe, Africa, and Central and South America (Desjeux, 2004). There is a remarkable increase in risk factors for leishmaniasis worldwide and the disease burden is increasing (Reithinger *et al*., 2007). Visceral leishmanisis is endemic in the tropical and sub-tropical regions of Africa, Asia, the Mediterranean, Southern Europe, South and Central America. The distribution of VL in these areas however is not uniform; it is

patchy and often associated with areas of drought, famine and densely populated villages with little or no sanitation (Fig. 2.3).

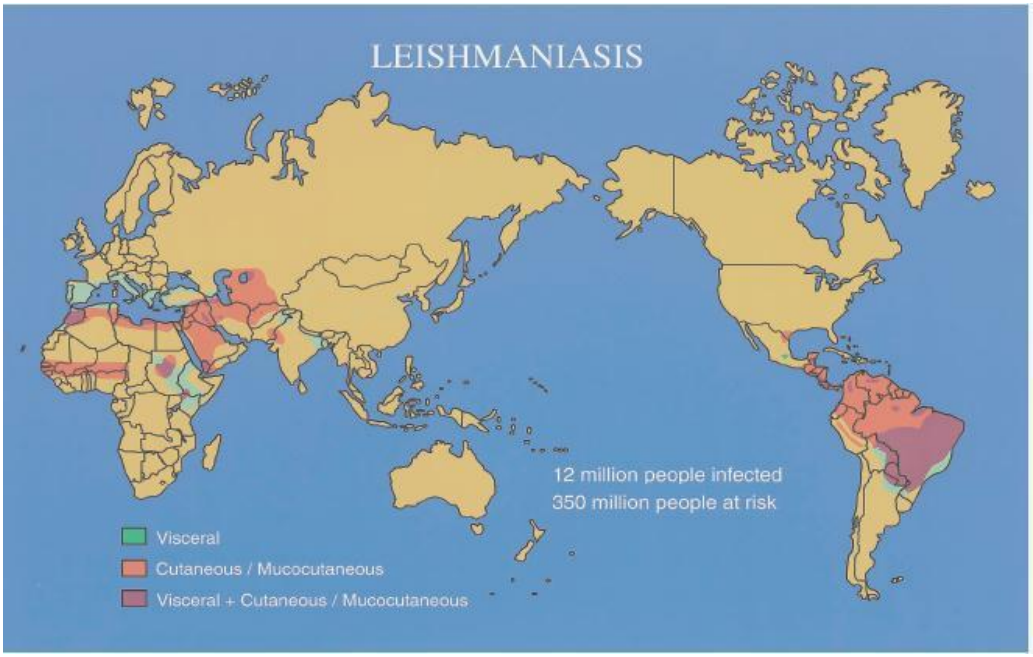


**Figure 2.2:** Global distribution of leishmaniases. (Source: Handman Emmanuela, Leishmaniasis: Current state of Vaccine Development, 2001).

The global estimate for new cases of visceral leishmaniasis is 500,000 cases per year out of which 90% of the cases arise in just five countries. These include Bangladesh, Brazil, India, Nepal and Sudan (Desjeux, 2004). Each year, there are 1.5 million new cases of CL in more than 70 countries worldwide with 90% of the cases reported in Afghanistan, Algeria, Brazil, Islamic Republic of Iran, Peru, Saudi Arabia and Syria (Ghalib and Modabber, 2007). Ninty percent (90%) of MCL cases occur in Bolivia, Brazil and Peru (Desjeux, 2004). In India, visceral leishmaniasis is endemic in the districts of Bihar, Uttar Pradesh, Orissa, Tamil, Nadu and Gujarat. Geographical distribution of leishmaniasis is restricted to tropical and temperate regions (Conjivaram and Ruchir, 2007).

In Africa, VL is a significant problem in South Sudan, Kenya, Sudan, Ethiopia, Uganda and Eritrea (Wasunna *et al*., 2005). Sudan is the most affected country, being one of the five countries that constitute 90% of all global cases of VL (Hailu *et al*., 2005). Sudan also has the highest incidence of Post Kala-azar Dermal Leishmaniasis (PKDL) in the world (Ghalib and Modabber, 2007). Visceral leishmaniasis has been known in Sudan since 1904 to be endemic along the Blue Nile where it enters Ethiopia and its tributaries (Ngure *et al*., 2009; Al-Salem *et al*., 2016). Since the first reported case of VL in Sudan, the disease has become wide spread and is endemic in south and eastern parts of the White Nile and Upper Nile states. Other areas affected include the provinces of Kasala, Jonglei and Kapoeta in the south, El Fasher and El Nahud in the west and north of Khartoum (Al-Salem *et al*., 2016)**.**

In Ethiopia, there is the presence of a significant number of patients co-infected with HIV and VL (Ngure *et al*., 2009). The etiologic agent associated with the epidemic is *L. donovani* and the sand fly vector is *Phlebotomus orientalis* (Al-Salem *et al*., 2016). Ethiopia recorded the initial VL case in 1942 in areas around the southern parts of the country. Since then the disease has spread to become endemic in the Segen, Woito and Gelana river valleys. The highest incidence has been recorded in the Aba Roba area (Lemma *et al*., 2015). The VL cases have also been reported in the villages close to the Segen river valley.

In Somalia, sporadic cases of VL first appeared in 1934, mainly in the Middle Shabelle and Lower Juba areas. Children below the age of 15 years are at the highest risk of

infection and males are over three times more susceptible than females (Shiddo *et al*., 1995). In Israel VL is rare and the few cases that have been reported are largely confined to the run down Arab villages in western Galilee, indicating that the disease is linked to poverty, poor sanitation and sub-standard housing.

In West Africa, leishmaniasis is endemic although it is one of the less recognized or under-reported parasitic infections (WHO, 2018). Cases of leishmaniasis have been reported in Niger, Mali, Nigeria, Senegal, Cameroon, Burkina Faso, Mauritania, Gambia, and Guinea. There is high prevalence of both HIV and leishmaniasis co-infection in Burkina Faso (Niamba *et al*., 2007). Algeria is among the eight countries that contribute 90% of worldwide cases of CL (Reithinger *et al*., 2007).

### 2.6 Leishmaniasis in Kenya

Leishmaniasis has been endemic in Kenya for a long time. The most prevalent forms are cutaneous leishmaniasis, visceral leishmaniasis and post kala-azar dermal leishmaniasis (PKDL) (WHO, 2015). The occurrence of CL is rare compared to VL, and the two forms of diseases do not tend to overlap geographically. Visceral leishmaniasis is found predominantly in the arid, low-lying areas of the Rift Valley, Eastern and North Eastern provinces, whereas CL occurs over a wide range of environmental conditions from semi-arid lowlands to high plateaus in the Eastern, Rift Valley, Central and Western provinces (malaria consortium, 2010). Visceral leishmaniasis is endemic in Baringo, Koibatek, Turkana, West Pokot, Kitui, Meru, Keiyo, Marakwet, Mwingi, Tana River, Kajiado, Marsabit, Isiolo, East Pokot, Wajir, Mandera and Machakos districts (Wasunna *et al.*, 2005). In 2001, there was an outbreak of VL in Wajir and Mandera districts of

North Eastern Kenya with 904 patients diagnosed between May 2000 and August 2001 (Marlet *et al*., 2003) (Fig. 2.4).

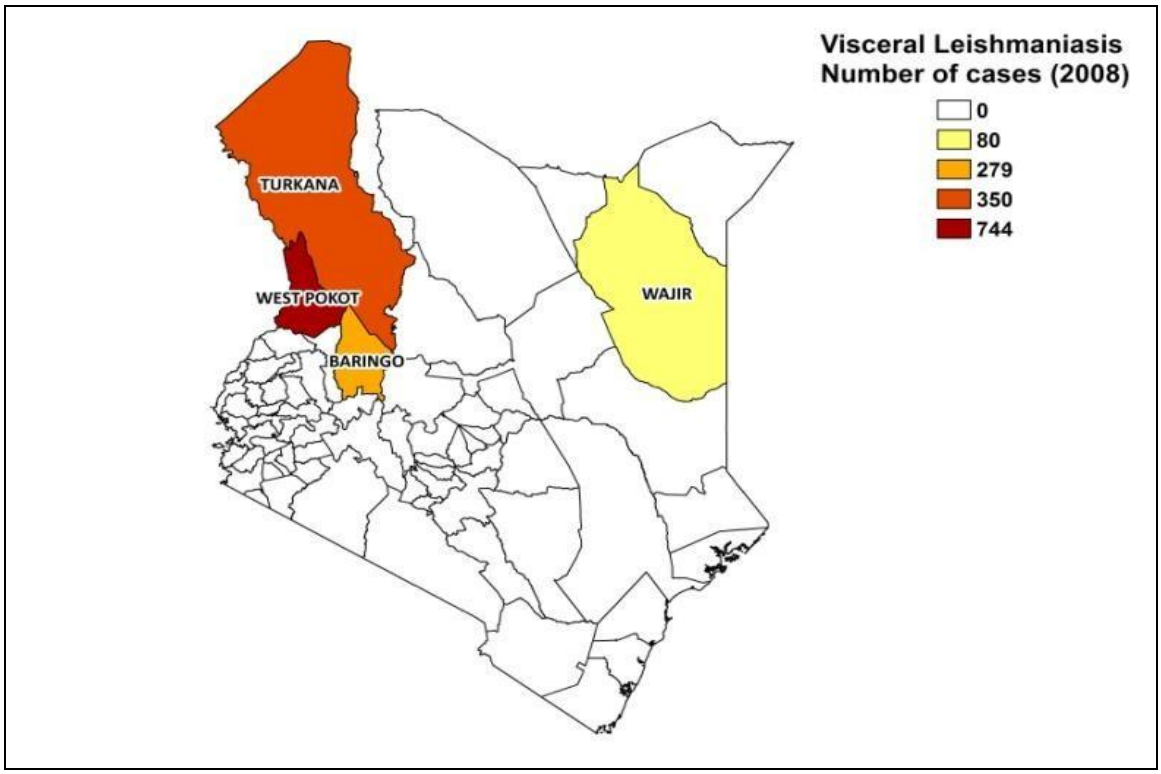


**Figure 2.3:** Distribution of reported cases of visceral leishmaniasis in Kenya.
(Source: http:// www.who.int/ leishmaniasis/resources/ KENYA)

In Kenya, cutaneous leishmaniasis is caused by *L. major*, *L. aethiopica and L. tropica* (Ngure *et al.,* 2009). Cutaneous leishmaniasis due to *L. major* is transmitted by *P. duboscqi.* It is mostly found in animal burrows, here it feeds on frequently infected rodents. Diffuse cutaneous leishmaniasis (DCL) was first reported in Kenya in 1969 in Bungoma district and Mount Elgon area (Kungu *et al*., 1972). *Leishmania aethiopica* has been identified as the etiological agent, rodents as the animal reservoirs and *P. pedifer* Lewis as the vector of DCL in Mt. Elgon region (Ashford, 2000). The PKDL in Kenya was first described in 1959 by Manson-Bahr (Ngure *et al.,* 2009). Although various

aspects of the transmission and control of leishmaniasis have been studied in Kenya, the impact of the disease and particularly VL is still enormous (Tonui, 2006).

### 2.7 Reservoir hosts of Leishmaniasis

Vertebrate animals, other than man, act as animal reservoirs. The infection is, therefore, mostly zoonotic, where man is an accidental host. There are, however, situations where man may be the reservoir and the cycle then is anthroponotic. Each species of *Leishmania* favors one or more animal reservoir, except *L. donovani* and *L. tropica*, which are thought to be mainly, if not exclusively, anthropomorphic (Bari and Rahman, 2016).

The parasite is hosted by a large number of animals called the wild reservoir or the animal hosts, the vector sand fly is the intermediate host and domestic dogs and man are domestic or accidental hosts. In anthroponotic (urban or dry type) cutaneous leishmaniasis, caused by *L. tropica*, the transmission is generally from man to sand fly to man again. Although in some countries dogs and small rodents have been found to be infected with this parasite and these may be serving as the reservoir host. Therefore, dogs are the principle reservoir of these parasites and play a central role in the transmission cycle to man by phlebotomine sand flies (Emami and Yazdi, 2008).

In zoonotic cutaneous leishmaniasis (rural, wet type) caused by *L. major*, the transmission of infection, i.e. rodent to sand fly to rodent cycle, is maintained in wild rodent/gerbil colonies as sand flies breed in abundance in the cool and shady burrows

(Githure *et al*., 1996). *Rhombomys opimus*, *Meriones* spp. and *Psammomys obesus* are the three major reservoir species of the rodents that maintain infection in most of Central Asia, Middle East and North Africa. Others that are implicated in various parts of the world include dogs, cats, jackals, carnivores, fox, wolves, rats etc. A number of rodent species have been implicated as being the animal reservoir hosts in Pakistan, these include, *Meriones hurriane* or other species of gerbils, *Rhombomys opimus* and *Tatera indica* (Githure *et al.,* 1996). Other rodents that might be important in this respect include *Meriones persicus, M. crasus, M. lybicus erythrorus* and *Mus musculus* (Bari and Rahman, 2016). Dogs, cats, jackals, carnivores, fox, wolves and rats have been identified as reservoirs in other parts of the world. Identification and control of reservoir host has an immense influence on the epidemiology of the disease.

### 2.8 Control and treatment of leishmaniasis

#### 2.8.1 Chemotherapy

Current treatment options are very limited; most are expensive and problematic due to issues of resistance, toxicity and side-effects. Since anti-leishmanial vaccines are still being developed, the current control strategies for leishmaniasis rely on case management. However, case management is difficult to be conducted since it is restricted by factors like lack of access to affordable, active drugs, incorrect prescribing and poor compliance (Zavitsanon *et al*., 2008). The drugs currently recommended for the treatment of leishmaniasis include sodium stibogluconate, meglumine antimoniate and amphotericin B. However, these drugs have drawbacks such as serious side effects, long courses of treatment and rampant drug resistance especially of the antimonials (WHO, 2007). Amphotericin B, particularly its lipid formulation AmBisome®, is often used to

treat VL and CL, especially in areas where (Pentostam) sodium stibogluconate (SSG) resistance is increasing. However, with amphotericin B there are also serious issues with toxicity and its cost is often prohibitively high (Malaria Consortium, 2010). As a preventive measure some have reported that use of ITNs, treated wall cloths, repellents and other personal protective measures are not effective in preventing visceral leishmaniasis transmission in Kenya (Tonui, 2006).

### 2.8.2 Insecticides for vector control

The best method of interrupting any vector-borne disease is to reduce human-vector contact. Sand fly control programmes in most VL foci have advanced slowly when compared to that of other haematophagus arthropods like mosquitoes, ticks and black flies. Leishmaniasis specialists advocate for vector control especially for areas of anthroponotic transmission (Hailu *et al*., 2005). The commonly used vector control measures include indoor spraying of houses with insecticides where sand flies are endophilic and the use of both treated and untreated bed nets where sand flies are endophagic (Piscopo and Mallia, 2006). Personal protection using repellents and nets is an important aspect of control that reduces human-vector contact. In endemic areas, spraying with DDT and other residual insecticides is effective in sand fly control **(**Conjivaram and Ruchir, 2007).

In the zoonotic foci where carriers are involved and dogs are the main vertebrate host, the effective methods include destruction of dogs and elimination of sand flies by environmental and chemical control (WHO, 2005). Control measures are aimed at

reducing sand fly populations and man-vector contact by the use of residual spraying in houses and animal shelters, insecticide treated nets for human use, repellents applied on people's skin exposed to sand fly bites, and topical application of insecticides on dogs for prevention of canine leishmaniasis (Maroli *et al*., 2009).

Insecticides pose adverse effects to the user and the environment. The issue of concern is not only the effectiveness of these spraying programmes but also their side effects are of great importance on health and environment, and their potential for sustainability, which will rely on how much the insecticides will cost and their application. Sand flies seem to have developed a lot of resistance to the chemicals, especially to DDT and in some scenarios to Malathion and pyrethroids (Umkant and Sarman, 2008). The use of pheromones is another strategy of controlling insect vectors although this has not yet been exploited in the control of vectors of leishmaniasis.

**2.9 Phlebotomine sand flies as disease vectors**

Sand flies (Diptera: Psychodidae) belonging to genera *Phlebotomus* and *Lutzomyia* are the vectors of leishmaniasis in the Old and New World, respectively (Emami and Yazdi, 2008). They are principally present in the warm zones of Asia, Africa, Australia, southern Europe and the Americas (Maroli *et al*., 2013). The vector adapts easily to the peridomestic conditions of impoverished areas, exploiting the accumulation of organic matter produced by domestic animals and poor sanitary conditions (Amóra *et al*., 2009). Approximately 30 species of sand flies are proven vectors of about 20 *Leishmania* species. Those in the genus *Phlebotomus* are vectors of a bacterium (*Bartonella*

*bacilliformis*) that causes Carrion's disease (oroyo fever) in South America. In parts of Asia and North Africa, they transmit a viral agent pappataci virus (an arbovirus) that causes sand fly fever (pappataci fever) as well as protozoan pathogens (*Leishmania* spp.) that causes leishmaniasis (Umkant and Sarman, 2008). Moreover, sand flies are said to have been involved in arboviral infection transmission and suspected in transmitting Chandipura virus (Kumar *et al*., 2012).

One of the characteristics of the sand fly is that it feeds at dusk and always remains close to the area where it breeds, not too high from the ground, reason being it is weak when it comes to flying. Different species have different feeding and resting patterns. Control strategies can be formulated based on the above important characteristics (Piscopo and Mallia, 2006). The only proven vector of the *Leishmania* parasite is the blood-sucking female of the genus *Phlebotomus* and *Lutzomyia* (Murray *et al*., 2005). There are 30 or so of the over 500 species of phlebotomine sand flies known to transmit *Leishmania* parasites as shown in Table 2.1. These include *P. argentipes* on the Indian sub-continent, *P. duboscqi, P. martini* and *P. orientalis* in Africa and the Mediterranean basin, *P. chinensis* and *P. alexandri* in china. In the New World *L. longipalpis* is the only known vector of *L. chagasi* (Murray *et al*., 2005).

*Phlebotomus martini* and *P. celiae* are associated with the presence of termite mounds, soil moisture and a prolonged wet season while *P. orientalis* prefers drier habitats and is the main man-biter in *Acacia-Balanites* forests in Sudan and Ethiopia (Sadlova *et al*., 2013). Sand flies live in rodent burrows, crevices, holes in river banks, trees and houses

in the Old World while in the New World sand flies also dwell in the tree canopies and forest litter (Bari and Rahman, 2016).

**Table 2.1:** Human pathogenic species of *Leishmania* and their vectors in Old World

| **Sand fly vector** | **Geographical Distribution** | ***Leishmania* transmitted** | **Disease form** |
|---|---|---|---|
| *P. papatasi, P. duboscqi, P. salehi* | North Africa, central and West Asia | *L. major* | Rural, zoonotic, cutaneous leishmaniasis, or oriental sore |
| *P. sergenti* | Central & west Asia and western India | *L. tropica* | Urban, anthroponotic cutaneous oriental sore |
| *P. longipes, P. pedifer* | Ethiopia and Kenya | *L. aethiopica* | Cutaneous leishmaniasis, diffuse |
| *P. argentipes, P. orientalis, P. martini* | India, Nepal, Bangladesh and east Africa | *L. donovani* | Visceral leishmaniasis, kala-azar, post-kala-azar dermal leishmaniasis |
| *P. ariasi, P. perniciosus* | Mediterranean basin, central and west Asia | *L. infantum* | Infantile visceral leishmaniasis |

*****(Source: Sharma and Sarman, 2008)

### 2.10 Identification of sand fly species

Epidemiological studies on leishmaniasis start with identification of vectors, though there is some difficulty in taxonomic identification of adult insects. It has been estimated that there are currently 988 valid phlebotomine species and subspecies from all continents except Antarctica, including 29 fossils, with 512 extant and 17 fossil taxa found in the Americas (Bates *et al*., 2015). They are grouped in the suborder Nematocera of the order Diptera, family Psychodidae, Sub-family Phlebotominae. Taxonomy of sand flies relies

mainly on the morphological and anatomical characteristic features, which require dissection and mounting of freshly collected or preserved sand fly specimens (Kumar *et al*., 2012). Based on these features, some taxonomists have identified three genera of sand flies: *Phlebotomus, Sergentomyia and Chinius* that are widely accepted by modern Old World taxonomists. In the genus *Phlebotomus*, 11 subgenera, 96 species and 17 subspecies have been recognized by Lewis (Maroli *et al*., 2012). Still, some authors place sand flies in the family Phlebotomidae while others retain them in the sub-family Phlebotominae of Psychodidae. However, there is no general agreement on the number of genera but five have been identified: *Phlebotomus, Sergentomyia, Lutzomyia, Warileyia and Brumptomyia*.

There are several methods of vector identification but experienced scientists can reliably identify sand fly specimens on the basis of external features and behaviour like colour, size and other characteristics (WHO, 1982). However, it is necessary to confirm identities with the aid of a compound microscope during dissections by observing the cibarial armatures, spermatheca and the pharynx (Abonnenc and Minter, 1965). Currently, the classification of phlebotomine sand flies remains controversial and cumbersome. At the same time, taxonomy of sand flies is still an important component for getting to understand biodiversity, biology and behaviour of vector species of sand flies in order to plan appropriate intervention measures.

## 2.11 Distribution of old world sand flies

*Phlebotomus duboscqi* Neveu-Lemaire occurs in Burkino Faso, Ethiopia, Gambia, Ghana, Kenya, Mali, Mauritania, Niger, Nigeria, Senegal, Sudan, Togo, and Yemen. *Phlebotomus martini Parrot* is found in Ethiopia, Kenya, Sudan, Somalia and Uganda. *Phlebotomus orientalis* Parrot is distributed in Sahel region of Africa from Niger to Egypt; Ethiopia, Kenya, Rwanda, Uganda; Saudi Arabia and Yemen on the Arabian Peninsula. *Phlebotomus papatasi* Scopoli occurs in a broad swath from France across most of the Mediterranean Basin, and eastward to India, including the Arabian Peninsula and Ethiopia. *Phlebotomus sergenti* Parrot is one of the most widely distributed sand fly species in the Old World, ranging from Portugal and the Mediterranean Basin eastward to Ethiopia, the Arabian Peninsula, and India. *Phlebotomus argentipes* occurs in Nepal, India, Sri Lanka and Pakistan eastward to Myanmar, Thailand, Vietnam, Laos and Indonesia (Ngure *et al*., 2009: WHO, 2017).

## 2.12 Biology of sand flies

These are small, fragile, nocturnal insects without strong capabilities to fly (Alexander, 2000). Male and female sand flies both feed on nectar and sap from plants including honeydew from aphids and other homopterans, nectar from flowers and fruits, and other plant juices. However, females are blood feeders and are active at dusk and all through the night in search of blood. They usually cover a radius of a few to several hundred meters around their habitat (Sharma and Singh, 2008). Sand flies are “pool” feeders, and their bite is a bit painful and usually causes red spots that may blister. There are about 600 species in five genera within the subfamily *Phlebotominae.* Species in three genera,

*Phlebotomus*, *Lutzomyia* and *Sergentomyia*, suck blood from vertebrates; only the former two transmit disease to man (Bari and Rahman, 2016).

Adult phlebotomine sand flies are 2-5 mm long, have hairy appearance, relatively large eyes and relatively long and stilt like legs. When resting, its wings are held nearly erect and in a characteristic upright V-formation. They show nocturnal activity and have a characteristic hopping type of flights. Adults are weak fliers and do not usually disperse more than a few hundred meters from their breeding places (Fig.2.5).

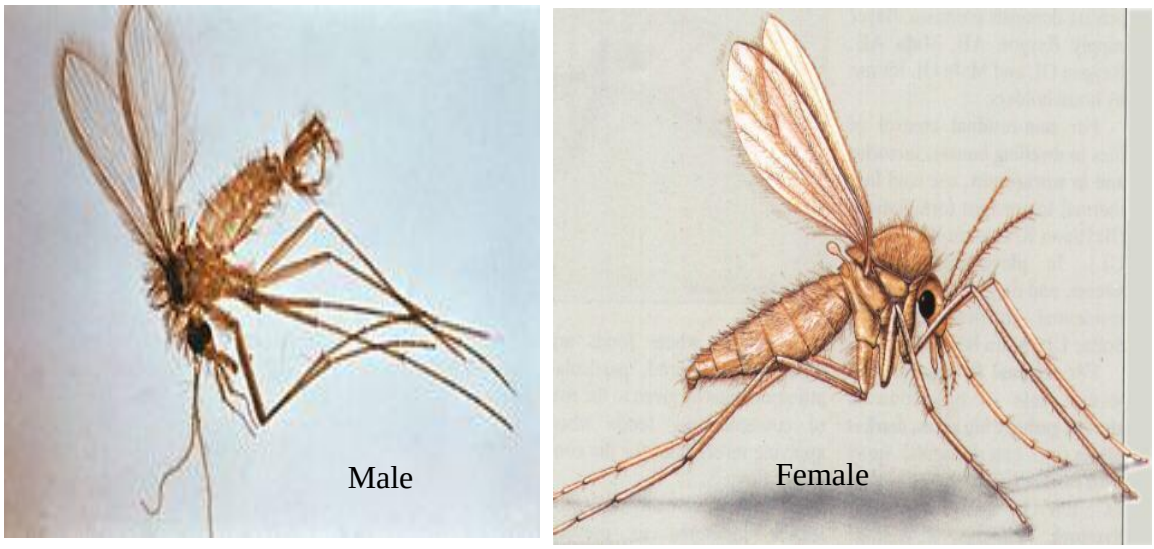


**Figure 2.4:** Adult male and female sand fly
**(*Source:http:// www.who.int/ leishmaniasis/ disease_epidemiology/en/,*)**

Sand flies live in termite mounds, rodent burrows, crevices, holes in river banks, trees and houses in the Old World while in the New World they also dwell in the tree canopies and forest litter (Kendrick, 1986). Only females bite since they need several blood meals before they can lay eggs. They mostly bite during the night and dusk although not all, for instance *Lutzomyia wellcomei* bites mainly during the day. Sand flies have short

mouthparts and are pool feeders (Umkant and Sarman, 2008). They can suck blood both from animals (cats, dogs, various rodents, cattle, birds and lizards) and human.

Adult sand flies shelter during the day in dark, humid places like tree holes, animal burrows or under rocks. Phlebotomine sand flies undergo complete metamorphosis through four developmental stages: egg, larva (four instars), pupa and adult (Maroli *et al*., 2012). Eggs are laid in terrestrial microhabitats rich in organic matter that provides food for larvae (Alexander, 2000). Eggs hatch between 7-11 days emerging as larva which feed on dead organic matter present in the breeding site.

Larvae molt and undergo development to second, third and fourth instar stages. Larvae are caterpillar-shaped with head capsules and small leaf-like antennae. They have long caudal setae that can help in their identification as sand fly larvae (Maroli *et al*., 2012). In the 4th instar, larvae bear a darker sclerotized plate on the dorsum of the last abdominal segment. The fourth instar larvae pupate and adults emerge and immediately look for a good blood meal (Fig. 2.6). Mating occurs at the site of emergence. The larval duration may take up to 18 days while the pupal stage usually lasts 7-12 days; males usually emerge before females (Armed Forces Pest Management Board). This life cycle could take 30-45 days depending on climatic conditions.

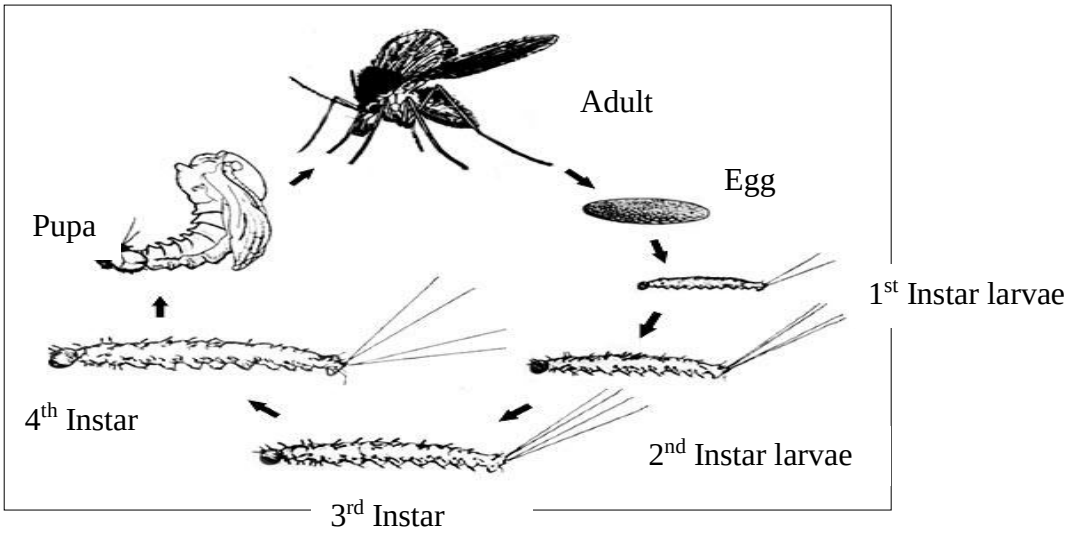


**Figure 2.5:** Illustration of the life cycle of a phlebotomine sand fly (adapted from Maroli *et al*., 2012)

### 2.13 Control of phlebotomine sand flies

Vector targeted strategies are particularly attractive, since the vectorial capacity to transmit infectious diseases to humans is related to vector density and in an exponential way, to vector survival (Umkant and Sarman, 2008). The main control measures for phlebotomine sand flies include environmental management, residual insecticide application, insecticidal plants and bioinsecticides. In the zoonotic foci where carriers are involved and dogs are the main vertebrate hosts, the effective methods of controlling sand flies will be the destruction of dogs and environmental management (Kishore *et al*., 2006)

### 2.13.1 Environmental management

The principle of environmental management is to make it unsuitable for the vectors to live and reproduce (Kishore *et al.,* 2006). Measures used include pruning trees in order to increase sunshine, thereby decreasing soil shading and preventing favorable conditions (temperature and humidity) for the development of sand fly larvae (Ngure *et al*., 2018) and modification of their ideal developmental habitats. In the use of houses to control sand flies, the walls which provide the resting sites can be plastered by filling all the cracks and crevices by mud and lime, thereby, stopping the breeding of sand flies. This approach has been shown to reduce sand fly density (Umkant and Sarman, 2008), although cracks and crevices may reappear after several months. Environmental management may also involve relocation of human settlements away from sand fly habitats and physical modification of these habitats.

### 2.13.2 Integrated vector management (IVM)

Integrated vector management (IVM) based vector control is encouraged by the World Health Organization (WHO). Vector-borne diseases account for about 17% of the estimated global burden of infectious diseases and exert an enormous toll on the continent of Africa (Townson *et al*., 2005). They result in loss of productivity, school absenteeism, and aggravation of poverty, high costs for health care and a burden on public health services (WHO, 2012). Past and current efforts aimed at controlling most vector-borne diseases have relied solely on disease management. As neither effective medication nor vaccines are available for some of these diseases, vector control remains pivotal. Vector control is crucial to reduce the extent to which drugs are needed to treat

the diseases, as the parasite can become resistant, or the drugs are often unaffordable for those most affected. The need to integrate efforts and optimize the use of limited available human and financial resources is evident (Townson *et al*., 2005).

In response to the challenges, the World Health Organization's (WHO) IVM strategy, a rational decision-making process to optimize use of resources, was established as a pivotal platform for combating these often chronic and debilitating diseases. The strategy is based on the premise that effective control is not the sole preserve of the health sector but of various public and private agencies, including communities (WHO, 2012). Salient attributes of IVM include: methods based on knowledge of factors influencing local vector biology, disease transmission and morbidity, use of a range of interventions, often in combination and synergistically, collaboration within the health sector and with other public and private sectors that impact on vectors, engagement of local communities and other stakeholders, a public health regulatory and legislative framework. An IVM–based process should be cost-effective, and subject to routine monitoring and evaluation of impact on vector populations and disease transmission (WHO, 2012).

### 2.13.3 Residual sprays

The effectiveness level of an insecticide relies on the class of the insecticide used, the susceptibility of sand flies to the insecticide, the type of surface treated, the dosage and method of application and overall coverage (WHO, 2010). Insecticides that have been used in controlling sand flies include products like organochlorines, organophosphates, carbamates and synthetic pyrethroids (Umkant and Sarman, 2008). The DDT still

remains insecticide of choice because of its low cost, high efficacy, long residual action and relative safety when used for indoor residual spray (Kishore *et al.,* 2006). However, resistance to the organochlorine DDT has been reported (WHO, 2010). The switch from the organochlorines and organophosphates to the newer pyrethroid insecticides provides several new potential chemicals for use as residual sprays. In Brazil, deltamethrin was used for both interior and exterior application. Sand fly populations inside houses were significantly reduced, but the exterior sprays were ineffective (Claborn, 2010). The newer pyrethroid insecticides (Deltamethrin and Permethrin) have provided several new potential chemicals for use as residual sprays. For example, a 90% reduction in sand fly populations was achieved following treatment of the termite mounds and animal burrows with pyrethroids. Unfortunately, the reduction lasted for two weeks only (Claborn, 2010). Residual sprays are also limited by several environmental factors including high summer temperatures, strong radiation and the accumulation of dust. These conditions can reduce the toxicity of insecticides (Claborn, 2010). It has also been shown that residual spraying is much more effective in urban situations when every house and animal shelter is treated than in rural areas where relatively few dispersed houses are sprayed and the sand flies represent a small proportion of the vector population (Zavitsanon *et al*., 2008). The sites where sand flies breed are not well known hence control measures that specifically target sand fly larvae are not feasible.

### 2.13.4 Prophylactic methods

These include self-protection by use of mosquito nets and repellents. The use of insect repellents like DEET (Diethyl-toluamide) or protective clothing has been used as a

prophylactic measure against leishmaniasis. Insecticide-treated nets are considered to be effective, relatively cheap and a sustainable method for sand fly control (WHO, 2010). However, studies have revealed that impregnated clothing to protect humans from sand fly vectors may be impractical because the use of ITNs is not effective in preventing VL transmission in Kenya (Tonui, 2006). The use of dog collars impregnated with pyrethroid insecticides in Iran reduced the incidence of visceral leishmaniasis in children although this technique led to many operational problems, such as loss of collars (WHO, 2010). People living in highly endemic foci should use personal protective measures to avoid bites by sand fly vectors of leishmaniasis.

### 2.13.5 Bioinsecticides

The indiscriminate use of insecticides induces insect resistance and disrupts food chains, affecting the population density of non-target species. These factors, associated with the many diseases transmitted by dipterans, calls for the development of new control strategies. An alternative is *Bacillus thuringiensis* var. *israelensis* or *Bacillus sphaericus*, currently used to control mosquito larvae and black flies (Amóra *et al*., 2009). *Bacillus sphaericus* was successfully used in the control of *P. martini* in Kenya where inhibitory effect of *B. sphaericus* on hatching of eggs of *P. duboscqi* was observed (Umkant and Sarman, 2008). However, the application of biolarvicides in the field is difficult due to diverse breeding habitat of sand fly (Kishore *et al*., 2006).

## 2.14 Malaria disease

Malaria is a mosquito-borne parasitic infectious disease affecting humans and other animals and causes a significant burden of disease at the global and regional level (Murray *et al.,* 2012). The disease is caused by parasitic protozoans of the genus Plasmodium namely; *P. vivax, P. malariae, P. ovale, P. knowlesi,* and *P. falciparum*. They are transmitted by female mosquito vectors of the *Anopheles* species (Murray *et al.,* 2012: WHO, 2014). Malaria results in symptoms that include fever, tiredness, vomiting, and headache (Caraballo, 2014). When the disease is severe it can cause yellow skin, seizures, coma, or death (Caraballo, 2014). Usually the symptoms start to be manifested 10 to 15 days after being bitten and if not properly treated, people may have recurrences of the disease months later (WHO, 2014). In those who have recently survived an infection, reinfection usually causes milder symptoms (Caraballo, 2014). This partial resistance disappears over months to years if the person lacks continuous exposure to malaria (Caraballo, 2014). The disease is most commonly transmitted by an infected female *Anopheles* mosquito (WHO, 2014). The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood (WHO, 2014). The parasites travel to the liver where they mature and reproduce (Caraballo, 2014).

Malaria continues to be an important disease in the tropics posing a major obstacle to sustainable development (Sachs and Melany, 2002). The burden of malaria mainly rests in sub-Saharan Africa where it is responsible for one in five childhood infections and acts in synergisms with other illnesses such as respiratory infections, diarrheal diseases and malnutrition to cause even higher proportion of childhood morbidity and mortality

(Breman *et al.,* 2001). Sub-Saharan Africa is greatly affected, and in 2015, the region had 88% of malaria cases and 90% of malaria deaths (WHO, 2016). In Kenya, malaria remains a major cause of morbidity and mortality with more than 70% of the population at risk of the disease (MOH, 2014). The malaria burden in Kenya is not homogenous. The areas around Lake Victoria and on the coast present the highest risk, and children under the age of five years and pregnant women are the most vulnerable to the infection. The overwhelming global burden of malaria is largely focused in the tropics and particularly in the sub-Saharan Africa owing to environmental risk factors that favour transmission of *Plasmodium* parasites (Killeen *et al.,* 2002; Snow *et al.,* 1999b).

Although malaria is both curable and preventable disease with many interventions available, its control in sub-Saharan Africa is constrained by poverty and insufficient health infrastructures (Sachs and Melany, 2002). This situation is further complicated by the emergence of drug resistance strains of malaria parasites (Trape, 2001) and insecticides resistant malaria vectors (Brook *et al.,* 2000; Chandre *et al.,* 1999; Hargreaves *et al.,* 2000; Hemingway *et al.,* 2002).

### 2.15 Global distribution of Malaria

Globally, an estimated 3.4 billion people in 91 countries and territories are at risk of being infected with malaria and developing disease, and 1.1 billion are at high risk (>1 in 1000 chance of getting malaria in a year) (WHO, 2016). Distribution of malaria depends mainly on climatic factors such as temperature, humidity, and rainfall. Malaria is transmitted in tropical and subtropical areas, where *Anopheles* mosquitoes can survive

and multiply, and malaria parasites can complete their growth cycle. Malaria parasite requires certain climatic conditions, both for its own development as well as for the survival of its vector mosquitoes (Caminade *et. al.,* 2014). As such, malaria transmission is concentrated in lowland areas of the tropics, where there is sufficient freshwater for mosquito breeding. However, malaria can also seasonably be transmitted in areas away from the tropics.

### 2.16 Global Public health importance of malaria

Malaria is perceived as the world's worst health problem, but Africa is an area in the world which suffers the greatest burden of mortality in early childhood and clinical disease have the least developed system of health reporting, the repeatedly quoted figures for annual deaths and clinical cases are best guesses (Snow *et al.,* 1999a; Snow *et al.*, 1999b). The global figures for deaths from malaria range from 1.5 to 2.7 million each year, most of who are children under five years of age and pregnant women (Schwartlander, 1997; WHO, 2014). Almost all these are caused by *Plasmodium falciparum*, one of the five species of malaria parasites in human. The others are *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi* (Murray *et al.,* 2012: WHO, 2014). It is estimated that a child dies from malaria every 12 or so seconds (MFI, 2003). This burden of mortality is not equally shared, falling most heavily on sub-Saharan Africa where more than 90% of these deaths occur and 5% of children die from the disease before reaching the age of five years (MFI, 2003).

Through a short communication to the *lancet,* Marsh *et al.,* (1998) stated that there is a malaria disaster in Africa, which is not just on its way but it is already happening. Malaria is responsible globally for 500 million cases of clinical disease and presents a public health problem for 2.4 million people, representing over 40% of the world's population in over 90 countries (WHO, 1998; WHO, 2014). Ten percent of the world's population will suffer a clinical attack of malaria each year. Fortunately, most will survive after an illness lasting 10 to 20 days, but during the clinical illness, they will be unable to attend school or work, diminishing education attainment and productivity (MFI, 2003). In some areas, the malaria situation is deteriorating as a result of environmental changes, civil disturbance, increasing level of travel and increasing drug resistance. Thus, the need for effective malaria control has never been achieved (Greenwood, 1997).

Insecticides have played an important role in the control of insect vectors of diseases since early $20^{th}$ century. Although important advances continue to be made in the development of alternative control measures, insecticides will remain a vital part of integrated control programs for the near future (Curtis, 1994). Indoor spraying is based on the observation that after blood feeding many endophilic vector species rest on walls until the eggs are fully developed, when the females fly outdoors in search for oviposition sites. During the resting phase of the gonotrophic cycle, the vectors absorb sufficient levels of insecticides which end up in killing them or reducing their longevity and thus their vectoral capacity. However, some female mosquitoes do not rest on the walls long enough to absorb toxic levels of insecticides (Khan *et al*., 1997). Despite the numerous control strategies, victory against malaria is yet to be achieved. Malaria is thus, returning

to areas from which it had been eradicated and entering new areas such as Eastern Europe and Central Asia (MFI, 2003). Consequently, this calls for enhanced additional research and development of new efficient control strategies.

### 2.17 Forms of Malaria

There are five species of *Plasmodium* that can infect humans (Caraballo 2014). Most deaths are caused by *P. falciparum* because *P. vivax, P. ovale*, and *P. malariae* generally cause a milder form of malaria (WHO, 2014; Caraballo, 2014). The species *P. knowlesi* rarely causes disease in humans (WHO, 2014). The risk of disease can be reduced by preventing mosquito bites through the use of mosquito nets and insect repellents, or with mosquito control measures such as spraying insecticides and draining standing water (Caraballo, 2014). The disease is widespread in the tropical and subtropical regions that exist in a broad band around the equator (Caraballo, 2014). This includes much of Sub-Saharan Africa, Asia, and Latin America (WHO, 2014). In 2015, there were 296 million cases of malaria worldwide resulting in an estimated 731,000 deaths (GBD 2015 a, b) with approximately 90% of these cases and deaths occurring in Africa (WHO, 2014). Rates of the disease decreased by 37% from 2000 to 2015 (WHO, 2014), but rose again thereafter from with 198 million cases reported in 2014 (WHO, 2014). Malaria is commonly associated with poverty and has a major negative effect on economic development (Gollin *et al.*, 2007: Worrall *et al.*, 2005). In Africa, it is estimated to result in losses of US$12 billion a year due to increased healthcare costs, lost ability to work, and negative effects on tourism (Greenwood *et al.*, 2005).

## 2.18 Biology of mosquitoes

The mosquito has four distinct stages of its life cycle: egg, larva, pupa, and adult. The adult is an active flying insect, while the larvae and pupae are purely aquatic. Depending on the species, eggs are laid either on the surface of water or are deposited on moist soil or other objects that will be flooded.

### 2.18.1 Life Cycle of mosquitoes

The mosquito goes through four separate and distinct stages of its life cycle: Egg, Larva, pupa, and Adult. Each of these stages can be easily recognized by its special appearance. Eggs are laid one at a time or attached together to form rafts.

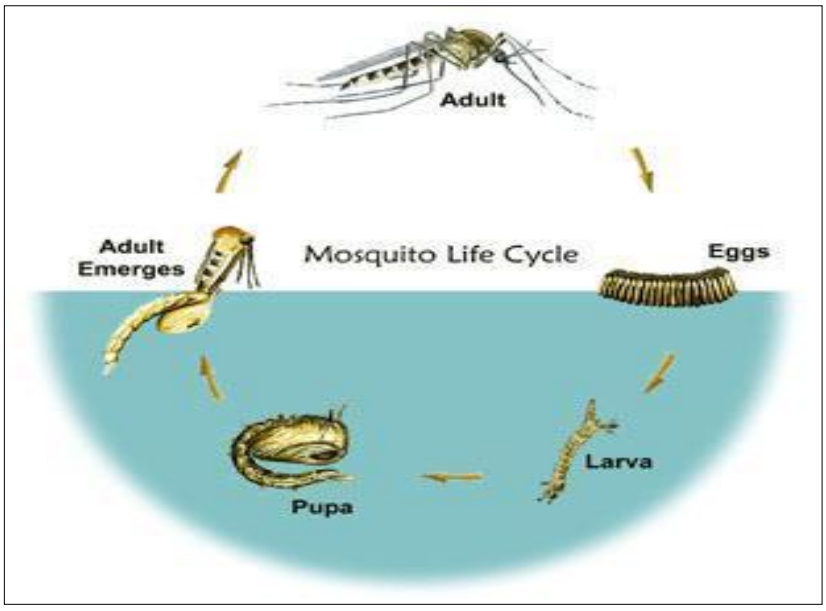


**Figure 2.6:** Illustration of the life cycle of a mosquito: (Adapted from American Mosquito Control Association (AMCA)

**a) Eggs**

One factor common to all the mosquito species is that eggs are laid in association with water or on a moist surface. They are white when first deposited, darkening to black or dark brown within 12-24 hours. Single eggs are 0.5mm long, and those of most species appear similar when seen with naked eye (except those of *Anopheles* spp. whose eggs have floats attached to each side of the egg). Eggs are laid singly by some species and others lay eggs to form rafts. The incubation period varies considerably among species. Eggs of permanent water mosquitoes when deposited on the water surface may hatch in 1-3 days depending on the temperature. Floodwater species deposit their eggs on moist soil or another wet substrate and have a wide variation in incubation periods. These eggs will not hatch until submerged by rising water caused by rainfall, melting snow in the spring or other floodwater. Depending on the species and conditions the next time they are flooded, they can take ten days or may not hatch until they are flooded a year or more (Mala *et al*., 2011).

**b) Larvae**

The larvae (wrigglers) of all mosquitoes have four developmental stages called instars and live in water. They are named $1^{st}$, $2^{nd}$, $3^{rd}$, and $4^{th}$ and have the following next stage bigger in size than the previous. The larva sheds its skin at the end of each instar, by molting. During the larval stage there is activated consumption of its food and feeds on particles that may be decaying inside water. In most spp, larvae consist of a tube that aides in respiration making it re surface for oxygenation. Species determines the size in

terms of length of the larval period, also water temperature with some taking 5-6 days. When they mature the 4$^{th}$ instar larva transforms to pupae

**c) Pupae**

Pupae exhibits high levels of viability in mosquito cycle unlike most other insects, and like the larva, lives in water. Appearance and shape makes the pupae differ from the larva in shape. Its body is divisible into two, and it is comma-shaped with two distinct body regions. Head and thorax comprise the first region, and it has a pair of respiratory trumpets also greatly enlarged. Periodically the pupa resurfaces for oxygenation**.** The second region is the abdominal region. It has flexible attachments segmenting up to the tip, together with peddle looking attachments. It lasts just a couple of days and the pupa does not consume any food and it is at this stage that all tissues of the larvae change into the adult tissues. From the pupal case on the surface of the water directly emerges a mature adult**.**

**d) Adult**

The adult mosquito is entirely terrestrial and is capable of flying long distances. Both females and males feed on nectars which they use for energy. Males and females mate during 3-5 days after they have emerged. Females mate only once and males live for only a week. Only the females feed on blood, which they take when they are biting. The blood meal is only for the egg development. Many mosquitoes feed on any warm-blooded bird or mammal. However, some prefer cold-blooded animals. Some species also prefer birds and seldom feed on mammals, which is the case with *Culex* spp. a known vector of West

Nile Virus (WNV). Unfortunately, many species feed on a wide range of warm-blooded mammals including humans. Once a female has completely engorged, she flies to a dark shaded place and rests there until her eggs are completely developed, usually three to five days. Once the eggs are developed, the female is called a gravid female and she begins to look for a place to lay her eggs. If a female survives her egg laying activities, she will soon start looking for another blood meal, after which she will lay another batch of eggs. No mating is needed at this stage. Normally a female will live long enough to lay one to three batches of eggs. Most mosquito species are actively searching for blood meal at night from dusk to dawn. During the daytime the females hide in dark, cool and humid places, where they are protected from drying. Females will often bite in the daytime if humans invade the wooded areas where they are resting. However, *Aedes albopictus* is an aggressive biter which prefers to feed during the daytime hours and is often a nuisance in urban areas. It is black with white spots on its body (Mala *et al*., 2011).

**2.19 Sampling of adult mosquitoes**

Many methods have been used in sampling mosquitoes including indoor resting catches and human-landing catches. Other methods include: light traps, mouth aspiration, sticky paper traps etc.

### 2.19.1 Indoor resting catches

Several malaria vectors are known to be endophilic, and rest inside houses before and / or after feeding on man or other hosts. Collection of indoors resting mosquitoes is usually done using aspirators or by knockdown pyrethrum spray catches (PSC) (Service, 1993).

Specimens sampled by pyrethrum spray catches are mostly fed females resting indoors in the morning.

### 2.19.2 Human-landing catches

Human landing catches are the most reliable and representative measure of human-vector contact that can be used for calculating the entomological inoculation rate (EIR). In a typical human-landing catch, one to three people acting as both bait and catchers sit down at a selected site and aspirate mosquitoes as they land on their exposed limbs. The advantages of this method is that it directly measures the biting rate of anthropophagic mosquitoes considered to be representative of the vector population responsible for malaria transmission (Davis *et al.,* 1995). This is because the mosquitoes are caught in precisely the act of biting an individual (Lines *et al.,* 1991). Furthermore, the age distribution, infection status and the life histories of mosquitoes captured in this way are representative of those biting humans. Unfortunately, this method has some major disadvantages and is facing increasing opposition (Ndiath *et al*., 2011).

First, an all-night human-landing catch requires well-motivated staff and close supervision if the results are to be reliable. Second, there is the element of the collector's subjectivity as a result of availability in human attractiveness and skills in catching mosquitoes and thus it is difficult to standardize the estimate based on biting catches. Third, and perhaps most important, it raises some ethical issues because the occupational exposure to host-seeking anophelines may place the collectors at an increased risk of being bitten by infectious mosquitoes (Service, 1977). The increasing numbers of drug

resistant malaria parasites strains further compound this (Trape, 2001). In addition, this method is cumbersome and labour intensive. There is need therefore to search for a satisfactory method of sampling Afro-tropical malaria vector that would reduce the need to use human landing catches. The possibility of making valid biting estimates from artificial traps (or any sampling device) is highly desirable.

### 2.20 Identification and processing of mosquitoes

As of today, personnel working as entomologists and technologists are assigned in most nations to trap samples and identify them visually. Unfortunately, the process of identifying the actual species of mosquito is currently a manual process requiring highly trained personnel to visually inspect each specimen one by one under a microscope to make the identification. The mosquitoes are normally collected by light traps in collection nets, aspirated, counted and the numbers recorded in field note books. The mosquitoes are desiccated in dry silica gel and transported to the laboratory where they are stored at room temperature awaiting identification. The identification is done using morphological identification key (Gillies and de Meillon, 1968; Gillies and Coetzee, 1987). Mosquito identification is carried out guided by taxonomic features in the identification key books; Example: Mosquitoes from Ethiopian region by Edwards, A supplement of the Anophelinae of Africa, South of the Sahara by (Gillies and Coetze, 1987). Key features used include: the pulps, wing patterns, i.e nomenclature of markings and veins, the legs, the tarsus, and general features on the thorax and abdomen.

## 2.21 Mosquitoes as disease vectors

Several mosquito species belonging to genera *Anopheles, Culex* and *Aedes* are vectors of pathogens of various diseases like malaria, filariasis, Japanese encephalitis (JE), dengue and dengue hemorrhagic fever, yellow fever among others (Mittal and Subbarao, 2003). According to WHO, (2002) report, *C. quinquefasciatus* and other human-biting mosquitoes of the *Culex pipiens* complex transmitted more than half of the world's burden of lymphatic filariasis (LF). These mosquitoes are responsible for bancroftian filariasis transmission in the Americas, Egypt, urban East Africa, the Indian subcontinent, Indonesia and Southeast Asia. Strategies for control of insect vectors have changed and evolved through time to the modern period depending on the overall epidemiology of the pathogen involved as well as the ecology of the vector (WHO, 2017). In order to accomplish long-range, intelligent and environmentally sound pest control, the management and manipulation of pests must be accomplished using not just one but all available pest control methods (Connelly and Carlson, 2009). This includes water management and sanitation programmes as well as use of larvicides and adulticides.

## 2.22 Global distribution of dominant species of malaria vectors

Anophelines are found worldwide except Antarctica (Fig.2.8). Malaria is transmitted by different *Anopheles* species, depending on the region and the environment. There are approximately 3,500 species of mosquitoes grouped into 41 genera. Human malaria is transmitted only by females of the genus *Anopheles*. Of the approximately 430 *Anopheles* species, only 30-40 transmit malaria (i.e., are "vectors") in nature (WHO, 2016). Anophelines that can transmit malaria are found not only in malaria-endemic areas, but

also in areas where malaria has been eliminated. The latter areas are thus constantly at risk of re-introduction of the disease.

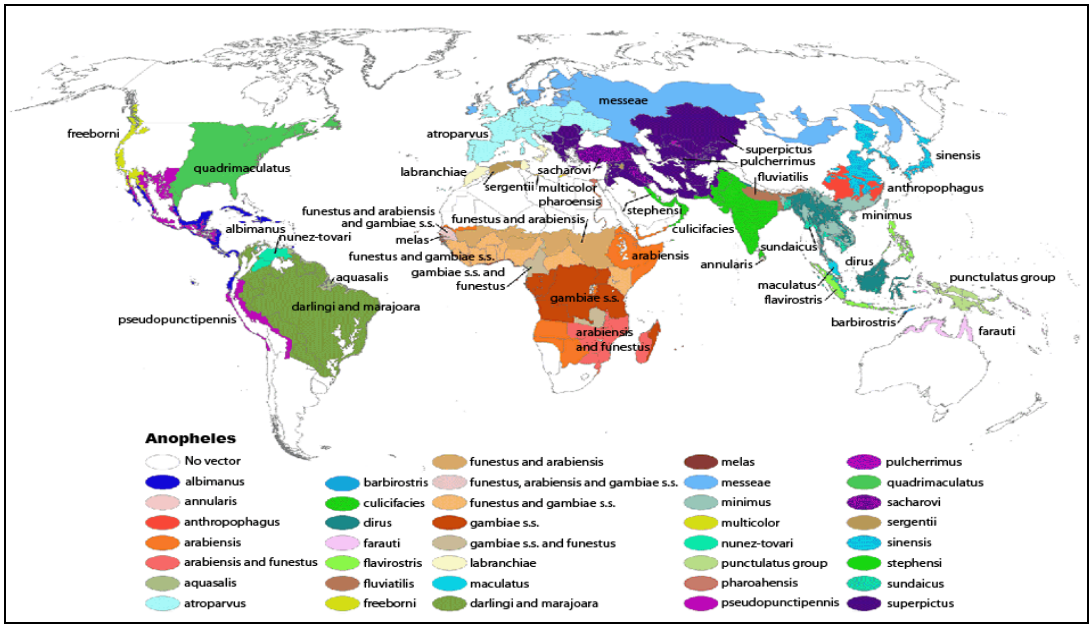


**Figure 2.7:** World distribution of *Anopheles* mosquitoes (source: Kiszewksi *et al*., 2004)

## 2.23 Mosquito vector control

Vector control refers to methods used to decrease malaria by reducing the levels of transmission by mosquitoes. For individual protection, the most effective insect repellents are based on DEET or picaridin (Sherwood *et al*., 2009). Insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS) have been shown to be highly effective in preventing malaria among children in areas where malaria is common (Lengeler, 2004; Tanser *et al*., 2010). Prompt treatment of confirmed cases with artemisinin-based combination therapies (ACTs) may also reduce transmission (Tanser *et al*., 2010).

Mosquito nets help keep mosquitoes away from people and reduce infection rates and transmission of malaria (WHO, 2008). Nets are not a perfect barrier and are often treated with an insecticide designed to kill the mosquito before it has time to find a way past the net. Insecticide-treated nets are estimated to be twice as effective as untreated nets and offer greater than 70% protection compared with no net (Enayati *et al.,* 2010). Communal application of controlled amounts of DDT is permitted under the Stockholm Convention that bans its agricultural use (van den Berg, 2009). One problem with all forms of IRS is insecticide resistance. Mosquitoes affected by IRS tend to rest and live indoors, and due to the irritation caused by spraying, their descendants tend to rest and live outdoors, meaning that they are less affected by the IRS (Pates and Curtis, 2005). There are a number of other methods to reduce mosquito bites and slow the spread of malaria. The efforts to kill mosquito larva by destroying the pools of stagnant water in which they develop or by adding substances (oil) to kill during their development is effective in some locations (Tusting *et al.,* 2013). Electronic mosquito repellent devices which make very high-frequency sounds that are supposed to keep female mosquitoes away, do not have supporting evidence (Hemingway *et al.,* 2007).

### 2.24 Larval control

Vector control measures directed against larvae may be the only effective approach especially when the mosquitoes bite outdoors. Larval control involves elimination of breeding places by drainage or filling, larviciding, biological control and microbial methods. The anti-larval measures tend to be more successful when breeding places are not extensive and thus cheap (WHO, 1985). The approach requires one to first ascertain

the adverse ecological changes that may follow the use of larvicides or biological agents to control larvae (Killeen *et al.,* 2002). Epstein, (1997) adds that larval control measures seem to be suitable in desert areas where breeding is limited and small quantities of larvicides that are applied at the appropriate season would have long lasting effects. Larval control by ecologically compatible biological agents' such as Gambusia fish and other predators or microbes like *Bacillus thuringiensis israeliensis* and *Bacillus sphaericus* have also been tried.

### 2.25 Integrated vector control methods

A marked increase in malaria has been noted in the African highlands, largely due to the rise of drug-resistant strains of *Plasmodium falciparum* parasites (Guyatt *et al.,* 2002; Hay *et al.,* 2005). Because of climatical changes, many water bodies are now exposed to the sun and provide ideal conditions for vector proliferation and increased malaria transmission (Briet *et al.,* 2006). Malaria control in highlands is based on insecticide-treated nets (ITNs), indoor-residual spraying (IRS) with insecticides and prompt and effective treatment of clinical malaria (WHO, 2005). Many believe integrated vector management (IVM), targeting both larval and adult mosquitoes, is the future for malaria control (WHO, 2003; Townson *et al.,* 2005; WHO, 2007). While ITNs are the priority strategy, there is growing interest in attacking the aquatic stages of malaria vectors with microbial larvicides, in conjunction with environmental management (Killeen *et al.,* 2006; Munga *et al.,* 2006; Fillinger *et al.,* 2008; Gu *et al.,* 2008).

It has been demonstrated that microbial larvicides reduced malaria vector mosquito larvae and adult females by more than 90% in a rural town in western Kenya (Fillinger *et al.,* 2006). However, the effectiveness of this approach for reducing the incidence of malaria among vulnerable children remains unproven. Insecticide Treated Nets are a firm favourite in the armamentarium against malaria and their protective efficacy is well known, (Hawley *et al.,* 2003; Lengeler *et al.,* 2007) with reductions of 40–70% in human mosquito biting rates (Geissbühler *et al.,* 2007; Killeen *et al.,* 2007).

**2.26 Malaria in Kenya**

In Kenya, malaria is the leading cause of morbidity accounting for approximately 30% of the outpatient illness reported in the health facilities. Approximately 6 million cases are reported annually according to Health Information System (HIS, 1993). Out of the malaria patients admitted in Kenya health facilities, 5.1% die and 10% of the survivors have severe lasting health effects according to the Ministry of Health reports (MoH, 1994). The disease remains a serious health problem where it is endemic. It is wide spread in Coast, most parts of Nyanza, Western and parts of Eastern, North eastern and Central and Rift valley regions. Efforts to control the diseases have been complicated as a result of the development of strains of *P. falciparum* that are resistant to chloroquine and other drugs which are widely used in the treatment of malaria. Of the five species affecting humans in Kenya, *P. falciparum* is the most prevalent. The species is found in all endemic areas and usually accounts for more than 80-90% of malaria infections. Most of the fatal cases of malaria are due to *P. falciparum*. Among the other four species, *P. malariae* is more prevalent than *P. ovale, P. knowlesi* and *P. vivax*. The latter is only

occasionally reported. In Kenya malaria endemicity can be grouped into: Stable and Unstable malaria.

### 2.26.1 Stable Malaria (Holoendemic)

Stable malaria occurs in areas where transmission is more or less continuous throughout the year. This is experienced in Coast, Nyanza and Western regions. Malaria prevalence in human population is generally between 50-70% and accounts for at least 30% of the outpatient mortality (HIS, 1993). Morbidity and mortality are especially severe on infants and children under 5 years. However, infants born in such areas acquire some degree of passive immunity from their mothers, which protects them against malaria in their first few months of life. Most adults and older children develop antibodies to malaria and can maintain high levels of clinical immunity. The immunity is lost to some degree by individuals under special circumstances which may include pregnancy, HIV infection, surgical operation and Sickle cell condition.

### 2.26.2 Unstable malaria (Mesoendemic)

Unstable malaria occurs in areas with seasonal increases in both morbidity and mortality. Areas in Kenya where malaria is mesoendemic include Mwea, Machakos, Kitui and Marigat. Malaria in these areas accounts for more than 40% of the childhood mortality and people of all age groups may suffer clinical attack. Epidemic malaria is defined as a situation where a sudden increase in malaria cases above 50% of those usually observed in a given population at a time. It occurs in highland areas with an altitude between

1500M-2000M above the sea level. All age groups are normally affected by epidemics but the disease is usually more severe in children (WHO, 1993).

### 2.27 Epidemiology of malaria in Kenya

More than four million cases of malaria are reported annually in Kenya. A 5.1% mortality rate has been reported among patients admitted with severe malaria. Although *Plasmodium falciparum* is the species most frequently associated with severe malaria and accounts for 80-90% of cases in Kenya, *P. malariae*, *P. ovale*, and *P. vivax* also exist in the country (Marsh and Snow, 1997). Vector species in Kenya are members of the *Anopheles gambiae* complex and *A. funestus*. Transmission patterns of the disease in Kenya are influenced by rainfall, vector species, intensity of biting, and altitude. Stable malaria occurs in most parts of Coast, Nyanza, and Western Provinces. Transmission is high in these areas with an average of one infective bite/person/week throughout the year. Unstable malaria occurs in areas of low endemicity such as Machakos, Embu, and Kitui of Eastern Province, and Marigat and Ngurumani of Rift Valley Province. Epidemic malaria occurs in highland areas bordering endemic zones. All land lying at altitudes above 1600 meters including Nairobi and Mount Kenya and its surroundings are malaria-free. Children under five years of age, pregnant women, and nonindigenous residents and visitors may experience severe malaria. In contrast to historically effective vector control in urban areas, the rapid expansion of peri-urban areas now constrains vector control activities. Finally, prevention and control activities need to be integrated vector management programs (MoH, 2014).

## 2.28 Impact of irrigation scheme on malaria endemicity

Rice is the most important crop in the developing world in terms of production and in contribution to diet. It is also the main source of employment and income for the rural population in those regions. Ninety-five percent (95%) of the world's harvested rice of 146 million is in the developing countries (FAO, 1981). In order to increase production of rice and other crops in the arid areas, it is usually necessary to develop irrigation capacities (Hargrove and Harlan, 1988). Ultimately, large-scale irrigation projects are often planned and implemented without any assessment of their negative impact on human health. Riceland agro-ecosystem, in which water is present on the land throughout much of the crop-growing season, may provide ideal habitats for mosquito vector of malaria, lymphatic filariasis and arboviruses.

Flood paddies in Kenya, Uganda and Tanzania produce significant numbers of *A. arabiensis* in areas hyper-endemic for malaria. In holoendemic areas such as Ahero in Kenya, asynchronous planting of fields maintains continuous emergence of adult mosquitoes during most of the year (Chandler and Highton, 1975). Studies have been carried out by several workers on the ecology of *A. arabiensis* sibling species in the Kano plains irrigation scheme at Ahero (Chandler and Highton, 1975; Highton, 1979; service, 1977) Parallel studies have been carried out in Mwea irrigation settlement scheme (Mutero, 1985; Mukiama, 1987). The difference between the two Kenyan irrigation schemes is that Ahero lies in an area with large numbers of *A. gambiae* species of mosquitoes throughout the year hence malaria transmission all the year round. On the other hand, paddies in Mwea are flooded for a better part of the year and so mosquito

numbers fluctuate periodically leading to seasonal transmission of malaria (Mukiama and Mwangi, 1989; Mutero *et al.,* 2000).

**2.29 Use of insecticides treated bed-nets**

The insecticides used on bed nets today called pyrethroids illustrate the point well. They were introduced in agriculture during the 1970s and in the following decades, (1980s) trials on bed nets started. In the mid-1980s, scientists working in Gambia and Tanzania published papers demonstrating the efficacy of the insecticides in the control of mosquitoes. In the early 1990s there were already publications confirming their efficacy in the protection of people. Unfortunately, it is only now, close to three decades after their introduction in agriculture, that we are witnessing attempts at their mass scale up for real public health impact. Furthermore, the impact of these insecticides cannot be expected to last forever; indeed, there is already good scientific evidence predicting their future failures. When they fail, if the global insecticides arsenal has not changed, there will be no fallback position as the chemical industry is least interested in developing protective products for the poor, particularly malaria endemic countries which need the alternative and are more or less incapacitated. Therefore, there might be real grave danger when pyrethroids resistance increases to the point of affecting control program.

Only 60% of those at risk of malaria, particularly pregnant women and children under five years of age benefit from the most effective combination of personal and community protective measures such as insecticides treated nets and other interventions. Of all the pregnant women, only 60% have access to chemoprophylaxis by presumptive

intermittent treatment. With respect to progress on prevention, the number of ITNs distributed has increased 10-folds during the past 3 years in more than 14 African countries. Subsidized or free-of-charge ITNs distribution has proved successful in increasing coverage of the most vulnerable population. In most African countries, many more households have mosquito nets not treated with insecticides than ITNs. Scaling up of insecticides re-treatment services will therefore be an important factor in increasing ITNs coverage. The recent introduction and manufacture of permanent treated nets is expected to greatly improve overall efficacy and effectiveness.

### 2.30 Insecticide resistance in malaria control

The past 15 years have seen unprecedented progress in malaria prevention and control. This has mainly been as a result of a significant scaling up of vector-control interventions, particularly in sub-Saharan Africa. However, these fragile gains are threatened by emerging resistance to insecticides among *Anopheles* mosquitoes and to antimalarial medicines among *Plasmodium* parasites (WHO, 2017). WHO, (2017) pointed out that if left unchecked insecticide resistance could lead to a substantial increase in malaria incidence and mortality. The global malaria community needs to take urgent action to prevent an increase in insecticide resistance, and to maintain the effectiveness of existing vector-control interventions. Resistance affects all major malaria vector species and all four recommended classes of insecticides. Since 2010, a total of 61 countries had reported resistance to at least one class of insecticide, with 50 of those countries reporting resistance to 2 or more classes (WHO, 2012).

## 2.31 Malaria control and intervention

Effective malaria vector control is reliant on knowledge of local vector species and their susceptibility to insecticides, as well as on vector and human behaviours that may allow mosquitoes to avoid contact with interventions and thereby maintain residual transmission. Periodic collection of such data is essential to inform vector control strategies and track their impact on malaria transmission.

Rotation of IRS by mode of action (MOA) on an annual basis currently is the best practice for resistance management in malaria vectors in most settings. A plan by WHO, (2014) was developed to counter insecticide resistance by formulating management strategies on the basis of existing vector control interventions, status of insecticide resistance and epidemiological context. In principle, good resistance management practice requires the application of multiple insecticides of different biochemical modes of action (MOA) in rotations, mixtures or by combining multiple interventions (Mnzava *et al.*, 2015).

# CHAPTER THREE: FECUNDITY AND SURVIVAL RATES OF SAND FLIES AND MOSQUITOES AFTER EXPOSURE TO LAMBDA-CYHALOTHRIN (ICON®) INCORPORATED INTO 1, 4-DICHLOROBENZENE (PCB®)

## 3.1 Summary

This chapter is composed of work on experiments that attempted to show feeding successes, fecundity, repellence, and survival rates of test insects (sand flies and mosquitoes) in the laboratory setting. Sand flies and mosquitoes were exposed to various treatments of lambda-cyhalothrin (ICON®), PCB® and a combination of ICON®/PCB®. The hamster was used as bait for the sand fly and mosquitoes in the laboratory experiments. The number of sand flies and mosquitoes that fed, those that died and those that were alive 48 hrs after treatments were recorded. The results were analysed to show which insecticide was better suited to control sand flies and mosquitoes in the endemic areas. The results from the laboratory showed that the lambda-cyhalothrin combined with PCB® was more effective in controlling the vectors of malaria and leishmaniasis as compared to ICON® and PCB® applied individually.

## 3.2 Introduction

Although leishmaniasis is estimated to cause the ninth largest disease burden among individual infectious diseases, it is largely ignored in discussions of tropical disease priorities (Alvar *et al*., 2012). This consignment to critical oblivion results from its complex epidemiology and ecology, the lack of simple, easily-applied tools for case management and control, and the lack of current incidence data, and this often results in a failure by policy-makers to recognize its importance (Alvar *et al*., 2006).

Leishmaniasis continues to be a major public health problem in Kenya. The disease is classified by the World Health Organization among neglected tropical diseases (NTDs) and mainly affects populations that are socio-economically disadvantaged (Alvar *et al.*, 2012). However, lately, there has been an increase in the prevalence and geographic distribution of leishmaniasis to areas that were previously unaffected mainly due to civil unrest, trade and climate change (Bashaye *et al.,* 2009; Razmjou *et al.,* 2009). This is further compounded by the emergence of leishmaniasis and HIV co-infection (Alvar *et al*., 2008) and resistance to synthetic chemicals by both the parasite and the vectors. This grim picture points to the need for sustainable control of the disease.

Leishmaniases in Kenya are caused by phlebotomine sand flies. Visceral leishmaniasis is caused by *Leishmania donovani* and transmitted by *Phlebotomus martini* (Perkins *et al*, 1988). Cutaneous leishmaniasis is caused by three types of *Leishmania* spp. *Leishmania major* is transmitted by *P. duboscqi* in Baringo County (Beach *et al*., 1984) and *L. tropica* by *P. guggisbergi* in Laikipia and Nakuru counties while *L. aethiopica* is transmitted by *P. pedifer* in Bugoma county (Tonui, 2006; Ngure *et al*., 2009).

The use of lambda-cyhalothrin (ICON®) and PCB® in this study may represent the most sustainable method of reducing disease transmission in communities living in malaria and leishmaniasis endemic regions. The insecticide treated blocks act as the killing agent when placed near the hamster. Sand flies and mosquitoes driven by desire to blood feed try to land on the hamster which is placed near the treated block and get into contact with

the effects of the insecticide. Pyrethroid group is one of the 4 classes of insecticides (organochlorines, carbamates, and organophosphates) is composed of permethrin, deltamethrin and lambda-cyhalothrin. These are lethal insecticides that are recommended for the control of adult malaria vectors (WHO, 2012). Control of sand flies and mosquitoes, the vectors of leishmaniasis and malaria respectively, has further been complicated by the emergence of insecticide resistance. It is because of these problems that transmission still goes on unabated necessitating the need to develop alternative control method.

The synthetic pyrethroids are increasingly being used in the malaria control programs (WHO, 2002). Lambda-cyhalothrin, available as 10 percent wettable powder formulation (WP), is used at dosages of 20-30 mg (a.i)/$m^2$ for indoor residual spraying in the control programs in some endemic countries (Elnaiem *et al*., 1999). It is a synthetic pyrethroid insecticide that has been shown to be effective in killing mosquitoes and phlebotomine sand flies when used in low doses (Sharp *et al.,* 1993; Elnaiem *et al.,* 1999). It has no smell and has a long residual period of 6 months (Sharp *et al.,* 1993; Davies *et al.,* 2000) unlike DDT it is not toxic to vertebrates (Elnaiem *et al.,* 1999; Sharp *et al.,* 1993; Asidi *et al.,* 2005). Also no resistance has been reported for sand flies (Amalraj *et al.,* 1999) and mosquitoes (Asidi *et al.,* 2005) unlike DDT. Kroeger *et al.,* (2002) in a study in Venezuela observed the efficacy of lambda-cyhalothrin in protecting people from bites of sand flies, it is also ready acceptance by users as was shown in Brazil (Kelly *et al.,* 1997), and its cost effectiveness make it a more useful insecticide for the control of malaria and leishmaniasis.

The 1, 4-dichlorobenzene (PCB®) when used as a deodorant and pesticide like lambda cyhalothrin lasts for six months. Combination of lambda-cyhalothrin and 1, 4-dichlorobenzene under the slow release mechanism will help not only to keep sand flies and mosquitoes away but also other household pests as well as air-freshening the houses. The use of the lambda-cyhalothrin fortified with 1, 4-dichlorobenzene, which are cheap will help repel the two vectors especially in the areas which they are sympatric. This kind of control method was evaluated to reduce the biting/feeding rate of the mosquitoes (*Anopheles*) and sand flies (*Phlebotomus*) species and also to suppress the egg-laying in the laboratory.

The study determined the feeding success of mosquitoes and sand flies and compared the efficacy of lambda-cyhalothrin fortified with 1, 4-dichlorobenzene in the control of endophilic phlebotomine sand flies and *Anopheles* mosquitoes.

### 3.3 Materials and methods

All the laboratory work and experiments were conducted at the Centre for Biotechnology Research and Development (CBRD) laboratory of Kenya Medical Research Institute (KEMRI). Lambda-cyhalothrin was acquired from an agrochemical company by the name King Quenson Industry Group, Shenzhen City, Guangdong Province of China. It was 98% Technical Grade; Formualtion: 5% EC, 10% WP. Application: Non-systemic insecticide with contact and stomach action and repellent properties. It gives rapid knockdown and long residual activity. It controls a wide spectrum of insect pests.

A total of 100 mosquitoes (four replicates containing 25 mosquitoes each) were used for each test concentration and for the control. Results were expressed as percentage mortality after 24 hours and corrected for any control mortality. Concentrations were chosen so that at least one concentration gives 100% mortality, at least two concentrations give between 50% and 99% mortality, and at least two give between 5% and 50% mortality (WHO, 2005). The concentrations are generally expressed as the percentage of active ingredient per unit area on the filter-paper e.g 60mg/$m^2$. Batches of 25 non-blood-fed females of sand flies or mosquitoes, aged 2-5 days, were introduced into the holding cage and held for one hour at 25°C ±2°C and 80% ±10% RH. At the end of the exposure time, sand flies or mosquitoes were transferred to another cage for 24 hours. Dead mosquitoes or sand flies were picked and counted after 24 hours.

### 3.3.1 Laboratory vectors and animals

a) Sand flies used in laboratory experiments were from the phlebotomine sand fly colonies that are reared and maintained in the Leishmaniasis Entomology Department of Centre for Biotechnology Research and Development (CBRD), Kenya Medical Research Institute (KEMRI). The species used was *Phlebotomus duboscqi* which is a hardy species that is capable of withstanding tough environmental conditions and is therefore easy to breed and colonize in the laboratory setting.

b) The mosquitoes used in the experiments were *Anopheles gambiae* from the mosquito colonies that are reared in the Malaria Molecular Entomology Insectary Department of CBRD, Kenya Medical Research Institute.

c) The hamsters used in all the experiments were acquired from the Animal House Section which is maintained by Centre for Biotechnology Research and Development in KEMRI. They were used alive but anaesthetized for the purpose of the experiment to provide a blood meal for the sand flies and mosquitoes.

### 3.3.2 Insecticides

Lambda-cyhalothrin (ICON®) which was used to perform insecticide experiments was acquired from an international company by the name King Quenson Industry Group, Shenzhen City, Guangdong Province of China, through a local chemical company that sells pesticide products in the country. It had a purity of 98% technical grade. Formulation: 5% EC, 10% WP. The WHO- recommended diagnostic concentrations for each group of vectors are chosen so that exposure for a standard period of time (usually 1 hour) followed by 24 hours, holding can be relied upon to cause 100% mortality of individuals of susceptible strains (WHO, 2005).

The 1, 4-dichlorobenzene (PCB®) also known as Paracide was locally purchased from a chemical company that manufactures them in Kenya. The known concentration of PCB® that was used in air conditioning and as a pesticide was 60 mg/$m^2$. The lambd-cyhalothrin dosage was prepared from a concentration of 10 mg/$m^2$ of active ingredient on netting material and was found to be effective against sand flies (Elnaiem *et al*., 1999) and 30 mg/$m^2$ worked well for *A. arabiensis* (Sharp *et al*., 1993).

Lambda-cyhalothrin (ICON®) combined with PCB® was prepared in the laboratory by mixing the two insecticides to test their synergy in controlling sand flies and mosquitoes as compared to individual insecticides (ICON® and PCB®). The combination of lambda-cyhalothrin and PCB® were therefore composed of concentrations of 60 mg/$m^2$ of PCB® and 40 mg/$m^2$ lambda-cyhalothrin to make a total of 100 mg/$m^2$ for experimental control of both sand flies and mosquitoes in the laboratory and in the field. In this study two formulations were tested at 40mg and 60mg (a.i)/$m^2$ for their effectiveness in terms of blood-feeding success, repellency, fecundity and mortality against *Phlebotomus duboscqi* and *Anopheles gambiae* in the laboratory.

### 3.3.3 Experimental design

A tunnel experiment was performed for the two vector species using a method adopted from Kasili *et al.,* (2009). These testing took place in tunnels made from plexi-glass cages with plaster of paris on their bases. Two cages with the measure of 25cm x 25cm x 40cm and 25cm x 25cm x 20cm were connected at their open ends with an adhesive tape to form a bigger cage measuring 25cm x 25cm x 60cm. Before taping them up, a disposable cardboard frame with a hole at the centre of the cardboard to facilitate insertion of a plexi glass cylindrical tube measuring 15cm long and a diameter of 4cm were put in place. The purpose of the cylindrical plexi-glass tube was to connect the two cages and provide easy movement of insects from one cage to the other as shown below: Fig 3.1. The treated block was placed close to the aneasthesized hamster to discharge maximum insecticidal effects to the hungry female sand flies and mosquitoes. The insects are known to be attracted to the hamster because of the exhaled carbon dioxide. The insects were introduced into the smaller cage with an aspirator and left free to move to the other cage where the treated block and the hamster were placed. The probing and blood feeding behaviours of the test insects were observed and scored.

In the process of the test, the cages were kept constantly at relative humidity of 26$^{o}$C and 80% (RH). To assess blood feeding and repellency the number of blood fed females (both alive and dead) in treated and control cages were compared. By pooling immediate and delayed mortalities of sand flies and mosquitoes from the two sections of the bigger cage the overall mortality was calculated. The tests were replicated four times and means calculated. The effects of repellency were calculated from a comparison of blood fed

females in the control and the blood fed in the test cages. Most of the sand flies and mosquitoes which entered in the cage with treated block did not manage a full blood meal. Some females did not even feed or land on the hamster.

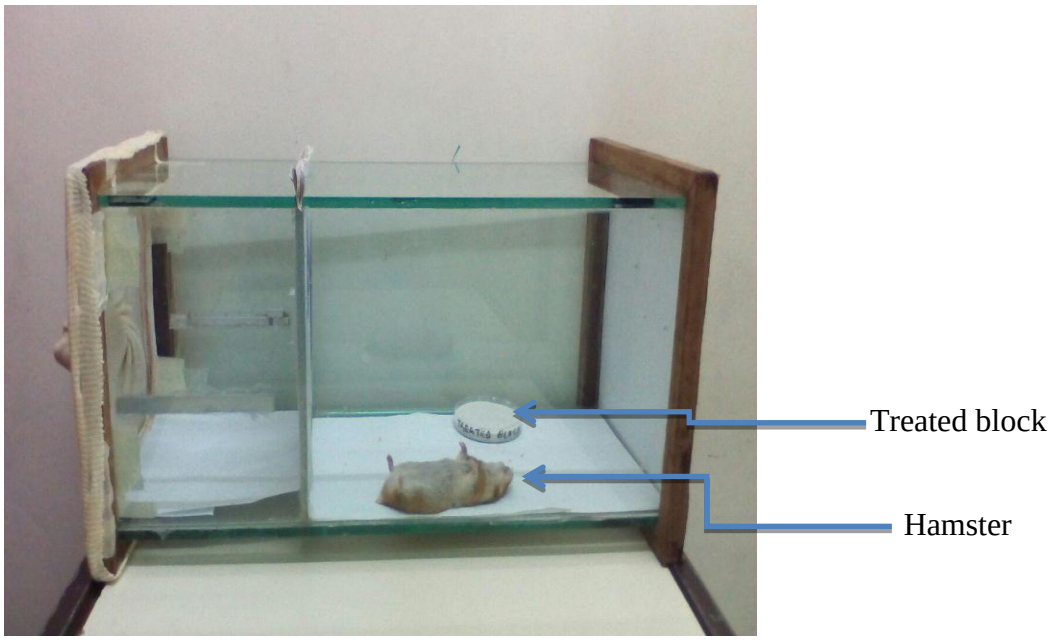


**Figure 3.1:** A plexi- glass tunnel layout used for sand fly and mosquito experiment (Adapted from Kasili *et al*., 2009)

Mosquitoes and sand flies were released into the tunnels in separate experiments. One side of the cage was closed using a stocky net while the other end also with also a stocky net for closing, was used to introduce either mosquitoes or sand flies. The two cages were attached to each other in the middle with a cardboard bearing a small hole at the centre to allow test insects to go through Fig. 3.1. The second cage was used to introduce the following ingredients:

1. An anesthetized hamster alone (control)
2. Hamster plus ICON® / PCB® block.

3. Hamster plus ICON® block alone (control for Number 2).
4. Hamster plus PCB® block alone (control for Number 2 and 3).

**Laboratory experimental study design**

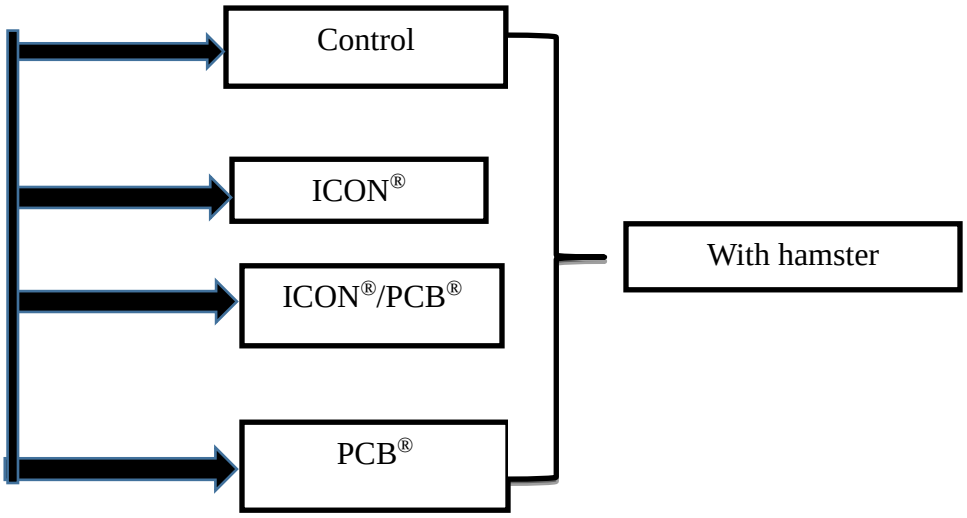


**Figure 3.2:** Experimental design **(Adapted from Kasili et al., 2009)**

The cages were only used as specified and were not re-used for any replicate. Movement of sand flies or mosquitoes was monitored, probing and feeding successes were observed in all the cages and the results recorded. Insects that died were picked, counted and recorded. Time taken by the insects to die following exposure to lambda-cyhalothrin positive block was recorded and determination of the mortality rates calculated. All fed females were aspirated using a mouth aspirator and transferred to different smaller cages individually and were maintained on sugar and monitored for oviposition. The number of eggs laid and hatching was recorded in order to determine the effect of the lambda-cyhalothrin positive blocks on the fecundity of the insects.

### 3.3.4 Sample size determination

All experiments were done in four replicates using 100 sand flies and split into batches of 25 per test (WHO, 2005). The test insects were non-blood-fed females aged 2-3 days old. Briefly, for each experiment (one) to (four) a total of 100 females' non-blood-fed *Phlebotomus dubosqui* and *Anopheles gambiae* were used separately.

### 3.3.5 Data analysis

The efficacy of the different treatments was compared using the final cumulative mortalities. Differences in mortality rates and adult longevity were analysed by ANOVA and the significance means differences were analysed by Student-Newman-Kuels-test (PROC MIXED procedure, SAS institute, 1997) and the probability level set at P=0.05. Percentage data and ratios were arcsine-transformed before analysis. For repellency tests, comparisons between and among groups subjected to different treatments were determined by t-test and ANOVA, respectively.

## 3.4 Results

### 3.4.1 Fecundity and survival rates of laboratory bred mosquitoes and sand flies exposed to ICON® incorporated into PCB®

Sand flies require blood meal to lay eggs and for most species they take 5-6 days to lay eggs post blood feeding (Lawyer *et al*., 2017). Since all vectors died after 48hrs of exposure to insecticides and their combination, there was no reported data on their ability to lay eggs. However, results demonstrated a steady decrease in survival of mosquitoes from 1$^{st}$ hour of treatment to 48 hours for all treatments. There was increase in mortality

of mosquitoes 1st hour of treatment with ICON® at a dose of 40mg for 24 hours and steady increase in mortality to 48 hours for all treatments. The mean difference of mortality of the vectors were analyzed using paired student t-test. The mean difference between exposure to ICON® and PCB® was not significantly different (-1.667±1.333, P=0.133). High mortality rates were observed in mosquitoes exposed to the combination of ICON® and PCB® than when exposed to PCB® (2.667±1.706, P=0.0894) and ICON® (4.333±2.789, P=0.0905), however not significant. The high mortality of mosquitoes exposed to a combination of ICON® and PCB® at a dose of 40mg: 60mg shows that this could be a better way of controlling *A. gambiae.* The increase of the rate of mortality shows that the slow release of the two insecticides facilitates the death of the insects within a few hours.

All dead and alive mosquitoes were counted, and their means plotted and analyzed using student t-test comparing combination against individual treatments. There was observed high mortality of mosquitoes exposed to PCB® as compared to ICON® but the mean difference was not significant (2.5±2.32, P=0.1652). However, there was a significant increase in mortality of mosquitoes exposed to PCB®/ICON® combination as compared to PCB® (2.166667 ±0.9098229, P=0.0315) and ICON® (2.5 ±1.056724, P=0.0321) alone (Figure 3.3).

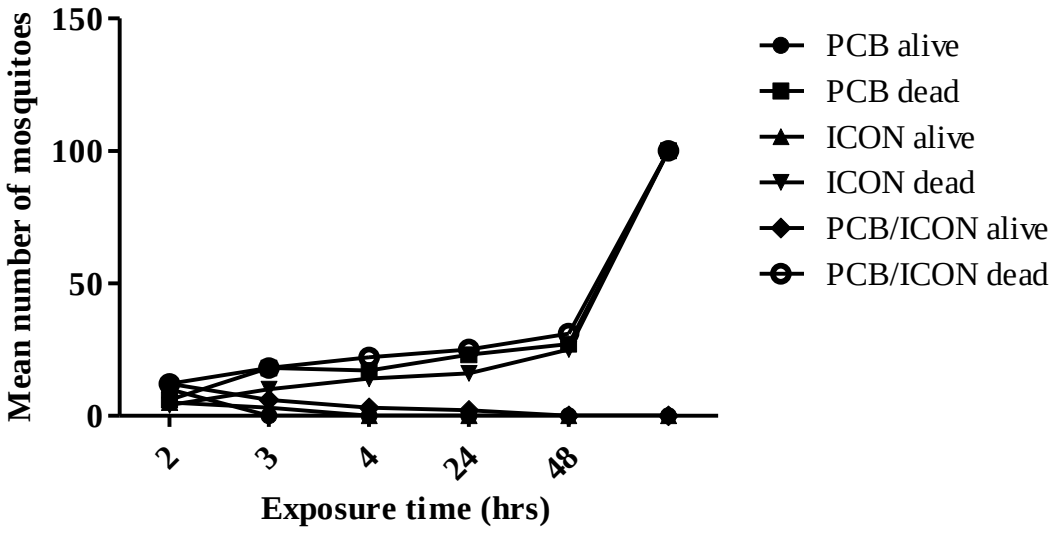


**Figure 3.3:** Trends of mosquito survival after treatment with PCB®, ICON® and PCB®/ICON® combination**.**

All dead and alive sand flies were counted, and their means plotted and analyzed using student t-test comparing combination against individual treatments. Like mosquitoes, there was an increase in sand fly density from the 1st hour of treatment to 24 hours and thereafter by steady increase in mortality to 48 hours for all treatments and a decrease in survival of sand flies from the first day of treatment to 48 hours for all treatments. The mean number of sand flies that survived was fewer in PCB®/ICON® combination than those in PCB® and ICON® put separately, respectively. The study showed high mortality of sand flies exposed to PCB® as compared to ICON® but their mean difference was significantly different (3.667±1.2824, P=0.0177). The mortality rates of sand flies exposed to PCB®/ICON® combination was higher as compared to PCB® (15.167±5.594, P=0.011)

and ICON® (17.66±6.955, P=0.0259). This could mean that, the mechanisms of insecticidal detoxification between sand flies and mosquitoes are different (Figure 3.3).

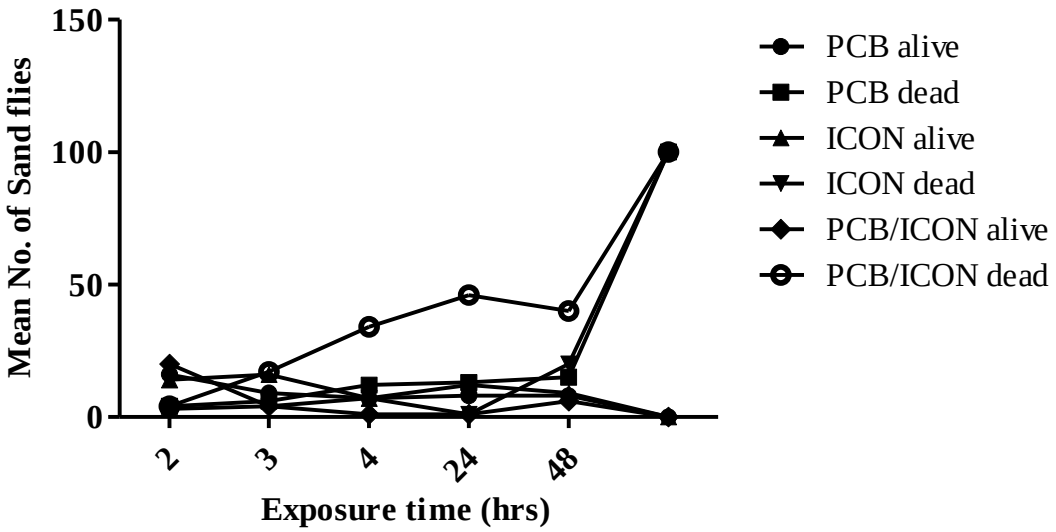


**Figure 3.4:** Mortality and survival rates of sand flies treated with ICON®, PCB® and other combination**.**

### 3.5 Discussion

Survival of arthropod vectors is one of the most important components of transmission of vector-borne pathogens (Garrett-Jones and Shidrawi, 1969; Reisen *et al*., 1980; Macdonald, 1956). Increased survival allows the vector to produce more offspring, to increase the chances of them becoming infected, to disperse over greater distances, to survive long enough to become infectious, and then to deliver more infective bites during the remainder of its lifetime. The 1, 4-dichlorobenzene (PCB®) can be absorbed through ingestion, inhalation or contact with skin. In mice, the oral route was found to be more rapid than inhalation in a study conducted with several human volunteers who were

subjected to p-DCB through inhalation (Lunde *et al.,* 2013). As a result, small changes in survival rate cause large changes in the rate of pathogen transmission (Macdonald, 1956; Parham and Michael, 2009; Barbazan *et al.,* 2010; Smith *et al.,* 2012; Lunde *et al.,* 2013). In this study there was an increase in mortality of mosquitoes from day one of treatment to 24 hours and steady increase in mortality to 48 hours for all treatments. A high number of mosquitoes exposed to ICON® survived as compared to PCB® but the mean difference was not significantly different. However, there was high mortality rates observed in mosquitoes exposed to combination than PCB® and ICON® independently. Conversely there was a steady decrease in survival of mosquitoes from the first day of treatment to 48 hours for all treatments. There was high mortality of mosquitoes exposed to PCB® as compared to ICON® but the mean difference was not significant. However, there was high mortality of mosquitoes which were exposed to PCB®/ICON® combination as compared to PCB® and ICON® independently and their mean difference was significantly different.

The high mortality of mosquitoes exposed to a combination of ICON® and PCB® at a dose of 40mg and 60mg, respectively shows that this could be a better way of controlling *A. gambiae* and probably other mosquito species. The increase of the mortality rates from 1st hour and full mortality rates in 24 hours shows that the slow release of the two insecticides facilitates the death of the insects. That mortality of sand flies exposed to ICON®/PCB® combination was lower than those exposed to ICON® and PCB® singly and this could mean that mechanisms of insecticidal detoxification differs between sand

flies and mosquitoes. Mosquitoes and sand flies' mortality rates were high when treated with ICON®/PCB® combination in the laboratory set up experiments.

The formulations or dosages differed only by causing mortalities. The higher mortality obtained with the combined ICON®/PCB® might be due to the higher concentration of the insecticide exposed to the mosquitoes and sand flies in the treatment cages. The decline in mortality rates in the other treatments could be due to the lower concentration of the dosage of the treatment when used singly. The findings of this study are similar to some studies carried out in Tanzania on the control of vectors of malaria using combinations of several insecticides (Okumu and Moore, 2011; Killeen *et al*., 2017). Considering the duration of exposure to the treatments of lambda-cyhalothrin and PCB® on blood-feeding success, repellency, fecundity and mortality dosages used of 40mg/$m^2$ and 60mg/$m^2$, lambda-cyhalothrin can be considered for use as a powerful control tool for malaria and leishmaniasis vectors.

# CHAPTER FOUR: FIELD EVALUATION OF LAMBDA-CYHALOTHRIN (ICON®) INCORPORATED INTO 1, 4-DICHLOROBENZENE (PCB®) AGAINST SAND FLIES AND MOSQUITOES

## 4.1 Summary

In this study, the tests were conducted in the field with lambda-cyhalothrin (ICON®), 1, 4-dichlorobenzene (PCB®) or a combination of the two against sand flies and mosquitoes. A comparative experimental design using the three treatments (ICON®, PCB® and ICON®/PCB®) was used. Their effectiveness was based on the total sand flies trapped prior to and post intervention. Efficacy of the different treatments was assessed based on their ability to inhibit sand fly or mosquito bites by repelling them and adult mortality. Trapping experiments were conducted using a randomized design with 30 households receiving different treated blocks in the rooms where people slept in Marigat and Mwea study sites. Pre-treatment vector collections were carried out to establish the vector numbers before intervention. The vectors were trapped for two nights at every study site. The traps laid in the evening at 1800 hr then picked the following morning at 0600 hr. The distance between households was approximately 100m in each of the two trapping study sites. The vectors were trapped with CDC light traps. The mean catches of sand flies in Mwea were higher in all the houses where the treatment blocks were later put in to show their efficacy. Before intervention the mean sand fly catch in Mwea was 148 sand flies and after intervention with ICON®/PCB® the mean catch reduced to 20 sand flies. Before intervention ICON® designated room catches had mean catches of 80 sand flies and this reduced to 15 sand flies after intervention. The reduction trend applied to both sand flies and mosquitoes.

Sand fly and mosquito densities were significantly reduced after intervention with all the three insecticide treatments with varying degrees of success. Intervention with ICON® alone reduced the population of sand flies and mosquitoes by 81% whereas ICON®/PCB® combined reduced them by 86% (table 4.1). The combination of ICON®/PCB® was more efficacious than the other two treatments: ICON® and PCB® each alone. The decrease of sand fly and mosquito population following exposure to ICON®, PCB® and ICON®/PCB® is a good indication that all the trial insecticides can be applied in controlling disease vectors of malaria and leishmaniasis. Molecular method of sand fly identification using PCR was useful in distinguishing a *P. martini* species that was identified using a compound microscope and aided by identification keys of Abonnenc 1965.

## 4.2 Introduction

The second largest parasite that kills people in the world after malaria is leishmaniasis (Mather's *et al*., 2007). It is vectored by peri-domestic sand flies which are mainly endophilic and endophagic (mainly feed and rest indoors) and most active when people are asleep (Dinesh *et al*., 2001). During daytime these flies are not active but seek shelter in dark, cool places. Sand flies feed on plant sugars, and only the female require a blood meal to acquire the necessary protein for egg production (Picado *et al*., 2012). Malaria is the leading parasitic killer in the world (WHO, 2017). The disease is one of the major severe public health problems globally. Close to half of the population in the world is at risk of malaria. In 2015, there were roughly 212 million malaria cases and an estimated 429,000 malaria deaths. Increased prevention and control measures have led to a 29%

reduction in malaria mortality rates globally since 2010. Sub-Saharan Africa continues to carry disproportionately high share of the global malaria burden. In 2015, the region was home to 90% of malaria cases and 92% of malaria deaths (WHO, 2016).

*Plasmodium* parasites cause malaria and are transmitted to human beings when bitten by infected *Anopheles* mosquito vectors. Of the 5 parasite species that cause malaria in humans, *Plasimodium falciparum* and *P. vivax* pose the greatest threat (WHO, 2017). The use of lambda-cyhalothrin, a synthetic pyrethroid, to control sand flies' dates back to the 1990s. For example, Kelly *et al*., (1997) applied lambda-cyhalothrin at 20 mg a.i/$m^2$ in all animal pens (blanket coverage) in a village in the Brazilian Amazon and realized a 90% reduction in *L. longipalpis* Lutz and Neiva. Davies *et al*., (2000) sprayed inside walls and ceilings of houses with 10% WP of lambda-cyhalothrin, at 25mg a.i/$m^2$, resulting in reduction of contracting cutaneous leishmaniasis in Kabul by 60% and reduction in the risk of cutaneous leishmaniasis in the Peruvian Andes by 54%. This pesticide proved effective against wild caught sand fly species of *L. verrucarum* in the Peruvian Andes and remained 100% lethal for up to six months. Both trials measured protection at the household level, and it is not known whether if the whole village was sprayed with the insecticide aerially, would produce mass killing of disease vectors both in the houses and in the bushes (Davies *et al.,* 2003). Nevertheless, it is noteworthy that residual treatment of human dwellings and animal shelters depends on availability of a suitable public health infrastructure, including adequate supplies of the insecticide, spraying equipment and trained personnel (Alexander and Maroli, 2003), which may be a challenge in many rural areas where leishmaniasis is endemic.

Studies on susceptibility of sand flies *P. argentipes* (Vector of Visceral leishmaniasis in India) and *P. papatasi* (vector of *L. major* in Middle East) showed that the sand flies are resistant to DDT and Malathion and susceptible to lambda-cyhalothrin (Amalraj *et al*., 1999). Other studies in Venezuela showed that curtains impregnated with 12.5 mg/$m^2$ lambda cyhalothrin are effective in protecting people from bites of sand flies that can transmit cutaneous leishmaniasis and sand flies reduced significantly in treated houses (Kroeger *et al*., 2002). With 2.5% EC lambda-cyhalothrin, no resistance or tolerance in *P. papatasi* Scopoli and *P. argentipes* Annandale was reported (Amalraj *et al.,* 1999). Asidi *et al*., (2005) evaluated nets treated with Chlorpyrifos-methyl 38.8% CS (100 mg/$m^2$) and lambda-cyhalothrin 25% CS (18 mg/$m^2$) in 11 huts in Cote d'Ivore and found out that estimated deterrence of *Culex* spp., ranged from 36% with Chlorpyrifos-methyl treated net to 58% with lambda-cyhalothrin

Lambda-cyhalothrin (ICON®) has also been used for the control of intra-domiciliary *Anopheles arabiensis* Patton in huts in South Africa (Sharp *et al*., 1993). Comparison of lambda cyhalothrin with DDT showed that the percentage survival of blood fed mosquitoes ranged from as low as 55% caught leaving lambda-cyhalothrin- sprayed huts, to 82% of those caught leaving DDT- sprayed huts. In Brazil, Charlwood *et al.,* (1995) conducted a field trial using lambda-cyhalothrin to control intra-domiciliary *A. darlingi* roots and DDT residual sprays inside surfaces in houses. This study showed that the number of people infected with *P. falciparum* were fewer in areas where houses were sprayed with lambda-cyhalothrin than with DDT- sprayed houses supporting the study of Sharp *et al.,* (1993) which showed that lambda-cyhalothrin is more effective than DDT.

In Sudan, Elnaiem *et al*., (1999) carried out investigations on the protective efficacy of lambda-cyhalothrin-treated bed-nets (10mg a.i/ $m^2$) against *P. orientalis* in the household. The Studies showed that *P. orientalis* is highly susceptible to lambda-cyhalothrin and resulted in providing complete protection against vector bites for 12 nights. Exposure of female's insects to nets treated with 10mg a.i/$m^2$ for 30 seconds showed that all females died within one hour.

The first factor to consider during formulation of any chemical control strategy against vectors solely depends on the selection of an insecticide. A hut scale field trial was carried out to study the effectiveness of house spraying with capsule suspension (CS) formulation of lambda-cyhalothrin in comparison with its wettable powder (WP) formulation on mortality, density and behaviour of malaria vectors in India (Kumar *et al*., 2010). Both capsule suspension and wettable powder formulations prevented the entry of *Anopheles fluviatilis* in the sprayed huts by more than 90% for more than 6 months. The exit rate increased 90-99% with different treatments and the feeding rate was reduced (91-97%). The formulations or dosages differed in causing mortalities. Overall, the total mortality rate of *A. fluviatilis* was higher in the huts sprayed with capsule suspension 30 (58%) than the huts sprayed with wettable powder 20 (48%).

In many tropical and subtropical countries across the globe, malaria is a major public health problem resulting into human morbidity and deaths (WHO, 2017). Lambda-cyhalothrin, available as 10% wettable powder formulation is used at a dosage of 20-30 mg a.i/$m^2$ for indoor residual control programs (Elnaiem *et al*., 1999). It was observed by

Charlwood *et al.,* (1995) and Kaburi *et al*., (2018) that the efficacy of lambda-cyhalothrin its readly accepted by the local populace, and its cost effectiveness make it a more useful insecticide for anti-malaria campaigns than DDT. Davies *et al*., (2000) sprayed houses with lambda cyhalothrin and protected residents in Peruvian Andes from cutaneous leishmaniasis. Sharp *et al*., (1993) assessed the residual effects of lambda-cyhalothrin with DDT for the intra-domicilliary control of *Anopheles arabiensis* in South Africa and found that lambda-cyhalothrin was more efficacious than DDT. Asidi *et al*., (2005) evaluated bed nets treated with organophosphate or lambda-cyhalothrin alone and in combination against insect-resistant *Anopheles gambiae* and *Culex quinquifasciatus* mosquitoes and found that lambda cyhalothrin alone was not as effective as when combined with organophosphate. Raghavendra *et al*., (2011) conducted field evaluation of lambda-cyhalothrin with ICON 10 CS indoor residual spraying against *Anopheles culicifacies* in India and found it effective in controlling the malaria vector. Choi *et al*., (2017) used combination of indoor residual spraying with insecticide treated nets versus insecticide treated nets alone for prevention of malaria and found that the combinations produced better results. Killeen *et al*., (2017) used insecticide-treated combinations of window screens and eave baffles to control mosquito vectors that were resistant to single insecticide treatment. Kaburi *et al*., (2018) evaluated the effects of lambda-cyhalothrin combined with 1, 4-dichlorobenzene on sand fly and mosquito vectors and found the combination ICON®/PCB® to be more efficacious than the individual treatments.

## 4.3 Materials and Methods

The two pesticides ICON® and PCB® were used in the field to control the vectors of malaria and leishmaniasis. They were mixed in the ratios of 40mg to 60mg. Determination of the dosage to be used was arrived at in the laboratory after a series of tests of the correct minimum amounts of each compound that could effectively controll the vectors. Comparisons of the treatments with those of other studies on the same vectors were done to make them as similar as possible. Treatment blocks of the mixtures were: ICON®, PCB® and ICON®/PC®B and were prepared in petri dishes and covered with aluminium foil to prevent them from losing potency before being used in the field. In this study, sand flies were exposed to ICON® and PCB® and their combination for three months (immediate exposure) and compared to long time exposure (6 months).

### 1, 4-dichlorobenzene

The 1, 4-Dichlorobenzene (p-PCB, PDB, PCB®, 1, 4-dichlorobenzene) is an organic compound with the molecular formula $C_6H_4C_{l2}$ and structure as shown below (Figure 4.1). This colourless solid has a strong sweet odour.

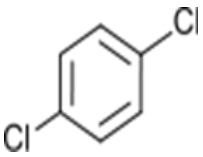


**Figure 4.1:** Chemical structure of 1, 4-dichlorobenzene

PCB® is produced by chlorination of benzene using ferric chloride as a catalyst.

$$C_6H_6 + 2\ Cl_2 \rightarrow C_6H_4Cl_2 + 2\ HCl$$

The compound scientifically made pure via fractional crystallization, relieng on its characteristics of having a high boiling point of 174°C and melting point of 53.5 °C. 1, 4-dichlorobenzene is poorly soluble in water (10.5mg/100ml at 20°C), do not decompose by action of bacteria that exist in the soil. As a hydrocarbon, PCB® is lipophilic and has affinity for fatty tissues. There is no direct evidence of carcinogenicity according to environmental protection agent (EPA). 1, 4-dichlorobenzene is registered by USA-EPA for water use at a concentration of 75µg per litre and also as a pesticide (O'Neil *et al*., 2001). There is no report of insecticidal resistance against 1, 4-dichlorobenzene in sand flies and mosquitoes in Kenya.

**b) Lambda cyhalothrin**

The chemical name of lambda-cyhalothrin is A-cyano-3-phenoxybenzyl-3-(2-chloro-3, 3, 3-trifluoro-1-propenyl)-2, 2- dimethylcyclopropanecarboxylate and the Molecular formula is $C_{23}H_{19}ClF_3NO_3$ (MW= 449.86) with the structures of the two isomers shown below (Figre 4.2).

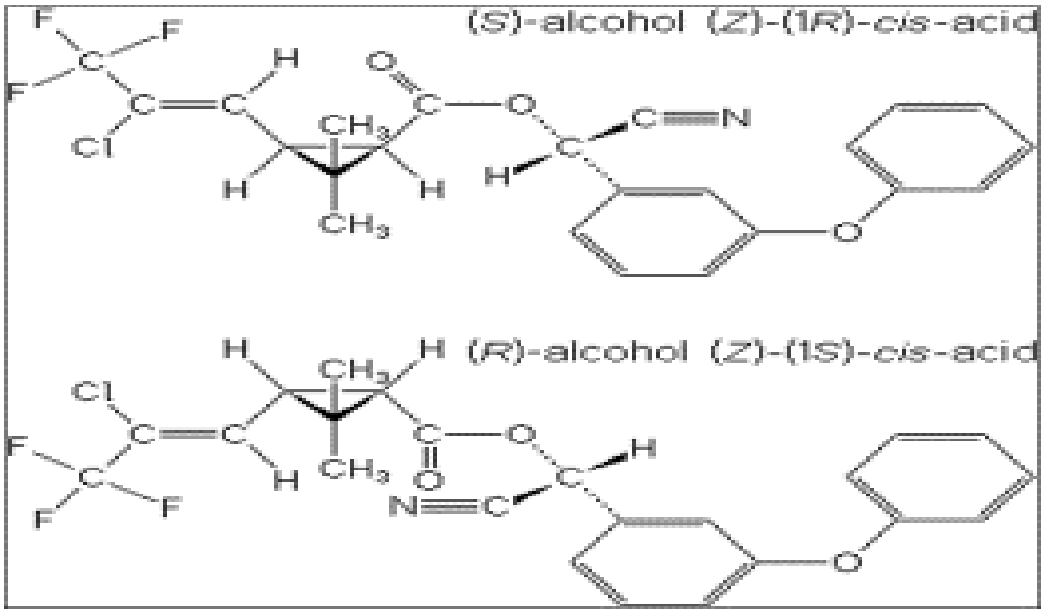


**Figure 4.2:** Chemical structures of Lambda-cyhalothrin

It was observed by Charlwood *et al.,* (1995) that the efficacy of lambda cyhalothrin, its readly accepted by the local people, and it cost effectiveness make it a more useful insecticide for anti-malaria campaigns than DDT. It is for this reason that lambda-cyhalothrin has been chosen for this study. Lambda-cyhalothrin, a pyrethroid, is another insecticide that has been used in sand fly control. Kelly *et al*., (1997) applied lambda-cyhalothrin at 20 mg a.i/$m^2$ in all animal pens in the village "blanket coverage" and detected 90% reduction in *L. longipalpis* Lutz and Neiva, abundance sheds in the Brazilian Amazon. The two pesticides were each tested separately and as a combination in the field. Before going to test them in the field, laboratory assays were conducted to show if they could be tried in the field. The results from the laboratory were quite encouraging and warranted the pesticides being tried in the field.

### 4.3.1 Study sites

Field studies were carried out in Marigat and Mwea sites.

**Marigat**

Marigat study site is an area that is endemic for both cutaneous and visceral leishmaniasis and was used to study the efficacy of the test repellents on phlebotmine sand flies and mosquitoes. Marigat is found in Baringo County, Rift Valley Province of Kenya (Figure 4.3), Baringo lies 250 km North West of Nairobi and covers an area of approximately 10,000 $km^2$ and is located north of the Equator in Kenya's Rift Valley Province. The area is semi-arid with subtropical climate. Rainy seasons are from March to September with peaks in May and August. Annual rainfall is below 300 mm while temperatures range from 17-42$^oC$. Natural vegetation in Marigat is mainly composed of *Acacia tortilis*, *Openchia polyacantha* and *Prosopsis juliflora* species either scattered or as forest in few cases, short bushes or patchy grassland. The ground is mostly bare soil or rocky with gullies in some areas. The Kenya government introduced *Prosopsis juliflora* to Baringo County from Tana River County to control desertification in the area and has changed the vegetation cover in the regions formerly devoid of vegetation. Rodent burrows are numerous in both vegetation covered and bare grounds. Termite mounts are common features in the area. There is a Perkerra irrigation scheme around Marigat that allows growth of various crops such as maize and vegetables. Animal husbandry is also practiced in the area whereby cattle, sheep and goats are kept.

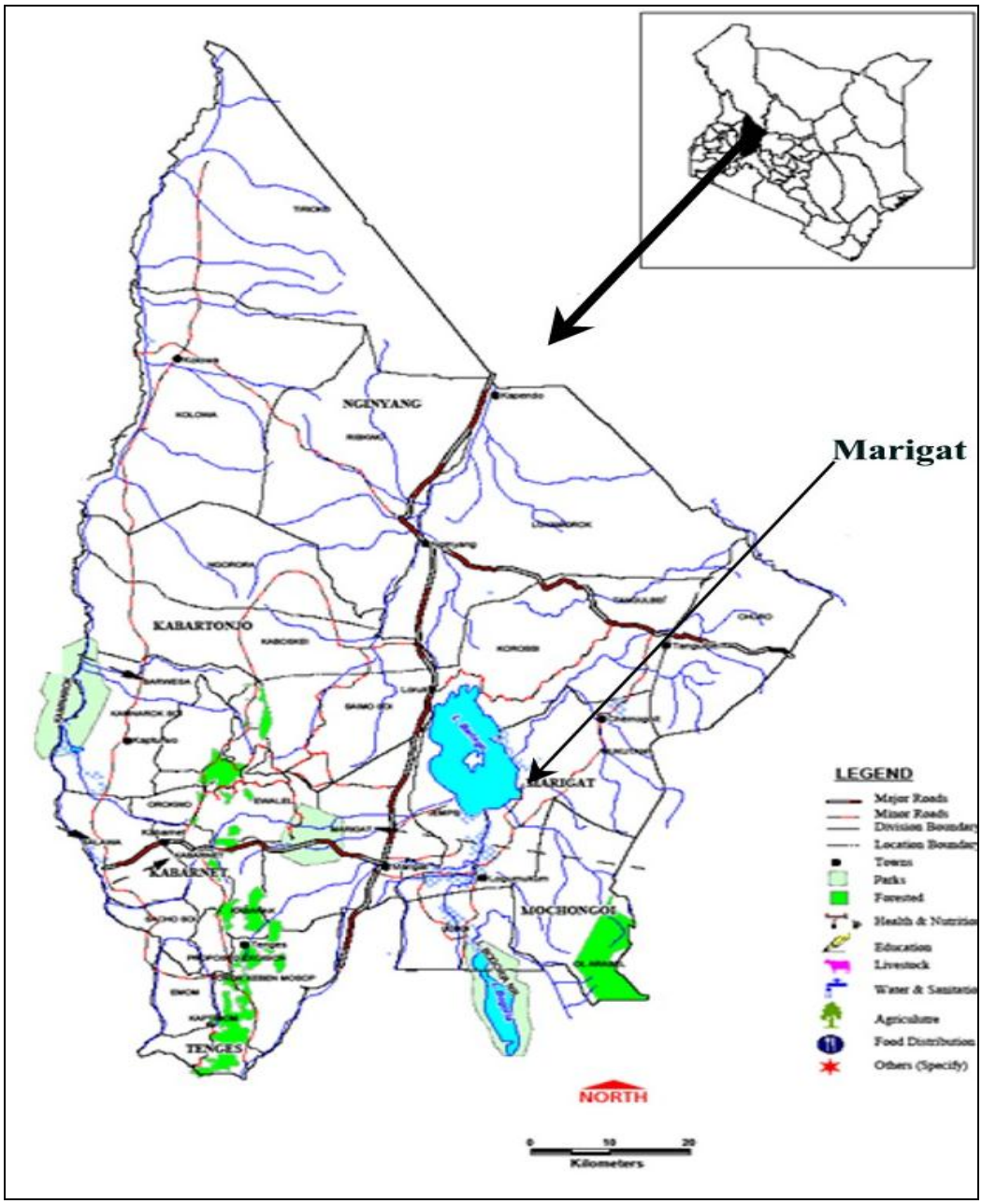


**Figure 4.3:** Map showing Marigat site within Baringo County **[Source: Office for the Coordination of Humanitarian Affairs, OCHA, and United Nations 2001].**

Mwea

Mwea a malaria endemic area that is not known to be endemic for leishmaniases was used to test the efficacy of the repellent on malaria vectors, particularly *A. gambiae* and

*A. funestus*. Mwea is found in Kirinyaga County, Kenya. Mwea Sub County is located in the Eastern side of Mount Kenya at an altitude ranging from 1100 to 1350 metres above sea level and is the home of Mwea Rice Irrigation Scheme. Seventy-five percent (75%) to 90% of the rice consumed in Kenya is produced from this settlement scheme (NIB report). It covers an area of about 12,000 hectares. The sub county can be generally grouped into 2 distinct ecological settings, the low-lying irrigated rice paddies to the south and the elevated upland area to the north. Mwea Sub County has 3,270 families living in 60 villages (Kenya Population Census Data, 1999) (Figure 4.4)

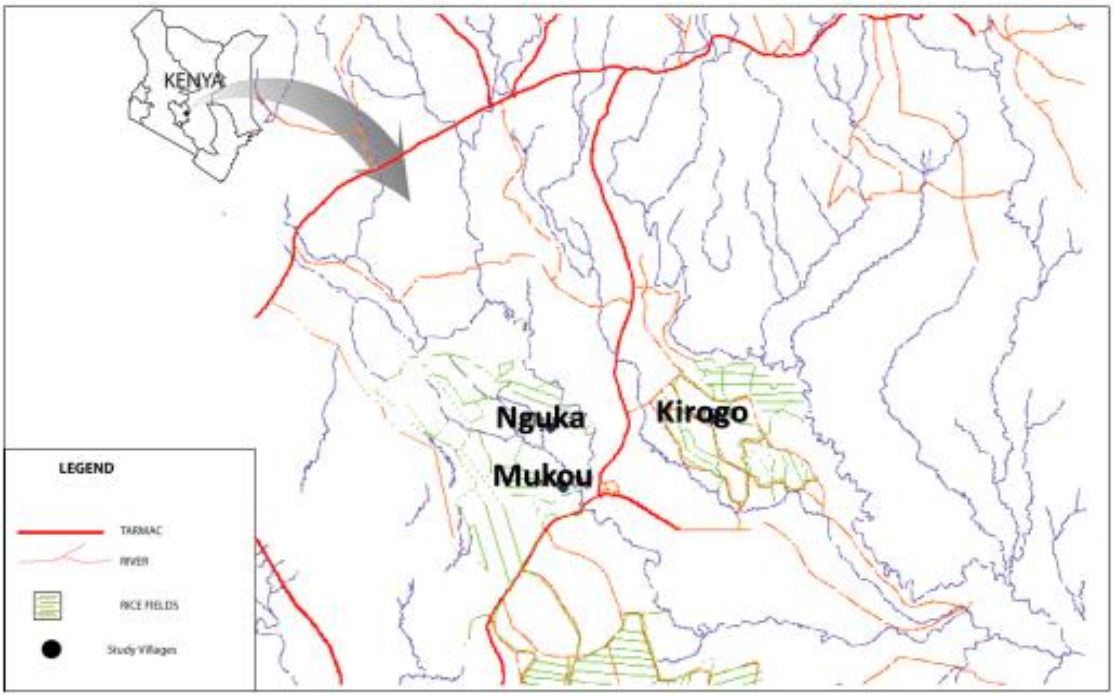


**Figure 4.4:** A Map showing Mwea sub county study villages, Kirinyaga County: [The green marking shows the canals running in the rice irrigation paddies (Adapted from Kaburi et al., 2008)]

### 4.3.2 Study Design

Thirty houses were randomly selected in Marigat and three villages (Kirogo, Nguka and Mukou) in Mwea study site (with an equal inter-house distance of 100 metres). The selection criteria were that houses made of stone walls and built with cement were classified as permanent houses with glass and steel windows. These types of houses were not used for the study because they were not easily accessed by the sand flies and mosquitoes. The other category of houses was those that were made of mud walls and raised caves which allowed the vectors to get in and out of the house with relative ease. The third types of houses were the ones thatched with grass and walls made of mud or sticks. These types of houses were mainly found in Baringo County. Most of the permanent stone houses were found in the Mwea study site. The windows were also made of wooden materials and in most cases allowed sand flies and mosquitoes to go in and out easily. The treated blocks were hang or placed at the window seals in the sleeping rooms. There were three types of treated blocks (ICON®, PCB® and ICON®/PCB®) which were used to cover 30 houses in Baringo and Mwea study sites. Each treatment was used in 10 houses per site. In the 30 experimental houses in Mwea and Marigat respectively, PCB-treated blocks were placed in all rooms where the inhabitants slept. For the experimental houses, the kitchen was avoided because of smoke. In the middle control houses untreated disinfectant blocks were used as described above, as recommended by EPA.


**Figure 4.5:** House types of the villages in a semi-arid area in Baringo County, Kenya study site

### 4.3.3 Morphological identification of sand fly and mosquito species

**a) Sand flies**

The CDC Miniature Light traps (John W. Hock, Gainesville, Florida) were used to trap sand flies. The traps were positioned in convenient places in 30 houses per room on a daily basis at 1800 hr and retrieved at 0600 hr the following morning for two consecutive days. The sand flies were aspirated, counted, recorded on excel data sheets. They were put in small vials with dessicant pebbles to provide dry condition and transported to KEMRI laboratory where they were dissected and mounted on microscope slides using chloral hydrate gum according to the method of Minter, (1963).

The collected sand flies were washed in 2% detergent solution to remove hairs and other debris. Thereafter, they were rinsed in Phosphate Buffer Solution (PBS) then transferred to a microscope slide for dissection and mounting. Head and genitalia were demonstrated on slides for species confirmation of the *Phlebotomus* spp. Excision of the heads was done and mounting done by aid of gum chloral. This was done on slides upside-down in order to expose the cibarium and pharynx. The coverslips were used to cover the mounted slides and they were left for one to two days to dry on the bench. By observing the cibarial armatures, spermatheca and the pharynx assisted by identification keys of Abonnenc and Minter (1965) species identification was achieved.

**b) Mosquitoes**

All the mosquitoes collected from the houses with CDC light traps were aspirated using mouth aspirators counted and the numbers recorded in field note books. The mosquitoes were desiccated in dry silica gel and transported to Kenya Medical Research Institute

(KEMRI), Leishmaniasis Laboratory, CBRD, where they were stored at room temperature awaiting identification. They were then identified using morphological features according to the identification keys of Gillies and de Meillon, (1968) and Gillies and Coetzee, (1987).

### 4.3.4 Molecular identification of the sand fly vectors

The DNA was extracted from the remaining parts of the specimens (thorax and abdomen) using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendation. Briefly, 180µl of buffer ATL was added to 1.5 ml microcentrifuge tubes containing each specimen and the tissues disrupted using micropestles before addition of 20µl of proteinase K. The mixture was vortexed and incubated at 56$^{o}$C for 1hour and 30 minutes followed by vortexing for 15 seconds, addition of 200µl of buffer AL, mixing by vortexing, addition of 200µl of 96%-100% ethanol and vortexing again. The mixture from the previous step was pipetted into the DNeasy mini spin column, put in 2ml collection tube, spined at 8000 rpm for 1 minute and the flow-through was collected was discarded. The DNeasy mini spin was transferred into a new 2ml collection tube, 500µl of buffer AW1 added, centrifuged at 8000rpm for 1 minute and the flow-through discarded as in the previous step. The DNeasy mini spin column was transferred into a new 2ml collection tube followed by addition of 500µl buffer AW2, centrifugation at14000 rpm for 3 minutes to dry the membrane and discarding of the flow-through and the collection tube as well. The DNeasy mini spin column was placed in a clean 1.5ml microcentrifuge tube and 100µl of buffer AE pipette directly onto the DNeasy membrane. This was incubated at room temperature for 1 minute followed by centrifugation at 8000 rpm to elute the DNA.

Molecular identification was performed by polymerase chain reaction (PCR) of the mitochondrial cytochrome oxidase subunit 1 (COI) genomic marker. The COI gene was amplified using DNA barcoding primers; forward LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse, HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). In a final volume of 15µl, the following was added: 7.5µl of 2x My Taq mix, 0.75 µl of each primer, 2µl of DNA extracted from the specimens (template) and 4µl of PCR grade water. Thermal cycling conditions included an initial denaturation step of 15minutes at 95˚C followed by 40 cycles of 95˚C for 1minute (denaturation), 52˚C for 30seconds (annealing) and 72˚C for 40 seconds (extension) and a final extension of 72˚C for 10minutes.

Further, the region was amplified using Uniminibar JV primers; forward primer, Uniminibar JV F (5'-ACAAATCATAARGATATTGGAAC-3') and reverse primer Uniminibar JV R (5'- AAAATTATAATAAAWGCATGAGC-3'), and the established 173bp PCR products resolved using High Resolution Melt (HRM) analysis (Ajamma *et al*., 2016) in Rotar gene Q HRM real time PCR thermal cycler (QIAGEN, Hannover, Germany). The PCR mixture consisted of 2µl of 5x Hot Firepol Evagreen HRM mix (Solis BioDyne, Tartu, Estonia), 0.25µl of each primer, 1µl of DNA template and PCR grade water in a final volume of 10µl. The thermal cycling conditions involved an initial denaturation at 95˚C for 15minutes followed by 40 cycles of denaturation at 95˚C for 30seconds, annealing at 52˚C for 45 seconds and extension at 72˚C for 45 seconds and a final extension at 72˚C for 10minutes. Without stopping the reaction, the PCR products were denatured at 95˚C for 1minute, held at 40˚C for another minute and melted by

gradually raising the temperature from 65˚C to 95˚C by 0.1˚C in each step, waiting for 90 seconds of pre-melt conditioning on first step and 2 seconds for each step afterwards. In both the amplifications, PCR grade water was used as the negative control while positive controls included DNA from known (molecularly identified) sand fly species. Sand fly species identification was done by comparing the HRM profiles generated to those of the reference control species. Representative samples of identified sand fly species with unique HRM curves were identified by purification, of about 650bp PCR products established in the LCO/HCO amplification above, using ExoSAP-IT (USB Corporation, Cleveland, OH) and submitted for sequencing using the Sanger method. To confirm the identity of PCR-HRM differentiated sand flies, the generated DNA sequences were edited using Geneious and queried against the Genbank database using the Basic Local Alignment Search Tool (BLASTN).

### 4.3.5 Determination of sand fly and mosquito density in the study sites (Pre-intervention studies)

The study was conducted in Baringo County and Mwea Irrigation Scheme in Kirinyaga County. In each study site, 30 houses were randomly selected and assigned numbers 1-30. The CDC light traps were used to trap sand flies and mosquitoes from 1800 hr in the evening to 0600 hr in the morning. Both sand fly and mosquito collections were carried out in the study households before interventions for a period of six months. This was done using the CDC light traps and exit traps of the Muirhead-Thomson, (1947). All the mosquitoes that got picked from traps were aspirated, counted and recorded in the record book. Female mosquitoes were aligned to species morphologically and preservation was

done in silica gel. Male anophelines were only identified, recorded and discarded. The sand flies were aspirated from the collection nets, counted and recorded in the record book. They were put in DMSO in small vials for processing later in the laboratory at Kemri, Leishmaniasis Lab. In the laboratory, the sandflies were dissected, mounted and identified to species level under a compound microscope with the aid of sand fly identification keys of Abonnenc and Minter, (1965). This process was carried out before the intervention to establish the size of vector populations in Baringo and Mwea study sites. The mean vector density per trap were calculated for each study site and graphs drawn representing the performance of each trap before intervention.

### 4.3.6 Intervention studies

In all the study houses in Marigat and Mwea, treated blocks were placed one in each room where people slept and exit traps by Muirhead-Thomson, (1947) were set for a minimum of 2 nights per month per study site and cleared before 0800 hr each day in the evening and collected the following morning at 0600 hr. The caught vectors were knocked down using ice. Collections were carried out for a period of 2 years in all the study sites. The sexes were distinguished (males and females) microscopically and recorded. All the mosquitoes and sand flies collected from the exit traps were preserved separately in collection cups. The number of fed and unfed females was also recorded. The mosquitoes were identified morphologically using the keys according to Gilles and De Meillon, (1968), and Gilles and Coetzee, (1987) while sand flies were mounted and identified using the standard sand fly keys (Abonnenc and Minter, 1965; Abonnenc, 1972). The classification of the blood meal status of mosquitoes and sand flies as unfed,

blood fed and gravid was based on the abdominal appearance aided by a dissecting microscope.

### 4.3.7 Data analysis

The data was entered in MS excel sheet and later imported to STATA 9.2, (STATACORP, TX USA) for the analysis. The average number of sand flies and mosquitoes per trap (house) for each treatment was used as the unit of analysis. Pre-intervention data were compared with the post intervention data using student t-test and among the different intervention groups using two-way ANOVA.

## 4.4 Results

### 4.4.1 Determination of sand fly and mosquito densities before and after intervention in the Marigat and Mwea Sub-counties

In Marigat, sand fly catches dropped from a pre-intervention mean of 2,301 flies/household to 224 flies/household immediately after intervention, but the number rose to 443 flies/household by the sixth month after intervention. Similarly, mosquito catches decreased from a high pre-intervention mean catch of 3,932 mosquitoes/household to 485 mosquitoes/household immediately after intervention with catch increasing to a mean of 588 mosquitoes/household by the sixth month (Table 4.1)

In Mwea, the sand fly catches decreased from a mean of 86.3 sand flies/household to 16.3 sand flies/household immediately after intervention, with the number decreasing further to 9.1 flies/household by the sixth month after intervention. Similarly, mosquito catches dropped from 91.5 to 30 mosquitoes/household immediately after intervention and decreased further to 18.5 mosquitoes/household by the sixth month (Table 4.1).

**Table 4.1:** Mean vector densities per household (±SE) before and after treatment in study site

| Site | Preintervention | | Intervention | Post-intervention | | | |
|---|---|---|---|---|---|---|---|
| | | | | Immediate | | 3-6 months | |
| | SF | MSQ | treatment | SF | MSQ | SF | MSQ |
| | 137.8±91.68 | 93.3±31.58 | PCB/ICON | 24±16.74 | 28.4±10.44 | 12.3±5.41 | 25.3±7.88 |
| Mwea | 36.7±14.77 | 82.9±28.39 | PCB | 5.9±4.57 | 25.1±7.28 | 3.9±2.04 | 12.4±3.4 |
| | 84.5±33.23 | 103.0±28.48 | ICON | 13.5±8.77 | 35.2±12.05 | 11.2±9.42 | 17.0±3.95 |
| | 120.1±44.2 | 115.8±44.55 | PCB/ICON | 4.5±1.86 | 16.1±3.47 | 8.5±3.5 | 20.1±6.25 |
| Marigat | 34.1±17 | 214.6±133.52 | PCB | 9.3±3.98 | 23.5±5.04 | 7.3±4.5 | 21.3±5.11 |
| | 75.9±48.71 | 62.8±16.45 | ICON | 8.9±2.08 | 8.9±3.72 | 8.5±4.66 | 17.4±5.23 |

*SF, Sand fly. MSQ, Mosquito. TT, Treatment.

### 4.4.2 Immediate effect of treatments on sand flies in Mwea site

From the study sites both sand fly and mosquitoes mean densities were higher in the pre-intervention than after treatmenmt with insecticdes. There was a significant difference in sand fly density after immediate exposure to ICON® alone (t=1.8125, P=0.0032), but the difference was not statistically significant after immediate exposure to PCB® or the PCB/ICON® combination (P>0.05) (Table 4.1).

### 4.4.3 Long term effects of treatments on sand flies after 3-6 months Mwea site

There was significant decrease in sand fly densities after treatment with ICON® (P=0.029007, t=1.812461, SEM±25.9868), PCB® (P=0.024838, t=1.812461, SEM±11.46322). The same was observed in households that received the treatment with the combination of PCB® and ICON®, but the difference was not statistically significant (P=0.113121, t=1.833113, SEM±66.29991). The decrease of sand fly populations following exposure to ICON®, PCB® and ICON®/PCB® is a good indication that all the trial insecticides can be used in the insects control (Fig. 4.6).

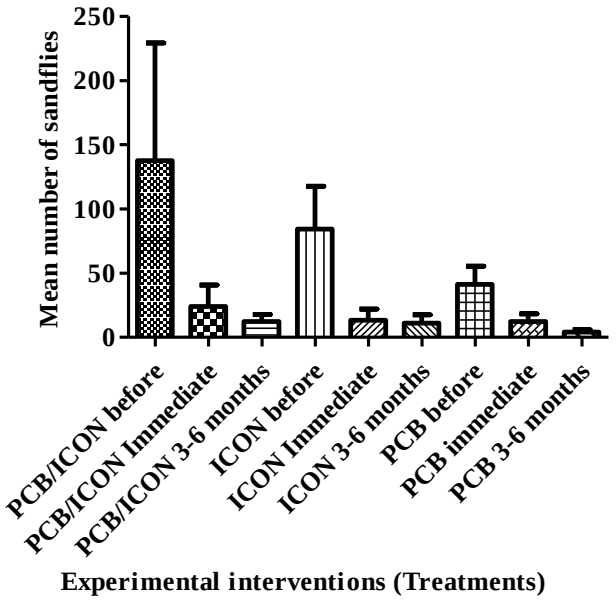


**Figure 4.6:** Sand fly densities in Mwea before and after intervention

### 4.4.4 Immediate effects of treatments to mosquitoes in Mwea site

There were a decreased in number of mosquitoes after exposure to ICON®, PCB® and combination - PCB®/ICON®. The mean differences of mosquitoes trapped before and after exposure to ICON®, PCB® and PCB®/ICON® were significantly different [(P=0.0117, 67.8±24.893, t=2.7237), (P=0.0266, 52.8±23.74, t=2.2235), (P=0.0138, 64.9±24.74, t=2.6229)], respectively (Fig.4.7).

### 4.4.5 Long term effects of treatments after exposure to mosquitoes in Mwea site

The mosquito densities in Mwea site were more as compared to Marigat site. There was a significant reduction of mosquito densities after treatment with ICON® (P=0.0232, 76.3±33.075, t=2.3069), PCB® (P=0.0194, 66.2±27.4, t=2.4158) and PCB®/ICON® combination (P=0.0178, 68±27.5, t=2.4685) as shown in Figure 4.7.

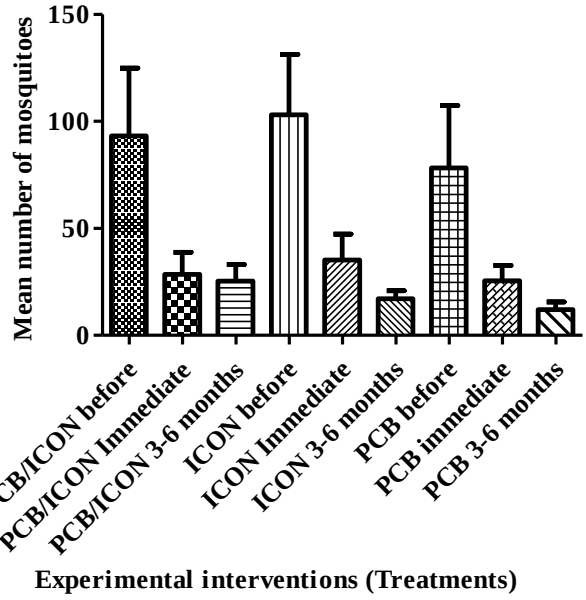


**Figure 4.7:** Immediate and long-term effects of ICON®, PCB® and PCB®/ICON® combination on mosquitoes in Mwea site

### 4.4.6 Immediate effects of insecticide treatments on sand flies in Baringo site

Results from this site shows that there was no significant decrease in sand fly densities after treatment with ICON® (67± 8.53, t= 1.3805, P=0.1004) and PCB® (24.8±16.2, t= 1.5309, P=0.0801). However, there was a significant decrease in sand fly population after treatment with PCB®/ICON® combination (115.6±42.98, t= 2.6894, P=0.0124) (Figure 4.8).

### 4.4.7 Long term effects of insecticide treatments on sand flies in Baringo site

There was no significant difference in sand fly densities following six months' exposure to ICON® (67.4±48.0389, t= 1.4030 P=0.0971) and PCB® (26.8±16.13, t= 1.6614, P=0.0655). Conversely the study found out that there was a significant decrease in sand

fly densities after treatment with PCB®/ICON® combination (111.6±41.434, t= 2.6934, P=0.0123) (Fig.4.8).

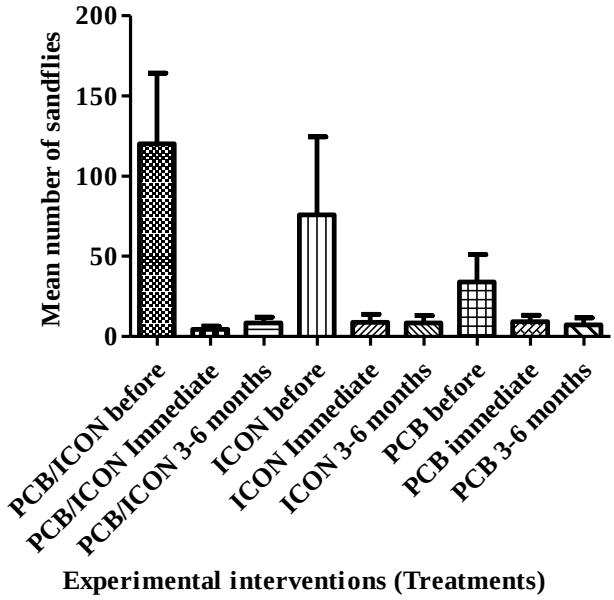


**Figure 4.8:** Effects of ICON®, PCB® and PCB®/ICON® combination exposure on sand flies in Marigat site, Baringo County.

### 4.4.8 Immediate effects of insecticide treatments on mosquitoes (Baringo County)

In this site, there was a significant difference in mosquito densities following zero to three months (immediate) exposure to the ICON® (53.9±16987 p=0.0057) and PCB®/ICON® (99.7±42.057, P=0.0209). However, there was no significant difference in mosquito densities after exposure to PCB® alone (191.1±131.17, P=0.0911) (Fig.4.9).

### 4.4.9 Long term effects of insecticide treatments on mosquitoes over time in Baringo County

The mosquito densities decreased significantly when exposed to ICON® and PCB®/ICON® as opposed to PCB® alone for 3-6 months' period (Figure 4.9). The houses which had ICON® and the combination of ICON®/PCB® recorded a reduction in mosquito densities as opposed to PCB® alone. This shows that CB® alone cannot be effective when used for more than 3 months.

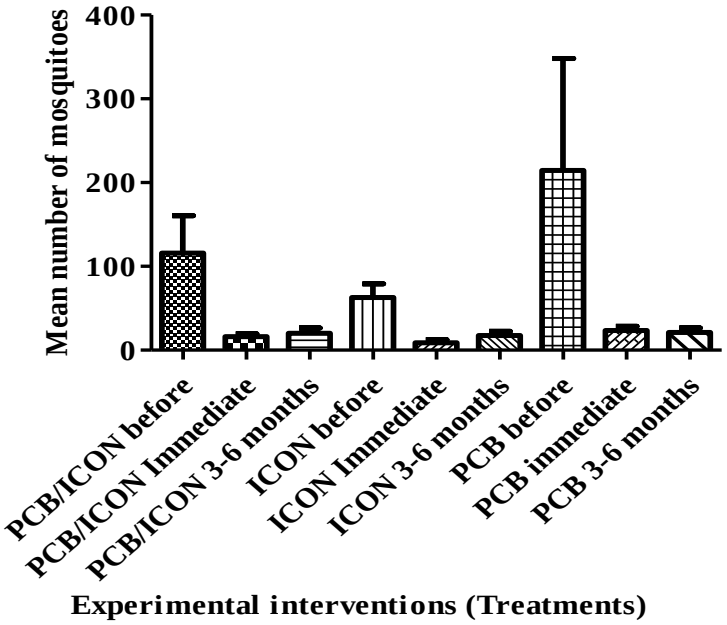


**Figure 4.9:** Mosquito densities in Baringo County before and after interventions.

Overall significance reduction of mosquito densities after treatment with ICON® (45.4±18.043, P= 0.0165) and PCB®/ICON® (95.7±43.147, P= 0.0269) was observed, however, there was no significant difference when mosquitoes were exposed to PCB® (193.3±131.94, P= 0.0885) (Figure 4.9).

### 4.4.10 Molecular identification of sand fly species collected from Kirogo, Nguka and Mukou villages in Mwea irrigation scheme

Polymerase Chain Reaction- High Resolution Melt (HRM) analysis revealed a HRM profile peak in sand fly sample B that was similar to *P. martini* positive control, whereas for samples C and A had profile peaks slightly outside that of *P. martini* positive control, suggesting that sand flies in sample B were more related to *P. martini* positive control (Figure 4.10 and 4.11).

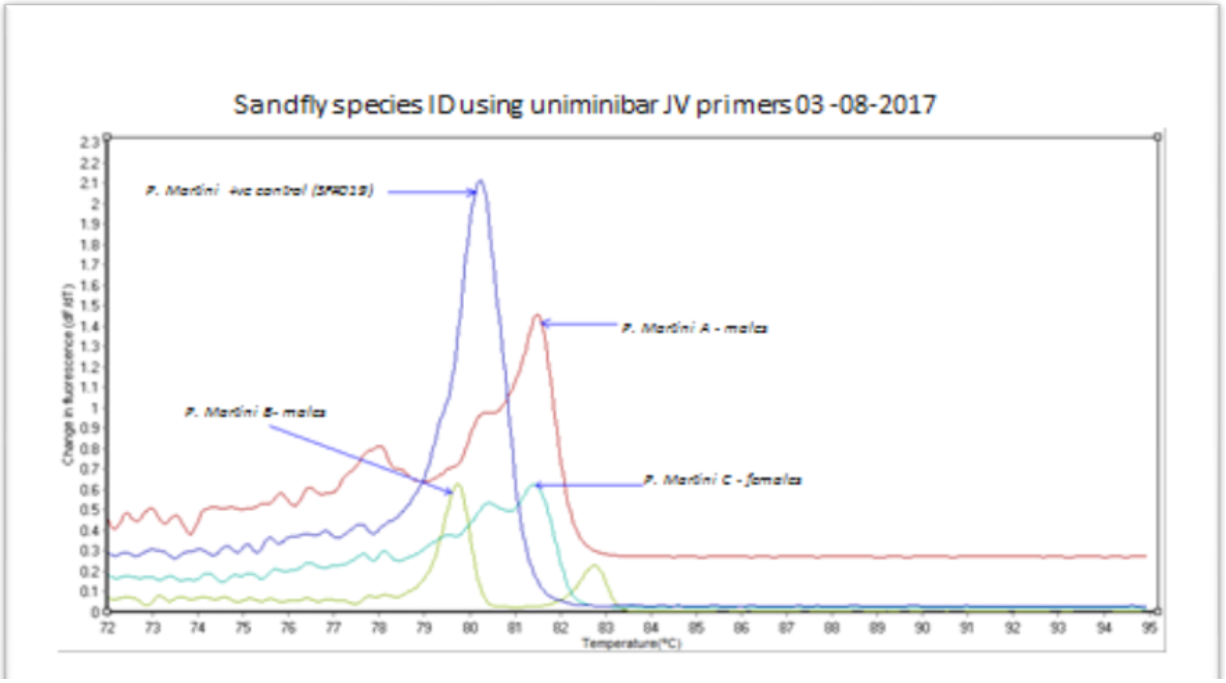


**Figure 4.10:** High Resolution Melt (HRM) profiles of sand fly samples A, B and C compared with P. martini positive control

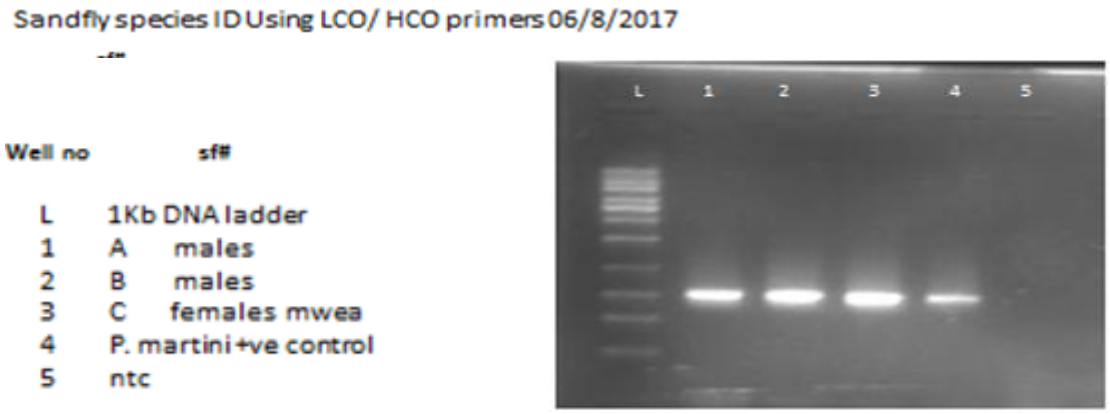


**Figure 4.11:** Electrophoretic analysis of PCR products from sand fly specimens for comparative identification with known primers

### 4.4.11 Morphological identification of sand flies collected from Marigat and Mwea irrigation Scheme

Out of 378 sand flies identified from Marigat study sites, seven species were represented with *Sergentomyia schwetzi* having the highest percentage (58%) followed by *S. antennatus* (19%) and *P. martini* (10%). *P. dubosqui* (1.6%) had the lowest percentage. Other species were; *S. clydei* (7.0%), *S. squamipleuris* (2.0%) and *S. bedfordi* (1.0%) (Table 4.2)

There were 251 sand flies identified from Mwea study sites, they were of two genera: Phlebotomus and Sergentomyia. The collection comprised of two *Phlebotomus* species and six *Sergentomyia* species (Table 4.2). The two *Phlebotomus* species were *P. martini* Parrot (4.8%) and *P. rodhaini* Parrot (0.8%) while *Sergentomyia* species were *S. schwetzi* Adler, Theodor and Parrot (6.0%), *S. bedfordi* Newstead (3.2%), *S. squamipleuris* Newstead (27.9%), *S. clydei* Sinton, (0.4%), *S. antennata* Newstead, (0.4%) and *S. inermis* Abonnenc, (56.6%). Female sand flies were three times the number of males

caught during the study. Out of the total number of sand flies caught, majority were *S. inermis* Abonnenc followed by *S. squamipleuris* Newstead.

**Table 4.2: Phlebotomine sand flies caught in households in Mwea Irrigation Scheme households**

| Study Area | Sand fly species | Total number collected | Percentage |
|---|---|---|---|
| **Mariga (N=378)** | *Phlebotomus martini* | 38 | 10.0 |
| | *Phlebotomus duboscqi* | 6 | 2.0 |
| | *Sergentomyia schwetzi* | 223 | 58.0 |
| | *Sergentomyia antennata* | 72 | 19.0 |
| | *Sergentomyia clydei* | 26 | 7.0 |
| | *Sergentomyia squamipleuris* | 8 | 2.0 |
| | *Sergentomyia bedfordi* | 5 | 1.0 |
| **Mwea (N=251)** | *Phlebotomus martini* | 12 | 4.8 |
| | *Phlebotomus rodhaini* | 2 | 0.8 |
| | *Sergentomyia antennata* | 1 | 0.4 |
| | *Sergentomyia bedfordi* | 8 | 3.2 |
| | *Sergentomyia clydei* | 1 | 0.4 |
| | *Sergentomyia schwetzi* | 15 | 6.0 |
| | *Sergentomyia squamipleuris* | 70 | 27.9 |
| | *Sergentomyia inermis* | 142 | 56.7 |

### 4.4.12 Morphological identification of mosquito species in Mwea irrigation Scheme and Marigat

A total of 2,694 mosquitoes were randomly sampled and identified from the Mwea site. The five most common mosquito vector species collected from this site were; *Anopheles arabiensis* Patton (1142; 52.5%), *Culex quinquefasciatus* Say (1035; 36.7%), *Anopheles pharoensis* Theobald (143; 5.2%), *Anopheles coustani* Laveran (38; 1.4%) and *Anopheles funestus* Giles (36; 1.3%). The species, *Anopheles arabiensis, Culex quinquefasciatus*, *Anopheles funestus, Anopheles coustani* Laveran and *An. pharoensis* were more abundant in the site which was within the rice agro-ecosystems (irrigation). Efficient surveillance is

essential for early detection of increased vector abundance and detection of pathogens in trapped mosquitoes. In total, 242 mosquito vectors were identified from the Marigat sites and the most abundant species was *Mansonia*, followed by *Culex* and *Anopheles*, respectively (Table 4.3).

**Table 4.3: Mosquito vector species collected in Mwea and Marigat**

| Study Area | Mosquito species | Total number collected | Percentage |
|---|---|---|---|
| **Mwea (N=2,694)** | *Anopheles arabiensis* Patton | 1,442 | 52.5 |
| | *Culex quinquefasciatus* Say | 1,035 | 36.7 |
| | *Anopheles pharoensis* Theobald | 143 | 5.2 |
| | *Anopheles coustani* Laveran | 38 | 1.4 |
| | *Anopheles funestus* Giles | 36 | 1.3 |
| **Marigat (N=242)** | *Mansonia africanus* | 135 | 55.8 |
| | *Culex vansomerini* | 38 | 15.7 |
| | *Culex pipiens* | 26 | 10.7 |
| | *Anopheles gambiae* | 22 | 9.1 |
| | *Mansonia uniformis* | 18 | 7.4 |
| | *Coquillittidia spp* | 3 | 1.2 |

### 4.5 Discussion

In Mwea exposure of sand flies to ICON® significantly reduced their densities unlike exposure to PCB® and ICON®/PCB® combination. This study therefore suggests that ICON® was effective on sand flies in Mwea site after exposure for a short period. The Mwea results contradicted the findings from the Baringo site, where ICON®/PCB® combination significantly reduced the sand fly densities as compared to ICON® and PCB® alone. The differences in efficacy of ICON® and PCB® in the two study sites could have been attributed to geographical location of the sites. Mwea and Baringo are two areas that have different climatical conditions. It has been shown that temperature affects

the activity of lambda-cyhalothrin. Exposure of sand flies to ICON® and PCB® for 6 months had a significant decrease in the densities as compared to three months' exposure. This study suggests that efficacy of ICON® and PCB® could be dependent on the lenth/period of exposure.

There was a high reduction in mosquito densities after exposure to ICON® and ICON®/PCB® combination for three months, however no significant decrease in their densities when exposed to PCB® was observed. This observation was also seen in exposure for 6 months. It is therefore suggested that PCB® alone cannot effectively kill mosquitoes but can synergize with ICON® to be effective. In Mwea there was a significant decrease in mosquito densities after exposure to ICON®, PCB® and their combination in both immediate and long-term exposure. This indicates that in Mwea site, PCB® and ICON® were able to kill mosquito individually the same way as thier combination, thus there was no synergy. This is a peculiar finding that needs further investigations on geographical location, climatic differences and probably the genetic material of the mosquitoes in the two sites.

The residual properties of lambda-cyhalothrin make it a very useful insecticide in the control of malaria and leishmaniasis vectors by being able to remain effective for 6 months. The results support the finding of Muturi *et al.,* (2006) who reported that different levels of habitat perturbations with regard to rice cultivation have different effects on mosquito diversity and abundance. Appropriate vector population sampling is required in the prediction of vector-borne disease outbreaks and determining when and

where to apply control measures to prevent/suppress such outbreaks. In the current study, two species of sand flies were collected from households using CDC light traps. *P. martini* and *P. rodhaini* contributed 4.8% and 0.8 % respectively of the total collection and the rest were from *Sergentomyia* species. In Sudan, *P. orientalis* is the vector for VL whereas in Kenya, *P. martini* is the suspected vector.

From the HRM profiles all the members of the group of interest are more closely related to each other. However, sand fly samples B were closely related to the known *P. martini* as compared to the sand fly samples C and A. Hence, there is a close relationship between sand fly samples B and *P. martini* species as was shown on HRM profiles.

# CHAPTER FIVE: MAMMALIAN TOXICITY OF LAMBDA-CYHALOTHRIN (ICON®) INCORPORATED INTO 1, 4-DICHLOROBENZENE (PCB®)

## 5.1 Summary

A total of twenty mice were used to find out whether the three treatments (PCB®, ICON® and ICON®/PCB®) had any toxic effects when administered orally to the mice. A group (five mice) of mice were treated with phosphate buffered saline (PBS), a placebo as control for the experiment. The behaviours were observed keenly and recorded for the first 6 hours and later 14 hours at the beginning of the experiment. At the end of the experiment, the body weights and their respective vital organs were measured and recorded. Comparably, there were no significant differences among the weights of the vital organs except the mean weight for the heart of the mice treated with ICON® which was significantly higher than that of mice treated with PBS. The mean weight of the lungs from the mice treated with PCB® was higher than that treated with PBS although their differences were not significant. The organs of the mice treated with combined (ICON®/PCB®) weighed more than those treated with either ICON® or PCB® independently. The control group (PBS treated mice) had generally lower mean weights of all the treatments. Therefore, the mean body weights of mice treated with (PCB®/ICON®) were more than those treated with PBS, PCB® or ICON® (Table 5.1).

## 5.2 Introduction

In general, *in vivo* toxicity study is the toxicological analysis of therapeutic products and its potency to evaluate qualitatively and quantitatively by histopathology and oral acute toxicity studies. Oral acute toxicity testing in mice have been used to evaluate natural

remedies for different pharmacological activities, taking into account the basic premise that pharmacology is simply toxicology at a lower dose (Sasidharan *et al*., 2008). A toxic substance might elicit interesting pharmacological effects at a lower non-toxic dose. The 1, 4 dichlorobenzene (PCB®) is an active ingredient of mothballs, deodorizers and fumigants. Due to the easy availability of this chemical, there is a considerable risk for accidental or intentional toxic exposure. Recently, multiple cases of PCB® toxicity due to mothball ingestion were reported and it was reported that toxicity due to PCB® can affect multiple organ systems including liver, kidney, skin, lung and the central nervous system (CNS) when misused (Dubey *et al*., 2014).

It has been reported that humans chewing toilet bowel deodorizing cakes consisting of 99.9% PCB® was toxic causing anxiolytic effect (Anderson and Anderson, 1997). The same was reported in rats and the median lethal dose ($LD_{50}$) of PCB® following acute oral toxicities was 3864 mg/kg and 3790 mg/kg in males and females, respectively, with dermal exposure $LD_{50}$ greater than 6000 mg/kg, and lethal concentration on inhalation at 6.0 mg/l (Cheong *et al.*, 2006). Orall ingestation in mice were reported to be more rapid than inhalation, according to a study conducted among several human volunteers who were exposed to PCB® (Yoshida *et al*., 2002) through inhalation. The 1, 4 dichlorobenzene (PCB®) has been shown to be metabolized in the liver by cytochrome P540 system to metabolite 2, 5 dichlorophenol 93, 5-DCP), and excreted through the kidneys (Hawkins *et al*. 1980). The fatal dose to a rat and a mouse is much less than the one that can injure a human, hence suggesting that PCB® is safe for use unless it is abused. Lambda-cyhalothrin toxicity levels have been documented. It is moderately toxic

in the technical form, but may be highly toxic via some routes in formulation such as Karate; Cyhalothrin K; Lambda-cyhalothrin [ICON®] and CCRIS 8987 (Kim *et al.*, 2015). Field studies found no significant adverse effects of lambda-cyhalothrin to fish (Dubey *et al*., 2014).

Available data indicate that lambda cyhalothrin is moderately toxic via the oral route in test animals. Reported oral $LD_{50}$ values are 79 mg/kg and 56 mg/kg for male and female rats, respectively (FAO, 2013). The rat oral $LD_{50}$ has also been reported as 144 mg/kg, although the vehicle used was corn oil. The reported rat $LD_{50}$ for the technical product is 64 mg/kg (Blackman *et al.,* 2015). This indicate moderate acute toxicity via the oral route of exposure. There is no available data regarding the acute toxicity of the technical compound via the inhalation route, but for Karate, the reported 4-hour inhalation $LC_{50}$ were 0.175 mg/L and 0.315 mg/L for female and male rats, respectively (Blackman *et al.*, 2015). These data indicate a moderate to high toxicity via the inhalation route for the formulated product Karate, another name of lambda-cyhalothrin. The technical product has reported dermal $LD_{50}$ of 632 mg/kg and 696 mg/kg for male and female rats - vehicle used was propane-1, 2-diol, (Ali, 2012; Blackman *et al.*, 2015). Since most pyrethroids are generally absorbed only poorly through the skin, the latter two systemic effects are unlikely unless the compound has been ingested. Effects are generally reversible due to rapid breakdown of the compound in the body (Ray & Richards, 2001). Like many compounds of the pyrethroid family, the observed toxicity of lambda cyhalothrin may vary according to not only the concentration of the active ingredient, but also to the solvent vehicle (Colovic *et al.,* 2013; Meister, 1992). The effectiveness of an insecticide

depends on several factors including chemical nature of the molecule, amount of active ingredients used, frequency of applications, time interval between applications, size and age of insect, area and time of contact with the insect (Cinthia *et al*., 2012).

### 5.3 Ethical considerations

Ethical clearance for this study was granted by the KEMRI Scientific Ethical Research Unit (SERU) and the KEMRI animal care and use committee. Safety in handling of the animals was observed during the experiment to minimize pain, suffering and stress.

### 5.4 Materials and methods

Mice weighing between 18-24 grams were acquired from the animal house in KEMRI. There were four experiments on toxicity namely: PCB®, ICON®, PCB®/ ICON® and PBS. Five mice were used for each experiment, making a total of twenty mice. While the first three comprised of insecticides, the PBS acted as a control. All the mice were weighed and their weights recorded. The experiments were carried out in the KEMRI Leishmaniasis laboratory where the mice were being administered with various doses of insecticides according to their body weights. The experimental mice together with the control mice were returned to the animal house where they were observed for a period of six months. Records on behavioural patterns of the animals were taken on regular basis. Measurements and weights of the animals were also taken regularly for each animal. After the stipulated time of the experiment, all the animals were euthanized in $CO_2$ and the biopsies of various body organs weighed and tested for toxicity. The organs that were weighed in grams included: the liver, lungs, spleen and heart. The measurements and weights were used to compare effects of treatments against the control ones.

## 5.5 Results

The toxic effect of PCB®, ICON® and combination on the appearance and the general behavioral pattern of mice are shown in Table 5.1. No toxic symptoms or mortality were observed in the animals. The behavioral patterns of animals were observed first for 6 hours followed by 14 hours after the administration and the animals in all the treatments were normal and did not display significant changes in behavior, skin effects, breathing, impairment in food intake and water consumption, postural abnormalities and hair loss.

Under laboratory conditions, both ICON® and PCB® showed no toxic effects to various organs of mice, which indicated that the two single treatments and the combination of ICON®/PCB® were safe for use. This is because the body weights as well as weights of vital organs of the mice were measured as shown in Table 5.1. There were no significant changes in body weight as ($P=0.118$, $F=2.307$). Comparably, there was no significant difference in weights of organs ($P>0.05$). The results revealed that, the essential organs such as liver, heart, lung and spleen were not adversely affected throughout the treatment. There was no significant diffence in the mean weight of heart from mice treated with PCB® and PBS ($t(3) =0.5157$, $P=0.3208$). Similarly, no significant difference in the mean weight of heart from mice treated with PCB® and ICON® [$t(5) =0.1.5325$, $P=0.093$], and the mean weight of heart from mice treated with PCB® and PCB®/ICON® combination [$t(5) =0.7381$, $P=0.2468$] were observed. The mean weight of heart from mice treated with ICON® was significantly higher than that of mice treated with PBS [$t(3) =95.2399$, $P<0.0001$]. There was no significant difference in the mean weight of lung from mice treated with PCB® and ICON® [$t(5) =0.8615$, $P=0.2142$], the mean weight of

heart for mice treated with PCB® was significantly lower than that of mice treated with PCB®/ICON® combination [t (5) =6.0035, P=0.0009]. The mean weight of the liver for mice treated with PCB® was significantly lower than that of mice treated with PBS [t (3) =0.0689, P=0.4747]. The mean weight of liver from mice treated with ICON® was significantly lower than that of mice treated with PBS [t (3) =2.2082, P=0.0571]. The mean weight of lung from mice treated with ICON® was significantly lower than that of mice treated with PBS [t (5) =7.0054, P=0.0005].

The mean weight of lung for mice treated with PBS was significantly lower than that of mice treated with ICON® [t (5) = 24.983, P<0.0001]. The mean weight of lung for mice treated with PBS was significantly lower than that of mice treated with PCB®/ICON® combination [t (5) =31.206, P<0.0001]. The mean weight of lung from mice treated with PCB® was higher than that of PBS [t (3) =2.3166, P=0.0517]. The mean weight of lung from mice treated with ICON® was higher than that treated with PCB®/ICON® combination [t (5) =0.5533, P=0.3019]. The mean weight of lung for mice treated with ICON® was higher than that of PBS [t (5) =1.7085, P=0.0741]. The mean weight of spleen for mice treated with PCB® was higher than that of ICON® [t (5) =1.3484, P=0.1177]. The mean weight of spleen from mice treated with PCB® was lower than that of PCB®/ICON® combination but there was no significant difference [t (5) = 0.053, P=0.4799]. The mean weight of spleen from mice treated with PCB® was higher than that of PBS but their difference was not significant [t (5) =1.2336, P=0.1361]. The mean weight of spleen for mice treated with ICON® was lower than that of PCB®/ICON® combination but not significantly different [t (5) = 0.6032, P=0.2863]. The mean weight

of spleen for mice treated with ICON® was higher than that of PBS but not significantly different [t (5) =0.3912, P=0.3559].

**Table 5.1: Mean weights of body and vital organs of mice treated with ICON®, PCB® and their combination**

| Treatment | Mean weights (g) | | | | |
|---|---|---|---|---|---|
| | Body | Liver | Lungs | Spleen | Heart |
| **PCB®** | 23.47±1.47874 | 1.293±0.10148 | 0.208±0.01014 | 0.1116±0.0128 | 0.23±0.08358 |
| **ICON®** | 23.4±0.853229 | 1.208±0.1025 | 0.2116±0.0246 | 0.098±0.00749 | 0.125±0.0847 |
| **PCB®/ICON®** | 25.233±0.695 | 1.25±0.10175 | 0.2016±0.013 | 0.113±0.021855 | 0.153±0.0055 |
| **PBS** | 24.4±1.71075 | 1.36±0.13472 | 0.175±0.015 | 0.1325±0.0111 | 0.1475±0.002 |

### 5.6 Discussion

According to the recently reported cases of PCB® toxicity due to mothball ingestion, the insecticides were investigated on vital organs including the liver, kidney, lungs and the spleen. Under laboratory conditions, both ICON® and PCB® showed no toxic effects of various organs of mice, which indicates that the two and the combination of ICON®/PCB® are safe for use. This experiment has demonstrated that mice treated with PCB® and ICON® independently reduced mean body weights in comparison with the PBS but their combination treated mice had mean weight above those of the control (PBS treated). This may suggest that combinations might have some minimal toxic effect as compared to individual products. The liver mean weights of the mice treated with PBS

had a higher value than that of ICON® and PCB® while the lungs, spleen and the heart had mean weight values higher than the PBS values. Although these mean weight values slightly vary with the placebo the difference was of no significance. The PBS treated mice recorded higher mean body weight (24.4g±1.71075), spleen (0.1325g±0.0111) and the liver (1.36g±0.13472). The lungs had mean weight of 0.2116g±0.0246 after treatment with ICON® and heart with 0.23g±0.08358 after treatment with PCB®. This concurs with the previous study by Schlenk *et al.,* (2001), which showed that, ICON® and PCB® were not toxic under laboratory conditions. This shows that ICON® and PCB® are safe for use as indicated by Hawkins *et al*., (2003) and Schlenk *et al.*, (2001), respectively.

# CHAPTER SIX: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

## 6.1 General discussion

This study utilized several approach in order to control the vectors of malaria and leishmaniasis which pose serious health problems to humans worldwide (WHO, 2017). The study involved laboratory experiments to test the concept of individual and combined insecticide treatments of ICON®, PCB® and ICON®/PCB®. The success of the laboratory work was followed by field activities in two areas that are endemic for leishmaniasis and malaria in Marigat sub-County, Baringo County and Mwea Irrigation Scheme, Kirinyaga County, respectively. The proof of concept not only worked in the laboratory but also produced good results in the field by killing and expelling the vectors from houses. Vector densities reduced by 80% after intervention with application of the test insectides and their combination. Synergistic effects were also found to produce remarkable performance to control the disease vectors as compared to the individual treatments. Nevertheless, even the individual insecticides controlled the vectors with varying levels of success hence can be used in their control.

Over the years, researchers have demonstrated that effectiveness of repellents over several hours can be enhanced by synergizing the repellent with a base or fixative materials such as vanillin, mothballs lotion, coconut oils, among others (Das *et al*., 2003). However, the effectiveness of the repellents depends on multiple factors including the type of repellents (active ingredients), formulation, mode of application, environmental factors (temperature, humidity and wind), the attractiveness of individual people to

insects, loss due to removal by perspiration and abrasion, the sensitivity of the insects to repellents, and the biting density (Singha and Chandra, 2011; Govindarajan *et al*., 2011; Ahmad *et al*., 2011).

Survival of arthropod vectors is one of the most important components of transmission of vector-borne pathogens (Reisen *et al*., 1980; Macdonald, 1956). Increased survival allows the vector to produce more offspring, to increase the chances of them becoming infected, to disperse over greater distances, to survive long enough to become infectious, and then to deliver more infective bites during their lifetime. As a result, small changes in survival rate cause large changes in the rate of pathogen transmission (Macdonald, 1956; Parham and Michael, 2009; Barbazan *et al.,* 2010; Smith *et al.*, 2012; Lunde *et al.*, 2013).

In this study there was an increase in mortality of mosquitoes from day one of treatment to 24 hours and steady increase in mortality to 48 hours for all treatments. A high number of mosquitoes exposed to ICON® survived as compared to PCB® but the mean difference was not significant. However, there was high mortality rates observed in mosquitoes exposed to combination than PCB® and ICON® alone. Conversely, there was a steady decrease in survival of mosquitoes from the first day of treatment to 48 hours for all treatments. High mortality of mosquitoes were observed when exposed to PCB® as compared to ICON® but the mean difference was not significant. However, there was high mortality of mosquitoes which were exposed to PCB®/ICON® as compared to PCB® and ICON® independently and their mean difference was significant. The high mortality of mosquitoes exposed to a combination of ICON® and PCB® at a dose of 40mg and

60mg, respectively shows that this could be a better way of controlling *A. gambiae* and probably other mosquito species. The increase of the mortality rates from 1st hour and full mortality rates in 24 hours shows that the slow release of the two insecticides facilitates the death of the insects. The mortality of sand flies exposed to ICON®/PCB® combination was lower than those exposed to ICON® and PCB® singly and this could mean that mechanisms of insecticidal detoxification differs between sand flies and mosquitoes. Mosquitoes and sand flies' mortality rates were high on treatment with ICON®/PCB® combination in the laboratory set up experiments.

The formulations or dosages differed only by causing mortalities. The higher mortality obtained with the combined ICON®/PCB® might be due to the higher concentration of the insecticide exposed to the mosquitoes and sand flies in the treatment cages. The decline in mortality rates in the other treatments could be due to the lower concentration of the dosage of the treatment when used singly. The findings of this study concurs to other similar studies carried out in Tanzania on the control of vectors of malaria using combinations of several insecticides (Okumu and Moore, 2011; Killeen *et al*., 2017). Considering the duration of exposure to the treatments of lambda-cyhalothrin and PCB® on blood-feeding success, repellency, fecundity and mortality dosages of 40mg/$m^2$ and 60mg/$m^2$, lambda-cyhalothrin can be considered for use as a powerful control tool for malaria and leishmaniasis vectors.

In this study sand flies were exposed to ICON® and PCB® and the combination of the two for three months (immediate exposure) and compared to long time exposure (6

months). In Mwea exposure of sand flies to ICON® significantly reduced their densities unlike exposure to PCB® and ICON®/PCB® combination. This study therefore suggests that ICON® was effective on sand flies in Mwea site after exposure for a short period. However, the Mwea study gave contrary findings with that of Baringo site, where ICON®/PCB® combination significantly reduced the sand fly densities as compared to ICON® and PCB® alone. It has been shown that temperature affects the activity of lambda-cyhalothrin. The efficacy of ICON® and PCB® can be attributed to geographical location since Mwea and Baringo are two areas that have different climatical conditions. This necessitates further studies on how climatical conditions affect the efficacy of the two insecticides. Furthermore, exposure of sand flies to ICON® and PCB® for six months had a significant decrease in their densities. In comparison with the three months' exposure, this study suggests that the efficacy of ICON® and PCB® is dependent on the length of exposure in time.

There was a significant decrease in mosquito densities after exposure to ICON® and ICON®/PCB® combination for three months, however there was no significant decrease in their densities when exposed to PCB® alone. This observation was also seen during the 6 months' exposure. It is therefore suggested that PCB® alone cannot effectively control mosquitoes but can synergize with ICON® to be effective. In Mwea there was a significant decrease in mosquito densities after exposure to ICON®, PCB® and their combination both in immediate and long-term exposure. This indicates that in Mwea site, PCB® and ICON® were able to control mosquitoes individually as well as when they were combined. This is a peculiar finding that needs further investigations on

geographical location, climatical differences and probably the genetic material of the mosquitoes in the two sites. An appropriate vector population sampling technique is required in the prediction of vector-borne disease outbreaks and determination of when and where to apply control measures to prevent/suppress such outbreaks.

The *P. martini* and *P. rodhaini* contributed to 4.8% and 0.8 %, respectively of the total collection and the rest were from *Sergentomyia* species. In Sudan, P. *orientalis* is the vector for VL whereas in Kenya, *P. martini* is the main vector for VL. Currently, the identification of phlebotomine sand flies is based on morphological characterstics. However, morphological identification requires considerable skills and taxonomic expertise. In addition, significant morphological similarities between some species, especially among females, may cause difficulties during the identification process. The DNA-based approaches have become increasingly useful and promising tools for estimating sand fly diversity and for ensuring the rapid and accurate identification of species. A partial sequence of the mitochondrial cytochrome oxidase gene sub-unit I (COI) is currently being used to differentiate species in different animal taxa, including insects, and it is referred to as barcoding sequence. The current study exploited the comparisons of HRM profiles from both loci for more robust species differentiation and identification. In this study HRM validation results (Fig 4.10) show high sensitivity in identifying both *P. martini* and sand fly sample B from Mwea site, however PCR-HRM could not distinguish between species within sand fly samples A and C and furthermore their relationship with *P. martini* species.

Oral acute toxicity testing in mice could be used to evaluate natural remedies for different pharmacological activities, taking into account the basic premise that pharmacology is simply toxicology at a lower dose (Sasidharan *et al*., 2008). A toxic substance might elicit pharmacological effects at a lower non-toxic dose. The 1, 4 dichlorobenzene (PCB®) is an active constituent of mothballs, deodorizers and fumigants. There is a high risk for accidental or intentional toxic exposure because the chemical is easily availabile. There have been multiple reported cases of PCB® toxicity due to mothball ingestion. The 1, 4 dichlorobenzene (PCB®) toxicity can affect multiple organ systems including liver, kidney, skin, lung and the central nervous system (CNS) when misused (Dubey *et al*., 2014). The 1, 4-dichlorobenzene (PCB®) can be absorbed through ingestion, inhalation or through contact with the skin. In mice, the oral route was found to be more rapid than inhalation in a study conducted among several human volunteers who were exposed to PCB® through inhalation (Yoshida *et al*., 2002).

Under laboratory conditions, both ICON® and PCB® showed no toxic effects of various organs of mice, which indicates that the two and the combination of ICON®/PCB® are safe for human use. This is because PCB® in humans chewing toilet bowel deodorizing cakes consist of 99.9% PCB® that are toxic when ingested and cause anxiolytic effect (Gonzalez *et al.*, 2016). It was reported that the median lethal dose ($LD_{50}$) of PCB® following acute oral toxicities in rats were 3864 mg/kg and 3790 mg/kg in males and females, respectively (Cheong *et al.,* 2006) and $LD_{50}$ was greater than 6000 mg/kg for dermal exposure with lethal concentration on inhalation at 6.0 mg/l (Elhalwagy *et al.*, 2015). In mice the oral route was reported to be more rapid than inhalation in a study

conducted among several human volunteers who were exposed to PCB® (Dubey *et al.*, 2014). The PCB® has been shown to be metabolized in the liver by cytochrome P540 system to metabolite 2, 5 dichlorophenol 93, 5-DCP, and excretion through the kidneys (Elhalwagy *et al.*, 2015). The dose fatal to a rat and a mouse is much less than the ones that can injure a human, suggesting that PCB® can be safe for use unless it is abused.

Lambda-cyhalothrin toxicity levels have been documented. Recorded data show that lambda cyhalothrin is moderately toxic through the oral route in test animals. Reported oral $LD_{50}$ values are 79 mg/kg and 56 mg/kg for male and female rats, respectively (Ali, 2012; Blackman *et al.*, 2015). The oral $LD_{50}$ has also been reported as 144 mg/kg for tha rats. The reported $LD_{50}$ for the technical product among the rats is similar, 64 mg/kg (Blackman *et al.*, 2015). These indicate moderate acute toxicity via the oral route of exposure.

### 6.2 Conclusions

Lambda-cyhalothrin and 1, 4 dichlorobenzene alone or in combination can be used safely for effective control of indoor-feeding sand fly and mosquitoes which are vectors for human diseases. There were synergistic effects observed when the two insecticides were used in combination and this demonstrated that the two can complement each other. It is anticipated that the cubes developed for vector control which are cost effective, feasible, fragrant friendly and protective, will provide control of leishmaniasis and malaria in endemic areas. This study observed that one of the insecticides used performed better in Marigat study site as compared to the same insecticide in Mwea sites.

The Mwea *Phlebotomus martini* sand flies were related to *Phlebotomus martini* known species. This shows that there could be other unknown/ undocumented breeding sites of sand fly vectors.

The current study has shown that ICON® and PCB® were not toxic to mice and this implies that they can be used safely in the control of malaria and sand fly vectors in houses/human settings.

### 6.3 Recommendations

- ✓ The combination of lambda-cyhalothrin and 1, 4 dichlorobenzene can be used to control mosquitoes and sand flies in malaria and leishmaniasis endemic regions in Kenya. The efficacy of the two test insectides needs to be availed to the policy makers so that the necessary steps can be taken to produce the insecticides for public use. Formulations of the blocks can be prepared with active ingredient levels that are environmentally friendly and non-toxic to humans.
- ✓ Further investigation is warranted on the effects of geographical location, climatic differences and probably the genetic material of the mosquitoes in the two sites.
- ✓ More studies on vectors of leishmaniasis including potential transmission in Mwea irrigation scheme are encouraged.
- ✓ Further studies need to be conducted to evaluate the effectiveness of ICON® and PCB® on other arthropod vectors in Kenya.

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# APPENDICES

## Appendix I: Informed consent form

This information was communicated to household heads orally in English, Swahili or other Kenyan dialect of potential participant's preference.

**Project title:** "Evaluation of lambda-cyhalothrin (ICON) incorporated into air fresheners and disinfectant blocks for the control of insect vectors for malaria and leishmaniases"

**Introduction:** My name is Josyline Cirindi Kaburi, a research officer from Kenya Medical Research Institute (KEMRI). I am with my research team to conduct a study on determining the efficacy and effectiveness of lambda-cyhalothrin, as an insecticide in control of malaria and leishmaniasis. Your area has been selected for study being a major endemic place for malaria/leishmaniasis disease(s) and I'm pleased to carry out this field study together with you. I would like to seek your permission, please read this consent form below.

**Procedure to be followed:** Houses will be selected randomly from the villages within Mwea and Marigat division. If your house has been selected for mosquito / sand fly trapping and you give consent, you will be visited by a field worker from your village and explained the purpose of the study in a language you can understand. If you agree to participate you will be required to sign the consent form, you will however be consulted a day before the activity so that you may plan with the Principal Investigator.

In addition, you will be assigned a special number to identify you and your household members. Traps will be set at 6.00 p.m and collected before 8.00 a.m.

**Risk:** There are no major risks involved in the proposed project. Lambda-cyhalothrin has no smell and irritating effects. However, for the mosquito collection in the houses, the

light trap may cause a little buzzing noise but this is not loud enough to distract your attention or cause you not to sleep. The study methodology that will be used has been used for trapping mosquitoes and sand flies in several studies without any recorded risks, hence no risks anticipated in the proposed study.

**Benefits:** The collection of the mosquitoes by the light trap method reduces the indoor mosquito and sand fly numbers. It also reduces the biting rates of endophagous and endophilic mosquitoes and sand flies that are known disease vectors. In addition, the houses which will be treated with lambda-cyhalothrin, we expect reduction of mosquitoes, sand flies and other pests like cockroaches, bedbugs, spiders for a period of 6 months. You will immediately benefit from reduced bites by the vectors and a reduction of other insect pests in your house.

**Confidentiality of the records:** Any information gathered in this study will be kept under confidentiality. Your names and of the household members will only be known to the PI and Co PI and will not be made publicly available and no other researcher will be able to associate your name or those of your household members with the data collected. At the end of the study, all names will be destroyed and will not be revealed in any publication.

**Obtaining additional Information:** Participants are encouraged to ask questions to get more information regarding this project. You will be notified of any new information developing in the course of research, if any, that might affect their participation. If you need further clarification with any matter of the project, please you may consult your area chief or the Ministry of health.

**Basis of participation:** You are free to withdraw your consent to participate in this study at any time and you will not be penalized by the PI. You will be allowed to re-enter at a later date if you so wish. You will be required to respond to questionnaires.

I state that I have read the information stated above, that I am over 18 years of age, in good physical health and wish to participate in a program of research being conducted by Josyline Kaburi in the leishmania section of the Centre for Biotechnology Resaerch and Development, KEMRI, Nairobi, P.O. Box 54840, 0200. Kenya. I understand that I am free to ask questions or to withdraw from participation at any time without penalty.

**For questions about the study, contact**:

Mrs. Josyline Kaburi

Research Officer, Leishmania section-CBRD

KEMRI

P.O. Box 54840

Nairobi 00200

Tel: (02) 722541 Fax: (02) 715105, email: jcirindi@kemri.org; jcirindi@yahoo.com

**Appendix II: Consent form for householders/household heads**

1, _____ (Name of parent/guardian) being the lawful head of this house hold do hereby give my consent to participate in the research project titled: **Evaluation of lambda-cyhalothrin (ICON®) incorporated into 1, 4-dichlorobenzene (PCB®) for the control of leishmaniases and malaria vectors,**

I have been explained the contents of this study and I have had the' opportunity to read the consent information for the study and the opportunity to ask questions concerning. this project. All the questions have been answered to my full satisfaction. I therefore consent to participate in the study and as a show of approval I have signed on the space provided below. Should any further questions arise concerning the study, I may contact (Josyline C. Kaburi, Dr Philip Ngumbi and Dr Chris Anjili, KEMRI, CBRD, P.O Box 54*840*-00200, Telephone: No. 2731192/3 or Prof J. M. Lucy Irungu and Prof Paul Ndegwa, University of Nairobi or your local area chief or the local MOH).

I also understand that I may revoke this consent at any time without penalty or loss of Benefits, if any.

Printed Name of Subject _____

Signature of Subject _____

Date _____

Printed Name of Researcher_____

Signature of Researcher _____

Date _____

Printed Name of Witness_____

Signature of Witness _____

Date _____

**Appendix III: Gum-chloral mountant**

**Puri's medium**

1) Distilled water …….10mls
2) Gum acacia (powder)…….8gms
3) Chloral hydrate (crystals) ……70gms
4) Glycerine ……..5mls
5) Glacial acetic acid …….....3mls

Ingredients should be dissolved in the above order at room temperature. A magnetic stirer may be used to help in mixing them well. Filter the fluid through cotton wool. After Minter: *Bulletin of Entomological Research,* 54: 483 (1963).

**Appendix IV: Standard operating procedure for preparation of solutions used in sand fly dissections**.

**Solution B**

These sand fly dissection solutions have a short shelf-life and therefore need to be prepared a fresh after every month.

**Formular for solution B: (Soak solution)**

1) Combine the following ingredients adding them to 111.8mls of sterile water.
2) Measure and add 6mls of freshly mixed Penicillin-Streptomycin solution.
3) Measure and add 1.2mls Gentamycin of sulfate (50mg/ml)
4) Weigh and add 60mg of 5-Flurocytosine.
5) Weigh and add 1ml (12mg of Amph-B powder in 20ml sterile water) of Amphotericin B.
6) Filter through 0.22-micron filter and put in a sterile bottle, add a label with the following information: Solution B, Soak solution, Date prepared, and your initials.
7) Store in a refrigerator for use in dissections of sand flies.

**Solution D.**

**Formular for Solution D: (Dissection solution)**

1) Combine the following ingredients adding them to 111.8mls of sterile saline.
2) Measure and add 6 mls of freshly mixed Penicillin-Streptomycin solution.
3) Measure and add 1.2ml of Gentamycin sulfate (50mg/ml).
4) Weigh and add 60mg of 5-Flurocytosine.
5) Filter through sterile 0.22-micron filter and put in sterile bottle(s).
6) Label bottle, Solution D, dissecting solution, date prepared and your initials.
7) Store the bottle (s) in a refrigerator for use during sand fly dissections.

**Appendix V: Raw data on number of mosquitoes and sand fly catches in marigat and mwea sites before and after interventions**

Pre- and post-intervention sand fly and mosquito catches at Marigat study site

| H/H# | Pre-intervention | | Treatment | Post-treatment1 Immediate | | Post-treatment 2 3-6 months | |
|---|---|---|---|---|---|---|---|
| | SF | MSQ | TT | SF | MSQ | SF | MSQ |
| 1. | 201 | 137 | PCB/ICON | 3 | 32 | 23 | 71 |
| 2. | 216 | 19 | PCB/ICON | 2 | 12 | 0 | 17 |
| 3. | 197 | 36 | PCB/ICON | 16 | 14 | 20 | 25 |
| 4. | 499 | 27 | ICON | 4 | 1 | 7 | 19 |
| 5. | 404 | 16 | PCB/ICON | 10 | 16 | 24 | 8 |
| 6. | 50 | 46 | PCB | 13 | 40 | 7 | 35 |
| 7. | 3 | 28 | ICON | 18 | 10 | 3 | 16 |
| 8. | 176 | 11 | PCB/ICON | 12 | 7 | 18 | 6 |
| 9. | 86 | 63 | ICON | 8 | 4 | 16 | 61 |
| 10. | 110 | 73 | ICON | 51 | 39 | 48 | 23 |
| 11. | 1 | 12 | PCB | 14 | 11 | 3 | 9 |
| 12. | 24 | 11 | PCB | 16 | 9 | 7 | 2 |
| 13. | 51 | 24 | ICON | 6 | 3 | 3 | 8 |
| 14. | 173 | 53 | PCB | 8 | 31 | 6 | 45 |
| 15. | 66 | 9 | PCB | 40 | 5 | 47 | 5 |
| 16. | 15 | 241 | PCB | 0 | 21 | 2 | 18 |
| 17. | 6 | 35 | ICON | 0 | 18 | 7 | 13 |
| 18. | 4 | 199 | PCB/ICON | 0 | 24 | 0 | 22 |
| 19. | 0 | 440 | PCB/ICON | 0 | 34 | 0 | 26 |
| 20. | 6 | 260 | PCB | 0 | 43 | 1 | 38 |
| 21. | 2 | 1386 | PCB | 1 | 31 | 0 | 32 |
| 22. | 0 | 144 | ICON | 0 | 5 | 0 | 6 |
| 23. | 0 | 40 | PCB/ICON | 0 | 0 | 0 | 13 |
| 24. | 1 | 14 | PCB | 0 | 2 | 0 | 2 |
| 25. | 0 | 55 | ICON | 0 | 0 | 1 | 17 |
| 26. | 0 | 22 | PCB/ICON | 1 | 7 | 0 | 0 |
| 27. | 1 | 14 | ICON | 0 | 6 | 0 | 7 |
| 28. | 3 | 114 | PCB | 1 | 42 | 0 | 27 |
| 29. | 3 | 238 | PCB/ICON | 1 | 15 | 0 | 13 |
| 30. | 3 | 165 | ICON | 2 | 3 | 0 | 4 |
| N | 30 | 30 | | 30 | 30 | 30 | 30 |
| Sum | 2,301 | 3,932 | | 227 | 485 | 243 | 588 |
| Mean | 76.7 | 131.1 | | 7.6 | 16.2 | 8.1 | 19.6 |
| Range | 0-499 | 9-1,386 | | 0-51 | 0-43 | 0-48 | 0-71 |

Pre- and post-intervention sand fly and mosquito catches at Mwea study site

| H/H# | Pre-intervention | | Treatment | Post-treatment1 Immediate | | Post-treatment 2 3-6 months | |
|---|---|---|---|---|---|---|---|
| | SF | MSQ | TT | SF | MSQ | SF | MSQ |
| 1. | 4 | 181 | PCB | 0 | 74 | 0 | 0 |
| 2. | 0 | 39 | PCB | 0 | 20 | 0 | 17 |
| 3. | 3 | 308 | PCB/ICON | 0 | 104 | 1 | 90 |
| 4. | 0 | 4 | PCB/ICON | 3 | 47 | 0 | 40 |
| 5. | 12 | 272 | PCB | 0 | 58 | 0 | 37 |
| 6. | 3 | 87 | ICON | 0 | 9 | 0 | 6 |
| 7. | 2 | 76 | PCB/ICON | 0 | 57 | 0 | 32 |
| 8. | 0 | 71 | ICON | 0 | 47 | 1 | 28 |
| 9. | 6 | 154 | PCB | 0 | 16 | 0 | 15 |
| 10. | 7 | 159 | ICON | 0 | 43 | 0 | 27 |
| 11. | 0 | 260 | ICON | 0 | 11 | 0 | 9 |
| 12. | 3 | 247 | PCB/ICON | 0 | 34 | 0 | 7 |
| 13. | 1 | 246 | ICON | 0 | 127 | 0 | 44 |
| 14. | 10 | 50 | PCB/ICON | 0 | 3 | 0 | 14 |
| 15. | 4 | 63 | PCB | 0 | 23 | 0 | 8 |
| 16. | 6 | 46 | PCB/ICON | 19 | 2 | 21 | 25 |
| 17. | 7 | 72 | PCB/ICON | 30 | 4 | 20 | 8 |
| 18. | 4 | 63 | PCB | 0 | 23 | 0 | 8 |
| 19. | 19 | 72 | ICON | 5 | 1 | 5 | 7 |
| 20. | 226 | 12 | ICON | 86 | 9 | 7 | 14 |
| 21. | 133 | 3 | PCB | 46 | 8 | 3 | 7 |
| 22. | 161 | 37 | ICON | 5 | 26 | 64 | 17 |
| 23. | 58 | 38 | PCB | 10 | 20 | 18 | 24 |
| 24. | 47 | 17 | PCB | 47 | 22 | 5 | 0 |
| 25. | 98 | 2 | PCB | 10 | 0 | 13 | 7 |
| 26. | 184 | 79 | ICON | 36 | 64 | 27 | 13 |
| 27. | 893 | 28 | PCB/ICON | 6 | 17 | 17 | 11 |
| 28. | 244 | 7 | ICON | 3 | 15 | 8 | 5 |
| 29. | 378 | 38 | PCB/ICON | 10 | 8 | 54 | 25 |
| 30. | 76 | 14 | PCB/ICON | 172 | 8 | 10 | 11 |
| N | 30 | 30 | | 30 | 30 | 30 | 30 |
| Sum | 2,589 | 2,745 | | 488 | 900 | 274 | 556 |
| Mean | 86.3 | 91.5 | | 16.3 | 30 | 9.1 | 18.5 |
| Range | 0-893 | 0-308 | | 0-172 | 0-127 | 0-64 | 0-90 |

H/H#, Homestead House Number. SF, Sand fly. MSQ, Mosquito. TT, Treatment.

MARIGAT SITE

| H/H# | Pre-treatment | | | Treatment | Immediate Effects | | Long term 3-6 months | |
|---|---|---|---|---|---|---|---|---|
| | MSQ | SF | | TT | MSQ | SF | MSQ | SF |
| 1. | 137 | 201 | | PCB/ICON | 32 | 3 | 71 | 23 |
| 2. | 19 | 216 | | PCB/ICON | 12 | 2 | 17 | 0 |
| 3. | 36 | 197 | | PCB/ICON | 14 | 16 | 25 | 20 |
| 8. | 11 | 176 | | PCB/ICON | 7 | 12 | 6 | 18 |
| 5. | 16 | 404 | | PCB/ICON | 16 | 10 | 8 | 24 |
| 18. | 199 | 4 | | PCB/ICON | 24 | 0 | 22 | 0 |
| 19. | 440 | 0 | | PCB/ICON | 34 | 0 | 26 | 0 |
| 23. | 40 | 0 | | PCB/ICON | 0 | 0 | 13 | 0 |
| 26. | 22 | 0 | | PCB/ICON | 7 | 1 | 0 | 0 |
| 29. | 238 | 3 | | PCB/ICON | 15 | 1 | 13 | 0 |
| | 1158 | 1201 | 161 | 45 | 201 | 85 | | |
| 6. | 46 | 50 | | PCB | 40 | 13 | 35 | 7 |
| 11. | 12 | 1 | | PCB | 11 | 14 | 9 | 3 |
| 12. | 11 | 24 | | PCB | 9 | 16 | 2 | 7 |
| 14. | 53 | 173 | | PCB | 31 | 8 | 45 | 6 |
| 15. | 9 | 66 | | PCB | 5 | 40 | 5 | 47 |
| 16. | 241 | 15 | | PCB | 21 | 0 | 18 | 2 |
| 20. | 260 | 6 | | PCB | 43 | 0 | 38 | 1 |
| 21. | 1386 | 2 | | PCB | 31 | 1 | 32 | 0 |
| 24. | 14 | 1 | | PCB | 2 | 0 | 2 | 0 |
| 28. | 114 | 3 | | PCB | 42 | 1 | 27 | 0 |
| | 2146 | 341 | | | 235 | 93 | 213 | 73 |
| 4. | 27 | 499 | | ICON | 1 | 4 | 19 | 7 |
| 7. | 28 | 3 | | ICON | 10 | 18 | 16 | 3 |
| 9. | 63 | 86 | | ICON | 4 | 8 | 61 | 16 |
| 10. | 73 | 110 | | ICON | 39 | 51 | 23 | 48 |
| 13. | 24 | 51 | | ICON | 3 | 6 | 8 | 3 |
| 17. | 35 | 6 | | ICON | 18 | 0 | 13 | 7 |
| 22. | 144 | 0 | | ICON | 5 | 0 | 6 | 0 |
| 25. | 55 | 0 | | ICON | 0 | 0 | 17 | 1 |
| 27. | 14 | 1 | | ICON | 6 | 0 | 7 | 0 |
| 30. | 165 | 3 | | ICON | 3 | 2 | 4 | 0 |
| | 628 | 759 | | | 89 | 89 | 174 | 85 |

Legend: H/H# =Homestead House Number, SF= Sand fly, MSQ= Mosquito & TT= Treatment

Table 4.4 Vector densities before and after treatment in Mwea study site

MWEA SITE

| H/H# | Pre-treatment | | | Treatment | Immediate Effects | | Long term 3-6 months | |
|---|---|---|---|---|---|---|---|---|
| | MSQ | SF | | TT | MSQ | SF | MSQ | SF |
| 1. | 181 | 4 | | PCB | 74 | 0 | 0 | 0 |
| 2. | 39 | 0 | | PCB | 20 | 0 | 17 | 0 |
| 5. | 272 | 12 | | PCB | 58 | 0 | 37 | 0 |
| 9. | 154 | 6 | | PCB | 16 | 0 | 15 | 0 |
| 15. | 63 | 4 | | PCB | 23 | 0 | 8 | 0 |
| 18. | 60 | 5 | | PCB | 10 | 0 | 9 | 0 |
| 21. | 3 | 133 | | PCB | 8 | 46 | 7 | 3 |
| 23. | 38 | 58 | | PCB | 20 | 10 | 24 | 18 |
| 24. | 17 | 47 | | PCB | 22 | 0 | 0 | 5 |
| 25. | 2 | 98 | | PCB | 0 | 3 | 7 | 13 |
| | 829 | 367 | 251 | 59 | 124 | 39 | | |
| 3. | 308 | 3 | | PCB/ICON | 104 | 0 | 90 | 1 |
| 4. | 54 | 0 | | PCB/ICON | 47 | 3 | 40 | 0 |
| 7. | 76 | 2 | | PCB/ICON | 57 | 0 | 22 | 0 |
| 12. | 247 | 3 | | PCB/ICON | 34 | 0 | 7 | 0 |
| 14. | 50 | 10 | | PCB/ICON | 3 | 0 | 14 | 0 |
| 16. | 46 | 6 | | PCB/ICON | 2 | 19 | 25 | 21 |
| 17. | 72 | 7 | | PCB/ICON | 4 | 30 | 8 | 20 |
| 27. | 28 | 893 | | PCB/ICON | 17 | 6 | 11 | 17 |
| 29. | 38 | 378 | | PCB/ICON | 8 | 10 | 25 | 54 |
| 30. | 14 | 76 | | PCB/ICON | 8 | 172 | 11 | 10 |
| | 933 | 1378 | | | 284 | 240 | 253 | 123 |
| 6. | 87 | 3 | | ICON | 9 | 0 | 6 | 0 |
| 8. | 71 | 0 | | ICON | 47 | 0 | 28 | 1 |
| 11. | 260 | 0 | | ICON | 11 | 0 | 9 | 0 |
| 13. | 246 | 1 | | ICON | 127 | 0 | 44 | 0 |
| 19. | 72 | 19 | | ICON | 1 | 5 | 7 | 5 |
| 20. | 12 | 226 | | ICON | 9 | 86 | 14 | 7 |
| 22. | 37 | 161 | | ICON | 26 | 5 | 17 | 64 |
| 26. | 79 | 184 | | ICON | 64 | 36 | 13 | 27 |
| 28. | 7 | 244 | | ICON | 15 | 3 | 5 | 8 |
| 10. | 159 | 7 | | ICON | 43 | 0 | 27 | 0 |
| | 1030 | 845 | | | 352 | 135 | 170 | 112 |

Legend: H/H# =Homestead House Number, SF= Sand fly, MSQ= Mosquito & TT= Treatment

**Appendix VI: Sequences**

>Samples AUNIMINIBAR

GAAGTCGAGCGTCTTGCGTTCGTAACTTTGTGAGACGCCAATTTCTTTCC

CCTTACCCTCCTTTTCTCCCTCTACTATTCTTCCCTATATCCTTCTTTCA

ACCCCCACTTCAAAATAAATCTTTCCTGCCAATGTCCTTATGATTATTAT

AATCTTATCATTTTCTTATTTAAGCTCCTAGAAATGCAGATCATAATCCC

CCCCTATAATATTAAGTTCCATTATCCTTATTATTCCTCCCACCAATTAA

ACCAAATTCCACCTTCTCCCCCCCTTCCCCCCTTTTTTTTCTCCAAACCC

CTTTAAACCCTCCCTTTTTTTCCAATTACACTCTTCCCCCCCCTCCTCTC

AACTCCCCCCCACACCCCCATCTCTTACTCCCCTTTTTTTTCCCCTTTTT

TCATTATTCCCTTTTTTACCCCCTCTTATCCCCCCCTTCCCCCCCCCCCC

CCCCCCCTCCCCCCCCCCCCCCCCACCCCACCCCCTTTTCCCCTTCCCCCT

TTT

>Samples B UNIMINIBAR

CCGACATCTACAGTGCCTTAGTAATGTGTAGAGAGCGAAGAAATTTTGCC

CCCTCCCCCTATTTTTCCCCCCAAAAAATTTCCTCTTTAATCCTCTTTGA

AGCCCCCAATTTAAATAGGTAATCCTACCGCCCCATGCTTTTGTGATTTT

TAAATATTATCTTTCTCCTTATTTTTCTTCAATATTTAAAACCTTATTCC

CCCCAAATACCAAAACCCCCCAAACCCTTTTCCCCAAAATTAAACAAAAT

TCCACTTCCCCCCCTTTTCCCCTTTTTTTTTCAAACACTTTAAAAACACC

ATTTCAGCAGAACACTCTTCCCCCCCCTTCCCCAATAATAACCACAAAAA

TCCCATCTATCATCCACCTTTTTCTACCTTTTTTATCATTTTTCAATTTT

TTTAACATCAATTTTTTAAAAAAATAAAAAAACAAGTACCCCCCCCAAAA

AATTTTTTTCTTTTTTAAAAAAAAAAAAAAAAAAAAAAAAAATTTCTTTT

TTTTCCAAAACCCCCCAAACCC

>Samples C UNIMINIBAR

GCGATGTTGTGGAGTTTGGTAGGGTGTATGAATAGTTTATACGCCGCCCT

CCTTCTTCTCGCTCACGATGCTTCCCTGTGTCCCTTTCTTGCCACCACCA

GCTTCCGAAATGTAATCGTACGGCCGATATCTTTAGGATTGGTATAATAT

TTCTTTCCTTTTCTTTTTTTTCCTTCTGATTTTTATATTTATTATTCCCC

TTTTTTCTCATACCCCCTTCTATTCCCTTTCCCCCCAAACTTATACCATT

TTTCCTTCTTCCCCTTCTTTTCCCTCTTTTTTTTACAAACCCTTTAAACTC

ACCTTTATCCCCTATACTTTTTTCCCCCCCTTTCCCTTATTTTCCCCCAA

TTATCCCCCTTTTCTTCCCTCTTTTTCTCCCTTTTCTTCATTTTTTCCTT

TTTCTACTCTTCTTTTTTTATTTTTTTTTTTTTTTTTTTTTTTTTTTTT

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**Appendix VII: List of publications from this study.**

**Peer reviewed Journals**

1. Kaburi Josyline, Ngumbi Philip, Irungu Lucy, Ndegwa Paul, Ingonga Johnstone, Bernard Osero, Dickson Libendi and Anjili Christopher (2018). Synergestic effects of Lambda-cyhalothrin incorporated into 1, 4-dichlorobenzene for the control of sand fly and mosquito vectors in Baringo and Kirinyaga Counties, Kenya. Asian Journal of Biological and Life Sciences, vol. 7(1): 1-27
2. Josyline Kaburi, Philip Ngumbi, Lucy Irungu, Paul Ndegwa, Johnstone Ingonga, Bernard Osero and Christopher Anjili (2018). Discovery of Phlebotomus species of sand flies in Mwea Irrigation Scheme, Kirinyaga County, Kenya: A possible leishmaniasis focus. Annals of Clinical Cytology and Parasitology, 4(3):1103
3. Kaburi Josyline, Ngumbi Philip, Irungu Lucy, Ndegwa Paul, Ingonga Johnstone, Ruttoh Reuben, Bernard Osero, and Anjili Christopher (2018). Effects of Lambda- cyhalothrin incorporated into 1,4-dichlorobenze on sand fly and mosquito vectors in endemic areas of Kenya; Journal of Zoological Research, vol. 2(1): 7-12

## Appendix VIII: Turnitin Report