OPTIMISING A RESTING BOX TO ENHANCE ADULT GRAVID FEMALE MOSQUITOE AUTO-DISSEMINATE LARVICIDE IN A SEMI FIELD

BY

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DECLARATION

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DEDICATION

I dedicate this work to my family, parents and friends for their moral and financial support.

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ABSTRACT

Malaria control strategies that target adult mosquitoes are challenged by the emergence of insecticide resistance and behavioral changes of vectors. The conventional approach of applying larvicides is limited by high operational costs and inadequate knowledge of mosquito breeding habitats especially in rural African communities. This study developed a potential mosquito contamination site, assessed the potential of adult female *Anopheles gambiae s.s* to pick up fluorescent dye and auto dissemination from resting box to their breeding habitats. A screened semi-field system (SFS) with and without a mud hut inside were used to evaluate the efficacy of a designed mosquito resting box for delivery of fluorescent dye to resting mosquitoes and subsequent auto dissemination to artificial habitats within the SFS. Mud hut was used as a replica of the community housing. Coloured cotton fabric (red, black, blue, white) were evaluated for resting preference inside and outside the hut. Different box sizes and shapes were designed, with an inner lining using preferred fabric colour. Laboratory reared blood fed *An. gambiae* were released in the SFS to establish the preferred resting box size, colour and shape. The effective cardboard box-2nd design (CB-2) was then dusted with 5g of non-toxic red fluorescent dye, in which mosquitoes were released nearby the box, examined to ensure they entered the box, and allowed mosquito to locate habitat. The visitation rate at larval sites was examined using an OviART gravid trap. Trapped mosquitoes were removed daily, examined for dust contamination and recorded. Black fabric generally had a high resting preference of 60%. CB-3(Rectangular shaped cardboard-3rd design) lined with black fabric had a higher percentage resting preference (61%) compared to red fabric lined box of similar design (39%).The box shape experiment revealed that rectangular box of dimension 45cmL×30cmL×45cmH had a significantly resting preference with a mean resting rate of 74.0 ± 4.406 compared to circular box (51.0 ± 3.947) of similar size, the difference was significant (F= 14.899, df= 1, P= 0.001). Auto dissemination demonstration showed a high proportion of mosquito visiting a Cedrol treated habitat 58(50.49-65.51) compared to control/tap water site 36 (29.05-42.62). In all the recaptured mosquitoes in both treated and control site, high proportion had full dye 39 (33.55-43.79) and a few, partial dye 19 (14.20-24.46). This was an indication of successful transfer of dye from the box to mosquito and from mosquito to oviposition site showing a possibility of mosquitoes to transfer lethal dose to the larval site. This study provides a proof of auto dissemination principle of using adult female *An. gambiae* to transfer chemical from effective passive contamination station to malaria vector habitat. The finding shade light on the potential of this approach to target and control immature stages of malaria vectors (*An, gambiae* s.s). Field studies using novel adulticides and larvicides are recommended to evaluate the utility of the MRB as a mosquito control tool for the management of malaria.

Keywords: Mosquito resting box, *Anopheles gambiae,* Semi-field system, auto dissemination.

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ABBREVIATIONS, ACRONYMS AND SYMBOLS

ACT	Artemisinin-based Combination therapy
ADAM	Auto-Dissemination Augmented by Male
ANOVA	Analysis of variance
CB-1	*1st* design of Cardboard box
CB-2	*2nd* design of Cardboard box
CB-3	*3rd* design of Cardboard box (Rectangular shape)
CB-4	*4th* design of cardboard box (Circular shape)
DF	Degree of freedom
DIFF	Different
FHS	Fabric harness system
IRS	Indoor residual sprays
IGR	Insect growth regulator
LLITN	Long lasting insecticide treated net.
MRB	Mosquito resting box
Obs	Observed
PB-1	*1st* design of perspex-box
PPF	Pyriproxyfen
P-value	Level of significance
SD	Standard deviation
SFS	Semi-field system
WHO	World health organization

CHAPTER ONE

INTRODUCTION

1.1 Overview

Malaria remains an important vector-borne disease in Africa despite the recent gains in its control (WHO, 2017, Kweka *et al*., 2017). About 3.2 billion people – almost half of the world's population are at risk of malaria (WHO, 2016). Sub-Saharan Africa carries a disproportionately high share of the global malaria burden. In 2015, the region had 88% of malaria cases and 90% of malaria deaths (WHO, 2016). One of the strategies of malaria control is to reduce the host-vector contact frequency which ultimately interferes with malaria transmission through infectious bites (Massebo *et al.*, 2015). The current frontline approaches to reduce malaria burden is the use of long lasting insecticide treated bed nets (LLINs), effective treatment using artemisinin-based combined therapy (ACTs), indoor residual sprays (IRS), repellent products for personal protection and larval source management (WHO., 2014). However, the predominant tools for control of adult malaria mosquitoes heavily revolve around the use of LLINs and IRS (Okumu and Moore, 2011). These tools target mainly indoor resting adult mosquitoes, ignoring outdoor vector populations and immature stages of malaria mosquitoes. They are highly effective when used correctly, but mosquitoes are developing both behavioral and physiological insecticide resistance to this form of control (Ong and Jaal., 2015). There is need to develop additional tools for malaria mosquito control that target both indoor and outdoor resting mosquitoes, adult and juvenile stages, especially for the current drive towards malaria elimination in Africa. The aquatic larval management is effective, but it is highly challenged by habitat identification, habitat accessibility especially the microhabitats, dangerous areas for human reach, labor intensity and high cost of sustainability (Mains *et al*., 2015). Optimized indoor/outdoor mosquito resting box is a potential tool that can be used as a contamination station targeting blood-fed and gravid malaria mosquitoes. The resting box designed in this work can be deployed in an attract-and-kill strategy for horizontal transfer and auto-dissemination of larvicide. This new approach control both adult mosquitoes and larvae without risk of toxicity to non-target species in the environment.

Importantly, the box can incorporate recently discovered novel lures for gravid mosquitoes such as Cedrol (Okal *et al*., 2015; Swale *et al*., 2018), leading to enhanced trapping rates of malaria mosquitoes. The goal of this work was to develop a cheap easy to use and efficient tool for malaria mosquito control that is scalable for adoption in rural African communities. Results obtained in semi-field studies indicate a great potential for the MRB to be used as a mosquito control tool, and the future open field studies will assess the potential impact of the MRB on mosquito densities and disease transmission dynamics.

1.2 Statement of the problem

Vector control is a critical component of the drive towards malaria elimination and eradication in Africa (Talisuna *et al*., 2015). Efficacy of malaria vector control strategies; LLINs and IRS is compromised by mosquitoes developing both behavioral changes and insecticide resistance (Raphemot *et al*., 2014). Treated mosquito nets can only protect when an individual is under it, means mosquito still bite before bed time. These treated nets can only kill mosquitoes that bites and rest indoors (endophilic) and fail to kill mosquitoes that bites and rest outdoor (exophilic). Treated mosquito nets and indoor sprays can only target and kill adult mosquitoes but not the juveniles in an aquatic habitat. Chemicals soaked in mosquito net and sprayed on walls kill even the non-targeted insects. Additionally, none of the tools used currently target gravid mosquitoes per se. There is need for additional tool that can control both adult mosquitoes and larvae as well as indoor and outdoor mosquitoes, without risk of toxicity to non-target species (Killeen *et al*., 2006).

1.3 Main objective

To optimize a resting box to enhance adult gravid female mosquitoes auto-disseminate larvicides in a semi-field condition.

1.4 Specific objectives

The specific aims of the project were to:

i) Determine the preferred colour by gravid mosquitoes in a semi-field environment.

ii) Determine the preferred size of the mosquito resting box (MRB).

iii) Determine the box shape preferred by blood fed and gravid mosquitoes during resting.

iv) Assess the success of dust transfer to resting mosquitoes in the MRB and auto dissemination of dust to the artificial oviposition sites.

1.5 Hypotheses

i) Dull colours attract more malaria mosquitoes than bright colours.

ii) Big box attract more malaria mosquitoes than a small box size

iii) Circular box attract more mosquitoes than a rectangular box shape

iv) Resting mosquitoes pick larvicide from the MRB and transfer them to oviposition sites.

1.6 Justification of the study

Controlling human-vector contact has a central role in efforts towards malaria elimination by protecting humans from potentially infectious mosquito bites, this reduce pathogen transmission (Seccacini *et al.,* 2008). Over the past decades, the use of long-lasting, insecticide-treated nets (LLINs) and indoor residual spraying (IRS) against indoor-biting and indoor-resting malaria vector has significantly lowered the burden of malaria transmission (Caputo *et al*., 2012). Despite these gains, there is still ongoing transmission, a significant proportion of which now occurs outdoors (Cator *et al*., 2013). This is alluded to the fact that the development and adoption of alternative mosquito control tools has been exceptionally slow over the past years. Even though the use of (LLINs) and (IRS) has a great progress in reducing malaria transmission in Africa, the future use of these interventions, are seriously threatened by the emergence and ongoing spread of insecticide resistance (Caputo *et al*., 2012). The conventional application of larvicides in the habitats is also labor intensive, complex to organize and expensive to run, hard to identify habitats and treat a significant breeding sites to reduce the vector population (Cohen *et al.*, 2012).

Targeting aquatic larval stages of the vector by auto-dissemination mechanism, facilitated by adult female mosquitoes can be an effective method to suppress vector density and vector competence (Coetzee *et al*., 2013, Oketch *et al*., 2007). Using gravid female mosquito as a vehicle to auto-disseminate larvicide has been demonstrated for the transfer of pyriproxyfen (PPF) by container breeding *Aedes* mosquitoes (Kamal *et al.*, 2010) and presents an appealing idea to explore for the control of other mosquito species when effective resting box is used. The auto-dissemination mechanism is a milestone in malaria control.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria disease

Malaria is a serious vector-borne disease and it affects millions of people, mainly in Africa. About 3.2 billion people, nearly half of the world's population are at risk of malaria (WHO, 2016). In 2015, there were roughly 214 million malaria cases and an estimated 438 000 malaria deaths. Increased prevention and control measures have led to a 60% reduction in malaria mortality rates globally since 2000 but Sub-Saharan Africa continues to carry a disproportionately high share of the global malaria burden (Okara *et al.,* 2010). In 2015, the region was home to 89% of malaria cases and 91% of malaria deaths followed by the South-East Asia Region-7% and the Eastern Mediterranean Region 2% (WHO, 2015). More than 90% of deaths resulting from malaria occur in children of 1-5 years (WHO, 2011). Malaria negatively affects the economy by weakening the human labor, particularly in countries with tropical and sub-tropical climates. Moreover, no part of the world is free from vector-borne diseases (Panneerselvam *et al.*, 2012). The female malaria mosquito feeds on blood and plant juice (Manda *et al.,* 2007). The blood sucking habit renders adult mosquitoes prone to acquire pathogens from one vertebrate host and transmit to another-the most notorious being malaria (Killeen *et al.,* 2003).

2.1.1 Malaria control

In the absence of a sufficiently efficient vaccine, the diagnosis and treatment of clinical cases, intermittent preventive treatment of targeted populations and vector control are the only tools available to combat malaria.

Most researchers agree that vector control has a central role in achieving the ambitious goal of malaria elimination (WHO, 2015; Greenwood, 2008). The World Health Organization (WHO) continue to recommend a range of combined strategies for malaria prevention with vector control, primarily through the use of insecticide-treated bed nets (LLINs) and indoor residual insecticide spray (IRS), a key component of these strategies (Okumu *et al.*, 2013; WHO., 2012).

Despite the great progress in reducing malaria transmission in Africa over the past decade, the future use of both of these interventions, and indeed any other approach that relies on chemical insecticide, is seriously threatened by the emergence and ongoing spread of insecticide resistance.

The main mechanisms responsible for the widespread levels of resistance have been identified as: those mediated by changes at the target site of the insecticide (e.g. knockdown resistance [*kdr*] mutations), those caused by increases in the rate of insecticide metabolism and the behavioral avoidance (WHO., 2016). A study conducted in Tokyo Japan revealed that *Aedes aegypti* when exposed to Pyrethroid can alter the target site and become insensitive to chemical, alter chemical penetration and increase its metabolism and hence become resistant (Kasai *et al*., 2014). Chemical resistance is the ability of mosquitoes to survive exposure to a standard dose of insecticide. The emergence of insecticide resistance in a vector population is an evolutionary phenomenon caused either by behavioral avoidance (e.g. exophilic instead of endophilic) or by physiological factors whereby the insecticide is metabolized, not potentiated, or absorbed less in resistant mosquitoes than in susceptible mosquitoes (WHO., 2016). Mutations in the target site proteins involve nonsynonymous mutations of the gene encoding the paratype voltage-gated sodium channel (VGSC) expressed in the insect central nervous system targeted by pyrethroids.

These mutations are often known as ‘knock down resistance’ (K*dr*) mutations due to their association with a reduction of the knockdown effect (i.e. temporary paralysis of the insect occurring shortly after contact with pyrethroids). K*dr* mutations can also be selected by and do confer cross-resistance to organochlorine (DDT), which also targets the insect VGSC. In African malaria vector *An. gambiae,* two distinct mutations in the S6 transmembrane segment of domain II of the VGSC at position 1014 have been identified, leading to amino acid residue changes from a leucine to a phenylalanine in West Africa and a leucine to a serine in East Africa (Nkya *et al.*, 2013). Metabolic resistance is a more dynamic process, involving potent regulation of the mosquito detoxification system to counteract the chemical aggression caused by insecticides. Metabolic resistance consists of elevated levels or enhanced activities of insecticide-detoxifying enzymes in resistant insects, resulting in a sufficient proportion of insecticide molecules being metabolized before reaching their target in mosquito nervous system (Nkya *et al.*, 2013).

Detoxification enzymes typically linked to insecticide resistance include 3 major gene families, the cytochrome P450 monooxygenases (P450s or CYPs), the carboxyl/choline esterases (CCEs) and the glutathione Transferases (GSTs). Cuticle resistance is characterized by a modification of the insect cuticle leading to a slower penetration of the insecticide reducing the amount of insecticide molecules within the insect. Such resistance mechanism has been evidenced in the cotton bollworm (*Helicoverpa armigera*) regarding pyrethroids. Cuticle thickening linked to pyrethroid resistance has also been identified in the oriental fruit fly (*Bactrocera dorsalis*). In mosquitoes, cuticular resistance is often mentioned, but has rarely been characterized.

A recent study demonstrated a better tolerance of *An. funestus* to pyrethroids in association with an increased thickness of the cuticle (Wood *et al.,* 2010). Studies have shown that mosquitoes change their behavior as a way of protecting themselves from chemicals. The behavioral changes identified include but not limited to: change in biting time, resting places (indoor/outdoor), feeding palces-endophagic/exophagic. Experimental hut studies indicated that bed nets treated with pyrethroids and walls sprayed with DDT dramatically increase the rate at which African mosquitoes leave huts and reduce the number of blood-fed mosquitoes compared to untreated controls (Gatton *et al.,* 2013; Ranson *et al.,* 2011; Coetzee *et al.,* 2013; Sokhna *et al*., 2013;WHO., 2011).

The shortcomings of accepted vector control methods have highlighted the need for integrated vector management (IVM) strategies that can be fully embraced and implemented by national malaria control programs (Beier *et al.,* 2008; WHO., 2004; WHO., 2008). While these conventional methods can reduce malaria parasite transmission rates and incidence of new infections, they do not consistently reduce malaria prevalence (Beier *et al.,* 2008). Moreover, sustainable use of LLINs and IRS are challenged by, high cost, improper use and lack of community interest (Cohen *et al*., 2012; Imbahale *et al*., 2013). Instead of such drawbacks, the development of additional tools and practical operational solutions which will complement existing methods for malaria vector control is of high propriety (Russell *et al.,* 2013). However, any method of malaria mosquito control relies more on vital aspects of malaria vector such as: vector species, life cycle, behavior, host seeking, matting, blood feeding, oviposition and resting (Day, 2016).

2.1.2 Biology of the Anopheles malaria mosquito

Mosquitoes of the genus *Anopheles* transmit malaria parasites to humans (Neafsey *et al.*, 2013). *Anopheles* mosquito species vary in their vector potential because of environmental conditions and factors affecting their abundance, blood-feeding behavior, survival, and ability to support malaria parasite development. *An. gambiae* is responsible for most transmission of malaria parasite (Okara *et al*., 2010). In this regard, *An. gambiae* is the major vector of interest in malaria research (Imwong *et al.*, 2011). Complex biological events take place during developmental process of the mosquito. Six developmental stages are evidenced, in which adult female laying 50-200 eggs in each oviposition. Eggs laid singly (about 0.5mm long and boat shaped) directly on water, having floats on either side. They hatch within 2-3 days, although hatching may take up to 2-3 weeks in colder climate, into early larvae (late third instars/early fourth instars) late larvae (late fourth instars), early pupae (<30 minutes after pupation), late pupae (after tanning), and adult female and male mosquitoes within 24 hours of post emergence (Harker *et al.*, 2012).

Although there are 3,200 species of mosquitoes belonging to 42 genera, only *Anopheles* genera can transmit human malaria (Paul *et al.*, 2004). Among the Anopheles species identified as major vectors in Africa are: *Anopheles gambiae, An. arabiensis, An. bwambae, An. melas, An. merus and A. quariannulatus.* However, *A. gambiae and A. arabiensis* are the two most important species for malaria transmission (Munhenga *et al.*, 2014).

2.1.3 Mosquito behaviour

Behavior is a way in which an organism adjusts to and interact with its environment (Cator *et al*., 2013; Glenn *et al.*, 2009). Behavior activities range from relatively simple actions to more complex such as mating, feeding, resting, oviposition and courtship.

Behavior pattern vary from one species to another but they are all aimed at increasing the survival of the species (Tome *et al.*, 2014). To be able to reduce vector capacity, it is therefore important to understand how they behave, this is possible if we get to study the basic life history, behavior patterns that directly or indirectly influence the insect vector competence (He *et al.*, 2015).

Temperature has been postulated to be a major factor controlling mosquito behavior. It is accounted that the microhabitat mosquito would prefer (indoor/outdoor) is determined by its temperature fluctuation (Paaijmans & Thomas., 2011).

It has been reported in earlier studies that mosquitoes can alter their behaviour when their normal operational routine is breeched by human intervention such as sleeping under long lasting insecticide treated bed nets (LLITN), indoor residual sprays (IRS), use of repellent and deterrents (Thomson *et al.*, 2016). It is well documented that mosquito behaviour is influenced by chemical cues such as carbon dioxide (Co_2), pheromones. Other studies report that mosquito behaviour can be altered by light rays. In this event, it is proposed that if light can alter their behaviour then light can be used to control mosquito instead of relying on chemicals that ends up polluting environment (Sheppard *et al*., 2017).

2.1.4 Mosquito Blood Feeding

The foraging behavior of blood-sucking arthropods is the defining biological event shaping the transmission cycle of vector-borne parasites. It is also a phenomenon that pertains to the realm of community ecology, since blood-feeding patterns of vectors can occur across a community of vertebrate hosts (Chaves *et al*., 2010). Mosquito use plant sugars and vertebrate blood as nutritional resources. When searching for blood hosts, some mosquito expresses preferential behavior for selected species.

Host preference is affected by myriad extrinsic and intrinsic factors. Inherent factors are determined by genetic selection, which appears to be controlled by adaptive advantages that result from feeding on certain host species (Takken *et al*., 2013). Host preference of mosquitoes, although having a genetic basis, is characterized by high plasticity mediated by the density of host species, which by their abundance form a readily accessible source of blood. Host-selection behavior in mosquitoes is an exception rather than the rule. Those species that express strong and inherent host-selection behavior belong to the most important vectors of infectious diseases, which suggest that this behavioral trait may have evolved in parallel with parasite-host evolution (Takken *et al*., 2013). Transmission between vertebrate hosts is achieved by the blood-feeding habit of mosquitoes, which enables the disease agents to successfully become established in and be transmitted by their arthropod hosts (Biere., 1998). Selection of a blood host that is essential for the parasite/pathogen to successfully complete its life cycle is therefore important.

2.1.5 Mosquito mating behavior facilitate transfer of larvicide between males and females

Mating is one of the critical behavior that characterize the mosquito life strategy. Mosquitoes depend on sexual reproduction for species maintenance. Newly emerged male mosquitoes are unfit for coupling with a female, as the external genitalia require a morphological change. This is accomplished by inversion of the terminalia within the first 24 hours following emergence (Takken *et al.*, 2006). In many species, male accessory glands mature during the first few days of adult life, and this is needed before sperm can be successfully transferred (Takken *et al.*, 2006). Thus, males of many mosquito species require several days to mature before a first successful mating can take place. In *Anopheles gambiae* Giles *sensu stricto* and *An. arabiensis* Patton, optimal mating occurs with 5–7-day old. Mosquitoes mate through a swarming behavior. Swarming is an insect behavior of forming a large group which is usually conspecific (Howell and Knols., 2009). Evening swarms starts 2 minutes late evening before sunset in sheltered sites and a minute or two later in exposed areas. It takes approximately 5 minutes for the arrival of the first male for the swarm to reach estimated maximum numbers. Mating pairs are first seen approximately 7 minutes after the start of swarming. Maximum numbers of pairs in copula occur 8 minutes later. Up to 270 pairings is seen in the 20-minutes period before darkness. Males are attracted to sounds that approximate the female flight tone (Charlwood *et al.*, 2002). A study conducted by Mains *et al.*, (2015) showed that contaminated male mosquitoes can be used as a vehicle to deliver the insecticide to the breeding site directly or indirectly. Direct transfer is when the contaminated males visit the oviposition site (Figure 2.1b) and indirect is by transferring larvicide to female (Figure 2.1c) through mating exercise (cross-contamination) which later visits the immature habitat. Mains approach highlighted the mating behavior as concept of reaching multiple habitats with insecticides when employing auto-dissemination technique even from a small dissemination station.

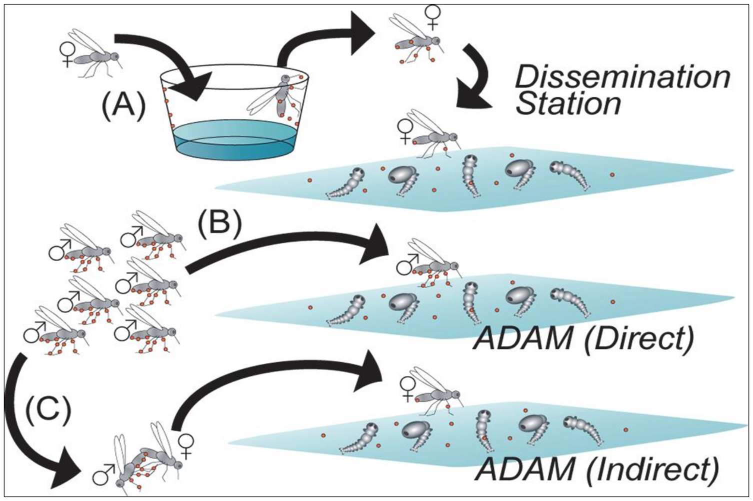

Figure 2.1 Transfer of larvicide by female alone may not be effective (A), contaminated male alone may not be effective (B), Cross contamination increase the number of habitats to be visited by both male and female (C), this assures effective auto-dissemination (Mains *et al*., 2015).

2.1.6 Host seeking and oviposition

Mosquitoes just like any other insect are thought to use a variety of cues in locating hosts, resting and oviposition sites. Literature explains more on chemical cues when locating a host but less information is available on visual cues more so when locating resting sites indoors and outdoors. Black color has been presumed as a preference color but nobody is certain about it and/or the optimal color combinations.The *Anopheles gambiae sensu lato* is ranked among the world's most efficient vectors of human malaria (Olayemi *et al*., 2011; Mweresa *et al.,* 2016). Their unique bionomics, particularly their anthropophilic, endophagic and endophilic characters, guarantee a strong mosquito-host interaction, favorable to malaria transmission. Mosquito uses both volatile and visual cues when seeking a resting and oviposition sites (Himeidan *et al.,* 2013; Spitzen *et al.,* 2016). Visual reflex guides mosquitoes to potential hosts where they are close enough to detect thermal cues (Breugel *et al*., 2015). The anthropophilic malaria mosquito *Anopheles gambiae sensu stricto* primarily takes blood meals from humans, whereas its close sibling *Anopheles arabiensis* is more opportunistic. Different odour baits elicit varying responses among mosquito species (Busula *et al.,* 2015). The preference to host for blood meal is host dependent and it has been discovered that some host (chicken) are repellent to some species of mosquitoes (Jaleta *et al.,* 2016). Studies done shown that mosquitoes are attracted to colors of different hue (Burkett and Butler, 2005) and they use color to locate their destinations. Similarly, their olfactory system is a communication center that receives and perceives physiochemicals (Cummins *et al*., 2012).

Lindh *et al*., (2015) recently discovered Cedrol, a noble oviposition chemical exploited by gravid mosquitoes to locate their oviposition site. So far, volatile compounds identified as oviposition attractants for mosquitoes include phenol, 4-methyl phenol, 4-ethyl phenol, indole, skatole, and p-cresol from hay infusions; 3-carene, α-terpene, α-copaene, α-cedrene, and d-cadinene released by copepods; alcohol and terpenoids including p-cresol from plants; ethyl acetate and hydrocarbon substances, probably released by filamentous algae; 3-methyl-1-butanol identified from bacteria (Himeidan *et al.,* 2013). Cedrol therefore adds to the list of oviposition attractants. Mosquitoes have temporal and spatial habitat distributions and a full understanding of the breeding habitats is important in planning effective anti-mosquito control measures (Varela *et al.,* 2014). Two aspects affect larval distribution in aquatic habitats; the oviposition choice of a gravid female and the survival of larvae in the aquatic environment (Gouagna *et al*., 2012).

When habitats are sampled 88% are found to have early instars of *Anopheles* larvae, while late instars occur only in 59% of sites.

This may indicate that oviposition occurs in a larger number of habitats perceived suitable by the ovipositing female but that the survival of larvae as expressed by larval density depends on factors associated with habitat size, stability and conductivity of water bodies (Fillinger *et al.,* 2009). The breeding of mosquito vectors indoor and outdoor containers suggests the need for public health enlightenment on the danger inherent on indiscriminate disposal or stack-up of containers and improper storage of water in and around the house (Idowu *et al.,* 2010).

2.1.7 Mosquito flight distance

Flight range has been recognized as an important factor in mosquito behavior. The ability to disperse, along with its host preferences, biting frequency, and transmission rates are crucial components of vector capacity and the ability to efficiently transmit a pathogen (Greenberg *et al.,* 2012). Humans living close to mosquito breeding habitats express concerns about future mosquito nuisance situations. There should be a considerable distance between human occupation and wetlands to avoid such problems. Such a distance can be useful in creating a buffer zone and setting a resting box. Extensive quantitative survey had been done to provide reliable information on mosquito flight distance and the relevant environmental conditions. Mosquitoes have an average maximum flight distance of between 50m-50km depending on the species (Verdonschot and Lototskaya., 2014). Long-distance or migratory flights are strongly related to species, ecological preferences and physiology. Jacob *et al*., 2012 performed an assessment of the post-blood meal flight distance of four mosquito species in a unique environment. Mosquitoes were trapped at the Rio Grande Zoo in Albuquerque and the blood source of blood-engorged mosquitoes was identified. The distance from the enclosure of the animal serving as a blood source to the trap site was then determined. It was found that mosquitoes captured at the zoo flew no more than 170m with an average distance of 106.7 m after taking a blood meal. This is the first study in which the flight distance of wild mosquitoes has been assessed using blood meal analysis and the first in which zoo animals have served as the exclusive source of blood meals.

2.1.8 Auto-dissemination of mosquitocidal compounds

Auto-dissemination is the approach that relies on adult mosquito behavior to spread larvicide to breeding sites at levels that are lethal to immature mosquitoes (Mains *et al*., 2015). Prior studies demonstrate that 'dissemination stations,' deployed in mosquito-infested areas, can contaminate adult mosquitoes, which subsequently deliver the larvicide to the breeding sites (Mains *et al.*, 2015). Larvicides are applied to aquatic habitats of developing immature mosquitoes and are demonstrated to reduce mosquito-borne disease transmission (Mains *et al.*, 2015). Auto-dissemination method is attractive to the species of mosquitoes breeding in small aquatic habitats, many of which can be small, sheltered and difficult to locate and treat (cryptic breeding sites). These are areas where chemicals are hard to reach when sprayed. Its potential to effectively counter the main challenge to conventional larviciding approaches, by effectively targeting the many cryptic breeding sites mosquitoes utilize, makes the technique more effective (Sihuincha *et al.*, 2005). This approach has been demonstrated to have advantages such as high level of residual activity of larvicide, it is also cost effective method of vector control because the dissemination station once set does not require frequent maintenance or frequent chemical applications (Caputo *et al*., 2012). Treatment of a small proportion of resting places (dissemination station) result in high coverage of aquatic sites (Devine and Killeen*.*, 2010).

This amplification is facilitated by an abundance of mosquitoes, the potential for multiple resting oviposition cycles (contamination events) over a mosquito lifetime and the persistence and potency of the larvicide used.

2.1.9 Dissemination station

Mosquito landing box provides an ideal resting environment for blood fed mosquitoes as blood is digested in the stomach. Researchers discovered that a resting mosquito can be contaminated with insecticides while resting in the resting box (Matowo *et al.,* 2016).

In Indonesia, scientists demonstrated the landing behaviour of mosquitoes on surfaces especially near their breeding sites. Landing of mosquitoes was shown to occur between the blood host and mosquito breeding sites. Burkot *et al*., 2013 decided to construct a barrier screen as a landing site to trace the movement of mosquitoes. It was constructed using polyethylene shade cloth netting, attached to two wooden or bamboo poles with polyester cord, set at 2m high from the ground and mosquitoes landing on it were searched at night (Plate 2.1-right). Barrier screen however is easy to make and set but can easily be dismantled by moving animals. Landing mosquitoes can easily escape because it lacks walls to restrict movement. If insecticide is applied, it can fade out more quickly due to high level of exposer to environment and elements of weather such as rain, wind among others.

Plate 2.1 Barrier screen for sampling mosquitoes in Indonesia, made from shade cloth attached to bamboo poles with polyester cord and searched at night; (adapted from Burkot *et al.*, 2013).

A prototype of auto-dissemination station topically contaminates oviposition-seeking container-dwelling mosquitoes with the insect growth regulator (pyriproxyfen), has been demonstrated (Wetoijera *et al.*, 2014). Odiere *et al*., 2007 demonstrated this idea with a clay pot on *Anopheles arabiensis* (Plate 2.2). Clay pots are readily available and relatively cheap, cool and offer conducive microenvironment for resting insects. However, elements of weather such as rain, sunshine, hailstone can destroy the pot. Pots are also valuable household equipment that are likely to be stolen when they are installed in the field for to control mosquito.

Plate 2.2 A clay pot as a dissemination station where adult insects pick chemicals (right) and an artificial oviposition site-left; (adapted from Wetoijera *et al*., 2014 and Odiere *et al*., 2007)

Caputo *et al.,* (2012) conducted a study in Rome using *Aedes albopictus.* They organized a dissemination station adapted from modified sticky trap (Plate 2.3). The four sticky surfaces replaced by four 1268 cm black cotton cloths and a thick net placed over the water to prevent mosquitoes from ovipositing. Before each experiment, each dissemination station (DS) is filled with 700 ml of tap water and each cloth dusted with 1g of powdered pyriproxyfen (ppf). This is obtained by manually grinding 0.5% or 5% PPF tablets to a granule average size of 40–80 micron. Water in the set up makes the condition inside the station conducive for mosquitoes. However, the materials used in its design are expensive, gravid mosquitoes can easily drop their eggs inside the water provided confusing it for oviposition site. This is likely to hinder auto-transfer because gravid mosquitoes may not leave the dissemination stations after oviposition.

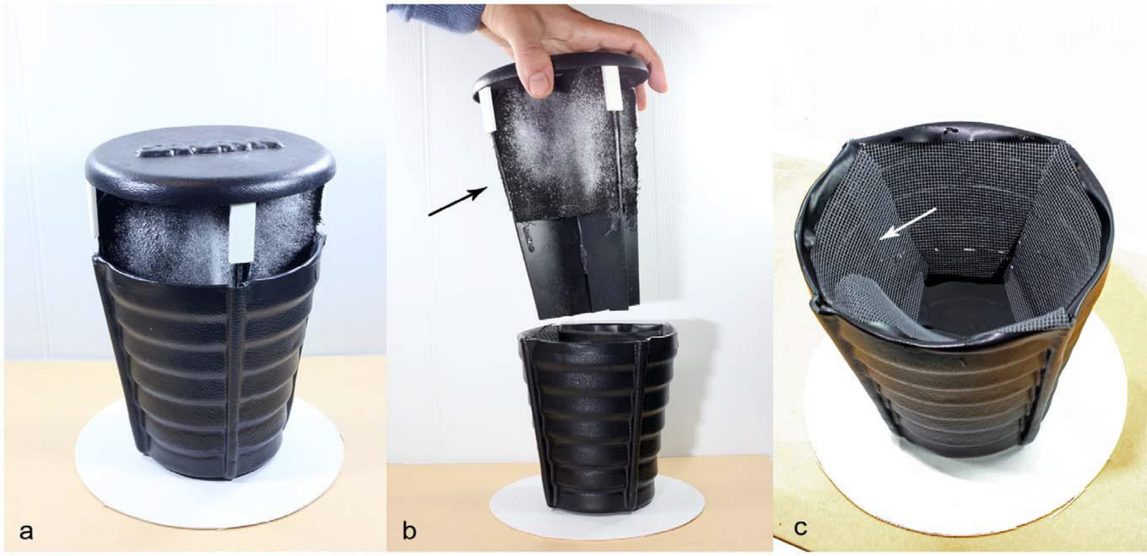

Plate 2.3 Complete assembly of dissemination station (DS); a) Two main parts of DS; b) Internal view of the DS; c); (Adapted from Caputo *et al.*, 2012).

Gaugler *et al*., (2012) designed an auto-dissemination station having a water reservoir to attract gravid females, joined to a transfer chamber designed to contaminate visiting mosquitoes. The unit is easily constructed by molding wet shredded cardboard using corn starch as a binder. Mmbando *et al*.,2015 developed mosquito landing box (MLB) baited with human odors and carbon dioxide. The MLBs is dusted with 10% pyriproxyfen (PPF) to mark mosquitoes physically contacting the devices. This design was tested on non-blood fed *An. arabiensis* mosquitoes with the aim of sampling mosquito densities. Mmbando *et al.,* (2015) designed a mosquito resting box (MRB) made of wood, solar-driven mosquito control box, measuring 0.7 m × 0.7 m × 0.8 m, and standing on short wooden pedestals, raised 10 cm above ground (Plate 2.4). It has side panels that are removable and multiple louvres forming mosquito landing or mosquito contact surfaces. However, wood can be affected by fluctuating weather condition. Solar panel is valuable and can be stolen. MLB use sola which is not readily available in local communities.

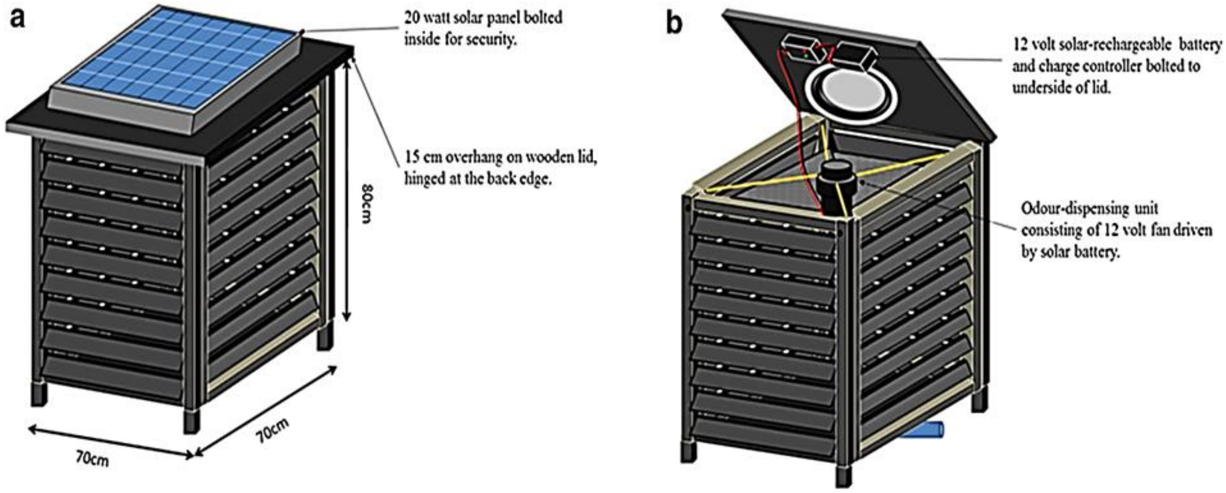

Plate 2.4 Outdoor baited mosquito landing box (MLB) with a solar on top and louvers in sides a), MLB with lid open to expose batteries and fan b); (Mmbando *et al.*, 2015).

A simple cow odour baited resting box design (45cm×30cm×45cm) was demonstrated as an effective sampling tool against *Anopheles arabiensis* (Plate 2.5). It is made of card box, wrapped with plastic transparent polythene to protect it from weather conditions. Inside was lined with black cotton cloth. The design was improved to create a dissemination station for horizontal transfer of insecticides. This resting box design provides small entry point for mosquitoes and was demonstrated using *Anopheles arabiensis* and not *Anopheles gambiae s.s* (Kweka *et al.*, 2009).

Plate 2.5 A resting box for An. arabiensis sampling; (Kweka *et al.*, 2009).

Okumu *et al.,* (2010), working in Ifakara Tanzania, designed the Ifakara Odor-Baited Station (OBS) to demonstrate the need to establish an additional tool to collect and contaminate mosquitoes in such a way that they can die even after leaving the target (Plate 2.6). The Ifakara OBS is a hut-shaped box made of canvas on a wooden framework, measuring (1.5 m × 1.5 m ×1.75 m). On one side, it has a round operator entry point (0.6 m diameter) fitted with a black cotton sleeve that prevent mosquitoes from exiting. Inside is lined with black cotton cloth and a plastic floor mat. It has exit traps made of ultraviolet-resistant netting on a wire frame However; the entire structure cannot be carried easily without dismantling it and assembled again on new site. On the other hand, ultraviolet-resistant netting used is relatively expensive.

Plate 2.6 The Ifakara Odor-Baited Station (OBS); (Okumu et al., 2010).

Pombi *et al.,* (2014) designed a sticky resting box (SRB) made of wood (45cm×33cm×35cm). It is easy to package and to transport (Plate 2.7). The inner sides are lined with A4 acetate sheets coated with rat-glue. The entry opening is 45cm×15cm.

This opening seems to be small for the movement of insects and release of attractant odour (water). The rat-glue and wood used in the set up may produce either repellent or attractant odour to mosquitoes. Mosquitoes that enter the box sticks on the glue hence cannot pick larvicide and transfer to the larval site. These may compromise the efficacy of this resting box design.

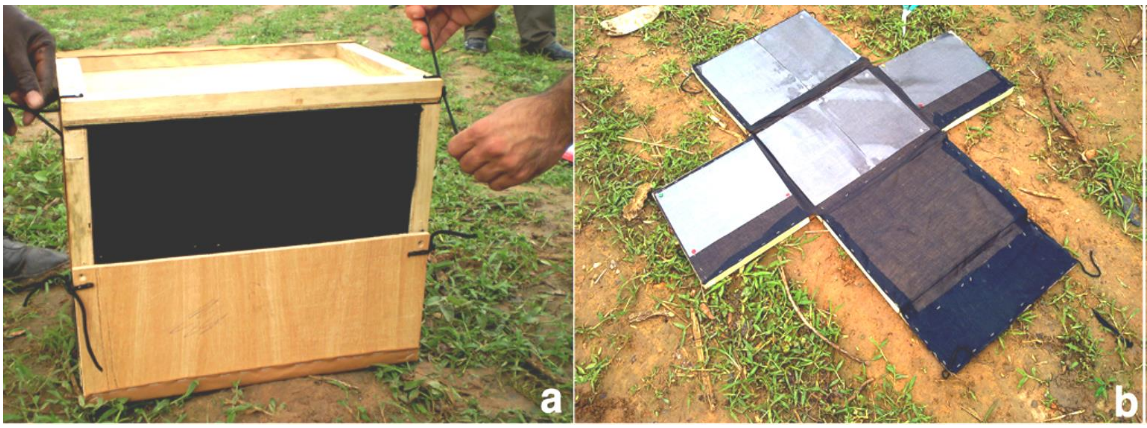

Plate 2.7 The sticky resting box (SRB). a) Fully assembled system showing entrance; b) Opened box exposing sticky sheets in the inner wall of the trap; (Pombi *et al.*, 2014).

The essential criteria that must be met to prove the efficacy of an auto-dissemination station require a demonstration of the effectiveness of a resting box in attracting mosquitoes inside, transferring the larvicide from the box to the resting mosquito, and facilitating the subsequent transfer of the larvicide from the mosquito to the target habitats at a lethal concentration (Geden and Devine., 2012). This approach is inexpensive and not complex in maintenance compared to conventional application of larvicides which is labor intensive (Fillinger *et al.*, 2008).

2.2.1 Mosquito marking techniques

Tracking the movement of mosquitoes require marking to be able to trace a behaviour pattern of interest. An individual marking method is a marking technique of placing spots of paint at different points on the thorax of mosquitoes (Tsudo and Kamezakia, 2014). Verhulst *et al.*, (2013) in their study discovered that marking neither reduced mosquito survival nor alter its behaviour. Dickens *et al.,* (2014). Conducted a study *Aedes aegypti* were marked using fluorescent dust, it revealed that dust storm allowed relatively high survival of mosquito, compared to unmarked controls.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study area

The study was conducted at International Centre of Insect Physiology and Ecology, Thomas Odhiambo Campus, Mbita point (*icipe*-TOC). *Icipe*-TOC is in Mbita municipality on the shores of Lake Victoria, western Kenya (0° 26’ 06.19” S, 34° 12’ 5313” E; altitude 1137 m above sea label).The Campus sits on 24.5 hectares of land, 40% of which consists of experimental fields and landscaped buildings. TOC’s research structures include state-of-the-art laboratories and offices, as well as insects and animal rearing facilities. The major malaria vectors in this area, which maintain holoendemic malaria, include *An. gambiae* Giles, *An. arabiensis* Patton, and *An. funestus* Giles (Kawada *et al*., 2011). The area has two rainy seasons; the long rains, which extend from March to June and the short rains which extend from August to December. Climatic conditions consist of temperature ranging from 17˚C to 34˚C. Annual rainfall ranges from 700mm to 1200mm.

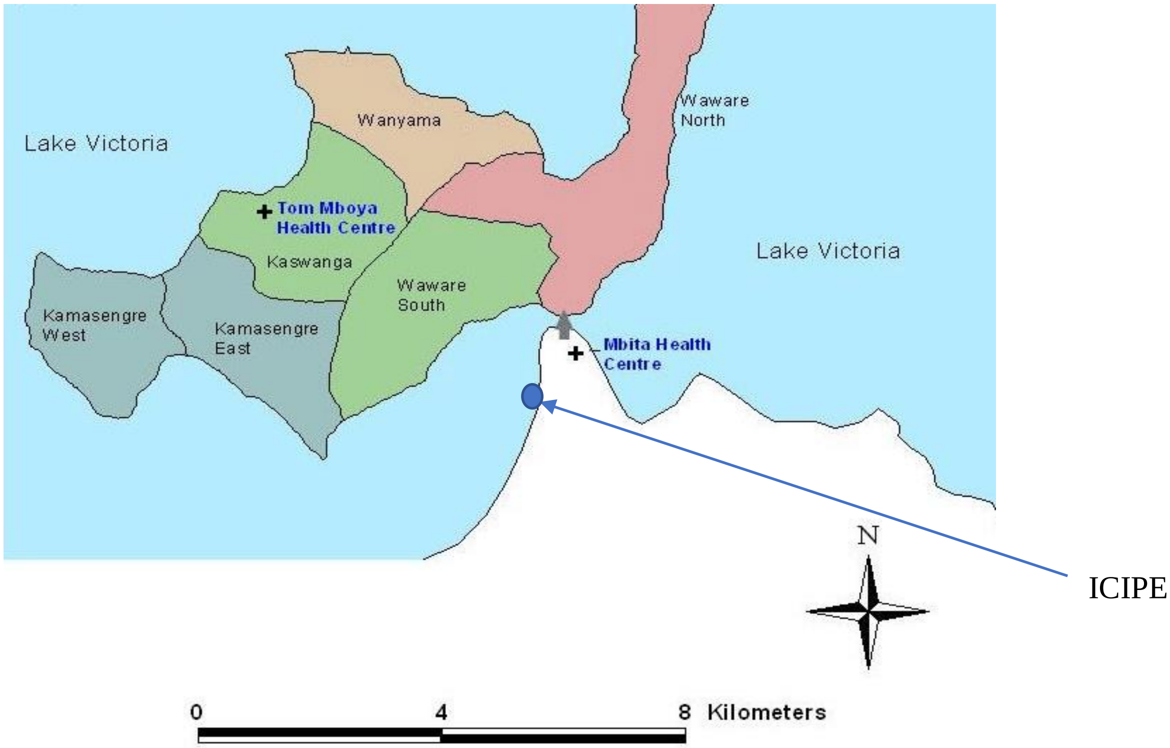

Figure 3.1 Map of Rusinga area and ICIPE Mbita Point (Source: Author, 2018)

Plate 3.1 Semi-field system (SFS) where experiments were conducted Labels: a= entrance, b= walls with fiber glass netting and c= polycarbonate roof (Source : Author, 2018).

3.2 Study design

The study had a laboratory component for bioassays, and semi-field component whereby the semi field system (SFS) comprised of an outdoor construction with mesh walls measuring 12 m × 7 m and a floor filled with sand (Plate 3.1). One of the SFS had a simple mud hut (2.5 m × 2.5 m × 2m) inside to provide a replica of a home. The preferred colour, size and shape of mosquito resting box (MRB) for gravid *Anopheles gambiae s.s* mosquitoes were tested under semi-field system (SFS) inside and outside the hut. A total of twelve (N=12) replicates, with a rotation program of fabrics were performed in the pursuit for colour preference. In each experimental day, two hundred (n= 200) gravid mosquitoes were released. A mouth aspirator was used to recapture mosquitoes that were found resting on the coloured fabrics. The dye transfer success from the box to mosquitoes in the resting box and transfer of dye to the artificial oviposition sites was done in semi field system. Its standardization was compared with the already done, efficacy studies of Pyriproxyfen (PPF) as a standard on *An. Gambiae* (Wetoijera *et al*., 2014).

3.3 Mosquito Colony

The primary focus of this research was female *An. gambiae* mosquito because they are hematophagous and able to transmit malaria parasite during their feeding on the suitable susceptible host. All proposed laboratory and semi-field studies were therefore conducted using already established *An. gambiae* s.s mosquito colony at Mbita point. Temperature and relative humidity in the insectary (colony room) varied between 26 °C and 30 °C and 67–73 % relative humidity. Mosquitoes (2–3 days old) held in a 30cm × 30cm × 30cm netting cage were maintained on 6 % glucose solution given as energy source *ad libitum* using absorbent paper wicks soaked in 25-ml vials.

A piece of cotton (50cm × 25cm) saturated with distilled water and positioned on top of the cage twice a day, ensured that mosquitoes remained hydrated throughout. Mosquitoes were fed on blood for 15 min from a human arm by volunteer staff at 0600 h after starving them for 6 h before the blood-meal. Mosquitoes were left undisturbed for 10 minutes after the blood meal. Gorged mosquitoes were selected through visual inspection and used for experiment. Females were presumed gorged when they had distended abdomens.

3.4 Experimental procedures

3.4.1 Experiment 1: Evaluating the visual acuity of mosquitoes to different colors

To assess the preferred color for a mosquito resting site, cotton cloths of four (4) different colors (Red, Blue, White and Black), were selected for study. Several designs were considered in designing the resting sites using the coloured fabrics as outlined below:

Design #1: Use of hut mud walls as anchors for fabrics to create resting sites.

A mud-walled iron sheet roofed hut (3 m x 3 m) constructed inside the semi field system (SFS) was used in this experiment (Plate 3.2a). Four cotton cloths of different colors were pinned on each side of the wall (4-walls) inside the hut using 1 inch ordinary nails (Plate 3.2bcd), fabrics were anchored on rotation to eliminate positional bias. The holes and positions relative to the cloths were maintained constant during all experiments to eliminate positional bias. Insectary reared (2-3 days old) freshly blood fed *A.gambiae s.s* malaria mosquitoes (*n*= 200) were collected from the insectary cage into a paper cup at 0630 h and released at the center of the hut at 0700 h then left undisturbed for 2hrs. Recovery of released mosquitoes was done in 2 h intervals post release, precisely at 0900 h, 1100 h, 1300 h and 1500 h. Mosquitoes found resting on the coloured sheets were recaptured using mouth aspirator and transferred into respectively colour coded cups.

Plate 3.2 A screen house with a hut inside a); sections of hut wall with anchored fabrics inside b-d) (Source: Author, 2018).

Design #2. Metallic frames (placed at 1.65 meters and 3.7 meters apart) as anchors for four fabrics to create coloured display resting sites in open SFS.

Fabrics were pinned on the metallic frames (1.83cm ×1.22m) and positioned inside an open screen house (12m x 7m). They were placed first at 1.65m and then 3.7m apart (Plate 3.3). Rotation of frames was done in each replicated experiments to eliminate bias to an experimental side of the screen house (SFS). Insectary reared (2-3 days old) freshly blood fed *A. gambiae s.s* malaria mosquitoes (*n*= 200) were collected from the insectary cage into a paper cup (enclosed with a white cotton netting, enforced by a rubber band) at 0630 h and released at the central point inside the open screen house (2.2m from each frame) at 0700 h then left undisturbed for a period of 2 hours. Mosquitoes found resting on the sheets were recaptured using mouth aspirator and transferred into collection cups labeled red, black, blue and white. Recaptured mosquitoes were placed in a freezer for 20 minutes to die, then counted and recorded.

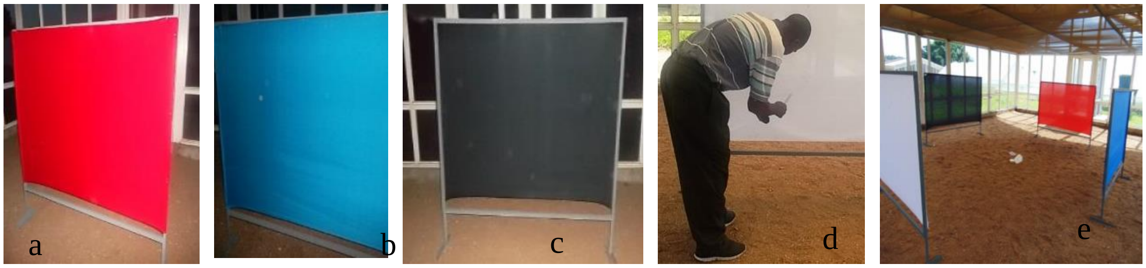

Plate 3.3 Fabrics anchored on frames (a-c), collecting resting mosquito (d), Arrangement of the frames inside an open screen house (e). This setup allows for equal distribution of light and for equidistant rotations of the panels (Source: Author, 2017).

3.4.2. Experiment 2: Optimizing the size of mosquito resting box (MRB).

Different box designs were constructed and evaluated to establish the preferred resting box size for blood fed mosquitoes.

Design 1: A prototype box adapted from Mmbando *et al.*, 2015 design.

To evaluate the optimal size of a mosquito resting box (MRB) for freshly blood fed and gravid *An. gambiae s.s,* experiments were conducted in a screen house (12 m x 7 m) fitted with mosquito netting on the roof and the side walls and with no hut inside (Plate 3.4a). A prototype box adapted from (Mmbando *et al.,* 2015) was assembled using translucent Perspex material and with an inner capsule fitted with black electrostatic netting (Plate 3.4bc). Four consecutive experimental nights, freshly blood fed laboratory reared (2-3 days old) mosquitoes (*n*= 200) were released 2 meters from the MRB at 0630 h. The release positions were varied in the three walls of the screen house. Recaptured mosquitoes were placed in a freezer for 20 minutes to die, counted and recorded.

Plate 3.4 An open screen house without hut inside a), Fist design, pers-Box (PB-1) with closed top b); PB-1 with top open showing the inner netting capsule c) (Source: Author, 2017).

Design 2:Optimizing mosquito entry opening into the MRB (45cm×22cm and 30cm×12cm) by comparing big (45cm×30cm×45cm), and small box (30cm×24cm×25cm) designs.

The cardboard material was used because it is easy to manipulate, cheap and readily available, but final design would use Perspex or any other material deemed appropriate. Cardboard boxes (*n*= 2) of different sizes were constructed; CB-1a of dimensions 45cmL×30cmW×45cmH with an opening of 45cm×22cm and CB-1b of dimensions 30cmL×24cmW×25cmH with an opening of 30cm×12cm (Plate 3.5). The inner walls of the boxes were lined with black cotton cloth. The box was suspended at 0.85 m from the ground (to avoid predation by ants) inside the hut. Freshly blood fed *An. gambiae s.s* (*n*= 200) were released 1m from the box at 0700 h. Recapture was done at an interval of 2 hours up to 1700 h. Recaptured mosquitoes were placed in a freezer for 20 minutes to die, then counted and recorded. Inner microclimate (humidity and temperature, data not shown) was recorded using a hobo data logger.

Design 3: Optimizing MRB (CB-2 design) with an enlarged entry point of 45cm×45cm.

We modified CB-1a to create a larger entry point for resting mosquitoes in which the entire box side was opened to create an entry of 45cm×45cm, lined with a black cotton cloth inside to make CB-2 design (Plate 3.5d). All other experimental conditions remained similar to the former experiment.

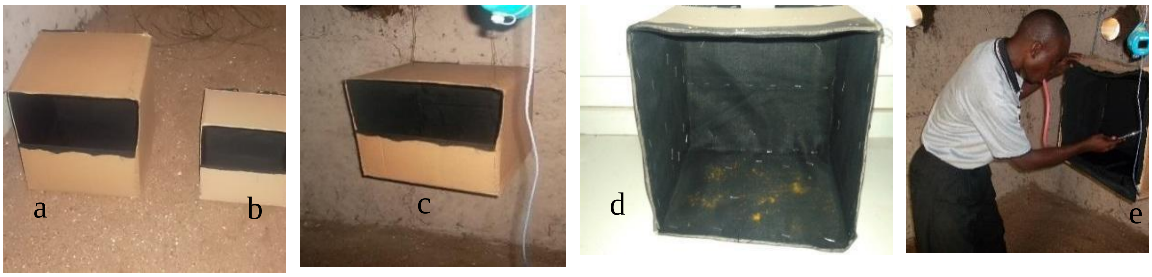

Plate 3.5 CB-1a= big box (45cm×30cm×45cm) with small opening (45cm×22cm), CB-1b= small box (30cm×24cm×25cm) with small opening (30cm×12cm), c= CB-1a tested in the hut and a visible Hobo data recorder nearby, d= CB-2 with enlarged entry(45cm×45cm), e= Collection (Source: Author, 2017).

Design .4 Optimization of preferred box color under semi-field conditions (CB-3 design)

Two boxes (CB-3) were constructed, measuring (94cm x 36cm x 48cm) and aligned with black and red cotton fabrics respectively (Plate 3.6). The two boxes were tested individually in the screen house. They were suspended at 0.85 m from the ground to avoid predation by ants inside the hut. Freshly blood fed *An. gambiae s.s* (*n*= 200) were released at 0700 h and recapture done at an interval of 2 h up to 1700 h.

 

Plate 3.6 Construction layout (left); CB-3 aligned with Black and red cotton cloth (right) (Source: Author, 2018).

3.4.3 Experiment 3: Optimizing a resting box shape (Rectangular and circular) for gravid mosquitoes.

A prototype box adapted from Sikulu *et al*, 2009 and Kweka *et al.*,2009 were used to evaluate the optimal shape of a mosquito resting box (MRB) for freshly blood fed and gravid *An. gambiae s.s.* Boxes were assembled (45cm×30cm×45cm) using cardboard material (Plate 3.7), and the inner surface fitted with black cotton cloth (Rectangular and Circular shapes) as informed by the previous experiment. The MRBs were positioned individually in the SFS at a corner and rotated in the four corners daily to eliminate aspects of positional biasness. For every experimental day, freshly blood fed insectary reared (2-3 days old) mosquitoes (*n*= 200) were released at the center of the SFS at 0630 h. The observations were done in an interval of 2 hours from 0900 h up to 1700 h.

  

Plate 3.7 CB-2 Rectangular (left), CB-4 Circular box construction (middle), Complete CB-4 box (right) (Source: Author, 2018).

3.4.4 Experiment 4: Contamination success of mosquitoes in the dissemination station (MRB) and transfer of Dye to artificial oviposition sites as an auto dissemination strategy.

The designed mosquito resting box (MRB) was dusted with 5g red fluorescent dye to create a contamination station. The fluorescent dye in this experiment was used as a proxy for larvicide. Artificial mosquito oviposition sites were planted at the back corners of the screen house by digging in 2 black troughs of 15 liters and filled them with tap water (10 liters). Cedrol (5ppm) was added in artificial oviposition site A (Treatment) as an oviposition attractant while site B had tap water only.

Sodium chloride (150g/l) was dissolved in both sites just 10 to 20 minutes before the beginning of experiment to facilitate the release of odour from artificial oviposition sites. The hut openings were closed using cotton wool. Laboratory reared adult gravid *A. gambiae* mosquitoes (*n*= 200) were released inside the hut at 1700 h and left for 2 hours. The closed hut windows were opened to allow mosquitoes resting in the box to freely fly out and access artificial oviposition sites within the screen house. OviART gravid mosquito trap developed by Dugassa (Dugassa *et al.*, 2013), was used to collect gravid mosquito attempting to land and lay eggs in the artificial oviposition site outside the hut (Plate 3.8b). The experiment was left to run till the following day at 0800 h. Mosquitoes found in the collection chamber of the OviART trap were put in the freezer for 20 minutes to die. Mosquitoes contaminated with dye were categorized as partial (when they have dye on wings and legs) and full (when they have dye on legs, wings and body).

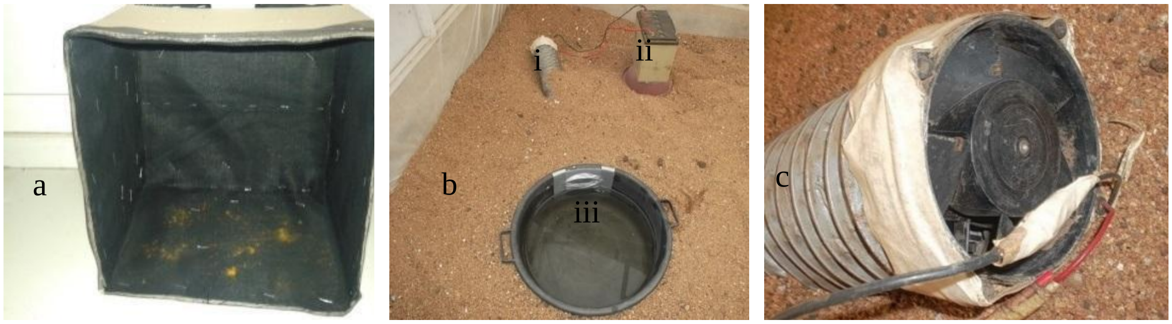

Plate 3.8. CB-2 a); OviART gravid trap set up in the screen house b); i=sucking fun, ii= battery, iii=basin with water. The fun that sucks mosquitoes c) ; (Dugassa *et al*., 2013).

3.5 Ethics statement

Ethical approval for this study was obtained from the Kenya Medical Research Institute's Ethical Review Committee (Protocol no. 422).

3.6 Data handling and analysis

Data entry and validation was done using MS-excel 2010 version. The attractiveness of four different colours was studied by displaying them on the hut mud walls and metallic frames and their values were compared by Post Hoc analysis using Turkey's test. The differences in total number of mosquitoes recapture from rectangular and circular box, contamination success of mosquitoes in the dissemination station (MRB) and transfer of larvicide to the artificial oviposition sites were determined by performing a one-way ANOVA, using SPSS version 20.0 with significance level of ≤ 0.05 for rejecting the null hypothesis. All values were expressed as mean ± standard error (SE).

CHAPTER FOUR

RESULTS

4.1. Experiment 1: Evaluating the visual acuity of mosquitoes to different colors shades

i) Resting preference when fabrics are anchored on the hut walls.

Table 4.1 Mosquito resting on fabrics anchored on the hut mud walls (HMWs) inside the semi field system

Fabric				**Attracted proportion (95% CI)**		
Colour	**Sum**	**Mean**	**N**	**(Mean ± SE)**	**SE**	**SD**
Red	337	28.08	12	28.08 ± 3.211	3.211	11.123
Black	331	28.00	12	28.00 ± 3.922	3.922	13.588
Blue	159	13.25	12	13.25 ± 2.168	2.168	7.509
White	56	4.67	12	4.67 ± 0.890	0.890	3.085

The mean resting proportion of mosquitoes across the coloured fabrics were as follows: Red cloth had (28.08 ± 3.211), black cloth had (28.00 ± 3.922), blue cloth had (13.25 ± 2.168), and white cloth had 4.67 ± 0.890. There was a significant difference between the mean number of resting mosquitoes across the four coloured-test fabrics (F= 16.811, df= 3**,** P = 0.000) with regard to preference as a resting site for malaria mosquito (Table 4.1).

Table 4.2 Multiple comparison of fabric colours mounted on the hut mud walls (HMWs) inside the SFS

Colour (t)	Colour (n)	MD (t-n)	Proportion recaptured	P-value
Fabric	**Fabric**	**Difference**	**(95% CI)**	**< 0.05**
Black (27.58)	Red (28.08)	-0.500	-0.500 (-8.46-7.46)	0.900
	Blue (13.25)	14.333*	14.333 (6.37-22.29)	0.001
	White (4.67)	22.917*	22.917(14.96-30.88)	0.000
Red (28.08)	Black (27.58)	0.500	0.500(-7.46-8.46)	0.900
	Blue (13.25)	14.833*	14.833 (6.87-22.79)	0.001
	White (4.67)	23.417*	23.417 (15.46-31.38)	0.000
Blue (13.25)	Black (27.58)	-14.333*	-14.333(-22.29- {-6.37})	0.001
	Red (28.08)	-14.833*	-14.833 (-22.79- {-6.87})	0.001
	White (4.67)	8.583*	8.583(0.62-16.54)	0.035
White (4.67)	Black (27.58)	-22.917*	-22.917(-30.88- {-14.96})	0.000
	Red (28.08)	-23.417*	-23.417(-31.38- {-15.46})	0.000
	Blue (13.25)	-8.583*	-8.583(-16.54- {-0.64})	0.035

(HMW)-Hut mud wall, MD-mean difference, CI-confidence interval, SFS-semi field system, t-first line of comparison, n-second line of comparison.

ii) Evaluating the effect of mud walls as an anchor for mosquito resting preference

A multiple comparison (Table 4.2) of all the test fabrics inside the hut indicates that black and red fabrics do not differ significantly (P= 0.900).

Table 4.3 Effect of the hut mud walls (MWs), labelled A-D when used as anchor for cotton fabrics on rotational basis to eliminate biasness.

Wall Side (z)	**Wall Side (x)**	**Difference MD(z-x)**	**mosquito recaptured (95% CI)**	**P-value < 0.05**
A (6.67)	B (20.83)	-14.167	-14.167(-24.18- {-4.15})	0.007
	C (24.83)	-18.167*	-18.167(-28.18- {-8.15})	0.001
	D (21.25)	-14.583*	-14.583(-24.60- {-4.57})	0.005
B (20.83)	A (6.67)	14.167*	14.167(4.15-24.18)	0.007
	C (24.83)	-4.000	-4.000(-14.01-6.01)	0.425
	D (21.25)	-.417	-.417(-10.43-9.60)	0.934
C (24.83)	A (6.67)	18.167*	18.167(8.15-28.18)	0.001
	B (20.83)	4.000	4.000(-6.01-14.01)	0.425
	D (21.25)	3.583	3.583(-6.43-13.60)	0.475
D (21.25)	A (6.67)	14.583*	14.583(4.57-24.60)	0.005
	B (20.83)	.417	.417(-9.60-10.43)	0.934
	C (24.83)	-3.583	-3.583(-13.60-6.43)	0.475

* ***The sides that differ significantly (p<0.05) in mean number of mosquito recaptured. z-first set of comparison, x-second set of comparison.***

A Post Hoc analysis using Tukey's test (Table 4.3) revealed that wall C attracted a mean proportion of (24.83±3.882), D(21.25±4.023), B(20.83±4.090) and A(6.67±1.183). The difference between the four hut walls was significant (*F*= 5.214, *df*= 3, *P*= 0.004) regarding their attractiveness to test mosquitoes, irrespective of the fabric colour displayed.

iii) Mosquito resting preference when fabrics were anchored on metallic frames, positioned at 1.65m apart.

Table 4.4 Mosquito resting preference when fabrics were anchored on metallic panels and positioned at a close proximity of 1.65 m apart inside a screen house.

Fabric	Mean	Recapture	Deviation		Recapture
Colour		(95%CI)	SD	SE	(%)
Black	23.88	23.88 ± 3.356	9.493	3.356	53.2%
Red	16.88	16.88 ± 2.263	6.402	2.263	37.6%
Blue	1.75	1.75 ± 0.773	2.188	0.773	3.9%
White	2.38	2.38 ± 0.885	2.504	0.885	5.3%

When frames were positioned at a distance of 1.65m apart, it revealed that the difference between colours was significant (F= 27.015, df= 3, P= 0.000), in which black colour had a mean recapture proportion of (23.88 ± 3.356), 53.2%, red (16.88 ± 2.263) 37.6%, blue (1.75 ± 0.773) 3.9% and white (2.38 ± 0.885), 5.3% (Table 4.4).

iv) Mosquito resting preference when coloured fabrics were anchored on metallic frames, positioned at 3.7m apart

Table 4.5 Mosquito Resting preference when colour fabrics were pinned on metallic panels, positioned at 3.7 m from each other.

		Recapture			**Recapture**
Cloth Colour	**Mean**	**(95% CI)**	**SD**	**SE**	**(%)**
Black	23.88	23.88 ± 2.302	6.512	2.302	43.4
Red	14.88	14.88 ± 2.191	6.198	2.191	35.6
Blue	7.43	7.63 ± 2.507	7.090	2.507	18.3
White	2.32	1.13 ± 0.350	0.991	0.350	2.7

When frames were moved far from the releasing center (3.7m), the difference between colours was significant (F= 14.006, df= 3, P= 0.000). Black colour recaptured a mean proportion of (18.13 ± 2.302) 43.4%, red (14.88 ± 2.191) 35.6%, blue (7.63 ± 2.507) 18.3%, white (1.13 ± 0.350) 2.7% of test mosquitoes (Table 4.5).

v) Effectiveness of the three (*n*= 3) fabric harness systems (FHS) used to study mosquito resting behavior.

Table 4.6 Compare resting preference when fabrics were anchored on hut wall, metallic frames positioned at 1.65m and 3.7m apart.

Display		**Recapture proportion**			**Percentage**
FHS	**Mean**	**(95% CI)**	**SD**	**SE**	**(%)**
Hut mud wall	220.75	220.75 ± 68.693	137.386	68.693	57.6
Frames (1.65m apart)	89.75	89.75 ± 43.820	87.641	43.820	23.4
Frames (3.7m apart)	72.75	72.75 ± 34.666	69.332	34.666	19.0

FHS=fabric harness system

In the comparisons of FHSs (Table 4.6), hut experiment had a recapture mean proportion of (220.75 ± 68.693), frame 1.65m (89.75± 43.820) and frame 3.7m (72.75± 34.666). However, there was no significant difference between the fabric harness systems (FHS) used (F= 2.510, df= 2, P= 0.136).

4.2 Experiment 2: Optimizing the size of mosquito resting box (MRB).

Table 4.7 Mosquito recapture using Pers-box design (PB-1) placed inside an open screen house.

Mosquitoes RL	Box position	Mean	Recapture (95%CI)	SD	SE
200	Site A	8.00	8.00 ± 1.000	1.414	1.000
200	Site B	6.50	6.50 ± 0.500	0.707	0.500
200	Site C	11.50	11.50 ± 0.500	0.707	0.500
200	Site D	6.50	6.50 ± 1.500	2.121	1.500

RL-number of adult mosquitoes released, SD-standard deviations, SE-Standard error, CI-confidence interval.

During experiment, the PB-1 design (Plate 3.4) recaptured 4% of released mosquitoes with proportion in site A(8.00 ± 1.000) site B(6.50 ± 0.500), site C(11.50 ± 0.500) and site D(6.50 ± 1.500) as showed in Table 4.7.

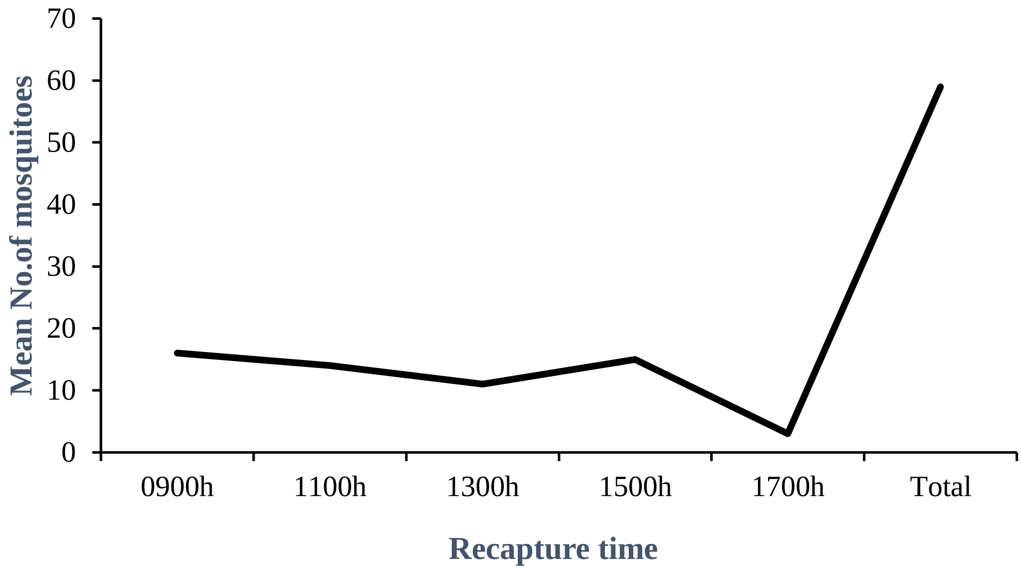

Figure 4.1 Mosquitoes recapture trend from 0900 h to 1700 h in a big box (CB-1a) of dimensions 45cm×30cm×45cm, having a narrow opened entry.

A big box design, having a narrow open entry (45cm ×22cm) as mosquito entry point, recaptured a proportion of (4.92 ± 0.398).This was approximately twice less compared CB-1b (17.58 ± 1.699) design (Figure 4.1).

Table 4.8 Mosquito resting behavior using CB-2 design (45cm×30cm×45cm) having an enlarged entry point (45cm×45cm), recaptured at different time interval.

Recapture		Recapture proportion		Recapture
Time	**Mean**	**(95%CI)**	**SD**	**(%)**
0700h-0900h	47.33	47.33 ± 3.673	12.723	63.7%
0900h-1100h	11.33	11.33 ± 1.010	3.499	15.2%
1100h-1300h	7.67	7.67 ± 0.732	2.535	10.3%
1300h-1500h	4.92	4.92 ± 0.712	2.466	6.6%
1500h-1700h	3.08	3.08 ± 0.802	2.778	4.1%

A big carton box (45cmL×30cmL×45cmH) was constructed, in which the entire side was opened for mosquito entry (45cm×45cm) as shown in (Plate 3.8a). In this experiment, the resting preference increased drastically with a proportion of (74.25 ± 4.406). This was far much compared to PB-1 (8.13 ± 0.854), CB-1b (17.58 ± 1.699) and CB-1a (4.92 ± 0.398).Collection variations in different time intervals are displayed in Table 4.8.

Table 4.9. Compare small entry (45cm×22cm) and large entry sizes (45cm×45cm) of CB-2 design box, dimension 45cm×30cm×45cm.

Box (45×30×45cm)		proportion			Recapture
Entry space	**Mean**	**(95%CI)**	**SD**	**SE**	**(%)**
Small entry size (45cm×22cm)	4.92	4.92 ± 0.398	1.379	0.398	6%
Larger entry size (45cm×45cm)	74.25	74.25 ± 4.406	15.262	4.406	94%

This experiment showed that the number of mosquitoes entering the resting box is significantly dependent on the space created for their entry into the MRB (F= 245.644, df= 1, P= 0 .000).Small entry size had a proportion of (4.92 ± 0.398) and large entry a proportion of (74.25 ± 4.406) as shown in Table 4.9.

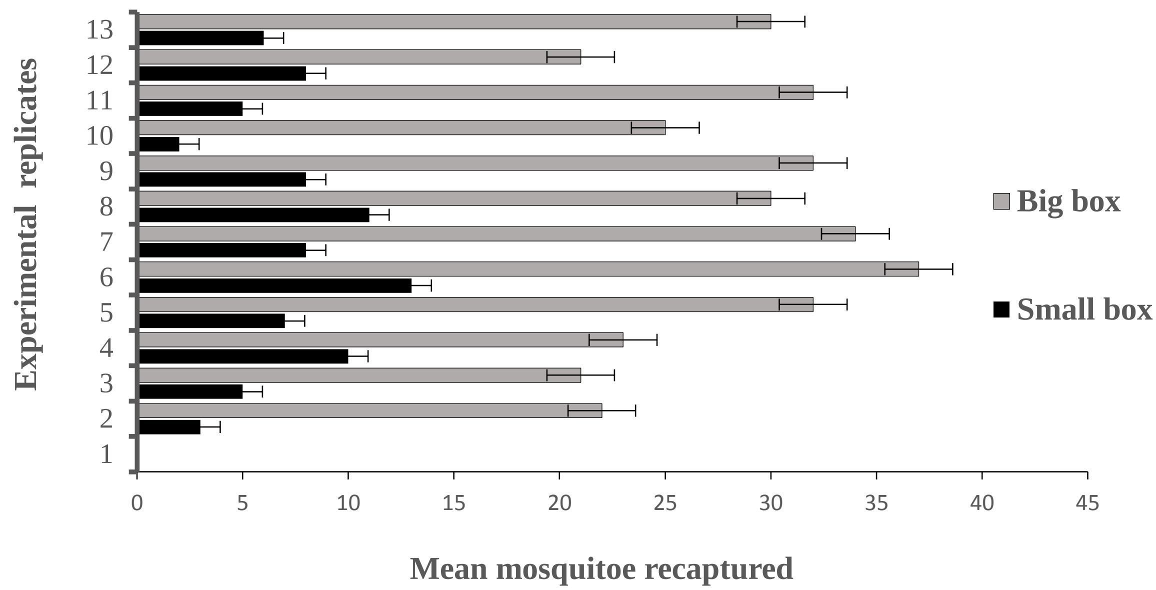

Figure 4.2. Effect of setting up small and big box with one sides fully opened, inside the experimental hut.

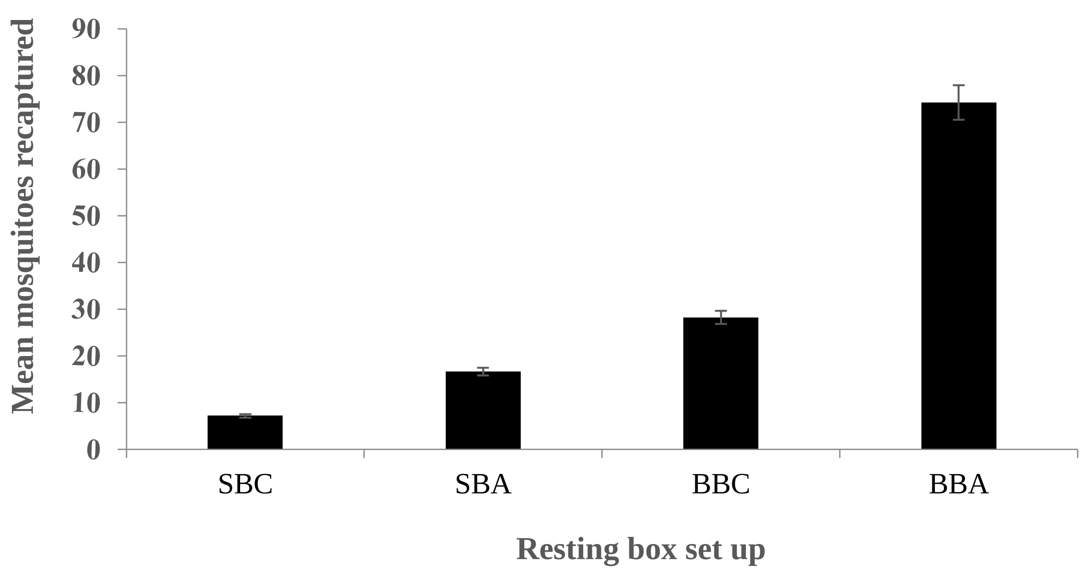

Figure 4.3 Resting boxes efficacy when set alone and when set together in the experimental hut.

(SBC-Small box combined, SBA- small box alone, BBC- Big box combined, BBA- big box alone)

Comparison experiments of small and big resting boxes (Figure 4.2 and 4.3) showed a recapture proportion of (28.25 ± 1.606) in big box and (7.17 ± 0.928) in small box.

Table 4.10 Mosquitoes resting preference in CB-3 of dimensions 94cmLx 36cmW x 48cmH, aligned with Black fabric.

Recapture time	Mean	Recapture proportion (95%CI)	SD	SE	Recapture (%)
0700h-0900h	40.08	40.08± 2.414	8.361	2.414	47.5 %
0900h-1100h	25.75	25.75 ± 1.789	6.196	1.789	30.5%
1100h-1300h	12.67	12.67 ± 0.980	3.393	0.980	15.0 %
1300h-1500h	5.00	5.00± 0.615	2.132	0.615	5.9 %
1500h-1700h	0.83	0.83 ± 0.207	0.718	0.207	1.0 %

Black lining box analysis recaptured a proportion of (84.33 ± 3.401). The differences between the recapture times were significant (F= 124.150, df= 4, P= 0.000) with high mosquito recapture at 0900 h (40.08± 2.414) 47%, 1100 h (25.75 ± 1.789) 31%, 1300 h (12.67 ± 0.980) 15%, 1500 h (5.00± 0.615) 6% and 1700 h (0.83 ± 0.207) 1% as shown in Table 4.10.

Table 4.11 Mosquitoes resting preference in CB-3 of dimensions 94cmLx 36cmW x 48cmH, aligned with Red fabric.

Recapture time	Mean	Recapture proportion (95%CI)	SD	Recapture (%)
0700h-0900h	29.33	29.33± 2.046	7.088	54.2 %
0900h-1100h	8.92	8.92 ± 0.848	2.937	16.5 %
1100h-1300h	7.67	7.67 ± 1.373	4.755	14.2 %
1300h-1500h	5.92	5.92± 1.011	3.502	10.9 %
1500h-1700h	2.25	2.25 ± 0.429	1.485	4.2 %

Resting box aligned with Red fabric recaptured a proportion of (54.08 ± 3.671). It revealed a significant difference in mosquito collection time (*F*= 70.945, *df*= 4, *P*= 0.000). The 0900 h recapture proportion was (29.33± 2.046) 54.2%, 1100 h (8.92 ± 0.848) 16.5%, 1300 h (7.67 ± 1.373) 14.2%, 1500 h (5.92± 1.011) 10.9% and 1700 h (2.25 ± 0.429) 4.2%. The collection mean decreases from 0900 h towards 1700 h (Table 4.11).

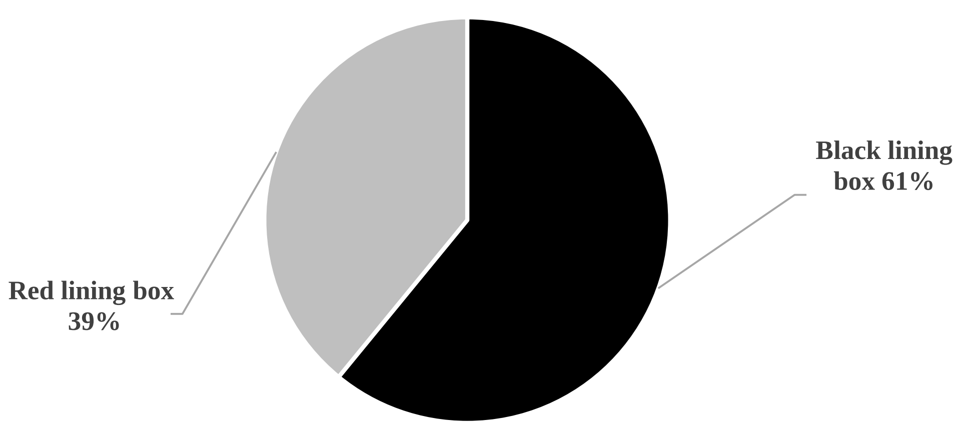

Figure 4.4 Compare red and black lining boxes for mosquito resting preference

In the analysis of black and red boxes of 94cmx 36cm x 48cm (Figure 4.4), black lining box recaptured 61 % (84.33 ± 3.401) of the released mosquitoes (*n*= 200) and red lining box 39 % (54.08 ± 3.671).

Black lining box collected almost twice as much as the red lining box. The colour used significantly affected the attraction of the box (*F* = 36.541, *df* = 1, *P*= 0.000).

Table 4.12. Compare resting preference in big box, small box and large box.

Design		**Proportion**		**Recap**
Box size	**Mean**	**(95%CI)**	**SD**	**(%)**
Big box (BRB-45×30×45)	74.25	74.25 ± 4.406	15.262	61 %
Large box (LRB-94x 36 x 48)	84.33	84.33 ± 3.401	11.781	69 %
Small box (SRB-30×24×25)	17.58	17.58 ± 1.699	5.885	15 %

BRB-big resting box, LRB-large resting box, SRB-small resting box

Approximately 10% of experimental mosquitoes were recaptured in a small box,42% in big box and 48% in larger box (Table 4.12).

Plate 4.1. Optimized mosquito resting box (MRB).

Cardboard box two (CB-2) of size 45cm × 30cm × 45cm, lined with black cotton cloth, with an entry point of 45cm × 45cm, having a recapture of (74.25 ± 4.406), is the mosquito resting box of choice in this study (Plate 4.1).

4.3 Experiment 3: Optimizing the box shape preferred by gravid mosquitoes for resting.

Table 4.13. Mosquito recaptured when using a circular resting box design in a semi field system (SFS).

Time Recapture	Mean	N	Sum	Proportion (95%CI)	SD	Recapture (%)
0700h-0900h	25.83	12	310	25.83 ± 5.056	17.513	49 %
0900h-1100h	13.75	12	165	13.75 ± 1.508	5.225	26 %
1100h-1300h	8.17	12	98	8.17 ± 1.086	3.762	16 %
1300h-1500h	3.75	12	45	3.75 ± 0.946	3.279	7 %
1500h-1700h	1.25	12	15	1.27 ± 0.179	0.622	2 %

In circular box design, the differences between the recapture time is significant (*F*= 15.958, *df*= 4, *P*= 0.000). In the morning hours (0700 h-0900 h) about 50% of mosquitoes were found resting in the box. This number reduces towards evening, up to around 2%. The circular box has a mosquito recapture proportion of (51.0 ± 3.947) accounting for 41% of the released mosquitoes (Table 4.13).

Table 4.14 Mosquito recaptured in a rectangular resting box design under semi field system (SFS)

Recapture time	Mean	N	Sum	proportion (95%CI)	SD	Recapture (%)
0700h-0900h	47.33	12	568	47.33 ± 3.673	12.723	64%
0900h-1100h	11.33	12	136	11.33 ± 1.010	3.499	15%
1100h-1300h	7.67	12	92	7.67 ± 0.732	2.535	10%
1300h-1500h	4.92	12	59	4.92 ± 0.712	2.466	7%
1500h-1700h	3.00	12	36	3.00 ± 0.826	2.860	4%

There is significant difference in the mosquito recapture time (*F*= 104.581, *df*= 4, *P*= 0.000). The first two hours in the morning account for 64% of the released mosquitoes. The number reduces as the day progress up to 4% (Table 4.14). The deviations are close from 1100 h to 1700 h (2.535, 2.466, 2.86 showing a consistent trend. Rectangular box had a mean proportion of (74.25 ± 4.406), approximately 59 %.

Table 4.15 Compare mosquito resting preferences in circular and rectangular box designs.

Box				Proportion			Recapture
Shape	**Sum**	**Mean**	**N**	**95%CI**	**SE**	**SD**	**(%)**
Rectangular	891	71.0	12	74.0 ± 4.406	4.406	15.262	59
Circular	617	51.0	12	51.0 ± 3.947	3.947	13.675	41

There was significant difference in resting preference of mosquitoes between rectangular box and circular box designs (F= 14.899, df= 1, P= 0.001). When the two box designs are compared (Table 4.1), rectangular design recaptured a proportion of (74.0 ± 4.406), accounting for approximately 60% of mosquitoes. Circular box recaptured a proportion of (51.0 ± 3.947) which is about 40% of released mosquitoes.

4.4 Experiment 4: Assessing contamination success of mosquitoes in the dissemination station (MRB) and transfer of larvicide (dye) to the artificial oviposition sites.

Table 4.16 Successful transfer of Dye by mosquitoes from the resting box to the artificial oviposition site.

Design					Proportion Oviposition			Recapture
	Site	**Sum**	**Mean**	**N**	**(95%CI)**	**SE**	**SD**	**(%)**
Treatment	A	696	58	12	58(50.49-65.52)	3.411	11.817	62
Control	B	430	36	12	36(29.05-42.62)	3.082	10.676	38

There was significant difference between Cedrol treated tap water site and untreated tap water oviposition site (*F*= 23.250, *df*= 1, *P*= 0.00). In this experiment, the treatment site (A) recaptured a proportion of 58(95% CI 50.49 - 65.51) of mosquitoes, accounting for 62% (Table 4.16). Untreated tap water site (B) accounted for 36(95% CI 29.05-42.62) of mosquitoes which constitutes 38%.

Efficacy of dust treated box in delivery of dye to the resting adult female mosquitos.

a) Treatment site

Table 4.17 Successful transfer of Dye from the treated box to the resting mosquitoes.

Design				**Proportion Oviposition**			**Recapture**
	Sum	**Mean**	**N**	**(95%CI)**	**SE**	**SD**	**(%)**
Partial dye	232	19.0	12	19(14.20-24.46)	2.330	8.072	33 %
Full dye	464	39.0	12	39(33.55-43.79)	2.327	8.060	67 %

Partial Dye: Dust sports on legs and wings. Full dye: Accumulation of dust on legs, wings and whole body.

The mosquito proportion having partial dye was 19(95% CI 14.20-24.46), this constitute 33% of mosquitoes visiting the treatment artificial oviposition site, while mosquitoes with full dye constitute a proportion of 39(95% CI 33.55-43.79), accounting for 67% of mosquitoes visiting the treatment site (Table 4.17). The difference between partial and full dye adsorption was significant (*F*= 34.470 *df*= 1, *P*= 0.000).

b) Control site

Table 4.18 Effectiveness of Treated box in releasing dust to the resting female mosquitos.

Design				**Proportion**			**Recapture**
	Sum	**Mean**	**N**	**(95%CI)**	**SE**	**SD**	**(%)**
Partial dye	151	13.0	12	13(8.14-17.02)	2.017	6.968	35
Full dye	279	23.0	12	23(17.99-28.51)	2.390	8.281	65

At the control oviposition site, a proportion of 13(95% CI 8.14-17.02) test mosquitoes recaptured had partial dye, proportionately 35%. Full dye constitutes 23(95% CI 17.99-28.51) which is 65% (Table 4.18).The difference between partial dye body contamination and full dye body contamination is significant (F= 11.632, df= 1, P= 0.003).

CHAPTER FIVE

DISCUSSION

5.1 Discussion

A resting box technology for attract and kill mechanism has been proposed as a potential new tool for mosquito control, especially outdoors (Pombi *et al.*,2014; Mmbando *et al.*, 2015). The current study was conducted under controlled conditions inside large semi-field structure to demonstrate the optimization of a passive resting box and its potential effectiveness in horizontal transfer of non-toxic fluorescent dye (Proxy for lavicide) to an artificial oviposition site by mosquitoes. The current study was designed first to explore the potential modulation of resting-site seeking behavior by colour, especially during early morning and late afternoon within the 12 hours of the day. Secondly, upon establishing the most attractive colour, the study wished to optimize a resting box size and shape conducive for a gravid mosquito. Since the broad aim was to enhance auto-dissemination mechanism, the last task was to demonstrate the possibility of auto-transfer of a non-toxic dye from the resting box to a planted larval site. The approach of attract and kill mechanism of disease vector has been used especially against other species of mosquito (Devine and Killeen., 2010), but less has been done on *Anopheles gambiae* mosquitoes. The study hypothesized that dull colour would appeal to a resting site-seeking gravid mosquito than bright colour. The current study presents additional evidence that it is possible to lure malaria mosquitoes to a preferred structure, contaminate them and transfer the contaminant to an oviposition site to continue killing the immature vector stages. In this study, the optimization of the four fabric colours were conducted in a hut and outside the hut. In hut bioassay, red and black fabric had high and equal mosquito recapture then blue and white.

In the process of analyzing fabric colours in the hut, particular wall sides appeared to be consistent in their high attraction to resting mosquitoes all through, irrespective of the fabric colour mounted. Wall C generally attracted high proportion of mosquitoes, followed by D, then B and lastly A. However, the influence of the wall side to mosquito was not pursued further though it would be of interest and may require more analysis.

Moreover, when fabrics were further analyzed by pinning on metallic frames and positioned at 1.65m apart, black colour appealed to mosquitoes (53%), then red at 38%, blue at 4% and white at 5%. Similar event was observed after moving frames 3.7m apart. Though, black fabric preference reduced to 43%, red 36%, white 3%, and blue increased to 18%. This experiment revealed that mosquitoes perceive colour more clearly when it is closer and tend to lose that ability as it moves away from the dissemination station.

This concept expands the idea that mosquitoes can recognize an object in their host seeking response (Burkot *et al.,* 2013; Hawkes *et al.,* 2016). It therefore implies that for more mosquitoes to get into the resting box, the MRB should be positioned closer, approximately 1m to 3m from the potential hiding zone or even from a potential host, especially when used indoors. However, this distance does not conflict the findings that approximate the maximum flight distance of mosquitoes, estimated between 50m to 50km because 1-3m is within that estimated (Verdonschot *et al*., 2014), also still within the flight range reported on post-blood meal flight distance experiment estimated at 106.7m (Jacob *et al*., 2012).

In an effort to understand the response of gravid mosquito to colour, the study examined the three approaches evaluated for colour preference.

There was high response in hut experiment, estimated at 58%, 23% was recorded when frames were positioned at 1.65m apart and 19% at 3.7m apart. However, in all cases observed, black colour attracted 60% of experimental mosquitoes followed by red at 40%.

Black fabric was therefore identified to be the most preferred colour by gravid mosquitoes and therefore fit for designing a mosquito resting box. This is in agreement with the report showing black cloth as a suitable surface that encourage mosquito landing and also effectively hold larvicide (Kimani *et al.,* 2006; Scholte *et al.,* 2005).

In the second experiment, the focus was on optimization of the mosquito resting box size. This experiment was based on the premise that mosquitoes need a large surface area for their movement into the constructed structure. The first design reflected Mmbando *et al*., 2012 prototype, but it had a capsule inside and five louvers at both four corners to let in as many mosquitoes as possible with minimal restriction. However, this design recaptured 4% of the released mosquitoes, and this was considered to be too low. In the second study, small box was constructed using a cardboard box (CB), a small entry point was created at one of the side. The design recaptured 10% of mosquitoes and this was almost twice as much compared to 4% recorded in the first design.

When entry size was enlarged using larger box construction, the box drastically increased the number of mosquito entering inside to 94%. This was a breakthrough in the pursuit for box size optimization. In the research design, the released mosquitoes were recaptured at an interval of 2 hours, as from 0700 h to 1500 h. It was evidenced that a high number of recaptures was possible between 0700 h to 1100 h, and the proportion decreased towards 1500 h .This trend was evidenced across all experimental days.

Although it could be argued that the drop was caused by the reducing number of mosquitoes in the SFS, but the practical idea was an observed scenario where the insects became more sluggish and tend to avoid continuous and active flight as time advanced in the day.

At some point, small and big boxes were set and tested together in the hut. The number of mosquitoes resting in each box dropped compared to when the similar boxes are set individually in the hut. This phenomenon probably explains a fact that when one box is offered in the hut for resting, more mosquito converges in it, but if more options are offered then mosquitoes randomly choose to rest in either of them, reducing the numbers that would have concentrated in one box, so this effect is a distribution factor.

A relatively larger carton box construction (CB-3) was tested, it increased mosquito entry into a box by 69% .In this study, it is clearly evidenced that size of the box has an effect in the resting preference of mosquito, because mosquito recapture tend to increase with increase in box size. In a close comparison, large box construction recaptured 69%, big box 61% and small box 15%. So far it is evidenced that an increase in the box size also increase the proportion of resting mosquitoes. The third experiment was conducted to optimize the box shape. Rectangular and circular shapes were designed, studied and extensively compared, simply because these are the dominant shapes in every environment. Circular box (CB-4) recaptured approximately 40% and rectangular shape 60% of the test mosquitoes, this is when both boxes were set together in the hut. The recapture was observed to be high in the morning and low in the afternoon. This variation in resting behavior of mosquito with respect to time, provide vital information that emphasizes on the appropriateness of setting the MRB in the morning, in order to maximize its resting potential. It also gives a general guide to any survey on mosquito behavior, more so the resting pattern.

This evidence moreover emphasizes that morning sampling of mosquito, would provide more success that in the afternoon. The difference observed between the two shapes was significant (P= 0.001). Meaning the choice made by mosquito to rest in a given box shape was not by chance but by design. Shape therefore is evidenced to affect the decision of mosquitoes when seeking for a resting station. So far, it is clear that most mosquitoes seem to prefer rectangular shape than a circular. This concur with the other authors in which most of their box designs were rectangular (Okumu *et al*.,2010;Pombi *et al*.,2014; Mmbando *et al.,* 2015).However, the reason for this preference is not known.

The possibility of gravid mosquito picking substances from the dissemination station to the larval habitat was demonstrated using non-toxic fluorescent dye as a proxy for larvicide. The intention was to demonstrate the possibility of a resting mosquitoes in the MRB picking the dye, fly out of the resting box, escape from the hut and visit an artificial oviposition site. This possibility is restricted by a sticky resting box that prevent resting mosquitoes from escaping out of the box to the larval site (Pombi *et al.*, 2014).

Generally, Cedrol (Oviposition attractant) treated oviposition site received 60% of site visiting mosquitoes and control (tap water only) received about 40%. More mosquitoes visited Cedrol treated site compared to untreated water. This significant level of Cedrol attraction, concur with the result obtained by Okal *et al.,* 2015.At the treatment site, 67% of recaptured mosquitoes were observed to have full dye and 33% had partial body dust contamination (Table 4.16). Similarly, 65% of mosquitoes had full dye and 35% partial dye at the control site (Table 4.17). More mosquitoes in this experiment were observed to carry full dye on their bodies. This is an indication of successful transfer of dye from the box to the resting insect and transfer of dust by insect to the oviposition site. Moreover, this indicates that a significant quantity of dye can be transferred by mosquito to the larval habitat and it begins to answer the question of the quantity of dust a mosquito is able to transfer in an auto-dissemination system.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This is the first study to investigate the potential for using a passive non-powered auto dissemination mechanism for the control of *An. gambiae s.s,* one of the most efficient African malaria vectors. The results are promising and indicate that this approach offers an opportunity to be considered in future malaria control strategies in Sub-Saharan Africa. The study identifies that dull colour (black) attract more adult blood fed and gravid *An. gambiae* mosquitoes, and are therefore recommended for use in a MRB. The *An. gambiae* mosquitoes visually perceive colour more clearly when it is closer than when it is at a distance of more than 3m.

A resting box with one side fully opened provides an excellent opportunity to allow more mosquitoes inside the box. Small entry route has a tendency of restricting mosquito movement; hence mosquitoes seeking a place to rest are not optimally attracted to such structures. Moreover, the bigger the box, the better for effectiveness of a MRB, but actual design should leverage on the aspects of assembly and cost of material and surface treatments. However, concentrating many resting boxes in one place encourages the spread effect that leads to dispersion of resting mosquitoes in boxes.

Mosquitoes prefer to rest in the morning hours than afternoon, probably due to their tendency to exit households in search of oviposition sites outside in the evening hours. It therefore means we expect to find more mosquitoes resting in the box in the morning and less at noon time, a duration that must be optimized for contamination of resting adults.

The shape of a mosquito resting box is an important factor for a resting site. Majority of adult female gravid and blood fed *An. gambiae s.s* prefer resting in a rectangular shaped resting box than in a circular resting box of similar dimensions. Such preference to rectangular shape was observed but the reason for preference is not clearly understood.

The ultimate idea of developing auto-dissemination system is to facilitate the transfer of IGRs from a resting station to the oviposition site. The effectiveness of designed MRB is actualized if resting mosquitoes can pick chemicals and move with it to the oviposition site, a phenomenon that was demonstrated using a red fluorescent dye as a proxy for chemical formulations. Mosquitoes successfully picked dye from the box and transferred them to the artificial oviposition site. Most mosquitoes had their legs, wings and body contaminated with the dye, a factor suggesting a possibility of achieving a contamination threshold and potentially lethal dose of the chemical.

6.2 Recommendations

The study recommends the use of black coloured fabric for constructing a resting box. The optimized box size for use should be 45cm×30cm×45cm and one of the side be fully opened, and it is should rectangular in shape. More studies are recommend on vector behavior to further evaluate some few key areas such as: i) The effectiveness identified in the semi-field system experiments should be demonstrated in real field conditions using a candidate larvicide and quantify the reduction of mosquitoes and the impact on disease transmission, ii) the quantitative study is required to determine the quantity of chemicals needed to constitute a lethal dose in a natural habitat and relate it to the number of mosquitoes visiting the oviposition site and iii) The impact of this technology on non-target organisms found in mosquito natural habitat.

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APPENDICES

APPENDICES I: Ethical approval, from KEMRI Ethical Review Committee, Protocol no. 422