EVALUATION OF THE REPELLENCY EFFECTIVENESS OF LINALOOL AND METOFLUTHRIN AGAINST *ANOPHELES GAMBIAE* S.L. IN KISIAN VILLAGE, WESTERN KENYA

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ABSTRACT

Malaria continues to pose a major public health threat. Despite the efforts that have been made to prevent humans from mosquito bites, changes in the vector biting behavior, and mosquitoes tending to bite earlier before bed time could lead to successful malaria transmission. Vector control for the prevention of malaria has relied mainly on the use of chemical insecticides with extended residual life on walls or nets. These insecticides act to some extent through repellent properties which has spurred increased interest in spatial repellents. In Kisian village, insecticide-treated nets (ITNs) have been scaled up. The main malaria vectors in this area are *Anopheles gambiae* s.l. The objective of this study was to test the effectiveness of two outdoor repellents; Linalool and Metofluthrin and four indoor formulations of Linalool repellent namely: natural Linalool, synthetic Linalool, 70-80% d-Linalool and 55% d-Linalool in selected houses in a village located west of Kisumu town near KEMRI's Center for Global Health Research. A cross-over design study was carried out in 80 pairs of houses within this site. Half of the randomly selected houses were provided with Linalool in a gel emanator placed on top of the partitioning wall inside the houses while the other half were not given any treatment. Natural Linalool and synthetic Linalool formulations were evaluated during the short rains while the 70-80% d-Linalool and 55% d-Linalool formulations were evaluated during the long rains. All houses were grouped in two sets. During the first evaluation, houses in set 1 received Linalool repellent and during the second evaluation, houses in set 2 were treated with Linalool repellent while the rest of the houses served as controls. Houses were sampled for mosquitoes each morning for two days in a week during the rainy season by pyrethrum spray catches (PSC) and the data collected from each house was entered into PDAs. To further assess the effectiveness in these spatial repellents in deterring outdoor host-seeking females from the host, three tents were used each night outside the houses. The tents were located at least 100 m apart. One repellent formulation (Linalool or Metofluthrin), was placed on poles approximately 1 meter high next to one of two tents and the third tent was not treated. Volunteers remained inside the tents from 7pm until 7am. At 7am, they collected mosquitoes from the tent traps using mouth aspirators and transferred them to paper cups labeled with date of collection and the tent from which the mosquitoes were collected. All the data entered in the PDA were downloaded into a database on a secure server and data from the outdoor collections were entered using visual CE and appended to the same database. Poisson regression was used for data analysis. Analysis was done using univariate procedure in SAS version 9.1. The major findings of this study is that ITN and 70-80% d-Linalool combined reduced *Anopheles* mosquito densities by 49% in treated houses relative to untreated controls (RR=0.49; CI, 95% 0.25-0.97; P=0.04). ITN and 70-80% d-Linalool combination reduced the density of fed *Anopheles* mosquitoes compared to ITN-55% d-Linalool treatment combinations (mean difference=-0.19; $P<$ 0.0001) compared with ITN only, 70-80% and ITN (RR=0.73; CI, 95% 0.53-0.99; P=0.04) and Natural linalool and ITNs (RR=0.61; CI, 95% 0.41-0.92; P=0.02) reduced densities of fed and half-gravid mosquitoes but all treatments had no effect on reducing the number of blood-fed *Anopheles* mosquitoes during the long rains. Tents treated with linalool had higher numbers of mosquitoes compared to tents with no treatment (RR=1.61; CI, 95% 1.27-2.05; P=0.0001). In conclusion, this study found Linalool and Metofluthrin repellents not effective in reducing indoor and outdoor densities of Anopheles mosquitoes while ITNs remain to be effective in the control of malaria vectors. The results from this study provide important information for malaria programs that aim at finding effective repellents for the control of malaria.

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background

Malaria affects an estimated 225 million people, killing as many as 781 thousand every year (WHO, 2009). In the tropics alone, malaria affects 300 to 500 million people resulting in 1 to 3 million deaths annually (Muturi *et al.,* 2008). Africans, particularly children, accounts for most of the burden of malaria (Breman *et al.,* 2004; WHO, 2010). Malaria is the most important vector borne disease in Kenya, and 25 million out of a population of 38.6 million Kenyans have been reported to be at risk (DOMC, 2010). Malaria accounts for 30-50% of all outpatient attendance and 20% of all admissions to health facilities and an estimated 170 million working days are lost to the disease each year (MOH, 2001). The Kenya Ministry of Public Health strategy for malaria control includes vector control, access to prompt and effective treatment, prevention of malaria during pregnancy, and epidemic preparedness and response (DOMC, 2011). The idea behind vector control is to reduce levels of mortality and morbidity by reducing transmission of the disease. Transmission reduction is achieved by interrupting the vector-human contact through the use of effective vector control tools such as insecticide-treated nets (ITNs), indoor residual spraying (IRS), larviciding and environmental management or the use of spatial repellents. ITNs and IRS are the primary tools for malaria prevention in sub-Saharan Africa (Lengeler, 2004a; Mabaso *et al.,* 2004). Although they are effective in reducing malaria transmission, morbidity and mortality, ITNs and IRS fall short of eliminating transmission.

The Global Malaria Action Plan that was launched by the WHO-Roll Back Malaria Partnership, targets universal coverage of all at-risk-populations with both preventive and curative measures (WHO, 2009).

The idea is to scale up preventive measures to full coverage then sustain them at that point for extended periods, thus shifting malaria control dynamics towards elimination and possibly thereafter, complete eradication (Okumu and Moore, 2011). If these recent calls for malaria elimination are to be heeded, additional vector control tools are necessary to act in synergy against the malaria vectors to further suppress transmission. Thus, the need to evaluate repellents as vector control tools

Although ITNs and IRS have considerable impact on malaria transmission by acting through direct mortality upon the adult female mosquitoes, many insecticides have both toxic as well as behaviuoral effects on insects (Grieco *et al.,* 2007). Nets treated with pyrethroid insecticides have been reported to reduce the number of mosquitoes through repellent effects, although it is thought that this is mediated through a phenomenon referred to as contact irritancy where the insect must physically touch the treated surface before it is driven back out of the house (Roberts *et al.,* 2000).

The findings that the effectiveness of existing tools for malaria prevention may rely partly or primarily on spatial repellency has spurred interest in several spatial repellents. Spatial repellents have many potential advantages over the existing tools. For example, Linalool is slow-release gel formulation in which a small package can be opened and the active ingredient emanates over a period of up to several months. These emanators could be delivered through health clinics or shops without the need for large, highly trained teams as required for IRS. In contrast to ITNs, a spatial repellent placed within a house may protect all residents and, if formulated well, would not require daily behavioral input from the residents.

New slow-release gel formulations are being developed in which a small package can be opened and the active ingredient emanates over a period of up to several months.

However, spatial repellents should not be viewed as replacements for existing vector control tools but as complementary tools to further reduce transmission and potentially as tools for insecticide resistance management. Several products are available on the market that are reported to be effective against a wide range of mosquitoes but have never been tested in sub-Saharan Africa where the primary vectors of malaria, *Anopheles gambiae* and *Anopheles funestus* are strongly anthropophilic (Garret-Jones, 1964; Gillies and Meillon, 1968) and there are doubts about the effectiveness of repellents against these mosquitoes. One of these spatial repellents is Linalool (Müller *et al.,* 2008), available under the trade name Conceal®. It has been formulated in several dosage forms including candles and gel emanators. The manufacturer has further developed gel emanators to last for up to 3 months. The other repellent is Metofluthrin and is available under the trade name Decmate®. Metofluthrin is a potent spatial Repellent that has been formulated in paper, gel or liquid emanators (Kawada *et al.,* 2004a; Kawada *et al.,* 2004b). However, by the time this study was conducted, no other study had been carried out to determine the effectiveness of Linalool and Metofluthrin in western Kenya, a region with high malaria vector densities. This study was therefore, designed to test these two spatial repellent active ingredients against *Anopheles gambiae* in this region and to determine their effectiveness in reducing outdoor biting as well as, the added value of one of these repellents (Linalool) to ITNs in reducing house entry and indoor biting of these malaria vectors.

1.2. Problem statement

Despite the increase in mosquito control efforts, malaria still claim many lives and a lot more people continue to suffer from the burden of the disease. Malaria control is difficult and cannot be accomplished by a single intervention (Shiff, 2002). The main tool in the global fight against malaria vectors is ITNs, which largely act by killing the vector on contact with the net and also have some irritancy/repellency attributes that increase the number of vectors exiting the house before acquiring a blood meal (Miller *et al.,* 1991). While interventions using only ITNs successfully reduce transmission intensity and the burden of malaria in many situations, it is not clear if these interventions alone will achieve those critical low levels that result in malaria elimination (Beier *et al.,* 2008). Malaria vectors can successfully bite and transmit the disease especially during the early evenings when people are still in their living rooms, kitchens or outdoors away from their ITNs. In fact, a study has been done that confirmed that *An. gambiae* s.l. has the ability to adapt to some extent to the presence of ITNs and that repeated washing of LLITNs resulted in reduced mortality of these mosquitoes and an increase in their survival rate (Atieli *et al.,* 2009). As a result, there is need for the implementation of other vector control tools that can work in synergy with ITNs. Several repellents are available in the market but there has been no randomized control trial of any of these products in Kenya. Spatial repellents can act synergistically with ITNs wherein people can be protected in their living rooms and outdoors before they go to bed where they will be protected by ITNs. This study was therefore, designed to test two active ingredient spatial repellents against *An. gambiae* in western Kenya and to determine their effectiveness in reducing outdoor biting of malaria vectors.

1.3. Objectives

1.3.1. General objective

To evaluate the effectiveness of Linalool and Metofluthrin spatial repellents against *Anopheles gambiae s.l.* in western Kenya.

1.3.2. Specific objectives

1. To evaluate the effectiveness of a combination of Linalool and ITNs in reducing the indoor density of mosquitoes in Kisian village, western Kenya.
2. To measure the effectiveness of Linalool and Metofluthrin in deterring host-seeking mosquitoes from entering human baited tent traps in Kisian village, western Kenya.

1.3.3. Research questions

1. What is the effectiveness of a combination of Linalool and ITNs in reducing the number of mosquitoes resting indoors as compared to the use of ITNs alone in western Kenya?
2. What is the reduction in the number of host-seeking mosquitoes entering human baited exposure free tent traps outside when Linalool and Metofluthrin are used as repellents in western Kenya?

1.4. Justification of the study

This study was designed to provide evidence for the effectiveness of which repellents when either used together with ITNs indoors or their independent use outdoors against local malaria vectors in Kenya. ITNs have been reported to sharply reduce malaria transmission (Gimnig *et al.,* 2003a; Gimnig *et al.,* 2003b), but they however, do not eliminate malaria transmission or biting of humans by mosquitoes since mosquitoes can still bite people before they take cover under ITNs. In addition, prolonged exposure of malaria vectors to insecticides may lead to changes in biting behavior expressed by outdoor biting and/or alteration in biting time (Takken, 2002). Furthermore, there are possibilities that mosquitoes may develop physiological resistance to pyrethroids and feed successfully on individuals sleeping under ITNs due to their sustained use.

In western Kenya, there is an observed reduction in the susceptibility of *An. gambiae* to pyrethroids which are used in ITNs (Vulule *et al.,* 1994; Mathenge *et al.,* 2001; Mathias *et al.,* 2011). When used in combination with ITNs, some repellents may improve the control of malaria transmission by protecting people when they are outdoors or not under their nets.

Spatial repellents may represent the next tool for malaria prevention and may serve as an ideal complement to ITNs by further reducing mosquito bites and because the mode of action of these repellents is different, they may provide a tool for insecticide resistance management.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Malaria Vectors

2.1.1. Species and their distribution

Mosquitoes are two-winged flies that belong to the family Culicidae and genus Anopheles. There are approximately 3,500 species and sub-species of mosquitoes worldwide. Out of these numbers, about 422 are Anopheles species with only about 70 being malaria vectors, of which 40 are vectors associated with man (Service, 1993). A detailed knowledge of the distribution of the main *Anopheles* malaria vectors is important as it guides national vector control strategies.

Mosquitoes are found throughout the world as long as there is some stagnant water needed for their immature stages. The *An. gambiae* s.l. also termed as *An. gambiae* complex, is considered to be the most efficient malaria vector mosquitoes in the world (Coetzee, 2004). There are six named and one unnamed species recognized within the complex (Hunt *et al.*, 1998). Although most of the mosquito species within this complex are found in fresh waters, a few can tolerate saline water. The two species that breed in salty water are *An. melas* in West Africa and *An. merus* in Eastern coast of Africa (Garret-Jones, 1964; Gillies and Meillon, 1968). Other three species of *An. gambiae* s.l. breed in freshwater (*An. gambiae* Giles, *An. arabiensis* Patton and *An. quadriannulatus* (White, 1973) and the sixth member, *An. bwambae,* has been described in the Semliki forest of Uganda to be associated with water containing high mineral content (White, 1973).

Malaria vectors found in western Kenya are *An. gambiae* s.s., *An. arabiensis* and *An. funestus*. The identification of these species and concomitant distribution records are not simply for academic interest, but are vital to effective malaria control (Coetzee, 2004).

2.1.2. Mosquito biology

To develop, mosquitoes require an environment of stagnant water. As a group, they have adapted to complete their life cycle in diverse aquatic habitats, including fresh water, salt water marshes, brackish water, or water found in containers, old tires, or tree holes.

The life cycle of the mosquito has four stages (Appendix I). The life cycle begins when a female mosquito lays eggs on or near water bodies. Some mosquito species lay their eggs on areas where flooding is likely to occur. Unhatched eggs of these species can withstand weeks to months of desiccation, remaining viable until the right conditions for hatching occur. The eggs of most species hatch into larvae in 2 to 3 days and develop through four growth stages (called instars) by moulting. The development time of the larval stages depend on water temperature, food availability and larval densities.

The larvae feed on organic matter in and the fourth instar larva may moult to become pupa. The pupal stage lasts 1 to 2 days after which they metamorphose to adults. Once the adult is ready to emerge, they climb out of a slit in the pupae casing onto the water's surface. At this point the adult is very soft. They must remain still until their bodies harden, at which time they are capable of flying. Once this is done, mating takes place and the life cycle begins again. Female mosquitoes must take a blood meal at least once per gonotrophic cycle in order to supply nutrients for egg development. Unfortunately, this comes in at the consequence pathogen transmission and spread of diseases (Javier et al., 2010). Male mosquitoes feed primarily on flower nectar (Foster, 1995) and both sexes use proboscis for their feeding. Female mouth parts are further modified for piercing the vertebrate skin and penetration of blood vessels. They usually feed every 3 to 4 days; in a single feeding, a female mosquito typically consumes more than its own weight in blood (Clements, 1963). Knowledge on the biology of disease vectors helps entomologists to design targeted control strategies.

2.1.3. Role of mosquitoes in malaria transmission

Malarial parasites are transmitted to humans chiefly by female A*nopheles* mosquitoes with a few cases of transplacental- and blood transfusion – associated transmissions. The malaria parasite has a complex, multistage life cycle occurring within two living beings, the vector mosquitoes and the vertebrate hosts. The survival and development of the parasite within the invertebrate and vertebrate hosts, in intracellular and extracellular environments, is made possible by more than 5,000 genes and their specialized proteins that help the parasite to invade and grow within multiple cell types and to evade host immune responses (Laurence *et al.,* 2002; Greenwood *et al.,* 2008).

These parasites are fertilized in the mosquito midgut and develop into motile zygotes, called ookinetes. Ookinetes invade the midgut epithelial cell from the luminal surface by rupturing the cell membrane and move through the cytoplasm to the side of the basal lamina, sometimes penetrating additional epithelial cells laterally.

On the basal lamina, ookinetes transform to oocysts and finally burst and release sporozoites into the body cavity of the mosquito, from where they travel to and invade the mosquito salivary gland. When the mosquito thus loaded with sporozoites takes another blood meal, the sporozoites get injected from its salivary glands into the human bloodstream, causing malaria infection in the human host. It has been found that the infected mosquito and the parasite mutually benefit each other and thereby promote transmission of the infection. The *Plasmodium*-infected mosquitoes have a better survival and show an increased rate of blood-feeding, particularly from an infected host (Ferguson and Read, 2004; Barillas-Mury and Kumar, 2005; Hill, 2006).

During the midgut invasion however, many ookinetes are killed by the insect's defense system, and the number of malarial parasites is greatly reduced. The midgut epithelium is, therefore, one of the most important biological barriers against malarial infection.

To breach this barrier and migrate to the site of further development, ookinetes have the ability to traverse the host cell (Sinton et al., 1926; Muirhead-Thomson, 1957).

2.2. Malaria

Malaria is a significant health problem in most parts of the world. It is a highly complex disease that is caused by members of the genus *Plasmodium,* apicomplexan which exhibit a life cycle involving a vertebrate host and an arthropod vector. *Plasmodium* species are generally host specific and vector specific in that each species will only infect a limited range of hosts and vectors. Four distinct species infected humans: *P. falciparum*, *P. vivax*, *and P.ovale* and *P. malariae*. The species differ with regard to their morphology, details of their life cycles, and their clinical manifestations. *Plasmodium falciparum* is responsible for almost all the observed mortality with over half the worldwide cases occurring in Africa (Snow *et al.,* 2005; WHO, 2005). Kenya's cases of malaria are caused primarily by this more virulent malaria species (Zhou *et al.,* 2011).

Human infection begins when the malaria vector, a female *Anopheles* mosquito, inoculates the sporozoites of plasmodia from its salivary gland into humans during a blood meal. The sporozoites mature in the liver and are released into the bloodstream as merozoites. These invade red blood cells, causing malaria fevers. Some forms of the parasites (gametocytes) are ingested by anopheline mosquitoes during feeding and develop into sporozoites, restarting the cycle (Jamison and Dean, 2006). More than 3 billion people live in malarious areas and the disease causes between 1 million and 3 million deaths each year worldwide (Breman *et al.,* 2004). In addition, malaria affects as many as 30,000 visitors to the tropics annually and it is estimated that 59% of the world's clinical malaria cases occur in Africa, 38% in Asia and 3% in the Americas (WHO, 2005). The same report indicated malaria as being responsible for 60% of fetal losses and over 10% of maternal deaths. Malaria mostly affects the poor tropical and sub-tropical countries and traps them in poverty (WHO, 2005).

Most malaria deaths occur in Africa and the disease has its largest impact on pregnant women and young children (Jamison and Dean, 2006).

In Kenya alone, approximately 23 million are at risk of malaria (PMI, 2008). The regular epidemics occur in the western highlands. The major malaria vectors in western Kenya are *An. gambiae* Giles, *An. arabiensis* Patton *and An. funestus* Giles (Zhou *et al.,* 2010). Given the variable nature of the disease, its vectors, and the vulnerability of human populations, control of the malaria-bearing mosquito is significantly important so as to reduce the rate and number of cases of both parasite infection and clinical malaria.

2.3. Vector control

2.3.1. General Information

Mosquitoes are the main vectors responsible for the transmission of human diseases worldwide (Lehane, 1991). Preventing mosquito bites is therefore an important factor in reducing disease transmission (Lucas *et al.,* 2005). There is however, no single method of malaria control that is completely effective in high transmission areas (Lengeler *et al.,* 1997; Beier *et al.,* 1999).

Vector control aims at decreasing contact between humans and vectors of human disease thus limiting the rate of disease transmission. However, vector populations can respond behaviorally, numerically, or evolutionarily to insecticides implemented against them in malaria control programs (Mathias *et al.,* 2011). With regard to behavior, females of some *Anopheles* species show elevated activity due to the excitation effects of the active ingredients in some insecticide formulations, resulting in their movement away from the sprayed walls or treated nets, with or without having obtained a human blood meal (Taylor *et al.,* 1981; Miller *et al.,* 1991; Mabaso *et al.,* 2004).

With regard to numeric responses to these interventions, malaria vector populations typically diminish in density and have reduced longevity (Fontaine *et al.,* 1978; Gratz, 1985; Beier *et al.,* 2008). To be able to effectively control mosquito populations, methods of control targeting their larval and adult stages need to be considered. For adult mosquito control, additional methods for reducing transmission of malaria—particularly those that might complement existing anti-adult methods are needed for *An. gambiae* (Mutuku et al., 2006a). Recent progress in reducing malaria morbidity and mortality in Africa is founded upon expanded coverage of ITNs, IRS, and its treatment of (Greenwood, 2008; Mendis *et al.,* 2009). For this progress to translate into the ambitious goal of malaria elimination, most agree that vector control has a central role (Greenwood, 2008; Mendis *et al.,* 2009).

2.3.2. Source Reduction (Larval Control)

Source reduction targets the immature stages of malaria vectors by eliminating their breeding sites. Most observations of the larval habitats of *An. gambiae* s.l. have noted a preference for temporary sunlight pools (Gillies and Meillon, 1968; Gillies and Coetzee, 1987) whereas *An. arabiensis* appear to exploit permanent, artificial habitats such as rice fields (Githeko *et al.,* 1996b) and garden wells (Robert *et al.,* 1998). Insecticides can also be applied to water bodies that harbor the larvae. Since larvae do not usually occupy the entire body of water, larvicides are applied where the larvae are, usually the areas near the shoreline of the lake, stream or ditch. Larvicides are classed as stomach toxins, contact larvicides, surface agents, natural agents and insect growth regulators (IGR).

There has been growing interest in the feasibility of controlling malaria by targeting immature stages (Killeen *et al.,* 2002a; Killeen *et al.,* 2002c). This interest has been attributed to the fact that adulticides often had negative impacts on human health and the environment while vectors targeted with these insecticides often developed resistance (IPEP, 2006).

Historically, control programs targeting the immature stages of malaria vectors, including *An. gambiae*, have been very successful. Source reduction activities in Zambia reduced malaria incidence by 50% (Utzinger *et al.*, 2001). Eradication of introduced *An. gambiae* s.l. from the northeast coast of Brazil and the Nile valley of Egypt (Killeen *et al.*, 2002a; Killeen *et al.*, 2002b) via antilarval measures provide additional examples where source reduction was successful. Some studies indicate that this tool may be effective even in rural Africa, however, larval control is more challenging to implement than adult control (Killeen *et al.*, 2002c) and the interventions that target larval stages have largely been dismissed as a potential tool for the control of malaria.

The major challenges to community involvement in larval source reduction activities are in educating people about the sources of the mosquitoes and motivating people to assume responsibility for controlling mosquitoes in and around their homes (Service, 1993; Gubler and Clark, 1996), responsibilities often assumed to be that of the government. This has been due partly to the relative ease and effectiveness of IRS and ITNs, and partly due to the presence of a wide range of potential larval habitats and the transient, unpredictable nature of many of these habitats (Mutuku et al., 2006b) rendering source reduction not feasible.

2.3.3. Insecticide-treated bed nets

The development and introduction of ITNs has been one of the major innovations in the control of mosquito borne diseases. ITNs have become an important tool in the prevention of malaria in highly endemic areas (Lengeler, 2004b) and an integral component of malaria control programs in Africa (Etang *et al.*, 2004). They reduce human-vector contact by either providing a physical barrier to mosquitoes or the insecticide having knock down effects on mosquitoes that attempt to feed (Miller *et al.*, 1991). In addition, many insecticides used in the treatment of net such as permethrin have exito-repellent properties that affect the behavior of mosquitoes by reducing the rate of entry into houses and increasing the rate of early exit

from houses (Lines *et al.,* 1987; Miller *et al.,* 1991). The effectiveness of ITNs in reducing morbidity and mortality due to malaria has been well documented (Alonso *et al.,* 1991; Binka *et al.,* 1996; Nevill *et al.,* 1996).

ITNs have been shown to reduce the number of malaria vectors in areas where this control strategy has been used. Studies in Asembo in western Kenya for instance, showed that ITNs significantly reduced the proportion of *An. funestus* (Gimnig et al., 2003a) and that of *An. gambiae* to levels much lower with a competitive proportionate increase in the population of *An. arabiensis* in the area with widespread ITN use (Mathias *et al.,* 2011).

Although reduced house entry and early exit may help to limit human-vector contact, mosquitoes may also respond to the use of ITNs in ways that compromise their effectiveness. Even in areas where ITNs are considered to be highly effective malaria control tools, it appears that their introduction may have caused behavioural shifts among malaria vectors, with outdoor and early evening feeding becoming more frequent among them. A case-control study in Colombia showed that ITNs provided only 50% reduction in malaria (Alexander *et al.,* 2005). This was consistent with a report from RBM that regular use of ITNs by children can reduce their overall risk of dying by 20% and the number of clinical malaria occurrence by almost 50% (RBM, 2008). While ITNs offer excellent levels of protection when sleeping, users are still exposed to mosquitoes that bite earlier in the evening, both inside and outside (Lucas *et al.,* 2005). Genetic changes in vector susceptibility to insecticides are of particular concern (Chandre *et al.,* 1999). Changes in mosquito behavior may also reduce the effectiveness of ITNs. This has been reported in a study previously carried out in coastal Kenya, whose findings indicated that *An. gambiae s.l.* was more likely to bite earlier and outdoors in villages with ITNs as compared to areas without the ITNs (Mbogo *et al.,* 1996). Shifts to vector species that are less likely to enter and feed indoors may result after the introduction of ITNs (Mathias *et al.,* 2011) limiting the

impact of these interventions. In addition, ITNs can be expensive for families at risk of malaria, who are among the poorest in the world (RBM, 2008; WHO, 2010). In such conditions, ITNs may be usefully synergised by an effective insect repellent (Okumu and Moore, 2011).

Untreated bed nets form a protective barrier around persons using them. However, it is widely believed that untreated nets alone do not afford much protection against severe malaria to users (Snow *et al.,* 1987; D'Alessandro *et al.,* 1995). Owing to the success of ITNs, which require impregnation at periodic intervals, long lasting insecticidal nets (LLINs), in which insecticide is incorporated into the net fibres was introduced (Dash *et al.,* 2007). The application of a residual insecticide greatly enhances the protective efficacy of bed nets. It has been observed that LLINs containing a repellent pyrethroid insecticide that can work for years and withstand multiple washings, offer one of the most promising tools for low cost and sustainable malaria control (Lucas *et al.,* 2005). Widespread access to ITNs has been advocated by Roll Back Malaria, but universal deployment require major financial, technical, and operational inputs (Lengeler, 2009).

2.3.4. Indoor Residual Spraying (IRS)

IRS is a major intervention for malaria control (WHO, 2006). Many malaria vectors feed indoors and rest inside the house after taking a blood meal, making them susceptible particularly to control through IRS, a vector control method that involves coating the walls and other surfaces of a house with a residual insecticide. This control method is based on the observation that after blood-feeding, many endophilic vector species rest on the walls or eaves of houses until eggs are fully developed, after which they fly outdoors in search of oviposition sites. During the resting phase of the gonotrophic cycle, the vectors absorb sufficient levels of insecticides which either kill them or reduce their longevity and hence their vectorial capacity.

Although IRS does not directly prevent people from being bitten by mosquitoes, it prevents further transmission of infection to other people. To be effective, IRS must be applied to a very high proportion of households in an area. IRS with DDT and dieldrin was the primary malaria control method used during the Global Malaria Eradication Campaign (1955-1969). Resistance to DDT and dieldrin and concern over their environmental impact led to the introduction of other, more expensive insecticides. As the eradication campaign wore on, the responsibility for maintaining it was shifted to endemic countries that were not able to shoulder the financial burden. The campaign collapsed and in many areas, malaria soon returned to pre-campaign levels. Interest in IRS has been rekindled in recent years, as it is increasingly considered to be a key component of integrated malaria management. In a study done in western Kenya, a reduction in vector prevalence and disease incidence was observed for at least six months following IRS (Zhou *et al.*, 2010).

2.3.5. Mosquito Repellents

Repellents make humans unattractive to a mosquito so that it will avoid areas of the body that have been treated with the product (Rutledge and Day, 2005), thus they play an important role in reducing human-vector contact (Das *et al.*, 2003). Although they do not kill mosquitoes, the best repellents will provide protection from bites for a long period of time from just one application. Mosquitoes have complex methods of detecting hosts and different types of mosquitoes react to different stimuli. Other methods used in mosquito control only reduce the chances of being bitten but they do not mosquito-proof a person. In this light, repellent use offers individuals added protection against mosquito-borne diseases. The mosquito repellents have not been met with great success because most candidate topical mosquito repellents have limited effectiveness. Consequently, there is a need for new, safe, and effective ways to control vector species of mosquitoes, and to deter blood seeking mosquitoes from humans and other hosts (Kline *et al.*, 2003).

Botanical insect repellents have become increasingly popular as viable alternatives to synthetic chemical pest repellents because they reputedly pose little risk to the environment or human health. However, the body of scientific literature documenting bioactivity of plant derivatives is sometimes contradicting and lacking in standardized testing protocols. Therefore, there is a need for increased research on the use of natural or herbal based repellents to ensure quality and determine the most effective means of application and use (Müller *et al.,* 2009).

Repellents do not all share a single mode of action, and surprisingly little is known about how repellents act on their target insects (Wright, 1975; Davis, 1985). Moreover, different species of mosquitoes may react differently to the same repellent (Rutledge, 1989).

Many factors play a role in how effective any repellent is, including the frequency and uniformity of application, the number and species of the organisms attempting to bite, the user's inherent attractiveness to blood-sucking arthropods, and the overall activity level of the potential host (Schreck, 1995). The N, N-diethyl-3-methylbenzamide (DEET) remains the gold standard of currently available insect repellents, however, there have been case reports of DEET toxicity in the literature (Zadikoff, 1979; Snyder *et al.,* 1986; Osimitz and Grothaus, 1995; Osimitz and Murphy, 1997) and consumer interest in natural alternative repellents is growing rapidly. The use of synthetic mosquito control compounds are becoming of greater concern to consumer health and responsible for ecological consequences (Müller *et al.,* 2009). At least fifty years of sustained struggle to control mosquitoes has resulted in cases of toxicity to non-target organisms(Croft and Brown, 1975), insecticide resistance(Brown, 1986), and ecological hazards (Hayes and Laws, 1991). Among alternative control strategies, the use of non-toxic plant essential oils such as citronella and essential oil derivatives such as Linalool and geraniol are being more widely considered for both industrial and household uses (Müller *et al.,* 2009).

The repellents evaluated in this study have been classified as spatial repellents. Spatial repellency is established when the repellents create a barrier by "saturating" a zone or space that contains a potential host (Gouck *et al.,* 1967).

In a previous study, spatial repellent was defined as an inhibiting compound, dispensed into the atmosphere of a three dimensional environmental space, which inhibits the ability of mosquitoes to locate and track a target, such as humans (Nolen *et al.,* 2002). Spatial repellents can provide a new technology for the protection of humans against mosquito bites (Kline *et al.,* 2003).

2.3.5.1. Linalool

Linalool (3, 7-Dimethyl-1, 6-octadien-3-ol) is a monoterpene compound reported to be a major component of essential oils of several aromatic plant species (Linck *et al.,* 2010). Linalool has been reported to exhibit spatial repellency (Kline *et al.,* 2003). Several Linalool-producing plant species are used in traditional medicinal systems for sedative purposes, including the interruption and prevention of seizures. Linalool has been used as a food additive and is also found in many perfumes and skin care products. It is generally considered safe to humans with only very minor side effects reported (Bickers *et al.,* 2003). At high concentrations, it is reported to be a mild sedative but the concentrations in the current formulations are expected to be much lower (Heuberger et al., 2004). It is expected that approximately 50-70 mg of Linalool would be released per day within homes that are estimated at 32 cubic meters (=32000 L), giving a concentration of 0.0021 mg/L, assuming the concentration were constant for the entire day.

Exposures to Linalool in fragrance compounds have been estimated at approximately 0.3 mg/kg body weight/day though the route of absorption was considered primarily dermal (Bickers *et al.,* 2003). Studies quantifying the side effects of inhaling Linalool in humans or animals are absent but mild sedative effects have been observed in animals after 1 hour

inhalation of concentrations of 1% Linalool (Linck *et al.*, 2009). Linalool's efficiency as a mosquito repellent has not been tested on all mosquito strains. Mosquito repellents that list Linalool as the active ingredient are questionable due to the absence of data on its efficacy as a mosquito repellent (Spencer, 2010).

In the current study, the indoor and outdoor efficacy of Linalool mosquito repellents was evaluated. The outdoor Linalool repellent was released from a battery powered cartridge (Appendix II), while the indoor Linalool was placed in selected houses.

2.3.5.2. Metofluthrin

Metofluthrin (2,3,5,6-tetrafluoro-4-methoxymethylbenzyl analog) is a vapor active synthetic pyrethroid, with strong bite inhibition and knockdown activity against mosquitoes (Ujihara *et al.*, 2004; Ishiwatar *et al.*, 2009). Metofluthrin is produced by Sumitomo Chemical Co. Ltd. in Osaka, Japan (Ujihara *et al.*, 2004). It belongs to the highly safe category of pyrethroids (Shono *et al.*, 2004) and has already been registered in several Asian countries such as Singapore, Indonesia, Myanmar, and Vietnam. It has been approved for indoor and outdoor use by the US EPA although currently, only products approved for outdoor use are available, and hence, this product will not be used indoors for this study. Maciver defined the "repellency" associated with pyrethroids as a reaction of mosquitoes at the threshold level when the neural activation and knockdown occur resulting in the loss of power to orient toward their hosts (Maciver, 1964).

The OFF! Clip-On Mosquito Repellent device consists of a blue, hard plastic case that contains a cartridge impregnated with Metofluthrin (31.2% w/v) and batteries to operate the fan (Appendix III). Metofluthrin volatilizes at ambient temperature, so heating is not required. An on-off switch can be toggled to turn on the fan, resulting in the suction of air into the top of the device, and the flow of air over the impregnated substrate in the cartridge causes the release of Metofluthrin through a grill of holes located on the front of the device.

It is estimated the evolution rate assessment is approximately 5–10 mg a.i. per hour at a continuous release rate. The manufacturer packaging claims the device provides protection against mosquitoes for 12 hours. The device has a clip that allows it to be worn on the waist or clipped onto a clothing accessory.

Although Metofluthrin has been studied on mosquitoes and other insects outside Kenya, there has been mixed observations about its efficacy. Paper or plastic strips, resin emanators, and coils impregnated with Metofluthrin have demonstrated spatial repellency against mosquitoes (*Culex*, *Aedes*, and *Anopheles* spp.) in the laboratory (Argueta *et al.,* 2004a; Kawada *et al.,* 2004a; Lucas *et al.,* 2007), indoor domestic environments (Kawada *et al.,* 2004a; Kawada *et al.,* 2005a; Kawada *et al.,* 2006) and various outdoor environments (Argueta *et al.,* 2004b; Shono *et al.,* 2004; Kawada *et al.,* 2005a) based on light trap catches or human landing counts. Studies on Metofluthrin-impregnated multilayer paper strips have shown a promising spatial repellent effect against mosquitoes in the laboratory and in field conditions and results show that mosquitoes were repelled by airborne Metofluthrin vapor in simulated outdoor conditions (Kawada *et al.,* 2004a). (Lukwa and Chiwade, 2008) observed that smoke from Metofluthrin-impregnated mosquito coils was repellent to *An. gambiae* mosquitoes in experimental huts for up to five hours. However, (Rapley *et al.,* 2009) did not observe spatial repellency of impregnated paper emanators against *Aedes. aegypti* mosquitoes in a controlled domestic setting despite a reduction in mosquito biting rates and increased mortality rates. Latest studies of the OFF! Clip-On fan vaporizer device releasing Metofluthrin include one that was recently evaluated against phletobomine sand flies in the Judean Desert, Israel whose results suggested that Metofluthrin from the device was not repellent against sand flies in a field environment despite showing insecticidal activity against flies collected in suction traps (Zollner and Orshan, 2011).

The vapor pressure of Metofluthrin is greater than 2-fold and 100-fold that of *d*-allethrin and permethrin, respectively. It vaporizes at normal temperature without heating, whereas the other conventional pyrethroids require heating to evaporation (Lucas *et al.,* 2005).

The high vapor pressure and insecticidal activity of Metofluthrin may lead to the development of new mosquito controlling devices, which require no external energy for vaporization, are low cost, and provide long-term efficacy and have excellent safety profile (Kawada *et al.,* 2005b). Findings from field trials by Kawada and others (Kawada *et al.,* 2004b) reported that mosquitoes were affected by airborne Metofluthrin vapors and not by direct contact with the chemical, thereby resulting in the spatial repellency. The spatial repellency is considered to occur through two main modes of pyrethroid action; knockdown activity and biting inhibition or disruption of orientation toward the host (Kawada *et al.,* 2005b). Among these, the latter may be categorized as a sub-lethal effect that results from neural excitement, which in turn appears to occur at an earlier stage of toxication or with a dosage that is lower than that required for knockdown or death (Birley *et al.,* 1987).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Study site

The study was conducted at Kisian village lying between longitude 34.6754 to 34.6895, latitude -9.8158 to -8.9098 and elevation 1088.6 to 1183.8m above sea level, near the Center for Global Health Research (CGHR), Kenya Medical Research Institute (KEMRI), approximately 10 km west of Kisumu city in western Kenya (Appendix IV). The population of Kisian comprises of 6446 people and 1367 households (KNBS, 2010). The region experiences a bimodal pattern of rainfall, with the heaviest rains falling from March through May and a smaller peak occurring in November and December each year. Malaria is highly endemic in this area with the hot and humid climate driving breeding of malaria vectors throughout the year (Mutuku et al., 2006a; Mala and Albert, 2011). The mean annual *Plasmodium falciparum* sporozoite inoculation rates has earlier been reported to range from 90 to 410 infective bites (Beier *et al.,* 1990; Githeko *et al.,* 1996a). The principal mosquito vectors in the area are *Anopheles gambiae*, *Anopheles funestus* Giles, and *Anopheles arabiensis* (Fontaine *et al.,* 1978). Recent studies indicate that *An. arabiensis* has become the primary vector in this area where insecticide treated nets have been scaled up (Mathias *et al.,* 2011).

3.2. Study population

The outdoor study population comprised of adult male volunteers while the indoor study comprised of consented households with houses of similar wall type, eave and roof types. Volunteers were provided chemoprophylaxis with Mefloquine during the entire period of the study.

3.2.1. Inclusion Criteria

Houses were eligible if they were located in a remote part of the village with open eaves. To ensure the safety of volunteers in the outdoor biting study, only males above the age of 18 years were eligible to participate.

3.2.2. Exclusion Criteria

Houses were ineligible if they were located in at a shopping centre or the semi-rural areas of the village or if they had closed eaves. Volunteers to participate in the outdoor biting study were ineligible if they were females or were below the age of 18 years.

3.3. Ethical considerations

3.3.1. Consent

Informed consent was obtained from household head for each enrollment in the study. Consent forms were translated into the local language (Luo) and back-translated into English to ensure accurate translation (Appendix V and VI). The form was read to each participant and, if they were unable to write, a witness signed on their behalf. For the tent trap collections, a form outlining the procedures was given to the volunteers to ensure that they understood the potential risks of a study of this kind (Appendix VII and VIII). Full ethical approval for this study was obtained from the ethical review board of KEMRI, the national Ethical Review Committee (Protocol number SSC 1750) (Appendix IX).

3.4. Sampling

3.4.1. Sample size determination

The sample size was estimated assuming that an average of 1 anopheline mosquito could be found in houses with insecticide treated nets during the rainy season and that the addition of a spatial repellent would further reduce that number by 80%. Using a paired t-test and estimating a standard deviation around the mean difference between the two treatments of 1, a sample size of 14 was estimated to detect a significant difference at α=0.05 at a statistical power of 80%. Since the data was not likely to be normally distributed, the sample size for a Wilcoxon Sign Rank test recommended as 15% higher than those estimated for a t-test was assumed (Erich and Lehmann, 2006). Furthermore, the data were expected to be strongly over dispersed with many pairs of houses having no mosquitoes.

The sample size was therefore multiplied by an over-dispersion factor (5) giving a final sample size of 80 ($14 \times 1.15 \times 5=80$) matched pairs of household. Similar numbers were estimated for the human baited tent traps.

3.4.2. Sampling procedure

All houses at the study site were visited and the house characteristics; wall type, presence of open eaves and roof type were recorded, as well as the presence or absence of an insecticide treated net (ITN). The distance between the houses was also recorded. A list of eligible houses was compiled for the site and a random sample of 80 pairs was taken from the list of eligible houses.

The study employed a cross-over design to meet the sample size criteria of 80 pairs of houses. Half of the selected houses were used as treatment houses and the other half as control houses during the initial evaluation effort while in the subsequent evaluation effort the houses were crossed over wherein the treatment houses now became the control houses and the control houses became the treatment houses.

A location to place the tents was identified within the same village. A distance of at least 100 m was allowed between the tents and the houses that were used for indoor collections.

3.4.2.1. Collections indoors

Each of the eighty pairs of selected houses was grouped into either of two sets (Set 1 and 2). Set 1 houses received treatment during the first evaluation effort while set 2 houses received treatment during the second evaluation effort. All treatment houses were provided with Linalool in a gel emanator placed on top of the partitioning wall inside the houses while the rest were not provided with any treatment and served as control houses. There were four indoor formulations of Linalool namely; natural Linalool, synthetic Linalool, 70-80% d-Linalool and 55% d-Linalool.

The natural and synthetic Linalool formulations were evaluated during the short rainy season while the 70-80% d-Linalool and 55% d-Linalool were evaluated during the long rainy seasons. During each round of these evaluations, half of the houses were provided with a treatment while the other half served as controls.

Indoor resting mosquitoes were collected by pyrethrum spray catches (PSC) 2 days after installing the treatments in the houses for 2 consecutive days. At the end of the PSCs the treatments were retrieved from all houses and securely stored for the second evaluation effort (Appendix X).

Pyrethrum spray catches (PSC) were performed in all houses (both treatment and control houses) using pyrethrum (0.025% v/v) and piperonyl butoxide (0.1% v/v) mixed in paraffin. The houses were visited early in the morning and all food, drinking water and animals were removed from the house and all residents asked to wait outside at least 30 minutes (Silve, 2008) . Sheets of clothing were then laid on the floors and over furniture that could not be removed and windows and doors closed (Appendix XI).

A collector sprayed along the eaves from the outside all round the house (Appendix XII) then moved to the inside and sprayed along the eaves from the inside and along the walls, corners and hangings inside the house. The house was then closed for 10 minutes, after which the sheets were taken outside and mosquitoes were collected from the sheets using a pair of forceps (Appendix XIII). The mosquitoes collected from each house were put on scintillation vials containing absolute ethanol and the vials were labeled with date of collection and house codes. The household questionnaire was filled accordingly using a PDA. All the samples were transported back to the entomology laboratory at CGHR in Kisian in a cooler box for ease of transportation.

3.4.2.2. Collections outdoors

To assess the effectiveness of these spatial repellents in deterring outdoor host-seeking females from the host, three human-baited exposure free tents were pitched each night outside houses. The tents were located at least 100m apart. The top portion of the tent was separated from the bottom portion by untreated netting material. A volunteer slept in the bottom portion of the tent.

There were 6 openings in the top portion of the net which allowed for entry of mosquitoes. The holes were approximately 10 cm in diameter and each was fitted with an inverted cone (made of netting material) to limit escape of mosquitoes (Appendix XIV). Mosquitoes were trapped in the top portion of the tent and were prevented from feeding on the person in the tent by netting material. The exposure free tent trap is a novel trap design which has shown promise in preliminary tests in several countries, including tests in western Kenya (Bayoh, unpublished data) and in Tanzania (Govella *et al.*, 2009).

Two treatment arms (Linalool gel and Metofluthrin paper) and one control arm of the study were used for the outdoor tent study and control. In the treatment arms, the Repellent was placed on a pole approximately 1 meter high from the ground next to the tent (Appendix XV) while there was nothing placed next to the control tent. Volunteers remained in the tents from 7pm until 7am. At 7am, they collected mosquitoes from the tent traps using mouth aspirators and transferred to paper cups labeled according to date of collection and the tent from which the mosquitoes were collected. Each paper cup was supplied with a collection code which was also recorded on the tent trap collection form. All the details of the tent trap collection form (Appendix XVI) were dully filled and the form was transported together with the mosquitoes to the entomology laboratory at CGHR in Kisian.

Treatments were removed and the tents were aired for one day between tests and the treatments were rotated after every night of collection. Volunteers were rotated through each tent after every week of valid data collection. This experiment ran each week for three weeks (Appendix XVII) for both short rainy and long rainy seasons.

3.5. Laboratory analysis

All the mosquitoes collected from the houses and the tents were morphologically identified with the aid of compound microscopes at a magnification of *100 and the numbers of the individual species were recorded on individual mosquito laboratory forms. The abdominal status of all female samples were determined as fed, unfed, half gravid or gravid depending of the physical nature and content of their abdomens.

3.6. Data management

3.6.1. Data collection

Data was collected from indoors using a household questionnaire that was administered to the household head to assess factors that may affect the number of mosquitoes in the house (ITN use, presence of animals, fire, other mosquito control measures like use of doom and mosquito coil) every time a PSC was done.

The questionnaires were made available in English and Luo translations (Appendix XVIII and XIX). PDAs were used to administer the household questionnaires.

3.6.2. Data storage

Household questionnaires in PDA data were synchronized on a secure server and the forms from the outdoor collections were entered using visual CE and stored into Access database and then kept in a locked cabinet within buildings that have restricted access. Once the data had been cleaned, any personal identifiers (name, compound and house numbers) were removed. A master list with personal identifiers was maintained to allow for follow-up visits throughout the duration of the study. At the end of data collection, the master list was destroyed.

3.6.3. Data analysis

The impact of the different repellents on the densities and feeding success (abdominal status) of *Anopheles gambiae s.l.* were analyzed using Poisson regression at the household or tent level (Proc Genmod in SAS version 9.1). Clustering at the level of household or tent was controlled for using generalised estimating equations with an exchangeable working correlation matrix. Variables found to have a $P > 0.1$ in univariate analysis were included in an initial multivariable model. Variables were removed using a backwards elimination procedure. The final model only retained variables that were significant at the level of $P < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Indoor study

4.1.1. Number of houses sampled for Linalool studies.

The number of houses sampled during the evaluation of each of the four different indoor Linalool formulations were between 140-151. The total number of houses used as intervention (with treatment) and as controls (without treatment) are shown in Table 1. More than 80% of the sampled houses had at least 1 ITN (Table1).

Table 1: Houses sampled for Linalool studies.

Treatment	Total number of houses sampled	Houses with treatment	Houses without treatment	% ITN coverage
Linalool-Natural	143	77	66	81.8
Linalool-Synthetic	140	70	70	86.4
Linalool-70-80%	151	73	78	92.1
Linalool-55%	147	76	71	85.7

4.1.2. Determination of the added impact of four indoor Linalool repellents used in houses with ITNs on malaria vector density.

In the determination of the added impact of indoor Linalool repellents deployed in houses, overall comparison of the densities of *Anopheles* mosquitoes that were collected from houses with each of the four repellents and ITNs to those with ITNs showed that there was a 49% reduction in *Anopheles* densities when a 70-80% d-Linalool combined with ITNs was compared to ITN only houses [RR=0.49 (0.25-0.97), P=0.04].

A combination of Natural Linalool and ITNs gave a slight reduction in the mean *Anopheles* density when compared to ITNs alone but this was not statistically significant (P=0.55). Indoor vector densities in houses with combination of ITNs and either the synthetic Linalool or the 55% d-Linalool were slightly higher than the houses with ITN alone, even though this was not statistically significant (mean difference; -0.039, 95% CI, -0.138-0.058, P=0.798 and mean difference; 0.04, 95% CI, 0.134-0.047, P=0.678 for synthetic Linalool and 55% d-Linalool, respectively) (Figure 2).

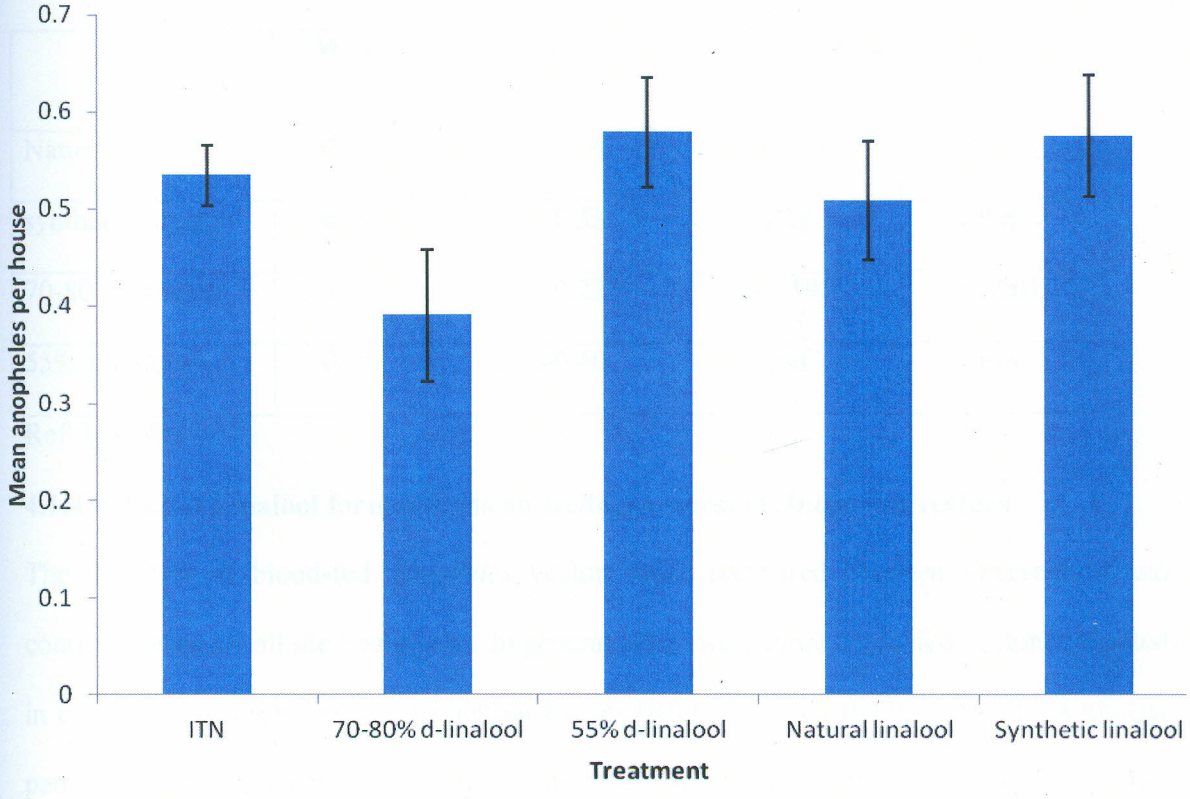


Figure 1: Comparison of indoor *Anopheles* densities between houses with the different Linalool formulations combined with ITNs and houses with ITNs only.

4.1.3 Comparison of the effects of Natural Linalool and Synthetic Linalool and that of 70-80% d-Linalool and 55% d-Linalool on feeding success of *Anopheles* vectors.

Comparing the density of fed *Anopheles* vectors in houses with natural Linalool plus ITNs and houses with synthetic Linalool plus ITNs using pairwise general linear model yielded no significant difference (mean difference = -0.07, $P = 0.35$).

There was however, a statistically significant reduction in the density of fed *Anopheles* vectors in houses with 70-80% Linalool plus ITNs compared to houses with 55% Linalool plus ITNs (mean difference = -0.19, $P<0.001$) (Table 2).

Table 2: Comparison of the mean density of blood fed *Anopheles* vectors.

	Mean	95% CI	Mean difference	*P*-value
Natural Linalool	0.21	0.16-0.26	-0.07	0.35
synthetic Linalool	0.28	0.22-0.33	Ref	Ref
70-80% Linalool	0.17	0.11-0.23	-0.19	**<.0001**
55% Linalool	0.35	0.30-0.40	Ref	Ref

Ref=base-line

4.1.4 Effect of Linalool formulations on feeding success of *Anopheles* vectors

The densities of blood-fed *Anopheles* vectors were compared between intervention and control houses for all the treatments. In general, there were more blood-fed vectors collected in control houses relative to intervention houses [RR=0.77 (0.61-0.97), P=0.02]. A similar pattern was also observed in all the four types of repellents, however, these were not statistically significant (Table 3).

Table 3: Density of blood fed *Anopheles* vectors in intervention and control houses for each of the Linalool formulations.

Treatment	Mean density		Risk Ratio	95% Confidence Interval	*P*-value
	Houses with treatment	Houses without treatment			
Linalool-Natural	0.87	2.04	1.12	0.61-2.06	**0.02**
Linalool-Synthetic	1.19	1.26	1.11	0.66-1.89	0.69
Linalool-70-80%	0.78	1.21	1.54	0.84-2.85	**0.04**
Linalool-55%	1.92	2.1	1.09	0.63-1.91	0.76

Comparing the densities of fed and half-gravid vectors collected from houses treated with either natural Linalool, synthetic Linalool, 70-80% d-Linalool or 55% d-Linalool repellent plus ITN to those collected from ITN only houses, revealed that all repellents and ITN houses had a lower *Anopheles* density except for 55% d-Linalool (Figure 3). However, only the 70-80% d-Linalool and natural Linalool were statistically significant [RR= 0.73(0.53-0.99), *P*= 0.04 and RR= 0.61(0.41-0.92), *P*= 0.02, respectively].

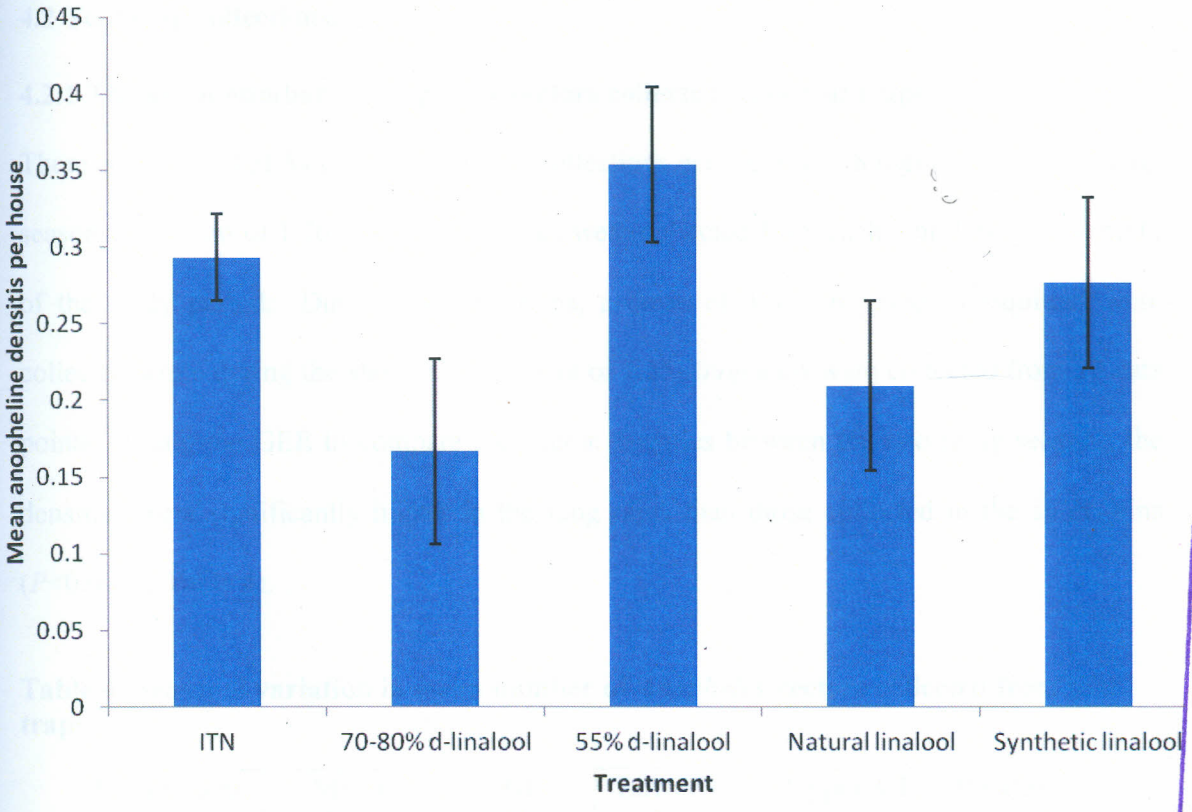


Figure 2: Comparison for the densities of blood fed *Anopheles* vectors in houses with a combination of Linalool and ITNs with ITN only houses.

4.2 Tent trap collections.

4.2.1 The mean number of *Anopheles* vectors collected from tent traps.

There were a total of 54 data points i.e., 3 collections per night for 18 nights during two rainy seasons. A mean of 1.76 *Anopheles* vectors were collected from each tent during each night of the study period. During the long rains, a mean of 3.80 *Anopheles* mosquitoes were collected while during the short rains, a mean of 1.35 *Anopheles* were collected from 27 data points. Using the GEE to compare the vector densities between the two rainy seasons, the densities were significantly higher in the long rains than those collected in the short rains ($P<0.0001$, Table 4).

Table 4: Seasonal variation in mean number of *Anopheles* vectors collected from tent traps.

Parameters	Mean	RR	Lower CI	Upper CI	*P*-value
Long rain	3.80	2.80	1.73	4.53	**<0.0001**
Short rain	1.35	Ref	Ref	Ref	Ref

RR: relative risk, CI= Confidence Interval, Ref=base-line

4.2.2 The effectiveness of Metofluthrin and Linalool repellents.

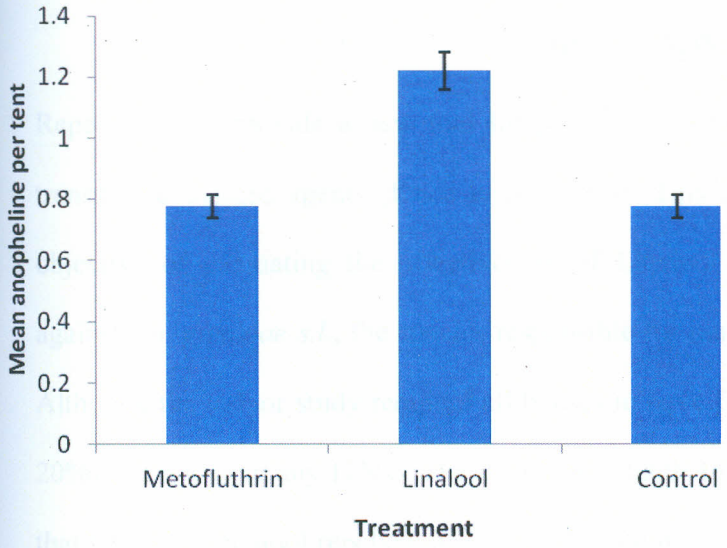
In comparing the efficacy of Linalool and Metofluthrin, the study arm with no treatment was used as the base-line while controlling for clustering at the individual tent level. During the short rains, both Metofluthrin [RR=0.99 (0.49-1.99)] ($P=0.98$) and Linalool [RR=1.58(0.83-3.03)] ($P=0.17$) had higher vector densities than the control although the differences were not statistically significant. However, in the long rains, tents with Linalool had 29% more vectors relative to the control tents and the difference was statistically significant [RR=1.61(1.27-2.05), $P=0.0001$]. Metofluthrin tents also showed an increase in vector densities although differences were not statistically significant [RR=1.28 (0.92-1.77, $P=0.14$)] (Table 5 and Figure 4).

Table 5: Mean numbers of *Anopheles sp.* mosquitoes collected from tent traps.

Treatment	Mean	Ratio (LCI-UCI)	*P*-value
Short rains			
Metofluthrin	0.78	0.99 (0.49-1.99)	0.98
Linalool	1.22	1.58(0.83-3.03)	0.17
No treatment	0.78	Ref	Ref
Long rains			
Metofluthrin	2.56	1.28 (0.92-1.77)	0.14
Linalool	3.22	1.61(1.27-2.05)	**0.0001**
No treatment	2	Ref	Ref

P-values in bold are statistically significant at $P<0.05$. LCI= Lower Confidence Interval, UCI=Upper Confidence Interval. Ref=reference

A) Short rains



B) Long rains

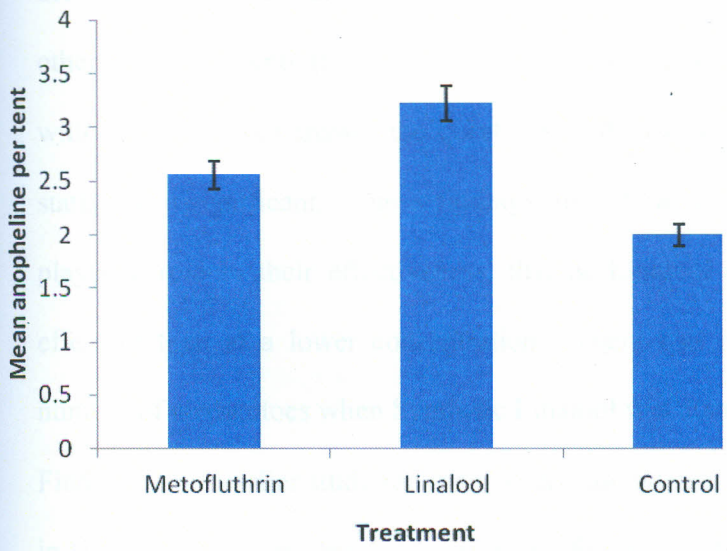


Figure 3: The performance of outdoor repellent formulations of Linalool and Metofluthrin during the A) short and B) long rains (error bars are 95% confidence intervals of the means).

CHAPTER FIVE

5.0 DISCUSSION

Repellents can provide a new tool for protection of humans and animals from mosquito-transmitted disease agents (Kline *et al.,* 2003). This study was designed with an overall objective of evaluating the effectiveness of Linalool and Metofluthrin spatial repellents against *An. gambiae s.l.*, the species responsible for malaria transmission in western Kenya. Although the indoor study required all houses to have ITNs, some of the sampled houses (< 20%) did not have any ITNs. The decrease in total *Anopheles* densities observed in houses that had both Linalool repellent and ITNs showed that out of the four repellents, only the 70-80% d-Linalool used in combination with ITNs was effective in reducing the mosquito densities to levels lower than those attained by ITNs alone. The differences observed for the other three repellents (i.e. 55% d-Linalool, Natural, and Synthetic Linalool) in combination with ITNs does not answer the questions of their effectiveness since these results were not statistically significant. These findings also showed that concentrations of the d-Linalool played a role in their effectiveness, that is, Linalool at a higher concentration was more effective than at a lower concentration. There was however, no difference in the mean number of mosquitoes when Synthetic Linalool was compared to Natural Linalool.

Findings in an earlier study reported synergistic interactions between insecticide impregnated in ITNs and repellents leading to enhanced feeding inhibition, mediated through a measurable reduction in mosquito biting (Eliningaya *et al.,* 2008). Even though the results presented in the current study generally showed a pattern towards reduction in the feeding success of *An. gambiae* mosquitoes in houses treated with the repellents as compared to their numbers in control houses, the differences were not statistically significant. It is important to note that even though not statistically significant, this pattern could be important in lowering transmission in houses with repellents are deployed.

In a laboratory evaluation, a mixture of repellents with deltamethrin, a pyrethroid used in some ITN formulations, inhibited blood feeding by *An. gambiae* mosquitoes (Pennetier *et al.*, 2007). In other studies performed in Pakistan, the combined use of repellents on skin and ITNs was proposed as a preventive measure in regions where vectors bite in the early evening or in emergency situations such as epidemics (Rowland *et al.*, 2004). This example of integrated vector control showed gains that can be achieved when interventions are used concurrently to cover for any limitation in individual interventions (Deparis *et al.*, 2004). Although the decrease in mosquito densities in houses with ITNs alone supports the earlier findings that ITNs have a profound impact on malaria vector populations by reducing their indoor resting densities (Lindsay *et al.*, 1989; Mbogo *et al.*, 1996), ITNs do not constitute a stand-alone solution to the problem of malaria in Africa (Hawley *et al.*, 2003). Thus house-based repellents contribute added protection from bites.

The results from the outdoor study showed that there were more mosquitoes collected from the tent traps during the long rains as compared to those collected during the short rains. During the short rains both Metofluthrin and Linalool did not show any significant increase in outdoor densities of *Anopheles* mosquitoes. However, it was not easy to make conclusions about the effectiveness of these repellents based on the short rainy season alone due to the low mosquito populations in the field. Although the number of host-seeking mosquitoes entering human baited exposure free traps outside during the long rains increased when Linalool and Metofluthrin was used, those in Linalool tents study arm increased significantly (P=0.0001). This result was similar to previous findings (Kline *et al.*, 2003) whereby Linalool was the most attractive compound to *Aedes aegypti* compared to deet and dehydro Linalool although all the candidate compounds caused an excitation and/or disorientation of mosquitoes to the chemical source.

Although these results were not consistent with those reported in another study which observed a rapid decrease in mosquito index after treatment with an indoor formulation of Metofluthrin tested on *Culex quinquefaciatus* and *Aedes aegypti* (Kawada *et al.*, 2005b), these results supported another recent finding that the use of Metofluthrin raise doubts about its ability to provide protection from outdoors (Zollner and Orshan, 2011).

5.1 CONCLUSIONS

1. There was a reduction in indoor mosquito densities only when 70-80% d-Linalool was used in combination with ITNs. This higher concentration of Linalool repellent also showed a pattern in reduction of the blood feeding success of *An. gambiae* mosquitoes in houses treated with the repellents as compared to their numbers in control houses.
2. The findings from the outdoor arm of this study showed that Linalool formulation for outdoor use is more of an attractant rather than a repellent while the Metofluthrin formulation for outdoor use is not effective in deterring host-seeking *Anopheles* mosquitoes from entering human baited tent traps outdoors.

5.2 RECOMMENDATIONS

1. Although there was a 49% added advantage of using 70-80% Linalool in addition to ITNs, more studies need to be done before it is fully recommended for public use.
2. There is need for the Ministry of health to evaluate the efficacy of repellents before they are released to the public domain.

REFERENCES

Alexander, N., Rodriguez, M., Perez, L., Caicedo, J., Cruz, J., Prieto, G., Arroyo, J., Cotacio, M. and Suarez, M. (2005) Case-control study of mosquito nets against malaria in the Amazon region of Colombia. The American journal of tropical medicine and hygiene 73, 140-148.

Alonso, P., Lindsay, S., Armstrong, J., Conteh, M., Hill, A., David, P., Fegan, G., Franisco, D., Hall, A., Shenton, F., Cham, K. and Greenwood, B. (1991) The effect of Insecticide-treated bednets on mortality of Gambian children. Lancet 337, 1449-1502.

Argueta, T.B.O, Kawada, H., Kubota, S. and Takagi, M. (2004a) Spatial repellency of metofluthrin-impregnated multilayer paper strip against *Aedes albopictus* under outdoor conditions, Nagasaki, Japan. Medical Entomology and Zoology 55, 211-216.

Argueta, T.B.O, Kawada, H., Sugano, M., Kubota, S., Shono, Y., Tsushima, K. and Takagi, M. (2004b) Comparative insecticidal efficacy of a new pyrethroid, metofluthrin, against colonies of Asian *Culex quinquefasciatus* and *Culex pipiens* pallens. Medical Entomology and Zoology 55, 289–294.

Atieli, H., Menya, D., Githeko, A. and Scott, T. (2009) House design modifications reduce indoor resting malaria vector densities in rice irrigation scheme area in western Kenya. Malaria Journal, 108.

Barillas-Mury, C. and Kumar, S. (2005) Plasmodium –mosquito interactions: a tale of dangerous liaisons. Cellular Microbiology 7, 1539–1545.

Beier, J., Copeland, R., Oyaro, C., Masinya, A., Odago, W., Oduor, S., Koech, D. and Roberts, C. (1990) *Anopheles gambiae* complex egg-stage survival in dry soil from larval development sites in western Kenya. Journal of the American Mosquito Control Association 6, 105–109.

Beier, J., Keating, J., Githure, J., Macdonald, M., Impoinvil, D. and Novak, R. (2008) Integrated vector management for malaria control. Malaria Journal 7.

Beier, J., Killeen, G. and Githure, J. (1999) Short report: entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. American Journal of Tropical Medicine and Hygien 61, 109-113.

Bickers, D., Calow, P., Greim, H., Hanifin, J., Rogers, A., Saurat, J., Sipes, I., Smith, R. and Tagami, H. (2003) A toxicologic and dermatologic assessment of linalool and related esters when used as fragrance ingredients. Food and Chemical Toxicology 41, 919-942.

Binka, F., Kubaje, A., Adjiuk, M., Williams, L., Lengeler, C., Maude, G., Armah, G., Kajihara, B., Adiamah, J. and Smith, P. (1996) Impact of permethrin impregnated bednets on child mortality in Kassena-Nankana district, Ghana: a randomized control trial Tropical Medicine International Health 1, 147-154.

Birley, M., Mutero, C., Turner, I. and Chadwick, P. (1987) The effectiveness of mosquito coils containing esbiothrin under laboratory and field conditions. Annals of Tropical Medicine and Parasitology 81, 163-171.

Breman, J., Alilio, M. and Mills, A. (2004) Conquering the intolerable burden of malaria: what's new, what's needed. A summary. American Journal of Tropical Medicine and Hygiene 71, 1-15.

Brown, A. (1986) Insecticide resistance in mosquitoes: pragmatic review. Journal of the American Mosquito Control Association 2, 123-140.

Chandre, F., Darrier, F., Manga, L., Akogbeto, M., OFaye, Mouchet, J. and Guillet, P. (1999) Status of pyrethroid resistance in *Anopheles gambiae sensu lato*. World Health Organization 77, 230-234.

Clements, A. (1963) The Physiology of Mosquitoes. Pergamon Press.

Coetzee, M. (2004) Distribution of the African malaria vectors of the *Anopheles gambiae* complex. American Journal of Tropical Medicine and Hygien 70, 103-104.

Croft, B. and Brown, A. (1975) Responses of arthropod natural enemies to insecticides.. Annual Review of Entomology 20, 285-335.

D'Alessandro, U., Olaleye, B.O., McGuire, W., Thomson, M.C., Langerock, P., Bennett, S. and Greenwood, B.M. (1995) A comparison of efficacy of Insecticide-treated and untreated bed nets in preventing malaria in Gambian children. Transactions of the Royal Society of Tropical Medicine and Hygiene 89, 596-598.

Das, N., Baruah, I., Talukdar, P. and Das, S. (2003) Evaluation of botanicals as repellents against mosquitoes. Journal of Vector Borne Diseases 40, 49-53.

Dash, A., Adak, T., Raghavendra, K. and Singh, O.P. (2007) The biology and control of malaria vectors in India. Current Science 92, 1571-1579.

Davis, E.E. (1985) Insect repellents: concepts of their mode of action relative to potential sensory mechanisms in mosquitoes (Diptera: Culicidae). Journal of Medical Entomology 22, 237-243.

Deparis, X., Lamizana, M., N'Guessan, R., Leroux, F., Lefevre, P., Finot, L., Hougard, J., Carnevale, P., Guillet, P. and Baudon, D. (2004) Efficacy of Permethrin-treated Uniforms in Combinations in Combination with DEET Topical Repellent for Protection of French Military Troops in Côte d'Ivoire. Journal of Medical Entomology 41, 914-921.

DOMC. (2010) The malaria situation in Kenya. In. Division of Malaria Control Kenya.

DOMC. (2011) Kenya Malaria monitoring and evaluation plan 2009-2017. In: M.o.P.H.a. Sanitation (Ed).

Eliningaya, J., Mosha, F., ALowassa, Mahande, A., Kitau, J., Matowo, J., Mahande, M., Massenga, C., Tenu, F., Feston, E., Lyatuu, E., Mboya, M., RMndeme, Chuwa, G. and Temu, E. (2008) Ethnobotanical study of some of mosquito repellent plants in north-eastern Tanzania. Malaria Journal 7, 152.

Erich, L. and Lehmann. (2006) Nonparametrics: Statistical Methods Based on Ranks, Revised In: D.A. HJM (Ed). Springer Science, New York, p. 56-57.

Etang, J., Chandre, F., Guillet, P. and Manga, L. (2004) Reduced bio-efficacy of permethrin EC impreganated bednets against an *Anopheles gambiae* strain with oxidasebased pyrethroid tolerance. Malaria Journal 3, 46.

Ferguson, H. and Read, A. (2004) Mosquito appetite for blood is stimulated by Plasmodium chabaudi infections in themselves and their vertebrate hosts. Malaria Journal 3, 12.

Fontaine, R., Pull, J., Payne, D., Pradhan, G., Joshi, G., earsonv, J., Thymakis, M. and Camacho, M. (1978) Evaluation of fenitrothion for the control of malaria. Bulletin of the World Health Organization 56, 445–452.

Foster, W.A. (1995) Mosquito Sugar Feeding and Reproductive Energetics. Annual Review of Entomology 40, 443-474.

Garret-Jones, C. (1964) The human blood index of malaria vectors in relation to epidemiological assessment. Bulletin of the World Health Organization 30, 241.

Gillies, M. and Coetzee, M. (1987) A supplement to the Anophelinae Africa South of the Sahara. In: Medical Research Institute, Johannesburg.

Gillies, M. and Meillon, B.D. (1968) The Anophelinae of Africa South of the Sahara. In: South African Institute of Medical Research, Johannesburg.

Gimnig, J., Kolczak, M., Hightower, A., Vulule, J., Schoute, E., Kamau, L., Phillips-Howard, P., Kuile, F., Nahlen, B. and Hawley, W. (2003a) Effect of permethrin-treated bed nets on the spatial distribution of malaria vectors in western Kenya. American Journal of Tropical Medicine and Hygien 68, 115-120.

Gimnig, J., Vulule, J., Lo, T., Kamau, L., Kolczak, M., Phillips-Howard, P., Mathenge, E., Kuile, F.t., Nahlen, B., Hightower, A. and Hawley, W. (2003b) Impact of permethrin-treated bednets on entomological indices in an area of intense year-round malaria transmission. American Journal of Tropical Medicine and Hygien 68, 16–22.

Githeko, A., Adungo, N., Karanja, D., Hawley, W., Vulule, J., Seroney, I., Ofulla, A., Atieli, F., Ondijo, S., Genga, I., Odada, P., Situbi, P. and Oloo, J. (1996a) Some observations on the biting behavior of Anopheles gambiae s.s., Anopheles arabiensis, and Anopheles funestus and their implications for malaria control. Experimental Parasitology 82, 306–315.

Githeko, A.K., Service, M.W., Mbogo, C.M. and Atieli, F.K. (1996b) Resting behaviour, ecology and genetics of malaria vectors in large scale agricultural areas of western Kenya. Parassitologia 58, 307-316.

Gouck, H., McGovern, T. and Beroza, M. (1967) Chemicals tested as space repellents against yellow-fever mosquitoes. Journal of Economic Entomology 60, 1587–1590.

Govella, N., Chaki, P., Geissbuhler, Y., Kannady, K., Okumu, F., Charlwood, J., Anderson, R. and Killeen, G. (2009) A new tent trap for sampling exophagic and endophagic members of the *Anopheles gambiae* complex. Malaria Journal 8, 157.

Gratz, N. (1985) The future of vector biology and control in the World Health Organization. Journal of the American Control Association 1, 273-278.

Greenwood, B. (2008) Control to elimination: implications for malaria research. Trends Parasitology 24, 449-454.

Greenwood, B., Fidock, A., Kyle, D., Kappe, S., Alonso, P., Collins, F. and Duffy, P. (2008) Malaria: progress, perils, and prospects for eradication. Journal of Clinical Investment 118, 1266–1276.

Grieco, J., Achee, N., Chareonviriyaphap, T., Suwonkerd, W., Chauhan, K., Sardelis, M. and Roberts, D. (2007) A new classification system for the actions of IRS chemicals traditionally used for malaria control. PLoS One 2, 716.

Gubler, D. and Clark, G. (1996) Community involvement in the control of *Aedes aegypti*. Acta Tropica 61, 169–179.

Hawley, W., Feiko, O., Kuile, T., Steketee, R., Nahlen, B., Terlouw, D., Gimnig, J., Shi, Y., Vulule, J., Alaii, J., Hightower, A., Kolczak, M., Kariuki, S., Penelope, P. and Phillips-Howard. (2003) Implications of the western Kenya permethrin-treated bed net studyfor policy, program implementation and future research. American Journal of Tropical Medicine and Hygien 68, 168-173.

Hayes, W. and Laws, E. (1991) Handbook of Pesticide Toxicology. In: General Principles, Vol. 1. NY, Academic Press, New York, p. 497.

Heuberger, E., Redhammer, S. and Buchbauer, G. (2004) Transdermal absorption of (-)-linalool induces autonomic deactivation but has no impact on ratings of well-being in humans. In. University of Vienna, Vienna, Austria.

Hill, A. (2006) Pre-erythrocytic malaria vaccines: towards greater efficacy. Nature Reviews Immunology, 6:21-32

Hunt, R., Coetzee, M. and Futtene, M. (1998) The *Anopheles gambiae* comlex: a new species from Ethiopia. Transactions of the Royal Society of Tropical Medicine and Hygiene 92, 231-235.

IPEP. (2006) Approaches to effective malaria control that avoid DDT in Kenya: use of *Bacillus thuringiensis israelensis* (Bti). In, Nairobi.

Ishiwatar, T., Sugano, M., Lucas, J. and Shono, Y. (2009) Biological efficacy of metofluthrin, a new pyrethroid insecticide, highly effective against mosquitoes. Advance in Human Vector Control 1014, 161–169.

Jamison and Dean, T. (2006) Disease control priorities in developing countries. Oxford University Press, 420-423.

Javier, K.N., Song, C. and Janson, L.R. (2010) Vitellogenin gene expression in autogenous *Culex tarsais*. Insect Molecular Biology 19, 423-429.

Kawada, H., Iwasaki, T., Loan, L.L., Tien, T., Mai, N., Shono, Y., Katayama, Y. and Takagi, M. (2006) Field evaluation of spatial repellency of metofluthrin-impregnated latticework plastic strips against *Aedes aegypti* (L.) and analysis of environmental factors affecting its efficacy in My Tho City, Tien Giang, Vietnam. American Journal of Tropical Medicine and Hygiene 75, 153-1157.

Kawada, H., Maekawa, Y. and Takagi, M. (2005a) Field trial of the spatial repellency of metofluthrin-impregnated plastic strip against mosquitoes in shelters without walls (Beruga) in Lombok, Indonesia. Journal of Vector Ecology.

Kawada, H., Maekawa, Y., Tsuda, Y. and Takagi, M. (2004a) Laboratory and field evaluation of spatial repellency with metofluthrin-impregnated paper strip against mosquitoes in Lombok Island,Indonesia. Journal of the American Control Association 20, 292-298.

Kawada, H., Maekawa, Y., Tsuda, Y. and Takagi, M. (2004b) Trial of spatial repellency of metofluthrin-impregnated paper strip against Anopheles and Culex in shelters without walls in Lombok, Indonesia. Journal of the American Control Association 20.

Kawada, H., Yen, N.T.I., Hoa, N.T., Sang, T.M., Dan, N.V. and Takagi, M. (2005b) Field evaluation of spatial repellency of metofluthrin impregnated plastic strips against mosquitoes in Hai phong city, Vietnam. American Journal of Tropical Medicine and Hygien 73, 350-353.

Killeen, G., Fillinger, U., Kiche, I., Gouagna, L. and Knols, B. (2002a) Eradication of Anopheles gambiae from Brazil: lessons for malaria control in Africa? Lancet Infectious Diseases 2, 618–627.

Killeen, G., Fillinger, U., Kiches, I., Gouagna, L. and Knols, B. (2002b) Eradication of Anopheles gambiae from Brazil. Lancet infectious Diseases 2, 618- 627.

Killeen, G., Fillinger, U. and Knols, B. (2002c) Advantages of larval control for Africa malaria vectors: low morbidity and behavioral responsiveness of immature mosquito stages allow high effective coverage. Malaria Journal 1, 8-13.

Kline, D., Bernier, U., posey, K. and Barnard, D. (2003) Olfactometric Evaluation of Spatial Repellents for *Aedes aegypti*. Journal of Medical Entomology 40, 463-467.

KNBS. (2010) Kenya Population & Housing census In: K.N.B.o. Statistics (Ed).

Laurence, F., Michael, P., Washburn, J. and Dale, R. (2002) A proteomic view of the Plasmodium falciparum life cycle. Nature 419, 520-526.

Lehane, M. (1991) Biology of blood sucking insects In. Harper Collins Adademic, London.

Lengeler, C. (2004a) Insecticide-treated bednets and curtains for preventing malaria. In: Cochrane Database of Systematic Reviews 2004.

Lengeler, C. (2004b) Insecticide-treated nets for malaria control: real gains. Bulletin of the World Health Organization 82, 2.

Lengeler, C. (2009) Insecticed-treated bed nets and curtains for preventing malaria. In: C. collaboration (Ed) John Wiley and sons. Cochrane library.

Lengeler, C., Smith, T. and Armstrong-Schellemberg, J. (1997) Focus on the effect of bednets on malaria control morbidity and mortality. Parasitology Today 13, 123-124.

Linck, V., Silva, A.D., Figueiró, M., Caramão, E., Moreno, P. and Elisabetsky, E. (2010) Effects of inhaled Linalool in anxiety, social interaction and aggressive behavior in mice. Journal of Phytotherapy and Phytopharmacology 17, 679-683.

Linck, V.d.-M., da-Silva, A., Figueiro, M., Piato, A., Herrmann, A., Birck, F., Caramao, E., Nunes, D., Moreno, P. and EElisabetsky. (2009) Inhaled linalool-induced sedation in mice. Phytomedicine 16, 303-307.

Lindsay, S., Snow, R., Broomfield, G., Janneh, M., Wirtz, R. and Greenwood, B. (1989) Impact of permethrin treated bednets on malaria transmission by theAnopheles gambiae complex in The Gambia. Medical and Veterinary Entomoly 3, 263–271.

Lines, J.D., Myamba, J. and Curtis, C.F. (1987) Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. Medical and Veterinary Entomology 1, 37-51.

Lucas, J., Shono, Y., Iwasaki, T., Ishiwatari, T. and Spero, N. (2005) Field efficacy of metofluthrin-A new mosquito repellent. In: L. Chow-Yang and W. Robinson (Eds), Fifth International Conference on Urban Pests, Malaysia, p. 301-307.

Lucas, J., Shono, Y., Iwasaki, T., Ishiwatari, T., Spero, N. and Benzon, G. (2007) U.S. laboratory and field trials of metofluthrin (SumiOne) emanators for reducing mosquito biting outdoors. Journal of the American Control Association 23, 47–54.

Lukwa, N. and Chiwade, T. (2008) Lack of insecticidal effect of mosquito coils containing either metofluthrin or esbiothrin on Anopheles gambiae sensu lato mosquitoes. Tropical Biomedicine 25.

Mabaso, M., Sharp, B. and Lengeler, C. (2004) Historical review of malarial control in southern African with emphasis on the use of indoor residual house-spraying. Tropical Medicine and International Health 9, 846-856.

Maciver, D.R. (1964) Mosquito coils Part II. Studies on the action of mosquito coil smoke on mosquitoes. Pyrethrum Post 7.

Mala and Albert, O. (2011) Factors influencing differential larval habitat productivity of Anopheles gambiae complex mosquitoes in a western Kenyan village. Journal of Vector Borne Diseases.

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

Mathias, D., Ochomo, E., Atieli, F., Ombok, M., Bayoh, N., Olang, G., Muhia, D., Kamau, L., Vulule, J., Hamel, M., Hawley, W., Walker, E. and Gimnig, J. (2011) Spatial and temporal variation in the kdr allele L1014S in Anopheles gambiae s.s. and phenotypic variability in susceptibility to insecticides in Western Kenya. Malaria Journal 10.

Mbogo, C.N., Baya, N.M., Ofulla, A.V., Githure, J.I. and Snow, R.W. (1996) The impact of permethrin-impregnated bednets on malaria vectors of the Kenyan coast. Medical and Veterinary Entomology 10, 251-259.

Mendis, K., Rietveld, A., Warsame, M., Bosman, A., Greenwood, B. and Wernsdorfer, W. (2009) From malaria control to eradication: The WHO perspective. Tropical Medicine International Health 4, 802-809.

Miller, J.E., Lindsay, S.W. and Armstrong, J.R.M. (1991) Experimental hut trials of bed-nets impregnated with synthetic pyrethroid and organophosphate insecticides for mosquito control in The Gambia. Medical and Veterinary Entomology 5, 465-476.

MOH. (2001) Malaria in Kenya. National malaria strategy 2001-2010. . In: M.o. Health (Ed), Nairobi.

Muirhead-Thomson, R. (1957) The malarial infectivity of an African village population to mosquitoes (*Anopheles gambiae*). American Journal of Tropical Medicine and Hygien 6, 971-979.

Müller, G., Amy, J., Butler, J., Kravchenko, D., Edita, E., Revay, E., Weiss, W. and Schlein, Y. (2009) Efficacy of the botanical repellents geraniol, linalool, and citronella against mosquitoes. Journal of Vector Ecology 34, 2-8.

Müller, G., Junnila, A., Kavchenko, V., Revay, E., Butler, J., Orlova, O., Weiss, R. and Schlein, Y. (2008) Ability of essential oil candles to repel biting insects in high and low biting pressure environments. Journal of the American Mosquito Control Association 24, 154-160.

Mutuku, F., Alaii, J., Bayoh, M., Gimnig, J., Vulule, J., Walker, E., Kaburi, E. and Hawley, W. (2006a) Distribution, description, and local knowledge of larval habitats of Anopheles gambiae s.l. in a village in western Kenya. American Journal of Tropical Medicine and Hygien 74, 44–53.

Mutuku, F.M., Bayoh, M.N., Gimnig, J.E., Vulule, J.M., Kamau, L., Walker, E.D., Kabiru, E. and HawleyY, W.A. (2006b) Pupal habitat reproductivity of *Anopheles gambiae* complex mosquitoes in rural villages in western Kenya. American Journal of Tropical Medicine and Hygien 74 1 54-6.

Muturi, E., Burgess, P. and Novak, R. (2008) Malaria vector management: where have we come from and where are we headed? The American journal of tropical medicine and hygiene 78, 536-7.

Nevill, C., Some, E., Mung'ala, V., Mutemi, W., New, L., Marsh, K., Lengeler, C. and Snow, R. (1996) Insecticide-treated bednets reduce mortality and sever morbidity from malaria among childrenon the Kenyan coast. Tropical Medicine International Health 1, 139-146.

Nolen, J., Bedoukian, R., Maloney, R. and Kline, D. (2002) Method, apparatus and compositions for inhibiting the human scent tracking ability of mosquitoes in environmentally defined three dimensional spaces. In, Vol. 6, U.S., p. 235.

Okumu, F. and Moore, S. (2011) Combining indoor residual spraying and insecticide-treated nets for malaria control in Africa: a review of possible outcomes and an outline of suggestions for the future. Malaria Journal 10, 208.

Osimitz, T. and Grothaus, R. (1995) The present safety assessment of DEET. Journal of the American Control Association 11, 274-278.

Osimitz, T. and Murphy, J. (1997) Neurological effects associated with use of the insect repellent N,N-diethylm- toluamide (DEET). Journal of Toxicology-Clinical Toxicology 35, 435-441.

Pennetier, C., Corbel, V., Boko, P., Odjo, A., RN'Guessan, Lapied, B. and Hougard, J.M. (2007) Synergy between repellents and non-pyrethroid insecticides strongly extends the efficacy of treated nets against Anopheles gambiae. Malaria Journal 6, 38.

PMI. (2008) Malaria Operation Plan(MOP) Kenya FY 2008. Technical guidance on the prevention and control of malaria in Africa. In: President's Malaria Initiative.

Rapley, L., Russell, R., Montgomery, B. and Ritchie, S. (2009) The effects of sustained release metofluthrin on the biting, movement, and mortality of Aedes aegypti in a domestic setting. American Journal of Tropical Medicine and Hygien 81, 94–99.

RBM. (2008) Insecticide treated nets. In: The Global Malaria Action Plan. For a Malaria-Free World

Robert, V., Awono-Ambene, H.P. and Thioulouse., J. (1998) Ecology of larval mosquitoes with special reference to *Anopheles arabiensis* (Diptera: Cullicidae) in market-garden wells in urban Dakar, Senegal. Journal of Medical Entomology 35, 948-955.

Roberts, D., Alecrim, W., Hshieh, P., Grieco, J., Bangs, M., Andre, R. and Chareonviriphap, T. (2000) A probability model of vector behavior: effects of DDT repellency, irritancy, and toxicity in malaria control. Journal of Vector Ecology 25, 48-61.

Rowland, M., Downey, G., Rab, A., Freeman, T., Mohammad, N., Rehman, H., Durrani, N., Reyburn, H., Curtis, C., Lines, J. and Fayaz, M. (2004) DEET mosquito repellent provides personal protection against malaria: a household randomized trial in an Afghan refugee camp in Pakistan. Tropical Medicine and International Health 9, 335-42.

Rutledge, C. and Day, J. (2005) Mosquito Repellents. In. University of Florida, Vero Beach.

Rutledge, C.R. (1989) Laboratory evaluation of controlled-release repellent formulations on human volunteers under three climatic regimens. Journal of American Mosquito Control Association 5, 52-55.

Schreck, C.E. (1995) Protection from blood-feeding arthropods. In: Auerbach PS, ed. Wilderness Medicine: Management of Wilderness and Environmental Emergencies. In, St. Louis: Mosby, p. 813-830.

Service, M. (1993) Community participation in vector-borne disease control. Annals of Tropical Medicine and Parasitology 87, 223–234.

Shiff, C. (2002) Intergrated approach to malaria control,. Clinical Microbiology Review 15, 278-93.

Shono, Y., Kubota, S., M.Sugano, Yap, H.H. and Tsushima, K. (2004) Field evaluation of papers strip and mosquito coil formulation impregnated metofluthrin for mosquito control in Malaysia. In: A.M.C. Association (Ed) The Abstract Book, 70th Annual Meeting of American Mosquito Control Association. Eatontown, Savannah, GA., p. 40.

Silve, J.B. (2008) Mosquito Ecology: Field Sampling Methods.

Sinton, J., Baily, J. and Chand, D. (1926) Studies in malaria, with special reference to treatment Part IV. The occurrence of sexual forms of *Plasmodium falciparum* in the peripheral circulation. Indian Journal of Medical Research 13, 895-916.

Snow, R., Guerra, C., Noor, S., Myint, H. and Hay, S. (2005) The global distribution of clinical episodes of Plasmodium falciparum malaria. Nature Letters 434, 214-217.

Snow, R.W., Rowan, K. and Greenwood, B.M. (1987) A Trial of Permetrin-treated bed-nets in the prevention of malaria in Gambian Children. Transactions of the Royal Society of Tropical Medicine and Hygiene 81, 563-567.

Snyder, J., Poe, R., Stubbins, J. and Garrettson, L. (1986) Acute manic psychosis following the dermal application of N,N-diethyl-m-toluamide (DEET) in an adult. Clinical Toxicology 24, 429-439.

Spencer, W. (2010) Linalool mosquito repellent. In: Tech-faq.

Takken, W. (2002) Do insecticide-treated bednets have an effect on malaria vectors? Tropical Medicine International Health 7, 1022- 1030.

Taylor, R., Hill, M., Stewart, D., Slatter, R. and Gichanga, M. (1981) A field evaluation of permethrin for residual control of mosquitoes 41, 423-433.

Ujihara, K., Mori, T., Iwasaki, T., Sugano, M., Y.Shono and Matsuo, N. (2004) Metofluthrin: a potent new synthetic pyrethroid with high vapor activity against mosquitoes. Bioscience Biotechnology and Biochemistry 68, 170–174.

Utzinger, J., Tozan, Y. and Singer, B. (2001) Efficacy and cost effectiveness of environment for malaria control. Tropical Medicine and International Health 6, 677-687.

Vulule, J.M., Beach, R.F., Atieli, F.K., Roberts, J.M., Mount, D.L. and Mwangi, R.W. (1994) Reduced susceptibility of Anopheles gambiae to permethrin associated with the use of permethrin impregnated bednets and curtains in Kenya. Medical and Veterinary Entomology 8, 71-75.

White, G.B. (1973) Comparative studies on sibling species of *Anopheles gambiae* Giles complex (Diptera-Culicidae). III. The distribution, ecology, behaviour and vectorial importance of species D in Bwamba County, Uganda, with analysis of biological, ecological, morphological and cytogenetical relationships of Ugandan species D. Bulletin of Entomological Research 63, 65-97.

WHO. (2005) World Malaria Report. In: WHO and UNICEF (Eds).

WHO. (2006) Indoor residual spraying. In: use of indoor residual spraying for scaling up global malaria control and elimination., Geneva.

WHO. (2009) Global Malaria Action Plan. In: W.H. Organization (Ed).

WHO. (2010) World Health Organization World Malaria Report. p. 1-238.

Wright, R. (1975) Why mosquito repellents repel. Scientific American 233, 104-111.

Zadikoff, C. (1979) Toxic encephalopathy associated with use of insect repellent. Journal of Pediatrics 95, 140-142.

Zhou, G., Afrane, Y. and Vardo-Zalik, A. (2011) Changing patterns of malaria epidemiology between 2002 and 2010 in western Kenya: The fall and rise of malaria. PloS one 6, e20318.

Zhou, G., Githeko, A., Minakawa, N. and Yan, G. (2010) Community-wide benefits of targeted indoor residual spray for malaria control in the western Kenya highland. Malaria Journal 9, 67.

Zollner, G. and Orshan, L. (2011) Evaluation of Methofluthrin fan vaporizer device against phlebotomine sand flies (Diptera: Psychodidae) in a cutaneous leishmaniasis focus in the Judean Desert, Israel. Journal of Vector Ecology 36, 157-165.